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Baclofen blocks yohimbine-induced increases in ethanol-reinforced responding in rats

Keith L. Williams [a,*], Melissa M. Nickel [b], Justin T. Bielak [a]

[a] Oakland University, Dept. of Psychology, 224 Pryale Hall, Rochester, MI 48309, United States
[b] Middle Tennessee State University, Dept. of Psychology, 1301 East Main Street, Murfreesboro, TN 37132, United States

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ABSTRACT

Chronic or repeated stress increases alcohol consumption. The GABA-B agonist baclofen decreases alcohol consumption and may be most effective for individuals with comorbid anxiety/stress disorders. The present study sought to determine if baclofen blocks stress-induced increases in ethanol self-administration as modeled by repeated yohimbine injections in rats. Rats were trained to respond for 15% *w*/*v* ethanol in operant chambers using a method that applies neither water deprivation nor saccharin/sucrose fading. Following training, the rats received 6 injections of 1.25 mg/kg yohimbine were given immediately prior to the operant sessions during a 2-week time period. Subsequently, some rats were pair-matched to receive either 1.25 mg/kg yohimbine or saline in the presence of 0.3, 1, and 3 mg/kg baclofen prior to sessions. Acquisition of ethanol self-administration was poor. Pretreatment with yohimbine consistently increased responding across repeated injections. Yohimbine's effect on ethanol intake unexpectedly diverged from the effect on responding as the rats failed to consume all reinforcers earned. Smaller doses of baclofen paired with saline injections had no effect on ethanol responding; only 3 mg/kg baclofen reduced ethanol self-administration. The smallest baclofen dose of 0.3 mg/kg failed to block the yohimbine-induced increase in self-administration. The large baclofen dose of 3 mg/kg continued to suppress ethanol self-administration when given with yohimbine. Baclofen 1 mg/kg blocked the effect of yohimbine even though it had no effect when given in the absence of yohimbine. Exposure to high ethanol concentrations may induce self-administration only in certain conditions. The dissociation between responding and intake suggests that repeated yohimbine injections may initiate other behavioral or physiological mechanisms that confound its effects as a pharmacological stressor. Furthermore, an optimal baclofen dose range may specifically protect against stress-induced alcohol self-administration, highlighting a specific contribution of GABA-B receptors and a potential therapeutic efficacy of GABA-B agonists at a non-sedating dose.

1. Introduction

Chronic or repeated stress and stress disorders contribute to the development and persistence of drug and alcohol use (McHugh, 2015; Logrip et al., 2012). Animal models demonstrate the involvement of stress hormones and brain pathways in the escalation of drug use and alcohol intake (Lu and Richardson, 2014; Zorrilla et al., 2014). The adrenergic system may provide an avenue to model the effects of stress on drug and alcohol consumption. For example, the presynaptic alpha-2 adrenoreceptor antagonist yohimbine increases adrenergic activity and has been called an anxiogenic drug (Ghitza et al., 2006; Shepard et al., 2004). In humans, yohimbine increases anxiety-like hormonal and subjective responses (Gurguis et al., 1997; Krystal et al., 1997). The drug also induces perceived withdrawal while increasing craving in opiate addicts (Stine et al., 2002). In rats, yohimbine simulates stress-like effects such as reinstatement of responding for methamphetamine (Shepard et al., 2004), food pellets (Ghitza et al., 2006), and ethanol (Le et al., 2005). Repeated injections of yohimbine may be useful in simulating repeated stress. Accordingly, Kupferschmidt et al. (2009) repeatedly injected yohimbine during cocaine extinction days and found marked attenuation of subsequent reinstatement when given yohimbine. The authors suggested that the rats became tolerant to the effects of stress in their model. Simms et al. (2011) repeatedly injected rats with yohimbine and found continued reinstatement of responding for ethanol across the treatment period although the magnitude of responding may differ based on ethanol training experience. Thus, yohimbine may be a useful tool to explore the interaction of repeated stress and pharmacotherapies used to reduce alcohol consumption and craving.

Baclofen, a GABA-B metabotropic receptor agonist, decreases many alcohol-related behaviors in preclinical models. For example, baclofen reduces ethanol consumption in a 2-bottle choice paradigm (Colombo et al., 2000; Daoust et al., 1987), consumption in 2-bottle alcohol deprivation effect (Colombo et al., 2003a) and responding in an operant paradigm (Anstrom et al., 2003; Janak and Gill, 2003). Baclofen also

* Corresponding author.
E-mail address: william9@oakland.edu (K.L. Williams).

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reduces extinction responding or reinstatement of responding (Colombo et al., 2003b; Maccioni et al., 2008). However, most of these studies utilized Sardinian alcohol-preferring rats as subjects. These data should be further explored in outbred rats. In humans, baclofen may measures of alcohol-related behaviors and may be utilized safely in alcoholics with cirrhosis (Addolorato and Leggio, 2010). However, the data are less convincing when randomized controlled trials are examined (Brennan et al., 2013; Gorsane et al., 2012; Ponizovsky et al., 2015) which leads some to suggest a more individualized and titrated high dose to be a more effective approach (Muller et al., 2015). Stress or anxiety may play a role in predicting the efficacy of baclofen. In a double-blind, placebo-controlled randomized clinical trial of baclofen in the treatment of alcohol dependence, baclofen was most effective in patients with comorbid anxiety disorders (Morley et al., 2014). These data suggest that the therapeutic effects of baclofen may emerge in certain subpopulations or in conditions of higher stress or anxiety.

The purpose of these experiments was first to determine if yohimbine would consistently increase ethanol self-administration and, thus, support the use of repeated yohimbine as a model of repeated stress. Second, we wanted to determine if baclofen could block the yohimbine-induced increase in ethanol self-administration.

2. Materials and methods

2.1. Animals and housing

Outbred male Wistar rats ($n = 40$) weighing 100–125 g upon arrival (Harlan Laboratories Inc., Indianapolis, IN) and were habituated for 48 h prior to training. Rats were pair-housed until adulthood and were maintained in a temperature- and humidity-controlled room with a reversed 12-h light/dark cycle (lights off at 7 am). All operant sessions began after 8:30 am. Rats had unrestricted access to food (Harlan Teklad rodent diet 2018 from Harlan Teklad, Chicago, IL) and water except during shaping sessions where food pellets were delivered as a reinforcer. Prior to the first experiment, one rat was euthanized due to health issues. This protocol was approved by Institutional Animal Care and Use Committee and the rats were treated in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

2.2. Apparatus

Experiments were conducted in standard operant chambers (Med Associates, St. Albans, VT) housed in melamine sound-attenuated cubicles. Each grid-floor chamber (30.5 × 24.2 × 29.2 cm) contained 2 rolled-edge standard levers on the right wall. One lever was located near the back of the chamber while the other was located near the front of the chamber. During self-administration, responding on one lever was reinforced while the other lever was inactive. A receptacle cup was located on the center of the right wall between the levers. The cup was fitted to receive food pellets or fluid deliveries from a syringe pump via 18 gauge stainless steel tubing connected to the cup. A white stimulus light was located above the cup. The house light was on the center of the left wall near the top of the chamber. Operant chambers were controlled with programs written in Med-PC Medstate Notation version IV (Med Associates).

2.3. Acquisition of ethanol self-administration

Rats were food deprived for 24 h before shaping to lever-press for 45 mg food pellets (Bio-Serv #F0021, Frenchtown, NJ). Rats began lever-pressing during 10–20 min shaping sessions over the course of 4 days during which rats were food-restricted but had free access to water in the home cage. Once rats sufficiently lever pressed for food, the food reward was replaced with a 15% *w*/*v* ethanol solution made from 99.98% ethanol (Pharmco Products Inc., Brookfield, CT) and mixed in distilled water. Simple exposure to the high ethanol concentration (20% *v*/*v* made from 95% ethanol) in an operant paradigm has previously been shown to elicit intake levels higher than 0.75 g/kg during 30 min sessions at the end of a 2-week training period (Augier et al., 2014). When comparing ethanol concentrations across studies, researchers would find it useful to state the concentrations of ethanol by weight (% *w*/*v*) as calculated using the specific gravity of ethanol which is approximately 0.79 g/ml (for review, see Brick, 2006). Differences in ethanol stock solutions affect the calculations for mixing ethanol solutions. For example, a 20% *v*/*v* solution made from a 95% stock solution contains less ethanol than a 20% *v*/*v* solution made from a 99.98% stock solution. Because 20% *v*/*v* ethanol made from 95% ethanol contains approximately 15 g of ethanol per 100 ml of water, we gave our rats an equivalent concentration of ethanol but stated it as 15% *w*/*v* so that ethanol concentrations can be standardized across different ethanol stock solutions. Rats were given free access to food and water in the home cage. A house light in the operant chamber indicated an active session for 30 min. Each lever-press resulted in 0.1 ml fluid reinforcer and a 4 s time out during which the house light was off and responding had no consequence. Accuracy of volume delivery was confirmed after each session by measuring the remaining fluid in the syringe pump and the receptacle cup. Fluid in the receptacle cup was measured using a 16 gauge needle connected to a 3 ml syringe. Operant sessions were conducted 7 days per week. A stimulus light was illuminated during the post-reinforcer time out above the receptacle cup on day 5 to increase responding. By day 9, the intakes dropped below 0.4 g/kg. To stimulate more responding, the fluid reinforcer volume was decreased to 0.05 ml on day 14, but intake remained low. On day, 19, the reinforcer volume was returned to 0.1 ml and the ethanol concentration was decreased to 10% *w*/*v*. Rats self-administered this ethanol concentration and fluid volume for 30 min per day on fixed ratio 1 (FR 1) schedule until adulthood (approximately post-natal day 77) at which time we began the first experiment.

2.4. Experiment 1: repeated yohimbine injections

This experiment was designed to test the ability of yohimbine to consistently increase ethanol-reinforced responding and intake (g/kg) across repeated injections. Rats ($n = 39$) received injections of saline 7 times during the 2 weeks leading up to yohimbine injections to habituate the rats to the injection procedure. The injections were given as intraperitoneal injections each in a volume of 1 ml/kg. Yohimbine HCl (Sigma Aldrich, St. Louis, MO) was dissolved in 0.9% saline and given in a dose of 1.25 mg/kg immediately prior to the operant sessions on 6 days spread over 2 weeks (Mondays, Wednesdays, and Fridays). Operant sessions were conducted on all other days. Pre-session saline injections were given 3 times interspersed among the yohimbine injection regimen (i.e., after 2nd yohimbine injection, after 5th yohimbine injection, after last yohimbine injection) to provide data for responding and intake after saline injection. Other days were non-injections baseline days. Although yohimbine is often given 30 min prior self-administration, yohimbine has been shown to be effective at shorter pretreatment intervals. For example, yohimbine substantially increased activity, heart rate and blood pressure within the first 15 min following injection (Zaretsky et al., 2015) and yohimbine given 10 min prior to a session increased responding for food (Liu, 2015). Pilot studies in our lab showed that yohimbine increased responding for ethanol given immediately prior to the session which lead us to choose this approach for the experiments presented here.

2.5. Experiment 2-baclofen–yohimbine interaction

This experiment was designed to test the ability of various doses of baclofen to reverse the effects of yohimbine on ethanol-reinforced responding and intake (g/kg). *RS*-baclofen (Tocris Bioscience, Minneapolis, MN) was dissolved in 0.9% saline and given via intraperitoneal