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Activation of extrasynaptic δ-GABA$_A$ receptors globally or within the posterior-VTA has estrous-dependent effects on consumption of alcohol and estrous-independent effects on locomotion

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ABSTRACT

Recent reports support higher than expected rates of binge alcohol consumption among women and girls. Unfortunately, few studies have assessed the mechanisms underlying this pattern of intake in females. Studies in males suggest that alcohol concentrations relevant to the beginning stages of binge intoxication may selectively target tonic GABAergic inhibition mediated by GABA$_A$ receptor subtypes expressing the δ-subunit protein (δ-GABA$_A$Rs). Indeed, administration of agonists that interact with these δ-GABA$_A$Rs prior to alcohol access can abolish binge drinking behavior in male mice. These δ-GABA$_A$Rs have also been shown to exhibit estrous-dependent plasticity in regions relevant to drug taking behavior, like the hippocampus and periaqueductal gray. The present experiments were designed to determine whether the estrous cycle would alter binge drinking, or our ability to modulate this pattern of alcohol use with THIP, an agonist with high selectivity and efficacy at δ-GABA$_A$Rs. Using the Drinking-in-the-Dark (DID) binge-drinking model, regularly cycling female mice were given 2 h of daily access to alcohol (20%*v*/v). Vaginal cytology or vaginal impedance was assessed after drinking sessions to track estrous status. There was no fluctuation in binge drinking associated with the estrous cycle. Both Intra-posterior-VTA administration of THIP and systemic administration of the drug was also associated with an estrous cycle dependent reduction in drinking behavior. Pre-treatment with finasteride to inhibit synthesis of 5α-reduced neurosteroids did not disrupt THIP's effects. Analysis of δ-subunit mRNA from posterior-VTA enriched tissue samples revealed that expression of this GABA$_A$ receptor subunit is elevated during diestrus in this region. Taken together, these studies demonstrate that δGABA$_A$Rs in the VTA are an important target for binge drinking in females and confirm that the estrous cycle is an important moderator of the pharmacology of this GABA$_A$ receptor subtype.

1. Introduction

Binge drinking behavior among women and girls has increased dramatically in recent years (Keyes et al., 2008). In the United States, a recent report from the Centers for Disease Control and Prevention highlights this "under-recognized problem," finding that almost 14 million women aged 18 through 34 binge drink an average of 3 times per month (Centers for Disease Control and Prevention (CDC), 2013). This trend is also seen elsewhere; for example, in the United Kingdom binge-drinking rates among young women have doubled in the past decade (Smith and Foxcroft, 2009). The prevalence of this risky pattern of alcohol intake among women may be of concern given clinical reports suggesting that women display a telescoped development of addiction upon initial drug use (Randall et al., 1999; Hernandez-Avila et al., 2004; Johnson et al., 2005). Even when sex differences in the rate of the development of problems from alcohol use are challenged (Keyes et al., 2010; Lewis and Nixon, 2014), clinical findings continue to support differences in the maladaptations that occur from alcohol use (Squeglia et al., 2011; Smith et al., 2015) and in the mechanisms that may drive problematic use (Perry et al., 2013; Foster et al., 2015), alcohol seeking (Cyders et al., 2016) and binge alcohol intake (Skinner et al., 2015). Unfortunately, few preclinical studies have specifically

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explored the neurochemical mediators of binge alcohol intake in females.

A growing number of reports support inhibition mediated by extrasynaptic $GABA_A$ receptors as a target for the effects of binge alcohol intoxication and consumption (Nie et al., 2011; Ramaker et al., 2011, 2012, 2014a, 2014b; Fritz and Boehm, 2014). Unlike the classic synaptic subtype of $GABA_A$ receptors that mediate phasic inhibition following vesicular release of GABA, extrasynaptic $GABA_A$ receptors mediate a constant tonic inhibition, responding to the low to moderate concentrations of ambient GABA found in the extrasynaptic space (Wei et al., 2003). Many of these extrasynaptic $GABA_A$ receptors express the δ subunit protein (δ-$GABA_A$R; Mody and Pearce, 2004). Inclusion of this subunit appears to alter lateral diffusion of the receptor across the membrane and its ability to cluster at the synapse (Jacob et al., 2008). The tonic inhibition mediated by these δ-$GABA_A$R subtypes has been shown to be enhanced by the 17–30 mM alcohol concentration range achieved during a binge drinking session (Glykys et al., 2007; Olsen et al., 2007). Indeed, genetic inactivation of δ-$GABA_A$R in the nucleus accumbens reduces alcohol-drinking behavior in male mice (Nie et al., 2011). Further, pharmacological manipulation using 4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridine-3-ol (THIP/gaboxadol), an agonist with high efficacy and selectivity for δ$GABA_A$Rs (Boehm et al., 2006; Bhattarai et al., 2011; Meera et al., 2011; Mortensen et al., 2010), reduces binge alcohol consumption in mice (Moore et al., 2007; Fritz and Boehm, 2014; Quoilin and Boehm, 2016). However, no study has explored the effect that estrous cycle may have on the role that δ-$GABA_A$Rs play in mediating binge drinking in females.

Findings thus far suggest important interactions between extrasynaptic $GABA_A$ receptors and gonadal hormones. Many neurosteroids-in particular the progesterone derived allopregnanolone and THDOC as well as the androgen derived androstanediol-work as positive allosteric modulators, increasing efficacy of GABA at $GABA_A$ receptors, especially at δ containing GABAA receptors, where GABA acts as a weak agonist. Thus, the δ subunit may be said to confer sensitivity to the neurosteroid-induced enhancement of GABAergic inhibition (Belelli et al., 2002; Brown et al., 2002; Mihalek et al., 1999; Spigelman et al., 2003; Wohlfarth et al., 2002; Stell et al., 2003). Previous reports demonstrate that allopregnanolone may be altered following voluntary consumption of alcohol (Finn et al., 2004b). As GABA has low efficacy at δ containing $GABA_A$ receptors, low doses of allopregnanolone act as positive allosteric modulators by increasing the efficacy of GABA for opening δ containing channels (Bianchi and Macdonald, 2003). High doses of allopregnanolone have also been shown to work as direct agonists at $GABA_A$ receptors (Hosie et al., 2006). This relationship between neurosteroid level and alcohol intake may be bidirectional. In particular, pretreatment of mice with finasteride, a compound that blocks activity of 5α reductase and synthesis of 5α-reduced neurosteroids like allopregnanolone, dampens alcohol drinking behaviors (Ford et al., 2005). Although this effect might be sex specific (see Ford et al., 2008; Finn et al., 2010), it is possible that neurosteroid availability and potential changes to the expression and function of δ-$GABA_A$ receptors that parallel changes in neurosteroid levels are relevant to binge drinking behavior in females. Indeed, recent findings suggest that neurosteroids acting at δ-$GABA_A$Rs can drive synaptic plasticity of dopaminergic neurons at the start of the reward circuit, at the level of the ventral tegmental area (Vashchinkina et al., 2014). Availability of neuroactive steroids oscillates across the estrous cycle much the same as their hormone precursors. For example, allopregnanolone shows a dip in availability during diestrus (Koonce, Walf and Frye, 2012). This reduction in the levels of neuroactive steroids that can act as positive allosteric modulators of δ containing $GABA_A$ receptors-like allopregnanolone-would be associated with an apparent drop in the efficacy of drugs that target this receptor during diestrus. For example, we could expect that a particular dose of THIP-a compound that works as a super-agonist at δ-$GABA_A$ receptors - would be more effective at producing GABAergic inhibition at δ-$GABA_A$ receptors at times when neuroactive steroids that act as positive allosteric modulators of δ-$GABA_A$ receptors are at their highest circulating levels, like proestrus and estrus when compared to its effects at diestrus. However, diestrus is also a time when the expression of δ-$GABA_A$ receptors peaks in various regions. Thus, it is possible that the contrasting shifts in availability of neuroactive steroids and expression of δ-$GABA_A$ receptors would result in maintenance of sensitivity to compounds acting on this receptor subtype.

Given the important role that GABAergic signaling in the VTA plays in alcohol intake generally (Nowak et al., 1998) and in binge consumption specifically (Moore and Boehm, 2009; Melón and Boehm, 2011), estrous-dependent changes in neurosteroid availability also suggests interactions between estrous cycle, neurosteroidogenesis and the mechanisms that underlie binge alcohol consumption.

In addition to oscillations in the synthesis and availability of neurosteroids, expression of δ-$GABA_A$Rs fluctuates across the ovarian cycle. Changes in the expression of δ subunit protein occur across the estrous cycle in the hippocampus (Maguire et al., 2005) and periaqueductal gray (Griffiths and Lovick, 2005; Lovick et al., 2005). Transcription of *Gabrd* mRNA in the hippocampus also alternates across the ovarian-cycle in a subregion selective manner (Wu et al., 2013). Of course, changes in these extrasynaptic δ-$GABA_A$Rs may be driven by the neurosteroid fluctuations described earlier (Maguire et al., 2005; Wu et al., 2013). Still, compounds that target this $GABA_A$ subtype in order to modulate alcohol-related behaviors may suffer from ovarian-related changes in their efficacy. Furthermore, behaviors that may be driven by activity of δ-$GABA_A$Rs may be altered across the estrous cycle (Barth et al., 2014; Cushman et al., 2014; Sabaliauskas et al., 2015).

The goal of this series of experiments was to determine whether the estrous cycle affects our ability to modulate binge drinking by targeting the extrasynaptic $GABA_A$ receptor system. Additionally, we wanted to clarify whether the ovarian cycle would change characteristics of this specific pattern of alcohol consumption in female mice and whether changes in the expression of extrasynaptic $GABA_A$ receptors was associated with any of the estrous-dependent effects found. Finally, to clarify whether changes in availability of the 5alpha reduced pregnane-derived neurosteroids also played a role in any changes in sensitivity to THIP across the cycle, we pretreated a cohort of animals with finasteride. We hypothesized that the murine estrous cycle would be an important regulator of both baseline binge drinking behavior and the ability to pharmacologically disrupt this pattern of intake in a high-bingeing inbred strain, due to estrous-associated changes in the expression of δ-containing $GABA_A$ receptors.

2. Methods and materials

2.1. Animals

Female C57BL/6J inbred mice were purchased from Jackson Laboratory (Bar Harbor, ME) and maintained at the Indiana University-Purdue University-Indianapolis (IUPUI) School of Science Animal Resource Center. Mice were 70–80 days old at the start of each experiment. Animals were individually housed in standard shoebox cages and habituated to a 12-h reverse light/dark schedule for at least 7 days prior to initiation of experiments. The temperature of the colony room was maintained around 21 °C. In order to preserve regular estrous cycling in the females, non-manipulated, socially-housed males were also housed in the colony room. Food was available ad libitum in the home cage. Water was available ad libitum in the home cage except when ethanol was made available as per the Drinking-in-the-Dark (DID) protocol (see below). The IUPUI School of Science Institutional Animal Care and Use Committee approved all procedures prior to initiation of experiments.