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Sex- and hormone-dependent alterations in alcohol withdrawal-induced anxiety and corticolimbic endocannabinoid signaling

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ABSTRACT

Alcohol dependence is associated with anxiety during withdrawal. The endocannabinoid (ECB) system participates in the neuroendocrine and behavioral response to stress and changes in corticolimbic ECB signaling may contribute to alcohol withdrawal-induced anxiety. Moreover, symptoms of alcohol withdrawal differ between sexes and sexual dimorphism in withdrawal-induced ECB recruitment may be a contributing factor. Herein, we exposed intact male and female rats and ovariectomized (OVX) female rats with or without estradiol (E_2) replacement to 6 weeks of chronic intermittent alcohol vapor and measured anxiety-like behavior, ECB content, and ECB-related mRNA in the basolateral amygdala (BLA) and ventromedial prefrontal cortex (vmPFC). Acute alcohol withdrawal increased anxiety-like behavior, produced widespread disturbances in ECB-related mRNA, and reduced anandamide (AEA) content in the BLA and 2-arachidonoylglycerol (2-AG) content in the vmPFC of male, but not female rats. Similar to males, alcohol-exposed OVX females showed reductions in *Napepld* mRNA in the BLA, decreased AEA content in the BLA and vmPFC, and reductions in all ECB-related genes measured in the vmPFC. Importantly, E_2 replacement prevented withdrawal-induced alterations in ECB content (but not mRNA) in OVX females, and although alcohol-exposed OVX females failed to exhibit more anxiety compared to their respective control, chronic alcohol exposure abolished the anxiolytic properties of E_2 in OVX rats. These data indicate that ovarian sex hormones (but not E_2 alone) protect against withdrawal-induced alterations in corticolimbic ECB signaling but do not impart resilience to withdrawal-induced anxiety. Thus, the mechanisms implicated in the manifestation of alcohol withdrawal-induced anxiety are most likely sex-specific.

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1. Introduction

Alcohol use disorders (AUD) are the third leading cause of preventable death in the U.S. (Mokdad et al., 2004), costing the U.S. approximately $224 billion a year (Bouchery et al., 2011). Maladaptive patterns of alcohol consumption are typically associated with increased activation of the amygdala and decreased activation of the medial prefrontal cortex (mPFC), which fundamentally contribute to negative emotional states (Menzaghi et al., 1994; Sommer et al., 2008) and cognitive impairment (George et al., 2012) that are associated with alcohol withdrawal, respectively. At the neurobiological level, an increase in glucocorticoids and extrahypothalamic corticotropin-releasing factor (CRF) activity

Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, anandamide; ANOVA, analyses of variance; BAL, blood alcohol level; BLA, basolateral amygdala; CB1R, cannabinoid-type 1 receptor; CIA, chronic intermittent alcohol; CORT, corticosterone; CRH, corticotropin-releasing hormone; DAGLa, DAG lipase alpha; EPM, elevated plus maze; E_2, estradiol; ECB, endocannabinoids; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; NAPEPLD, *N*-acyl phosphatidylethanolamine phospholipase D; OVX, ovariectomized; USV, ultrasonic vocalization; vmPFC, ventromedial prefrontal cortex.

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have been linked to symptoms of alcohol withdrawal in preclinical models (Koob et al., 2014).

In recent years, the endocannabinoid (ECB) system has been identified as a critical component of the stress response and is tightly regulated by both CRF and glucocorticoids (see Morena et al., 2016 for review). CRF, which is mobilized immediately after exposure to stress, rapidly increases the hydrolytic activity of fatty acid amide hydrolase (FAAH), the enzyme responsible for metabolism of anandamide (AEA), in both the basolateral amygdala (BLA) and central nucleus of the amygdala, which then mediates the generation of a behavioral state of anxiety (Gray et al., 2015; Natividad et al., 2017). During stress recovery, elevations in corticosterone (CORT) mobilize the other major ECB, 2-arachidonoylglycerol (2-AG), in the ventromedial prefrontal cortex (vmPFC) to disinhibit pyramidal neurons that promote termination of the stress response (Hill et al., 2011). Furthermore, repeated exposure to stress or persistent elevation in CORT, produce sustained elevations in extrahypothalamic CRF signaling, which in turn drives persistent changes in ECB function in the amygdala and mPFC (Gray et al., 2015, 2016; Hill et al., 2013). Given that CRF and glucocorticoids modify neuronal excitability and emotional states via rapid alterations in the ECB system (Di et al., 2016, 2003; Hill et al., 2011; Natividad et al., 2017; Wamsteeker et al., 2010; Wang et al., 2012), it is likely that alterations in the ECB system within stress-responsive brain regions contributes to the expression of withdrawal-induced anxiety during abstinence from alcohol. This is supported by both preclinical and clinical reports. For instance, acute alcohol intoxication or exposure to alcohol-related cues increases ECB levels in humans (Mangieri et al., 2009) and rodents (Alvarez-Jaimes et al., 2009; Caillé et al., 2007; Robinson et al., 2015; Vinod et al., 2006), while CB1R blockade reduces alcohol drinking in preclinical models of alcohol dependence (Arnone et al., 1997; Rodriguez de Fonseca et al., 1999; Freedland et al., 2001; Serra et al., 2001) and prevents stress-induced ethanol consumption (Racz et al., 2003). Furthermore, chronic intermittent alcohol (CIA) exposure dysregulates the ECB system itself, with CIA exposure resulting in a decrease in CB1R and *N*-acyl phosphatidylethanolamine phospholipase D (NAPE-PLD) mRNA expression (Sanchez-Marin et al., 2017; Serrano et al., 2012) and CB1R function on both GABAergic and glutamatergic terminals (Robinson et al., 2015; Varodayan et al., 2016a, 2016b) throughout the amygdala during withdrawal. Thus, alcohol intoxication and withdrawal produce dynamic fluctuations in the ECB system that may contribute to the emergence of negative emotional states during abstinence.

However, the vast majority of studies have focused exclusively on male subjects despite the fact that important sex differences exist in the manifestation of alcohol dependence and withdrawal (Ceylan-Isik et al., 2010; Sharrett-field et al., 2013). For instance, men are diagnosed with AUDs more often than women and report more severe symptoms of alcohol withdrawal (Deshmukh et al., 2003), while women report more alcohol-related medical and psychiatric problems, as well as fertility issues and menstrual cycle disruptions (Erol and Karpyak, 2015; Wilsnack et al., 1984). The ECB system and ovarian hormones also interact in a bidirectional manner (Gorzalka and Dang, 2012), and the ECB system has been implicated in the ability of ovarian hormones to modulate emotional behavior (Hill et al., 2007). Thus, sex differences observed in the symptoms of alcohol dependence may result, in part, from sex differences in the ECB system due to fluctuations in ovarian hormones.

Using a rodent model of CIA vapor exposure, we tested the hypothesis that the induction of alcohol dependence produces sex-specific alterations in anxiety-like behavior, CORT secretion, and ECB content/mRNA expression during acute withdrawal. We further tested the hypothesis that alcohol withdrawal in ovariectomized (OVX) females is sufficient to recapitulate the anxiety-like phenotype and ECB alterations observed in alcohol dependent male rats, and examined whether estradiol (E_2) replacement is capable of preventing these changes in OVX females. Our data indicate that alcohol withdrawal produces sexually dimorphic effects on anxiety-like behavior and ECB expression in the BLA and vmPFC and point to a potential mechanism by which sex-specific affective symptoms emerge during alcohol withdrawal.

2. Methods

2.1. Animals

Adult male and female Wistar rats (arriving at 60–65 days old; Simonsen Laboratories, Santa Clara, CA) were used for all experiments. Animals were group housed (2–3 of the same sex per cage), and kept in a temperature-controlled (21 ± 2 °C) vivarium on a 12 h reverse light-dark cycle with *ad libitum* food and water access. All animals were handled for at least 5 days prior to the beginning of the experiment, and the Washington State University Institutional Animal Care and Use Committee approved all procedures.

2.2. Alcohol exposure regimen

Male and female rats were exposed to CIA vapor (14 h on/10 h off; 7 days/week for 6 weeks), or air, as described previously (Williams et al., 2012; Vendruscolo and Roberts, 2014; Henricks et al., 2016). CIA vapor exposure induces robust alterations in motivational and affective states indicative of alcohol dependence, including escalated alcohol self-administration and negative affective behaviors (Nealey et al., 2011; Walker and Koob, 2008; Williams et al., 2012). The alcohol vapor chamber allows blood alcohol levels (BALs) to be easily titrated by the experimenter by adjusting the rate of 95% alcohol that is vaporized and introduced into the airflow. During the first week, BALs were determined daily by collecting blood from the tail vein (~50 μl), starting 30 min prior to the vapor turning off, until the desired BAL range had been achieved (175–250 mg%). Thereafter, BALs were determined 1–2 times per week, as well as on testing days. After centrifugation, plasma samples were assayed for alcohol content using an Analox GL5 alcohol analyzer (Analox Instruments, Lunenburg, MA). All behavioral testing and tissue harvest occurred during acute withdrawal (6–8 h after the vapor turned off), as we have previously shown that this time point is associated with increased negative affect in male rats (Williams et al., 2012; Henricks et al., 2016).

2.3. Elevated plus maze

Withdrawal-induced anxiety was assessed using the elevated plus maze (EPM) test, a well-validated model of anxiety-like behavior in rodents (Pellow et al., 1985; Williams et al., 2012). The EPM consisted of a raised Plexiglas platform (28.5 inches high) with two open arms and two closed arms of equal length (21.5 inches/arm). The floors consisted of clear Plexiglas, and the walls of both closed arms were white. Each animal was placed in the center of the maze at the start of the test and allowed to explore the maze for 5 min. All tests were run in dim lighting (~10 lux), and behaviors were recorded with Noldus behavioral tracking software (Leesburg, VA). The number of entries and amount of time spent in the open and closed arms, as well as the total distance traveled in the EPM