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Assessment of GABA-B, Metabotropic Glutamate and Opioid Receptor Involvement In An Animal Model of Binge Drinking

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Abstract

Drinking to intoxication or binge drinking is a hallmark characteristic of alcohol abuse. Although hard to model in rodents, the Scheduled High Alcohol Consumption (SHAC) procedure generates high, stable ethanol intake and blood ethanol concentrations in mice to levels consistent with definitions of binge drinking. The purpose of the current studies was to determine the effects of pharmacological manipulation of the opioidergic, glutamatergic and GABAergic systems on binge drinking with the SHAC procedure. Parallel manipulations were conducted in mice trained in operant self-administration of either sucrose or ethanol. For the SHAC procedure, genetically heterogeneous Withdrawal Seizure Control (WSC) mice were given varying periods of fluid access, with a 30 min ethanol session every 3rd day (total of 7). Mice were pretreated intraperitoneally with naltrexone (0, 0.6 or 1.25 mg/kg), baclofen (0, 2.5 or 5.0 mg/kg), or 2-methyl-6-(phenylethyl)-pyridine (MPEP; 0, 3.0 or 10.0 mg/kg) prior to each ethanol session. For the operant self-administration procedure, separate groups of C57BL/6 mice were trained to complete a single response requirement (RR16; 16 presses on the active lever) to gain 30 min of access to an ethanol or sucrose solution. Mice received pretreatments of the same doses of naltrexone, MPEP or baclofen prior to the self-administration sessions, with saline injections on intervening days. Naltrexone produced a dose-dependent decrease in binge drinking, and the highest dose also significantly decreased operant self-administration of ethanol and sucrose. Both doses of baclofen significantly decreased binge alcohol consumption, but the higher dose also tended to decrease water intake. The highest dose of baclofen also significantly decreased operant self-administration of sucrose. MPEP (10 mg/kg) significantly decreased binge alcohol consumption and sucrose self-administration. These results indicate that manipulation of the opioidergic, glutamatergic and GABAergic systems significantly decreased binge drinking.

INTRODUCTION

Alcoholism is a complex disorder that is impossible to capture in its entirety within a single animal model. Therefore, multiple models are commonly employed to increase our understanding of the mechanisms that underlie specific aspects of this disease, such as

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acquisition, maintenance of drinking behavior, dependence, and relapse (Rhodes et al., 2005; Rodd et al., 2004). Recent reports by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) indicate that 17.6 million people in the United States are alcohol dependent or abuse alcohol (NIAAA, 2007a). Additional epidemiological studies indicate that the percentage of individuals to engage in binge drinking ranged from 14–18% for adults or from 25–33% for adolescents (9th–12th grade), with even higher proportions in 12th graders alone (32–42%; Cozzoli et al., 2009, NIAAA, 2007b, 2007c). Binge drinking has been defined by NIAAA as 5 or more drinks (male) or 4 or more drinks (female) on at least one occasion in the past month or blood alcohol concentrations of ≥ 0.8 mg/ml within a 2 hour drinking period (NIAAA, 2007a).

With respect to binge drinking, it is often difficult to model either gross excess or obvious loss of control in a rodent model. Two contemporary methods that demonstrate animals drinking to the point of intoxication and achieving blood ethanol concentrations (BECs) consistent with that defined for binge drinking by NIAAA are the “Drinking in the Dark” (DID; details in Rhodes et al., 2005, 2007; Sharpe et al., 2005) and “Scheduled High Alcohol Consumption” (SHAC; Cozzoli et al., 2009; Cronise et al., 2005; Finn et al., 2005) procedures. We chose to use the SHAC procedure as a model of binge drinking, since recent work in our laboratory has shown that scheduling periods of fluid availability generated high and stable ethanol intake and a physiologically-relevant BEC in both C57BL/6 and genetically heterogeneous (i.e. Withdrawal Seizure Control; WSC) mice (Finn et al., 2005). It is noteworthy that mouse genotypes with an innate high alcohol preference (C57BL/6) as well as a low alcohol preference (WSC) maintained elevated BECs (≥ 0.8 mg/ml) and ethanol intakes of ≥ 2.0 g/kg within the 30-minute period of ethanol access. Animals under this procedure drink on a schedule that enables them to maintain high levels of ethanol consumption without forming a taste aversion (Finn et al., 2005; Szumlinski et al., 2007). An additional study established that the SHAC procedure produced quantifiable signs of intoxication in mice (Cronise et al., 2005).

An animal model that produces elevated BECs and generates behavioral intoxication possesses essential attributes for the examination of the neurochemical mechanisms that underlie binge-like intake as well as the development of pharmacological interventions for this drinking phenotype. Based on preclinical data and results with therapeutic drugs (i.e., naltrexone and acamprosate), a large body of evidence indicates that there are several different neurochemical systems that modulate alcohol’s rewarding and reinforcing properties (reviewed in Koob and LeMoal, 2006). With this in mind, the present studies focused on the opioid, glutamate and γ -aminobutyric acid (GABA) neurotransmitter systems.

The opioidergic system has been implicated as a major player in brain reward pathways, given that antagonists such as naltrexone reportedly reduce the reward value of abused drugs (McCaul et al., 2000; Anton and Swift, 2003; Kiefer et al., 2003; Littleton and Zieglgänsberger, 2003). Preclinical and clinical studies found that naltrexone reduced ethanol preference, consumption, and the risk for relapse (e.g., Stromberg et al., 1998, 2001, 2002; Höltér and Spanagel, 1999; Heyser et al., 2003). With regard to the DID model of binge drinking, naltrexone dose-dependently decreased ethanol intake at doses that did not influence water intake (Kamdar et al., 2007).

Baclofen is a GABA_B receptor agonist that inhibits the acquisition of ethanol consumption, decreases alcohol-induced craving and withdrawal symptoms, and promotes abstinence (discussed in Heilig and Egli, 2006). Numerous preclinical and clinical studies have documented that GABA_B receptor agonists suppress numerous alcohol-related behaviors, including reinforcement and conditioned place preference in rodents (Colombo et al, 2000,

2002, 2003aColombo et al, 2003b; Addolorato et al., 2002; Bechtholt and Cunningham, 2005; Walker and Koob, 2007; Maccioni et al., 2008). GABA_B receptor agonists also selectively alter binge drinking. While baclofen selectively increased ethanol intake with the DID procedure, the GABA_A receptor agonists muscimol and gaboxadol (THIP) reduced both ethanol and water intake (Moore et al., 2007).

Ethanol-induced alterations in glutamate neurotransmission within drug reward-related structures also are believed to contribute to the motivation to drink ethanol as well as to other behavioral effects of ethanol (reviewed in Gass and Olive, 2008; Szumlinski et al., 2008). Metabotropic glutamate receptor subtypes mGluR1 and mGluR5 have been studied extensively since they are localized in brain regions associated with drug reinforcement (Shigemoto et al, 1993, Romano et al, 1995; Lu et al, 1999). The mGluR5 antagonist MPEP [2-methyl-6-(phenylethyl)-pyridine] reduces the rewarding effects of ethanol in various animal models of ethanol self-administration and reinstatement (Bäckström et al., 2004; Olive et al., 2005; Schroeder et al., 2005; Hodge et al., 2006; Lominac et al., 2006) and decreased ethanol intake with the DID procedure (Gupta et al., 2008). Additionally, microinjection of MPEP into the nucleus accumbens (NAC) significantly decreased alcohol consumption with the SHAC procedure at doses that had no effect on water consumption (Cuzzoli et al., 2009).

The purpose of the present studies was to pharmacologically validate the SHAC procedure as a model of binge drinking by examining the effects of systemic drug injections of naltrexone, baclofen and MPEP in this model. The overall goal was to determine whether the effects of pharmacological agents on binge ethanol intake were comparable to results achieved with a second model of limited access ethanol intake, namely, operant self-administration of ethanol with the “sipper” procedure. The advantages to the “sipper” procedure, over the traditional fixed ratio (FR) procedures, are that the appetitive and consummatory phases of ethanol self-administration can be procedurally separated (e.g., Samson et al., 1998, 2000) and that we had documented in C57BL/6 mice that phase separation yielded a heightened appetitive drive to acquire ethanol access and a two-fold increase in ethanol consumption versus responding on a FR schedule (Ford et al., 2007a). In addition to determining the relative efficacy of naltrexone, baclofen and MPEP in these two models of ethanol intake, a secondary goal was to determine the specificity of the drug effects by measuring water intake (SHAC procedure) and sucrose self-administration.

METHODS

Animals

Two replicate lines of genetically heterogeneous WSC mice (i.e., WSC-1 and WSC-2) were kindly supplied by Dr. John C. Crabbe and were used in the studies with the SHAC procedure. These animals were bred in the Veterinary Medical Unit at the Veterans Affairs Medical Center (Portland, OR). Based on availability of the animals, studies were conducted on male WSC-1 (n=50), male WSC-2 (n=51), and female WSC-2 (n=20) mice. These animals were 6–8 weeks of age upon receipt and drug-naïve at the time of testing. Mice were group housed (4 per cage, separated by sex and genotype) in clear, polypropylene cages (28 × 18 × 13 cm) on corn cob bedding. WSC mice were used, as we had previously shown that the SHAC procedure maintained elevated BECs (≥ 0.8 mg/ml) consistent with the definition of binge drinking (Finn et al., 2005) and produced quantifiable signs of intoxication (Cronise et al., 2005) in this genotype.

C57BL/6J male mice (Jackson Laboratory West, Davis, CA; n = 26) were used in the operant self-administration studies, based on our prior experience with this genotype in operant self-administration studies (e.g., Ford et al., 2007a, 2007b, 2009). Specifically,

C57BL/6 mice readily acquire operant ethanol self-administration behavior, which remains stable across many months of testing. The animals were approximately 8 weeks old upon purchase and had approximately 6–8 months of experience with either ethanol (n = 12) or sucrose (n = 14) self-administration prior to testing. Mice had been previously tested for mecamlamine hydrochloride effects on ethanol and sucrose self-administration, and each mouse had the same history of treatment exposure (Ford et al., 2009). Further, mice were maintained on an operant self-administration procedure without any drug injections for four weeks prior to the initiation of this study. For this study, animals were pair-housed in clear, polypropylene cages (28 × 18 × 13 cm) on corncob bedding.

In all cases, animals were allowed to acclimate to a temperature (22 ± 1° C) and humidity-controlled environment on a 12-hour light/dark cycle (lights on at 0600 hrs) for a minimum of 1 week prior to experimentation. Rodent chow (Purina 5001) was provided *ad libitum* throughout the study. Fluid access was varied according to experimental demands for the SHAC procedure (details below). All procedures followed the United States Public Health Service/National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals* and the *Principles of Laboratory Animal Care*, and were approved by the local Institutional Animal Care and Use Committee.

SHAC procedure

The SHAC procedure is described in detail in Finn et al. (2005). Briefly, mice were acclimated to individual housing and 24 hours access to rodent chow and 1 bottle (inverted 25 ml graduated cylinder with sipper tube) containing tap water for 2 days. At the conclusion of the second day, all water bottles were removed at 1700 hrs to initiate fluid restriction. For the remainder of the study, mice were given varying periods of fluid access ranging from 4–10 hours per day starting at 0800 hrs for 21 consecutive days (see Table 1). Every 3rd session (starting on Day 3), mice were given access to a 5% v/v ethanol solution in tap water (5E) only during the initial 30 min of fluid availability, followed by access to water for the remaining period of fluid availability. For all intermittent sessions (i.e., Days 1–2, 4–5, etc.) water was the sole fluid available.

WSC-1 and WSC-2 mice were run in 3 passes, with each pass consisting of 40–41 animals. The naltrexone study used male and female mice, but the baclofen and MPEP studies used only male mice due to lack of female mice availability. For each pass, animals were separated into 3 different groups (vehicle, and two different doses of drug) that were matched for their 4 hr water consumption on the day prior to the first ethanol drinking session (i.e., Day 2). On ethanol drinking days (every 3rd day), mice were pretreated with drug or saline at either 15 min (naltrexone, baclofen) or 30 min (MPEP) prior to ethanol access.

To control for evaporation and spillage, bottles containing ethanol or tap water were placed on empty control cages and any displaced volume was subtracted from all treatment tubes. Body weights were monitored every day. Fluid intake volumes were recorded to the nearest 0.2 ml. Ethanol dose (g/kg) was calculated using the volume consumed (ml) and body weight of each mouse (g).

Operant Ethanol and Sucrose Self-Administration

Daily sessions were carried out Monday – Friday in 8 modular chambers (21.6 × 17.8 × 12.7 cm) with stainless steel grid floors (Med Associates Inc., St. Albans, VT). Each chamber contained a house light, two ultra-sensitive retractable levers, and a retractable sipper apparatus for extending or retracting a 10 ml graduated pipette with a double ball bearing sipper tube. The position of the active lever was counterbalanced between the left and right

sides of the operant chambers, to control for side preferences. In addition to measuring the volume consumed from the sipper tube (to the nearest 0.05 ml), a lickometer circuit was connected to each metal sipper to monitor lick patterns. Circuits were interfaced to an IBM-compatible computer operated by MED-PC software (Med Associates, Inc.). Each chamber was housed within a sound-attenuating wooden cabinet (61 × 38 × 33 cm; Fisher Custom Woodworking, Portland, OR), and a fan was installed to ensure sufficient air flow.

The procedure for initiating operant self-administration with the “sipper” model is described in detail elsewhere (Ford et al., 2007, 2009). Briefly, separate groups of C57BL/6 male mice were trained to respond for access to either a 10% v/v ethanol (10E) or a 5% w/v sucrose (5S) solution in tap water. Upon completion of training, a single response requirement of 16 presses on the active lever (RR16) resulted in 30 min of continual access to the fluid reinforcer. A 20 min time limit to complete the RR16 schedule was imposed. One advantage in the use of the “sipper” procedure is that the appetitive and consummatory phases of ethanol self-administration can be procedurally separated (e.g., Samson et al., 1998, 2000), which allows for an examination of drug effects on each phase of ethanol self-administration. Importantly, we have documented in C57BL/6 mice that phase separation yielded a heightened appetitive drive to acquire ethanol access and more than a two-fold increase in ethanol consumption, when compared with responding on a FR schedule (Ford et al., 2007a). Thus, the higher ethanol self-administration in C57BL/6 mice performing on a RR versus a FR schedule was an additional advantage in the use of the “sipper” procedure in the present studies.

As mentioned, mice had 6–8 months of self-administration experience prior to being used in the present study. After a one month period of no injections, mice were habituated to saline injections (i.e. 2 sessions; 15-minute pretreatment) and quickly exhibited stable baselines of 10E or 5S intake with less than 10% variability between sessions. Mice received one drug treatment per week, and saline injections were administered on intervening days to assess a return to baseline drinking levels. The order of treatments was as follows: naltrexone (0.6 mg/kg, then 1.25 mg/kg), MPEP (3 mg/kg, then 10 mg/kg), and baclofen (2.5 mg/kg, then 5 mg/kg). Pre-treatment times were the same as with the SHAC procedure (15 min for naltrexone and baclofen, 30 min for MPEP).

Drug Solutions

Ethanol (Pharmco Products, Brookfield, Conn., USA) was prepared as a 5% v/v or 10% v/v solution in tap water. Sucrose (Sigma-Aldrich, St. Louis, MO) was prepared as a 5% w/v solution in tap water. Naltrexone hydrochloride (0.6 or 1.25 mg/kg; Sigma-Aldrich, St. Louis, MO), baclofen (2.5 or 5.0 mg/kg; RCI, Natick, MA), MPEP hydrochloride (3.0 mg/kg or 10 mg/kg; Sigma-Aldrich), or saline was administered intraperitoneally in a volume of 0.01-ml/g of body weight.

Data Analysis

For the SHAC procedure, the dependent measures analyzed were ethanol dose (g/kg), volume of ethanol or water consumed (ml), and body weight (g). Analysis of variance (ANOVA) was used to examine drug dose and period of fluid access (repeated measures) effects on 5E intake (volume and dose) and total fluid consumption. For the operant self-administration data, ANOVA with repeated measures was used to examine dose effects on active and inactive lever responses and intakes of 10E and 5S (volume and dose). In all cases, significance was set at $p \leq 0.05$, significant interactions were pursued with ANOVA and post-hoc tests, and data are presented as the mean $\pm$ SEM.

RESULTS

SHAC Procedure

For each drug tested, all groups were matched for fluid consumption across sex (naltrexone only) and replicate line. The ethanol dose consumed in the saline-injected animals varied slightly across studies, and was lowest in the baclofen study.

Naltrexone—Scheduled high alcohol consumption was selectively decreased by pretreatment with naltrexone. As depicted in Figure 1A and 1B, naltrexone produced a significant and dose-dependent reduction in the ethanol dosage consumed during each 30 min access period [$F(2,38) = 63.88, p < 0.001$]. The significant interaction between naltrexone dose and total fluid access time on ethanol intake [$F(6,114) = 3.52, p < 0.005$] appeared to be due to the fact that the dose dependent reduction in ethanol intake was enhanced across time (see Figure 1A). Importantly, naltrexone did not significantly alter total fluid intake (Figure 1C).

Baclofen—Baclofen produced a significant and dose-dependent reduction in ethanol intake [$F(2,38) = 27.19, p < 0.001$] (Figure 2A and 2B), and significantly decreased total fluid intake [$F(2,38) = 4.13, p < 0.05$] (Figure 2C). Post-hoc tests indicated that there was a trend for the 5-mg/kg dose of baclofen to decrease total fluid intake. There was a significant interaction between dose and total fluid access time on ethanol intake [$F(6,114) = 9.04, p < 0.001$], which appeared to be due to the finding that baclofen was less effective as the period of fluid availability was increased from 4 to 10 hrs (see Figure 2A). Subsequent analyses confirmed that baclofen did not significantly alter ethanol intake when fluid availability was 10 hr/day.

MPEP—MPEP significantly decreased ethanol intake [$F(2,36) = 3.62, p < 0.05$] (Figure 3A and 3B) without significantly altering total fluid intake (Figure 3C). There was no significant interaction between dose and total fluid access time, suggesting that MPEP produced a comparable effect on ethanol intake across the various periods of fluid access. However, visual inspection of the data suggested that the 10-mg/kg dose of MPEP was more effective at decreasing ethanol intake in animals when fluid availability was 4 hr or 10hr fluid/day.

Operant Self-Administration Procedure

Baseline responding on the active and inactive levers (refer to saline treatments in Table 2), as well as 5S and 10E intakes (refer to saline treatments across panels in Figure 4), remained stable throughout the treatment time courses, indicating that there were no carry-over drug effects between subsequent tests. The range in baseline 5S intake (g/kg) across the treatment time courses was as follows: 1.97 ± 0.13 (naltrexone), 1.69 ± 0.16 (baclofen) and 1.85 ± 0.13 (MPEP). The range in baseline 10E intake (g/kg) was: 0.68 ± 0.07 (naltrexone), 0.72 ± 0.18 (baclofen) and 0.78 ± 0.15 (MPEP).

In general, the appetitive measure of responding on the active lever to gain access to the 10E or 5S solutions was not influenced consistently by any of the drugs administered (Table 2). Mice typically were able to complete the RR16 (16 presses on the active lever) to gain 30 min access to the sipper tube, with a few exceptions noted (see text below). The one significant decrease in frequency of active lever responses was observed following pre-treatment with the 5-mg/kg dose of baclofen (see text below). Inactive lever responding also was unaffected by drug pre-treatments, with a mean $\pm$ SEM of 0.60 ± 0.15 and 1.45 ± 0.31 inactive lever presses throughout testing in sucrose- and ethanol-reinforced mice, respectively.

Naltrexone—One 5S-reinforced mouse was unable to complete the RR16 schedule following pre-treatment with the 0.60 mg/kg dose, while two 10E-reinforced mice failed to gain access to the sipper tube following pre-treatment with the 0.6 and 1.25 mg/kg doses. Naltrexone pre-treatment significantly decreased both 5S [$F(2,25) = 17.26, p < 0.001$] and 10E [$F(2,16) = 5.12, p < 0.05$] intakes. The 1.25 mg/kg dose significantly reduced 5S intake by 47% ($p < 0.001$) and 10E intake by 46% ($p < 0.05$), when compared to within-group saline pre-treatment (Figure 4A).

Baclofen—In contrast to the effects of naltrexone on appetitive responding, baclofen pre-treatment significantly attenuated the frequency of active lever responses in ethanol-reinforced mice [$F(2,22) = 14.85, p < 0.001$]. Specifically, the 5-mg/kg baclofen dose significantly reduced active lever responding for 10E by 54% ($p < 0.001$) versus the saline pre-treatment condition, resulting in 7 of the 12 ethanol-reinforced mice being unable to complete the RR16 schedule and gain access to the 10E-filled sipper. Although two sucrose-reinforced mice also failed to complete the RR16 schedule following pre-treatment with the 5-mg/kg dose, the overall effect on active lever responses did not meet statistical significance in this group.

Baclofen pre-treatment significantly decreased intake of 5S [$F(2,24) = 14.41, p < 0.001$] but not 10E, which was likely due to the low number of ethanol-reinforced animals able to complete the RR16. In particular, the 5-mg/kg dose significantly decreased the dose of 5S consumed by 43% ($p < 0.001$) and the dose of 10E consumed by 24%, when compared to within-group saline pre-treatment (Figure 4B).

MPEP—Although one sucrose-reinforced mouse failed to complete the RR16 schedule, no overall effects of MPEP on the frequency of active lever responses was observed. One ethanol-reinforced mouse experienced technical difficulties with the sipper, so his data were not included in the analysis. MPEP significantly decreased 5S intake [$F(2,25) = 11.16, p < 0.001$], with the 10-mg/kg MPEP dose significantly reducing the dose of 5S consumed by 17% ($p < 0.05$) versus saline pre-treatment (Figure 4C). While MPEP pre-treatment did not significantly alter the dose of 10E consumed, the highest MPEP dose did reduce 10E intake by 19%.

DISCUSSION

The purpose of the present studies was to pharmacologically discern the neurotransmitter systems that contribute to binge drinking by examining the effects of naltrexone, baclofen and MPEP in the SHAC procedure. All three drugs significantly reduced ethanol intake, with differences in the magnitude of the effect as the period of fluid availability was increased. Repeated administration of naltrexone appeared to be more efficacious across time with regard to its suppression of ethanol intake, whereas baclofen appeared to be less efficacious. In contrast, the modest suppression of ethanol intake with MPEP was consistent across time.

Drinking to intoxication or binge drinking is a facet of alcoholism that has been particularly difficult to model in rodents because they typically do not voluntarily consume consistently high amounts of ethanol that produce BECs ≥ 100 mg%. We chose to use the SHAC procedure in the present studies since scheduling fluid access previously resulted in high, stable levels of ethanol intake and BECs (Finn et al., 2005). Based on evidence that binge drinking with the SHAC procedure elevated extracellular levels of glutamate, GABA, and dopamine in the NAC (Szumlinski et al., 2006, 2007) and that these similar effects were shown to be important in other drinking models (Bäckström et al., 2004; Olive et al., 2005; Schroeder et al., 2005; Szumlinski et al. 2005, 2008), the overall goal was to determine

whether the effects of pharmacological agents on high ethanol intake were comparable to results reported with other models of ethanol intake and self-administration.

Naltrexone

Many clinical and preclinical studies have shown evidence for a contribution of central endogenous opioid receptors to the motivation for alcohol consumption (Stromberg et al., 1998), with a fairly consistent reduction in ethanol intake and relapse risk by the opioid receptor antagonist naltrexone (e.g., Anton et al., 2004; Escher and Mittleman, 2006; Froehlich et al., 2003; McCaul et al., 2000; Middaugh et al., 1999, 2000, 2003; O'Malley et al., 1992, 1996; Phillips et al., 1997; Volpicelli et al., 1992, 1995; Williams and Woods, 1999a, 1999b; Williams et al., 1998). Consistent with the preclinical data, we found that naltrexone produced a significant dose dependent reduction in binge-like ethanol intake throughout the SHAC procedure (i.e., during each ethanol session). Notably, the reduction in ethanol intake by both naltrexone doses was enhanced over the 21-day duration of the study, with the 1.25 mg/kg dose of naltrexone completely suppressing ethanol consumption during the last two ethanol sessions (Figure 1A). Furthermore, there was a compensatory increase in water consumption so that the total fluid intake was unaltered. These results indicate that repeated administration of naltrexone may enhance the effects of the drug to selectively and completely suppress ethanol consumption over time. While early work suggested that there was a reduced efficacy of repeated naltrexone administration on ethanol intake in mice (Phillips et al., 1997), it is possible that the intermittent naltrexone injections prior to the binge ethanol sessions produced a conditioned aversion to subsequent ethanol intake. Consistent with this idea, the delta opioid receptor antagonist naltrindole produced an aversion to a previously non-aversive dose of ethanol at doses that did not produce a conditioned taste aversion when administered alone (i.e., without ethanol; Froehlich et al., 1998). Since naltrexone is a non-selective opioid receptor antagonist, it may have increased the aversive properties of a binge-like alcohol dose via an antagonism of delta opioid receptors.

The present results indicate that naltrexone selectively decreased binge ethanol intake by 66–93%, rather than suppressing overall fluid intake. This finding is consistent with the results of additional studies that used experimental manipulations to produce high, excessive ethanol intake. Systemic administration of comparable naltrexone doses (0.5 and 1.0 mg/kg) in a DID procedure (which results in drinking to the point of intoxication, without deprivation) produced a 17–28% reduction in ethanol intake without reducing the intake of tap water or a 10% sucrose solution (Kamdar et al., 2007). When mice were responding in an operant procedure for ethanol under high or low thirst motivation, the 1.25 mg/kg dose of naltrexone selectively decreased responding for ethanol (versus water) by 80% (Middaugh et al., 1999). However, using a schedule-induced polydipsia model of high ethanol intake (which elicits excessive fluid consumption without fluid deprivation), a 1.0 mg/kg naltrexone dose decreased ethanol and water intake by 43% (Escher and Mittleman, 2006). Collectively, these results suggest that naltrexone produces a relatively selective reduction in excessive ethanol drinking behavior.

Alternately, the operant self-administration results indicated that the high dose of naltrexone (1.25 mg/kg) produced a significant and non-selective decrease in consumption of both sucrose (5S) and ethanol (10E). Work conducted by Maccioni et al. (2005) showed that naloxone also produced a very similar decrease in operant self-administration of ethanol (15E) and sucrose (0.3S) intake in Sardinian alcohol-preferring (sP) rats. Earlier work found that low doses of naltrexone (0.1 and 0.32 mg/kg) significantly decreased operant reinforced responding for ethanol and sucrose solutions in rhesus monkeys (Williams et al., 1998). Notably, naltrexone shifted the ethanol concentration-consumption curve downward in an insurmountable manner. Subsequent studies determined that similar doses of naltrexone

produced a conditioned taste aversion for a paired sweetened solution (Williams and Woods, 1999a) and reduced responding for the preferred solution (either ethanol or water, which depended on the concentration of the ethanol solution), when animals were responding for ethanol or water concurrently (Williams and Woods, 1999b). While studies examining limited access home cage ethanol preference drinking reported a significant decrease in ethanol consumption following administration of opioid receptor antagonists (e.g., Lê et al., 1993; Phillips et al., 1997; Stromberg et al., 1998), water intake was low under these conditions. Given that early work suggests that the effects of opioid receptor antagonists are not specific for ethanol (see Williams et al., 1998 and references therein), the apparent differential selectivity of naltrexone in the SHAC versus operant self-administration procedures may be due in part to whether the animal was able to respond for a preferred solution.

Baclofen

The GABA_B receptor direct agonist baclofen has been shown repeatedly in rodent preclinical models to reduce the acquisition and maintenance of ethanol drinking behavior, the enhanced drinking following a period of abstinence (alcohol deprivation effect; ADE, a model of relapse-like drinking), symptoms of ethanol withdrawal, as well as ethanol's reinforcing, motivational and locomotor stimulating properties (see reviews by Colombo et al., 2000, 2004; Maccioni and Colombo, 2009 and references therein). Clinical evidence also suggests that baclofen reduced symptoms of alcohol withdrawal and craving in small cohorts of alcoholic subjects (see reviews by Addolorato et al., 2000, 2009; Colombo et al., 2004 and references therein). However, while the majority of data document that baclofen suppressed ethanol self-administration and consumption (Czachowski et al., 2006; Petry, 1997; Smith et al., 1992, 1999) and binge drinking with the DID procedure (Moore et al., 2007). It was suggested that the timing of baclofen injection and subsequent access to ethanol might be critical in determining how the drug would affect ethanol intake (Moore et al., 2007), since biphasic effects of baclofen have been reported (i.e., initial decrease followed by an increase; Smith et al., 1999). Nonetheless, the present results indicate that the 2.5 mg/kg dose of baclofen selectively reduced binge drinking with the SHAC procedure, while the highest dose of baclofen (5.0 mg/kg) significantly decreased ethanol consumption but also tended to decrease water consumption. The non-selective decrease in fluid consumption may be due to baclofen's sedative effect, given that a 5 mg/kg dose significantly decreased rotarod performance and locomotor activity in mice (Cryan et al., 2004).

An interesting finding was that the baclofen-induced reduction in binge ethanol consumption diminished as the total fluid access period increased (Figure 2A). This finding was in contrast to naltrexone's significant reduction in ethanol intake throughout all fluid access periods (Figure 1A), suggesting that tolerance may have developed to the effect of repeated baclofen injections on ethanol intake. Consistent with this idea, the ability of baclofen to reduce the maintenance phase of ethanol intake was maximal over the first 3–5 days of treatment and then tended to reduce with continuing administration (Colombo et al., 2000). Likewise, tolerance to the suppression in food intake with repeated baclofen injections developed after 8 days of treatment (Colombo et al., 2000).

With regard to the operant self-administration results, baclofen pre-treatment (5 mg/kg) significantly decreased sucrose self-administration by 43% and produced a non-significant decrease (24%) in ethanol self-administration. Several studies in rats have also reported that a baclofen-induced decrease in lever pressing for ethanol was accompanied by a proportional decrease in responding for sucrose (Anstrom et al., 2003; Janak and Gill, 2003; Maccioni et al., 2005; Petry, 1997). And, the overall selectivity of the reducing effect of

baclofen on ethanol-reinforced responding had a narrow window (only observed following 1 mg/kg but not following 3 mg/kg doses; Janak and Gill, 2003), suggesting that there is a lack of specificity with regard to the effects of baclofen on alcohol self-administration. It should be noted that only 5 (of 12) mice were able to complete the response requirement following pre-treatment with the 5 mg/kg dose of baclofen in the present study, consistent with the sedative effect of this dose that has been reported in mice (Cryan et al., 2004). Thus, the lack of a significant suppression in ethanol self-administration in the present study is likely due to the low number of C57BL/6 mice that were able to gain access to the 10E solution. In another study in C57BL/6 mice, a higher dose of baclofen (10 mg/kg) was required to significantly decrease operant ethanol self-administration (Besheer et al., 2004), but this dose of baclofen also suppressed locomotor activity and potentiated the sedative effects of low and high ethanol doses. Collectively, the present findings suggest that baclofen produced a non-selective reduction in ethanol intake with the SHAC and operant self-administration procedures.

MPEP

The selective mGluR5 antagonist MPEP has been shown in numerous studies to attenuate rewarding aspects of alcohol in mice (Lominac et al., 2006; Hodge et al., 2006; Gupta et al., 2008; Szumlinski et al., 2008; Cozzoli et al., 2009) and rats (Bäckström et al., 2004; McMillen et al., 2005; Olive et al., 2005; Shroeder et al., 2005, 2008; Besheer et al., 2009). With regard to models of binge drinking, high MPEP doses (20 and 40 mg/kg) selectively reduced intake of a 20% ethanol solution in the DID procedure without altering consumption of water or a 10% sucrose solution (Gupta et al., 2008). Our studies using the SHAC procedure show that injection of a 10 mg/kg dose of MPEP significantly decreased binge ethanol consumption without altering total fluid intake. This is consistent with elegant work by Szumlinski and colleagues, which determined that intra-NAC manipulations of steps in the mGluR5-Homer2-PI3K (phosphatidylinositol 3-kinase) signaling cascade significantly altered binge ethanol consumption with the SHAC procedure (Cozzoli et al., 2009). Specifically, viral-mediated knockdown of NAC Homer2b expression decreased binge ethanol intake, as did intra-NAC infusions of the mGluR5 antagonist MPEP or the PI3K antagonist wortmannin. Binge drinking also was significantly reduced in transgenic mice with an F1128R point mutation in mGluR5 that reduces Homer binding to the receptor (Tu et al., 1998), whereas moderate home cage ethanol consumption was intact (Cozzoli et al., 2009). Collectively, these results support the idea that intact signaling through a mGluR5-Homer2-PI3K complex within the NAc is important for a binge alcohol-drinking phenotype. Additionally, protein expression of members of the group 1 mGluR-Homer2-PI3K signaling complex were upregulated in the NAC of mice following 6 bouts of binge drinking with the SHAC procedure as well as in mice selectively bred by us for binge alcohol consumption with the SHAC procedure (Cozzoli et al., 2009). Upregulation of PI3K signaling also was identified in pathway analysis of NAC ethanol-induced changes in adult male rats (McBride et al., 2009). These combined observations suggest that NAC PI3K signaling is altered by ethanol administration, but specific downstream cascades that mediate biological changes resulting from altered PI3K activity are not characterized.

The self-administration results were somewhat surprising, given that MPEP produced a 17 - 19% decrease in intake of sucrose and ethanol, but the reduction was significant only for sucrose intake. The different results in mice (present versus previous work) with regard to the effects of MPEP on ethanol-self-administration may be due in part to recent evidence suggesting that MPEP has non-specific actions (e.g., inhibition of N-methyl-D-aspartate receptors; positive allosteric modulator at mGluR4 receptor subtype) in addition to its non-competitive antagonism at the mGluR5 receptor subtype (Lea and Faden, 2006). Future studies will examine MTEP (3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine), as it is a

mGluR5 antagonist that has greater selectivity and bioavailability than MPEP (Anderson et al., 2002; Cosford et al., 2003). MTEP was recently shown to produce a dose-dependent decrease in both the consumption of ethanol and the appetitive responding for ethanol in an operant procedure (Cowen et al., 2007) as well as a reduction in the interoceptive effects of ethanol (Besheer et al., 2009).

In conclusion, the present findings pharmacologically validate the SHAC procedure as a model of binge drinking in rodents. While thirst may contribute to the motivation to consume ethanol with the SHAC procedure, pharmacological manipulations that decreased binge drinking had modest effects on operant ethanol self-administration. Importantly, the SHAC procedure allows the mice to consume intoxicating doses of ethanol (rather than injecting them with an intoxicating dose of ethanol to model binge drinking) and achieve BECs ≥ 100 mg% (or 1.0 mg/ml). These levels exceed the criteria established by NIAAA for binge drinking, so the high ethanol intake and BECs achieved model the binge drinking in which humans are known to engage. Notably, prior binge drinking experience has been shown to be a strong predictor of alcoholic dependence later in life (Hingson et al., 2006). An understanding of the neurobiological mechanisms contributing to a binge drinking phenotype may guide pharmacological treatment strategies that can be used to reduce the development of alcohol dependence.

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Figure 1. Systemic administration of naltrexone significantly decreased binge drinking in WSC mice, measured with the SHAC procedure

Panel A depicts the effect of naltrexone on ethanol intake during the 30 min ethanol sessions for each period of total fluid availability. **Panel B** depicts the ethanol dose consumed, collapsed across all ethanol sessions. **Panel C** depicts total fluid intake across all periods of access on the ethanol session days. Values represent the mean $\pm$ SEM for 10 (saline) or 15 (per drug dose). $**p < 0.01$, $***p < 0.001$, vs. respective saline group

Figure 2. Systemic administration of baclofen significantly decreased binge drinking in WSC mice, measured with the SHAC procedure

The effect of baclofen on the ethanol dose consumed is depicted in **Panel A** for each period of total fluid availability and in **Panel B**, collapsed across all ethanol sessions. **Panel C** depicts total fluid intake across all periods of access on the ethanol session days. Values represent the mean $\pm$ SEM for 10 (saline) or 15 (per drug dose). $^+p < 0.10$, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, vs. respective saline group

Figure 3. Systemic administration of MPEP significantly decreased binge drinking in WSC mice, measured with the SHAC procedure

Panel A depicts the effect of MPEP on ethanol intake during the 30 min ethanol sessions for each period of total fluid availability. **Panel B** depicts the ethanol dose consumed, collapsed across all ethanol sessions. **Panel C** depicts total fluid intake across all periods of access on the ethanol session days. Values represent the mean $\pm$ SEM for 10 (saline) or 15 (per drug dose). *** $p < 0.001$, vs. respective saline group

Figure 4. Effects of naltrexone, baclofen and MPEP on operant self-administration of sucrose and ethanol

Following pre-treatment with drug or saline, mice were provided 30-min of continuous access to a sipper tube containing 5S (n=14) or 10E (n=11–12) following completion of the RR16 schedule of reinforcement. Values represent the mean $\pm$ SEM for the number of animals in parentheses. * $p < 0.05$, *** $p < 0.001$, vs. within-subject saline treatment

Table 1

Ethanol access sessions during the SHAC procedure

	Days 3, 6 & 9		Day 12		Day 15		Days 18 & 21	
Fluid Available	5E	water	5E	water	5E	water	5E	water
Fluid Access Time	30 min	3.5 hrs	30 min	5.5 hrs	30 min	7.5 hrs	30 min	9.5 hrs
Total Fluid Access	4 hrs		6 hrs		8 hrs		10 hrs	

Table 2

Effects of naltrexone, baclofen, and MPEP on appetitive measures.

	<u>Sucrose-Reinforced</u>		<u>Ethanol-Reinforced</u>	
	Active	Inactive	Active	Inactive
Naltrexone				
<i>Saline</i>	16.0 ± 0.0	0.4 ± 0.2	16.0 ± 0.0	1.3 ± 0.5
<i>0.60 mg/kg</i>	14.9 ± 1.1	0.2 ± 0.2	14.4 ± 1.5	0.4 ± 0.3
<i>1.25 mg/kg</i>	16.0 ± 0.0	1.8 ± 1.1	13.6 ± 1.7	1.0 ± 0.6
Baclofen				
<i>Saline</i>	16.0 ± 0.0	0.6 ± 0.4	16.0 ± 0.0	0.6 ± 0.3
<i>2.5 mg/kg</i>	16.0 ± 0.0	1.0 ± 0.9	16.0 ± 0.0	1.5 ± 0.9
<i>5.0 mg/kg</i>	13.7 ± 1.6	0.6 ± 0.2	7.4 ± 2.3***	1.3 ± 0.5
MPEP				
<i>Saline</i>	16.0 ± 0.0	0.4 ± 0.2	16.0 ± 0.0	1.4 ± 0.6
<i>3.0 mg/kg</i>	16.0 ± 0.0	0.5 ± 0.2	16.0 ± 0.0	1.6 ± 0.8
<i>10.0 mg/kg</i>	14.9 ± 1.1	0.4 ± 0.2	16.0 ± 0.0	3.1 ± 2.0

Values represent the mean ± SEM of $n = 14$ sucrose-reinforced or $n = 11-12$ ethanol-reinforced mice. All mice were responding on a RR16 schedule of reinforcement.

 $P \leq 0.001$ versus within-subject saline treatment.