Alcohol 50 (2016) 19–25

Contents lists available at ScienceDirect

Alcohol

journal homepage: http://www.alcoholjournal.org/

Ethanol-induced anxiolysis and neuronal activation in the amygdala and bed nucleus of the stria terminalis

Amanda C. Sharko [a,b,*], Kris F. Kaigler [a,b], Jim R. Fadel [a], Marlene A. Wilson [a,b]

[a] Department of Pharmacology, Physiology and Neuroscience, University of South Carolina School of Medicine, Columbia, SC, USA
[b] WJB Dorn Veterans Affairs Medical Center, Columbia, SC, USA

ARTICLE INFO

Article history:
Received 27 March 2015
Received in revised form
2 November 2015
Accepted 3 November 2015

Keywords:
Anxiety
BNST
Amygdala
NPY
Individual differences

ABSTRACT

High rates of comorbidity for anxiety and alcohol-use disorders suggest a causal relationship between these conditions. Previous work demonstrates basal anxiety levels in outbred Long-Evans rats correlate with differences in voluntary ethanol consumption and that amygdalar Neuropeptide Y (NPY) systems may play a role in this relationship. The present work explores the possibility that differences in sensitivity to ethanol's anxiolytic effects contribute to differential ethanol self-administration in these animals and examines the potential role of central and peripheral NPY in mediating this relationship. Animals were first exposed to the elevated plus maze (EPM) to assess individual differences in anxiety-like behaviors and levels of circulating NPY and corticosterone (CORT). Rats were then tested for anxiety-like behavior in the light–dark box (LD box) following acute ethanol treatment (1 g/kg; intraperitoneally [i.p.]), and neuronal activation in the amygdala and bed nucleus of the stria terminalis (BNST) was assessed using Fos immunohistochemistry. EPM exposure increased plasma CORT levels without altering plasma NPY levels. Acute ethanol treatment significantly increased light–dark transitions and latency to re-enter the light arena, but no differences were seen between high- and low-anxiety groups and no correlations were found between anxiety-like behaviors in the EPM and LD box. Acute ethanol treatment significantly increased Fos immunoreactivity in the BNST and the central amygdala. Although NPY neurons were not significantly activated following ethanol exposure, in saline-treated animals lower levels of anxiety-like behavior in the LD box (more time in the light arena and more transitions) were correlated with higher NPY-positive cell density in the central amygdala. Our results suggest that activation of the CeA and BNST are involved in the behavioral expression of ethanol-induced anxiolysis, and that differences in basal anxiety state may be correlated with NPY systems in the extended amygdala.

Introduction

Anxiety disorders frequently co-occur with alcohol-use disorders (AUDs), with 75% of individuals that abuse alcohol having a current or previous diagnosis of an anxiety disorder (Kushner, Abrams, & Borchardt, 2000; Kushner et al., 2012; Swendsen et al., 2010). Anxiety relief is often cited as a motivation to consume alcohol, and both human and animal studies have demonstrated that acute ethanol reduces anxiety (Eckardt et al., 1998; Kushner et al., 2000; Wilson, Burghardt, Ford, Wilkinson, & Primeaux, 2004). Additionally, it has been suggested that chronic anxiety symptoms may promote alcohol abuse and the development of alcohol dependence (Koob, 2003), as preclinical studies show that elevated innate anxiety states are associated with increased drinking in two-bottle choice paradigms (Primeaux, Wilson, Bray, York, & Wilson, 2006; Spanagel et al., 1995). In contrast, repeated ethanol exposure and withdrawal can result in elevated measures of anxiety (Kliethermes, 2005; Valdez et al., 2002), suggesting that chronic, heavy ethanol use may contribute to the development of anxiety disorders. While many studies have examined the relationship between withdrawal-induced anxiety and the progression from moderate ethanol consumption to ethanol abuse and dependence, it remains unclear to what extent pre-existing anxiety disorders contribute to the development of alcohol-use disorders.

This work was supported by grants from the National Institute of Mental Health to JRF and MAW (MH063344) and the National Institute on Alcohol Abuse and Alcoholism to JRF and MAW (AA017361) and a VA Merit Award to MAW (1101 BX001374).

* Corresponding author. Department of Pharmacology, Physiology, & Neuroscience, University of South Carolina School of Medicine, Bldg. 1 D26, 6439 Garners Ferry Rd., Columbia, SC 29209-1638, USA. Tel.: +1 803 216 3503; fax: +1 803 216 3538.
E-mail address: amanda.sharko@uscmed.sc.edu (A.C. Sharko).

http://dx.doi.org/10.1016/j.alcohol.2015.11.001
0741-8329/

A number of preclinical studies suggest that ethanol preference may be related to anxiety-like behavioral phenotypes. Some of the rodent lines selectively bred for high or low ethanol consumption show differences in anxiety-like behaviors. Both alcohol-preferring (P) rats and Sardinian alcohol-preferring (sP) rats demonstrate more anxiety-like behavior on the elevated plus maze (EPM) than the associated non-preferring (NP and sNP) lines (Colombo et al., 1995; Stewart, Gatto, Lumeng, Li, & Murphy, 1993). Interestingly, outbred rats appear to show a similar association. Previous work from our laboratory has shown that Long-Evans rats show highly variable anxiety-like behavior on the EPM, and rats characterized as having a high-anxiety phenotype had higher preference scores for ethanol in a 24-h two-bottle choice paradigm, as compared to low-anxiety animals (Primeaux et al., 2006). A comparable relationship was found in Wistar rats, with high-anxiety animals having higher ethanol intake and ethanol preference than low-anxiety animals (Spanagel et al., 1995). However, the relationship between anxiety-like behaviors and ethanol consumption may be dependent on the ethanol drinking paradigm used. In an acute voluntary ethanol consumption paradigm based on the murine drinking-in-the-dark model (Rhodes, Best, Belknap, Finn, & Crabbe, 2005), Long-Evans rats characterized as having a high-anxiety phenotype consumed significantly less ethanol than low-anxiety animals (White, Ford, Fadel, & Wilson, 2009). Additionally, rats consuming different amounts of ethanol in this limited-access paradigm showed differential anxiolytic effects on the elevated plus maze (Sharko, Kaigler, Fadel, & Wilson, 2013). Although these data indicate that individual differences in anxiety measures are associated with differences in ethanol preference and consumption patterns, further work is needed to clarify if these behavioral phenotypes are related.

One of the primary factors thought to influence ethanol consumption levels is sensitivity to ethanol's subjective effects, and we hypothesized that differences in sensitivity to the behavioral and neurobiological effects of ethanol may contribute to the observed differences in drinking behavior between high- and low-anxiety animals. Therefore, the first goal of this study was to determine if individual differences in baseline anxiety (as measured on the EPM) influence the acute anxiolytic effects of ethanol in the LD box. This design allowed for comparisons of measures of anxiety-like behavior in the EPM and the LD box in both saline- and ethanol-treated animals. Two behavioral tests were used as prior studies have demonstrated that re-exposure to tests like the EPM cannot be used to test drug responses, since EPM exposure produces one-trial tolerance and reduces the observed anxiolytic-like effects of benzodiazepines and ethanol in subsequent trials (Bertoglio & Carobrez, 2002; File, Mabbutt, & Hitchcott, 1990). We chose to use the LD box to assess ethanol-induced changes in anxiety because percent open arm time (%OAT) on the EPM has been shown to correlate with anxiety-like behaviors in the LD box (McCool & Chappell, 2007). We also analyzed the peripheral stress responses following the initial exposure to the EPM to examine if stress-induced changes in CORT or NPY levels correlated with the individual differences in anxiety-like responses in the EPM and/or ethanol-induced responses. Current evidence demonstrates a critical role for the NPY system in mediating both anxiety-related responses and the acute effects of alcohol (Sharko, Fadel, & Wilson, 2013).

It has been hypothesized that overlapping neurobiological mechanisms may account for the high rates of comorbidity seen for anxiety disorders and AUDs. Many of the brain regions that mediate the behavioral effects of ethanol also mediate responses to anxiogenic stimuli, particularly the central (CeA) and basolateral (BLA) nuclei of the amygdala and the bed nucleus of the stria terminalis (BNST). Acute ethanol treatment has been shown to alter neurotransmission in each of these regions (Kash, Matthews, & Winder, 2008; Lack, Ariwodola, Chappell, Weiner, & McCool, 2008; Roberto, Madamba, Moore, Tallent, & Siggins, 2003; Silberman, Shi, Brunso-Bechtold, & Weiner, 2008; Weitlauf, Egli, Grueter, & Winder, 2004), suggesting that these regions may be of particular importance in mediating the relationship between ethanol and anxiety. The second goal of this work was to identify differences in amygdalar and BNST activation that may underlie any behavioral differences in response to an anxiolytic dose of ethanol. We previously found that activation of the central amygdala and BNST was positively correlated with behavioral measures of anxiolysis following voluntary ethanol consumption (Sharko, Kaigler, Fadel, & Wilson, 2013). Using immunohistochemical techniques, we examined expression of Fos, an immediate-early gene commonly used as a marker for neuronal activation (Curran & Morgan, 1995), in the amygdala and the BNST in control and ethanol-treated rats, as well as activation of NPY-containing neurons in these regions.

Methods

Animals

Adult male Long-Evans rats (175–200 g; Harlan, Indianapolis, IN) were singly housed and maintained on a 12-h light–dark cycle (lights on at 1:00 AM) with *ad libitum* access to food and water. Animals were habituated to daily handling before the experiment, and body weight, water consumption, and food consumption were monitored for the duration of the experiment. All procedures were approved by the University of South Carolina Institutional Animal Care and Use Committee.

Responses in the elevated plus maze

One week before examining the acute effects of ethanol in the LD transition box, animals were tested in the EPM to assess their pre-existing anxiety levels in a similar test of novelty. Blood samples were taken before and after EPM testing to analyze EPM-induced increases in both CORT and NPY. Animals ($N = 30$) were tested on the EPM during the late light portion of the light–dark cycle. The apparatus consisted of four arms (56×10 cm) made of black Plexiglas® with gray matting, elevated 50 cm above the floor. Open arms had a 1 cm lip and closed arms had 40-cm tall black walls. Rats were placed on the maze in the center square facing an open arm and test sessions lasted 5 min. Behavior was tracked using the EthoVision XT automated tracking system (Noldus, Leesburg, VA). Percent open-arm time (%OAT), the ratio of time spent in the open arms over the time spent in all arms, and percent open-arm entries (%OAE) were calculated to assess anxiety-like behavior. Distance traveled and closed-arm entries were used to assess spontaneous locomotor behavior. For analysis purposes, animals were divided into high- and low-anxiety groups based on median split of percent open-arm time.

One week prior to EPM testing, animals were lightly restrained and 100 μL of blood was collected from a small nick in the tail vein. A second 100 μL tail blood sample was collected in the same way immediately following EPM testing. All blood samples were collected in tubes prepared with aprotinin and EDTA, then centrifuged, and plasma was stored at −20 °C until extraction and analysis. Plasma corticosterone levels were assessed in whole plasma using a CORT-specific ELISA kit (Enzo, Farmingdale, NY). For plasma NPY analysis, plasma samples were extracted over C-18 spin columns (Thermo, Rockford, IL), and NPY protein levels were analyzed from extracts using an NPY-specific ELISA kit (Bachem, Torrance, CA).