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DIFFERENCES IN THE REINSTATEMENT OF ETHANOL SEEKING WITH GANAXOLONE AND GABOXADOL

M. J. RAMAKER,[a]* M. M. FORD,[a] T. J. PHILLIPS[a,b] AND D. A. FINN[a,b]

[a] *Department of Behavioral Neuroscience, Oregon Health & Science University, Portland, OR 97239, United States*

[b] *Department of Veterans Affairs Medical Research, Portland, OR 97239, United States*

Abstract—The endogenous neuroactive steroid allopregnanolone (ALLO) has previously been shown to induce reinstatement of ethanol seeking in rodents. ALLO is a positive allosteric modulator at both synaptic and extrasynaptic $GABA_A$ receptors. The contribution of each class of $GABA_A$ receptors in mediating reinstatement of ethanol seeking is unknown. The first aim of the present study was to determine whether ganaxolone (GAN), a longer-acting synthetic analog of ALLO, also promotes reinstatement of ethanol seeking. The second aim was to examine whether preferentially activating extrasynaptic $GABA_A$ receptors with the selective agonist gaboxadol (THIP) was sufficient to reinstate responding for ethanol in mice. Male C57BL/6J mice were trained to lever press for access to a 10% ethanol (v/v) solution (10E), using a sucrose-fading procedure. Following extinction of the lever-pressing behavior, systemic THIP (0, 4 and 6 mg/kg) and GAN (0, 10, and 15 mg/kg) were tested for their ability to reinstate ethanol-appropriate responding in the absence of 10E access. GAN significantly increased lever pressing on the previously active lever, while THIP did not alter lever-pressing behavior. The results of this study suggest that direct activation of extrasynaptic $GABA_A$ receptors at the GABA site is not sufficient to induce ethanol seeking in the reinstatement procedure. Future studies are necessary to elucidate the mechanisms and brain areas by which differences in the pharmacological activity of GAN and THIP at the $GABA_A$ receptor contribute to the dissimilarity in their effect on the reinstatement of ethanol seeking. Nonetheless, based on the increased use of these drugs in clinical trials across multiple disease states, the effects of GAN or THIP on alcohol seeking may be an important consideration if these drugs are to be used clinically in a population with a co-occurring alcohol use disorder.

*Corresponding author. Address: 3181 SW Sam Jackson Park Road (L-470), Portland, OR 97239, United States. Tel: +1-503-220-8262x58020; fax: +1-503-273-5351.

E-mail address: mjramaker@gmail.com (M. J. Ramaker).

Abbreviations: ALLO, allopregnanolone; ANOVA, analysis of variance; AUD, alcohol use disorder; BECs, blood ethanol concentrations; FR, fixed ratio; GAN, ganaxolone; RR, response requirement; THIP, gaboxadol.

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INTRODUCTION

The National Institute on Alcohol Abuse and Alcoholism estimates that nearly 18 million Americans suffer from an alcohol use disorder (AUD). AUDs are considered chronic relapsing disorders (Leshner, 1997; Volkow and Li, 2005). Nearly 90% of dependent individuals relapse at least once in a 4-year span (Polich et al., 1980), highlighting the need for a better understanding of the mechanisms underlying craving and relapse. Although relapse is difficult to model in animals, the reinstatement model provides a measure of drug seeking during abstinence from the drug (de Wit and Stewart, 1981; Shaham et al., 2003). The model has predictive validity in that re-exposure to drugs, drug-related cues, and stressors, all of which can provoke craving and possibly relapse in humans (Childress et al., 1993; de Wit, 1996; Sinha, 2001), also promote reinstatement of drug-seeking in animals (Epstein et al., 2006).

Allopregnanolone (ALLO) is an endogenous neuroactive steroid that can be increased in the brain and plasma by stress, estrus, pregnancy, and ethanol (Purdy et al., 1991; Concas et al., 1998; VanDoren et al., 2000; Barbaccia et al., 2001; Finn et al., 2004). Due to its potent positive modulation at $GABA_A$ receptors, ALLO shares many behavioral properties with ethanol, such as anxiolysis, sedation, and anticonvulsant properties (Kumar et al., 2009). ALLO substitutes for ethanol in drug discrimination procedures, indicating that it shares subjective stimulus properties with ethanol (Grant et al., 1996, 2008; Bowen et al., 1999). Exogenous ALLO alters ethanol intake in a biphasic manner and reinstates ethanol seeking in rodents (Janak et al., 1998; Sinnott et al., 2002; Janak and Gill, 2003; Nie and Janak, 2003; Ford et al., 2005; Finn et al., 2008), suggesting that it plays a role in both ethanol seeking and consumption.

Although ALLO may have clinical benefit in multiple disease states (epilepsy, premenstrual dysphoric disorder, depression, traumatic brain injury; clinicaltrial.gov), the therapeutic potential of ALLO is limited by its short half-life (Timby et al., 2006). Ganaxolone (GAN) is a synthetic analog of ALLO with an added methyl group that renders it more resistant to metabolism (Nohria and Giller, 2007). Although its primary pharmacological and behavioral properties are unaltered, when compared to ALLO (Carter et al., 1997; Ungard et al., 2000), the half-life

of GAN is three to four times that of ALLO (Reddy and Rogawski, 2000). The use of GAN in clinical trials has broadened in the last decade, to include potential treatment of epilepsy, post-traumatic stress disorder, and smoking cessation (clinicaltrial.gov).

$GABA_A$ receptors are chloride channels composed of five subunits from a pool of at least 16 possible subunits: α_{1-6}, β_{1-3}, γ_{1-3}, δ, ε, π, and θ. Importantly, inclusion of the δ subunit limits the location of the receptor to the extrasynaptic space, where it is postulated to contribute exclusively to tonic inhibition (Farrant and Nusser, 2005). *In vitro* work using recombinant receptors suggests that δ subunit-containing $GABA_A$ receptors may be particularly sensitive to the GABA-modulatory effects of ALLO (Belelli et al., 2002). Similarly, δ subunit-containing $GABA_A$ receptors have been proposed to be a more sensitive target of physiologically-relevant doses of ethanol than non-δ subunit-containing $GABA_A$ receptors (Olsen et al., 2007; Mody et al., 2007; but also Borghese and Harris, 2007). Consistent with the importance of the δ subunit in the actions of neuroactive steroids and ethanol, δ subunit knockout mice showed reduced sensitivity to some of the behavioral effects of both neuroactive steroids and ethanol, and the knockout mice self-administered less ethanol than their wild-type littermates (Mihalek et al., 1999, 2001). Although GAN and ALLO can act at both synaptic and extrasynaptic $GABA_A$ receptors (Belelli and Herd, 2003), the contribution of each class of receptors to the reinstatement of ethanol seeking has not been examined.

The primary aim of the present studies was to determine whether GAN reinstates ethanol seeking in mice, as previously demonstrated with ALLO (Finn et al., 2008). The second aim of the study was to examine whether preferentially activating extrasynaptic $GABA_A$ receptors with gaboxadol (THIP), a $GABA_A$ receptor agonist with selectivity for δ subunit-containing $GABA_A$ receptors (Wafford et al., 2009; Meera et al., 2011), was sufficient to induce reinstatement of ethanol seeking. Locomotor tests were performed with each drug to elucidate whether changes in lever-pressing behavior during extinction were accounted for by changes in general locomotor activity. Information regarding the ability of GAN or THIP to promote alcohol seeking may be of significant value considering the increasing use of these drugs in clinical trials across multiple disease states, such as smoking (GAN), post-traumatic stress disorder (GAN), and depression (THIP) (clinicaltrial.gov). Particularly in diseases that have a high comorbidity with AUDs, the effects of GAN or THIP on alcohol seeking may be an important consideration if these drugs are to be used in a clinical setting in a population with a co-occurring AUD.

EXPERIMENTAL PROCEDURES

Animals

Male C57BL/6J mice, approximately 8 weeks of age at the start of experiments, were purchased from The Jackson Laboratory-West (Sacramento, CA, USA). Mice were pair-housed for the reinstatement study or group-housed for the locomotor study in Biofresh bedding (Ferndale, WA, USA), except during individual testing. Mice were provided *ad libitum* access to rodent chow (LabDiet, St. Louis, MO, USA) and water (except where noted). Mice were maintained on a 12-h light–dark cycle (lights on at 0600); all experiments were conducted during the light cycle and were carried out Monday through Friday. All efforts were made to minimize animal suffering and to reduce the number of mice used, and all procedures were approved by the local Institutional Animal Care and Used Committee and complied with NIH guidelines.

Drugs

Ethanol (200 proof; Pharmco Products, Brookfield, CT) and sucrose (Sigma–Aldrich Company, St. Louis, MO, USA) solutions were prepared by dilution in tap water. GAN was purchased from Tocris Bioscience (Ellisville, MO, USA) and was solubilized in 20% (w/v) 2-hydroxypropyl-beta-cyclodextrin (β-CD; Cargill Inc., Cedar Rapids, IA) in Millipore water. THIP (4,5,6,7-tetrahydroisoxazolo-[5,4-c]pyridine-3-ol hydrochloride) was purchased from Tocris Bioscience and dissolved in saline. For all experiments, drugs were injected intraperitoneally (i.p.) in a volume of 0.01 ml/g body weight and with a 30-min pretreatment time. The pretreatment times were chosen to match our previous work with GAN and THIP (Ramaker et al., 2011, 2012).

Apparatus

Ethanol self-administration acquisition, extinction, and reinstatement sessions were carried out in eight operant conditioning boxes consisting of a 21″ L × 13.75″ W × 5” H inner chamber located inside a sound and light-attenuating chamber, as described previously (Ford et al., 2007a,b). Each conditioning box contained a house light, two retractable levers with a stimulus light above each one, a retractable sipper apparatus, and a stainless steel grid floor. The retractable sipper was made from a 10-ml graduated pipette with a double ball-bearing metal sipper tube. Each metal sipper was connected to a lickometer circuit, which interfaced to a computer operating with MED-PC software (Med-Associates Inc., St. Albans, VT, USA). The computer recorded time-stamped licks on the sipper throughout the acquisition phase as well as lever presses throughout all phases of the experiment.

Locomotor chambers have been described in detail previously (Gubner et al., 2013). Briefly, 16 automated locomotor chambers (40 × 40 × 30 cm; AccuScan Instruments Inc., Columbus, OH, USA) were used, which were equipped with pairs of eight photocell beams and detectors located 2 cm above the floor. VERSADAT software (AccuScan Instruments Inc.) was used to convert beam interruptions into horizontal distance traveled (cm). Each chamber contained a fan to provide ventilation and background noise, and each chamber contained a 3.3-W incandescent light bulb during activity testing.

Acquisition

Self-administration was acquired with the ‘‘sipper’’ procedure (Samson et al., 1998; Ford et al., 2007a,b).