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mGlu5-dependent modulation of anxiety during early withdrawal from binge-drinking in adult and adolescent male mice

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ABSTRACT

Binge alcohol-drinking elicits symptoms of negative affect such as anxiety upon cessation, which is a source of negative reinforcement for perpetuating this pattern of alcohol abuse. Binge-induced anxiety during early (24 h) withdrawal is associated with increased expression of metabotropic glutamate receptor 5 (mGlu5) within the nucleus accumbens shell (AcbSh) of adult male mice, but was unchanged in anxiety-resilient adolescents. Herein, we determined the role of mGlu5 signaling in withdrawal-induced anxiety via pharmacological manipulation using the mGlu5 negative allosteric modulator MTEP and the positive allosteric modulator CDPPB. Adult (PND 56) and adolescent (PND 28) male C57BL/6J mice binge-drank for 14 days under 3-bottle-choice procedures for 2 h/day; control animals drank water only. Approximately 24 h following the final alcohol presentation, animals were treated with 30 mg/kg IP MTEP, CDPPB, or vehicle and then tested, thirty minutes later, for behavioral signs of anxiety. Vehicle-treated binge-drinking adults exhibited hyperanxiety in all paradigms, while vehicle-treated binge-drinking adolescents did not exhibit withdrawal-induced anxiety. In adults, 30 mg/kg MTEP decreased alcohol-induced anxiety across paradigms, while 3 mg/kg MTEP was anxiolytic in adult water controls. CDPPB was modestly anxiogenic in both alcohol- and water-drinking mice. Adolescent animals showed minimal response to either CDPPB or MTEP, suggesting that anxiety in adolescence may be mGlu5-independent. These results demonstrate a causal role for mGlu5 in withdrawal-induced anxiety in adults and suggest age-related differences in the behavioral pharmacology of the negative reinforcing properties of alcohol.

1. Introduction

Both clinical and preclinical studies consistently report that chronic binge alcohol-drinking is associated with symptoms of negative affect and dysphoria during periods of abstinence. Binge-drinking is defined as a pattern of consumption sufficient to elevate blood alcohol concentrations (BAC) to ≥80 mg/dl, which relates to approximately 4–5 drinks in a 2-h period (NIAAA, 2004). Frequent binge-drinkers typically develop tolerance to the hedonic rewarding properties of alcohol, leading to an escalation of intake in order to reach a desired level of subjective intoxication. However, elevated consumption coupled with frequent bouts of intoxication/withdrawal exacerbates the severity and duration of subsequent withdrawal symptoms (Ballenger and Post, 1978; Becker and Hale, 1993; Carrington et al., 1984). Over time, withdrawal-induced negative affect fuels the transition to addiction by shifting the primary motivation for drinking from positive to negative reinforcement in order to alleviate this aversive state during periods of abstinence.

While both adults and adolescents engage in binge-drinking, it is especially prevalent amongst adolescents (CDCP, 2014). In fact, over 90% of alcohol consumed by underage drinkers is in the form of binge-drinking episodes (NIAAA, 2017). Adolescents typically consume larger quantities of alcohol than adults, yet adolescents are reportedly less susceptible to the negative consequences of acute intoxication (e.g., locomotor incoordination and sedation) and adolescents also experience fewer 'hangover'-like symptoms such as withdrawal-induced anxiety and dysphoria (Doremus et al., 2003; Spear and Varlinskaya, 2005; Varlinskaya and Spear, 2004; White et al., 2002). Recent work in our laboratory has successfully recapitulated these age-related differences in withdrawal-induced negative affect using a mouse model of voluntary binge-drinking. We have shown that adult alcohol-drinking mice exhibit increased behavioral indices of anxiety during early (24 h) withdrawal (Lee et al., 2016). This elevated anxiety coincides with increased expression of metabotropic glutamate receptor 5 (mGlu5) within the nucleus accumbens shell (AcbSh) during acute withdrawal, as indicated by western blotting (Cozzoli et al., 2012; Obara et al.,

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2009; Lee et al., 2016, 2017b). In contrast, adolescent drinkers were resilient to both withdrawal-induced hyperanxiety and increased mGlu5 expression within the AcbSh during early withdrawal (Lee et al., 2016, 2017b).

mGlu5 signaling is known to play a significant role in alcohol abuse, as systemic administration of mGlu5 antagonist reduces alcohol reinforcement and voluntary consumption (Hodge et al., 2006; Lominac et al., 2006; McMillen et al., 2005; Schroeder et al., 2005). Our lab has previously shown increased mGlu5 expression within both the AcbSh (Cozzoli et al., 2009, 2012; Lee et al., 2016; Obara et al., 2009; Szumlinski et al., 2008) and the CEA (Cozzoli et al., 2014; Lee et al., 2017b; Obara et al., 2009) during acute alcohol withdrawal in adult animals and intracranial administration of mGlu5 antagonist within the AcbSh (Cozzoli et al., 2009, 2012; Gass and Olive, 2009) and CEA (Cozzoli et al., 2014) reduces alcohol consumption. Both the CEA and AcbSh are components of the extended amygdala, and drug-induced dysregulation within this circuitry is known to mediate many of the negative affective consequences of drug abuse (reviewed in Gilpin et al., 2015; Koob, 2003).

Given the evidence of alcohol-induced upregulation of mGlu5 within brain regions implicated in anxiety, we hypothesized that this could be a causal mechanism involved in withdrawal-induced anxiety. Additionally, an age-dependent insensitivity to alcohol-induced upregulation of mGlu5 signaling in adolescent drinkers could constitute a neurobiological basis for their resilience to withdrawal-induced hyperanxiety. Indeed, glutamatergic dysregulation is implicated in the etiology of both addiction (reviewed in Cleva and Olive, 2012; Holmes et al., 2013; Kalivas et al., 2009; Tsai et al., 1995) and anxiety (reviewed in Bergink et al., 2004; Simon and Gorman, 2006; Swanson et al., 2005). Not only do mGlu5 receptor antagonists attenuate behavioral measures of both drug seeking and anxiety in animal models (e.g., Backstrom et al., 2004; Cozzoli et al., 2009, 2012, 2014; Klodzinska et al., 2004; Kumar et al., 2013; Lou et al., 2014; Sinclair et al., 2012), but they have also shown anxiolytic efficacy in human clinical trials (Pecknold et al., 1982; Porter et al., 2005) and mGlu5 antagonism is thought to contribute to the therapeutic efficacy of the alcoholism medication Acamprosate (De Witte et al., 2005; Harris et al., 2002; Mann et al., 2008). Thus, a mutual basis of glutamatergic dysfunction could contribute to the high comorbidity between addiction and affective disorders.

In the present study, we assessed the functional significance of mGlu5 signaling in withdrawal-induced anxiety in adult and adolescent binge-drinking mice using the mGlu5 negative allosteric modulator 3-[(2-Methyl-1,3-thiazol-4- yl)ethynyl]pyridine (MTEP) and the positive allosteric modulator 3-Cyano-*N*-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB), which have high potency and selectivity (Busse et al., 2004; Lindsley et al., 2004). Additionally, allosteric modulators are of particular interest for their pharmacotherapeutic potential due to their 'self-limiting' activity (Epping-Jordan et al., 2007). In contrast to direct competitive antagonists/agonists, allosteric modulators have no intrinsic activity, but instead enhance or suppress the activity of the receptor in the presence of a ligand (Conn et al., 2009).

We predicted that MTEP treatment would reduce early withdrawal-induced anxiety in adult drinkers, while treatment with the CDPPB should exacerbate alcohol-induced mGlu5 hyperactivation and increase anxiety. In adolescent alcohol-drinking mice, we hypothesized that increasing mGlu5 signaling with CDPPB would elicit a hyperanxious, adult-like phenotype during early withdrawal. This would suggest that withdrawal-induced anxiety is mediated by a common underlying mechanism in both adult and adolescent bingers, and a resistance to alcohol-induced neuroadaptations of mGlu5 could underlie the resilience to withdrawal-induced negative affect seen in adolescent drinkers. Based on the evidence supporting the anxiolytic properties of mGlu5 antagonism in both humans and laboratory animals (e.g., Kotlinska and Bochenski, 2008; Kumar et al., 2013; Varty et al., 2005), we also anticipated an anxiolytic effect of MTEP treatment in alcohol-naïve animals, although to a lesser extent than hyperanxious adult mice in alcohol withdrawal.

2. Materials and methods

The binge-drinking and behavioral testing procedures employed herein were nearly identical to those used in previous studies in our lab (Lee et al., 2015, 2016, 2017b) and are summarized briefly below. All procedures were conducted in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication No. 80–23, revised 2014) and approved by the IACUC of the University of California, Santa Barbara.

2.1. Subjects

The animals used in this study were male C57BL/6 mice that were either PND 28 (adolescents) or PND 56 (adults) at the onset of drinking, to maintain consistency with our previous experiments. C57BL/6 mice are commonly used in alcohol studies due to their propensity to consume alcohol (Hwa et al., 2011; Le et al., 1994). Animals were housed in a climate-controlled vivarium under a reverse light/dark cycle (lights off at 10am) in groups of 4 per cage. Animals were identified using small animal ear tags (Stoelting, Wood Dale, IL). Food and water were available *ad libitum*, except during the 2-h alcohol-drinking period. The study consisted of 2 age groups (adults and adolescents), 2 drinking groups (alcohol or water), and 3 treatment groups (MTEP, CDPPB, or vehicle); n = 11/group.

2.2. Drinking-in-the-dark (DID) procedures

Half of the animals from each age group were subjected to 14 consecutive days of binge-drinking under 3-bottle DID procedures. Control animals received a single water bottle only. Alcohol-access was restricted to 14 days in order to correspond to the estimated duration of early-mid adolescence in mice (Spear, 2000) and to maintain consistency across age groups. Each day prior to the drinking period, animals were separated into individual drinking cages and allowed to acclimate for approximately 45 min. Animals were then given concurrent access to 10, 20, and 40% (v/v) unsweetened ethanol solutions for 2 h, beginning 3 h into the circadian dark cycle- the time of peak daily fluid intake (Rhodes et al., 2005). At the conclusion of the drinking period, animals were returned to their original group cages. The amount of alcohol consumed each day was calculated by bottle weight immediately before and after the drinking period and expressed as a function of the animal's body weight (in kg).

2.2.1. Blood alcohol sampling

Submandibular blood samples were collected from all alcohol-drinking animals on day 11 of drinking, immediately upon conclusion of the 2-h drinking period. The scheduling of the blood sampling was selected to ensure that the animals' intakes had stabilized, while also allowing ample time for recovery prior to behavioral testing. BACs were determined using an Analox alcohol analyzer (model AM1, Analox Instruments USA, Lunenburg, MA).

2.3. Drugs

The initial study used a high 30 mg/kg dose of both MTEP (Sigma Aldrich; St. Louis, MO) and CDPPB (NIMH C-918; Bethesda, MD) dissolved in 90% sterile water: 10% Tween-80 (Sigma Aldrich; St. Louis, MO), injection vol = 0.01 ml/g. This dose was selected from the high end of the dose-range typically reported to be behaviorally effective in the literature. For example, anxiolytic effects of MTEP have been reported at 20 mg/kg (Klodzinska et al., 2004) and 30 mg/kg achieves 100% receptor occupancy (Busse et al., 2004), while a 30 mg/kg dose of CDPPB reverses amphetamine-induced prepulse inhibition deficits