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Lobeline attenuates ethanol abstinence-induced depression-like behavior in mice

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ABSTRACT

Evidence indicates that the brain nicotinic acetylcholine receptor (nAChRs) ligand lobeline reduces depression-like behaviors, ethanol drinking, and nicotine withdrawal-induced depression-like behaviors. The purpose of the present study was to determine the effects of lobeline on ethanol abstinence-induced depression-like behavior and associated neuroadaptive changes in mice. Adult C57BL/6J male mice were allowed to drink 10% ethanol for 4 weeks using a two-bottle choice procedure. Mice were tested after 24 h and 14 days of ethanol abstinence in a forced swim test (FST), a measure for depression-like behavior. Acute lobeline treatment (1 mg/kg) significantly reduced immobility time compared to controls after 24 h and 14 days of abstinence. In addition, abstinence from chronic ethanol exposure reduced serotonin levels in the hippocampus, which was reversed by acute lobeline treatment. Repeated lobeline treatment (1 mg/kg, once daily) for 14 days during ethanol abstinence also significantly reduced FST immobility in mice exposed to ethanol. Chronic ethanol exposure significantly reduced the number of 5-bromo 2′-deoxyuridine (BrdU)-positive cells in the dentate gyrus of the hippocampus, indicating decreased hippocampal cell proliferation. Abstinence from chronic ethanol exposure also decreased brain-derived neurotrophic factor (BDNF) in the dentate gyrus and CA3 region of the hippocampus. In contrast, repeated lobeline treatment significantly increased both BrdU- and BDNF-positive cells. Taken together, our results indicate that lobeline produced antidepressant-like effects, likely by targeting brain β2-containing nAChRs, serotonergic neurotransmission, and/or hippocampal cell proliferation. Therefore, lobeline may have therapeutic utility to treat alcohol abstinence-induced depression.

Introduction

Epidemiological studies have shown that alcoholism and depression commonly occur together (Sullivan, Fiellin, & O'Connor, 2005). Alcohol- (ethanol-) dependent individuals have a high prevalence of depression (Kessler et al., 1996). Prolonged exposure and subsequent abstinence from ethanol have been shown to induce depression in humans (Schuckit et al., 1997).

Brain nicotinic acetylcholine receptors (nAChRs) have been implicated in ethanol or nicotine addiction and co-morbid psychiatric disorders, including depression (Rahman, 2015; Rahman, Engleman, & Bell, 2016). Evidence suggests that lobeline is a non-selective antagonist with high affinity for α4β2 and α3β2 nAChRs (Dwoskin & Crooks, 2002; Parker, Beck, & Luetje, 1998). Furthermore, lobeline crosses the blood-brain barrier and decreases voluntary ethanol intake in rats (Bell, Eiler, Cook, & Rahman, 2009) and mice (Sajja, Dwivedi, & Rahman, 2010; Sajja & Rahman, 2011, 2012). We have previously demonstrated that the nAChR ligand lobeline has antidepressant-like properties in mice (Roni & Rahman, 2011, 2013, 2015a, 2015b). In addition, we have found that lobeline reduces nicotine withdrawal-induced depression-like behaviors in mice (Roni & Rahman, 2014). While our previous studies determined the effects of nAChR mediated signaling in depression-like behavior or ethanol-drinking behavior, the effects of nAChR signaling on ethanol abstinence-induced depression-like behavior and associated neuroadaptive changes remain unknown.

Depression and alcoholism seem to share similar neuroadaptive changes in the brain. A dysregulation in the serotonergic system

Abbreviations: ACh, acetylcholine; BDNF, brain-derived neurotrophic factor; BrdU, 5-bromo 2′-deoxyuridine; FST, forced swim test; nAChR, nicotinic acetylcholine receptor; PFC, prefrontal cortex; SVZ, subventricular zone.

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has been implicated in the development of depression (Ressler & Nemeroff, 2000) and subpopulations of alcoholic patients (Davis, 2008). Chronic ethanol is suspected to influence the occurrence of depressive mood symptoms during abstinence, as serotonin deficiency is also believed to be associated with depression (De Witte, Pinto, Ansseau, & Verbanck, 2003). *In vivo* animal studies have revealed that ethanol dependence can lead to a deficiency in the release of serotonin in different brain regions (Uzbay, Usanmaz, & Akarsu, 2000).

At the molecular level, brain-derived neurotrophic factor (BDNF) has been associated with depression and alcoholism. Human studies have shown significant reductions in peripheral levels of BDNF in patients suffering from depression (Karege et al., 2005; Lee, Kim, Park, & Kim, 2007) and ethanol dependence (Joe et al., 2007). Dysregulation in BDNF signaling affects neurogenesis, neuronal survival, and development. Imaging studies revealed reductions in hippocampal volume of both depressed and ethanol-dependent patients (Agartz, Momenan, Rawlings, Kerich, & Hommer, 1999; Gerritsen et al., 2011). Chronic ethanol exposure-induced decrease in BDNF expression in rat hippocampus has been associated with depression-like behavior (Hauser, Getachew, Taylor, & Tizabi, 2011). Likewise, mice abstinent from chronic ethanol have significantly less hippocampal neurogenesis, which is reversed by chronic desipramine, a classic antidepressant treatment (Stevenson et al., 2009). However, the neurobiological and behavioral changes that occur during protracted abstinence following chronic voluntary ethanol drinking are still less understood.

Based on our previous studies and published reports, we have hypothesized that lobeline will decrease ethanol abstinence-induced depression-like behavior and associated neuroadaptive changes. Therefore, the purpose of the present study was to determine the effects of lobeline on depression-like behavior in mice abstinent from chronic voluntary ethanol drinking. We have also measured the effects of lobeline on serotonin levels in the hippocampus. Finally, the effects of lobeline on ethanol abstinence-induced BDNF expression and cell proliferation in the hippocampus were determined.

Materials and methods

Animals

Male C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, ME). The animals were housed in individual Plexiglas® cages, under standard laboratory conditions (21 ± 2 °C, relative humidity 50–60%) and maintained on a 12-h light/dark cycle. Mice had free access to food and water. Mice were 10–12 weeks of age at the start of the experiment. All procedures were in compliance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at South Dakota State University.

Drugs and chemicals

Lobeline hydrochloride and 5-bromo-2′-deoxyuridine (BrdU) were purchased from Sigma-Aldrich (St. Louis, MO). Lobeline (1 or 4 mg/kg, subcutaneous) and BrdU (150 mg/kg, intraperitoneal) were dissolved in saline before injection in a volume of 0.01 mL/g body weight of animal. Doses, expressed as the salt form of the drugs, were selected based on previous studies (Mandyam, Harburg, & Eisch, 2007; Roni & Rahman, 2013). Ethanol drinking solution (10% v/v) was prepared daily by diluting ethanol (190 proof, Fisher Scientific, Waltham, MA) with tap water.

Experimental procedures

Mice were randomly assigned to two experimental groups: ethanol-drinking mice and water-only controls. Ethanol-drinking mice were allowed to voluntarily consume ethanol (10% v/v) or water for 28 days using a two-bottle drinking procedure (Stevenson et al., 2009). One bottle of ethanol and another bottle of tap water were provided in each cage through 15-mL plastic centrifuge bottles with stainless-steel sipper tubes. Mice were weighed and the fluid levels in the bottles were recorded to the nearest 0.1 mL to determine daily fluid intake. The position of the bottles was exchanged every other day to avoid side preferences. Ethanol-drinking mice were assigned to treatment groups such that mean ethanol intake was balanced across groups. Water-only controls had access to two bottles of water throughout the experiment. Ethanol solution-containing bottles were removed from the cages after 28 days of drinking. In the first experiment, we examined the effects of lobeline on ethanol abstinence-induced depression-like behavior and brain serotonin. Ethanol-drinking mice received saline or lobeline treatment before the FST during 1 or 14 days of abstinence (Fig. 1A). Mice in the water-only group received a saline injection before the FST. Twenty-four hours after the FST, mice again received saline or lobeline treatment and brains were harvested for serotonin assay as described below.

In the second experiment, we examined the effects of chronic lobeline treatment (14 days) on ethanol abstinence-induced depression-like behavior, BDNF expression, and hippocampal cell proliferation. Mice were divided into an ethanol-abstinent group and a water-only control group as discussed above. Ethanol-abstinent mice received once-daily injections of saline or lobeline during a 14-day abstinence period (Fig. 1B). The first injection was given 24 h after removal of ethanol bottles. The water-only group received daily injections of saline in parallel to the abstinent mice. The FST was performed on the last day of chronic treatment (5 h after the last injection). Mice were sacrificed 24 h and 26 h after the FST for BDNF and BrdU immunohistochemistry, respectively.

Forced swim test

The forced swim test (FST) is a widely used behavioral test to assess efficacy of antidepressants in rodents. The FST was conducted during the lights-on period of the daily light/dark cycle. Mice were placed individually in a cylindrical Plexiglas® tank (45 cm high × 20 cm diameter), which was filled with 25 cm of water (20–22 °C). The time spent immobile during the 6-min FST was recorded as described previously (Stevenson et al., 2009). Immobility was counted when no additional activities were observed other than those required to keep the head above water. Mice were removed from the cylinder immediately after the test, dried with paper towels, and kept under a heating lamp until completely dry before returning to their home cages.

Serotonin assay

The serotonin assay was performed as described previously (Roni & Rahman, 2013; Sajja et al., 2010). Twenty minutes after saline or lobeline treatment, mice were sacrificed by rapid decapitation. Prefrontal cortex (PFC) and hippocampus were dissected from 1-mm coronal sections using Allen brain atlas and mouse brain stereotaxic coordinates (Franklin & Paxinos, 2007). Some samples were discarded due to insufficient amounts of collected tissue, resulting in 4–5 samples/group. Samples were stored at −80 °C until analysis. Upon assay, tissues were diluted with 0.1 N perchloric acid (1:10 as g/mL); samples were homogenized and centrifuged (14,000 ×*g* for 30 min at 4 °C). Resulting supernatants