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Pharmacological characterization of the 20% alcohol intermittent access model in Sardinian alcohol-preferring rats: a model of binge-like drinking

Valentina Sabino, Ph.D.^{1,*}, Jina Kwak¹, Kenner C. Rice, Ph.D.², and Pietro Cottone, Ph.D.^{1,*}

¹Laboratory of Addictive Disorders (LAD), Department of Pharmacology and Experimental Therapeutics and Department of Psychiatry, Boston University School of Medicine, Boston, MA, USA

²Chemical Biology Research Branch, National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD, USA

Abstract

BACKGROUND—Binge drinking is defined as a pattern of alcohol drinking that brings blood alcohol levels to 80 mg/dl or above. In this study, we pharmacologically characterized the intermittent-access to 20% ethanol model (Wise, *Psychopharmacologia* 29 (1973), 203) in Sardinian alcohol-preferring rats to determine to which of the compounds known to reduce drinking in specific animal models their binge-like drinking was sensitive to.

METHODS—Adult male Sardinian alcohol-preferring (sP) rats were divided into two groups and allowed to drink either 20% v/v alcohol or water for 24 hours on alternate days (Monday, Wednesday and Friday) or 10% v/v alcohol and water for 24 hours every day. After stabilization of their intake, both groups were administered three pharmacological agents with different mechanisms of action, naltrexone –an opioid receptor antagonist-, SCH 39166 –a dopamine D1 receptor antagonist-, and R121919 –a CRF₁ receptor antagonist-, and their effects on alcohol and water intake were determined.

RESULTS—Intermittent, 20% alcohol (“Wise”) procedure in sP rats led to binge-like drinking. Alcohol drinking was suppressed by naltrexone and by SCH 39166, but not by R121919. Finally, naltrexone was more potent in reducing alcohol drinking in the intermittent 20% binge drinking group than in the 10% continuous access drinking group.

DISCUSSION—The Wise procedure in sP rats induces binge-like drinking, which appears opioid- and dopamine-receptor mediated; the CRF₁ system, on the other hand, does not appear to be involved. In addition, our results suggest that naltrexone, and perhaps also SCH 39166, is particularly effective in reducing binge drinking. Such different pharmacological responses may apply to subtypes of alcoholic patients who differ in their motivation to drink, and may eventually contribute to treatment response.

Keywords

Naltrexone; Opioid; CRF OR CRH OR corticotropin-releasing; Ethanol; Addiction

*Address correspondence to: Valentina Sabino, Ph.D. or Pietro Cottone, Laboratory of Addictive Disorders, Boston University School of Medicine, 72 E Concord St, R-612, Boston, MA 02118, T. 617-638-4327 or 617-68-5662, vsabino@bu.edu or cottone@bu.edu.

Disclosure/Conflict of Interest

The authors report no conflict of interest.

INTRODUCTION

Binge drinking is defined as a pattern of alcohol drinking that brings blood alcohol levels (BALs) to 80 mg/dl or above, according to the National Advisory Council of the National Institute on Alcohol Abuse and Alcoholism (NIAAA, 2004). For an adult, this pattern is equivalent to approximately four and five drinks in males and females, respectively, consumed within a 2-hr time frame.

Binge drinking has important negative consequences at a personal, medical and social level. A recent survey (NESARC nationwide survey) has shown that 8.5% of adults drink to a level exceeding the NIAAA binge guidelines 1 or 2 times a week, and that this seems to be much more frequent in children and adolescents (Dawson et al., 2008).

Analogous to the definition employed in humans, in preclinical research binge-like drinking has been operationalized as a pattern of alcohol drinking that produces BALs of 80 mg/dl or above. Several preclinical models have been developed in rodents to mimic the binge-like drinking phenotype; however, a heated debate persists regarding the nature of the neurobiological processes which underlie this type of excessive consumption.

Indeed, binge drinking may be considered either as the initial phase of a condition that will eventually lead to alcohol dependence, or as an independent unit, demonstrated by the fact that animal models of binge-like drinking have recently begun to be classified and differentiated based on whether or not the binge-like drinking is accompanied by alcohol dependence (Crabbe et al., 2011). In the first case, excessive drinking has been hypothesized to be driven by the aversive, negative effects of withdrawal (negative reinforcement), while in the latter case by the pleasant, positive reinforcing effects of alcohol (positive reinforcement). Examples of binge-like drinking models with alcohol dependence are those in which withdrawal-associated drinking follows chronic exposure to either alcohol vapor or alcohol-containing liquid diets that act as a sole caloric source (Lopez and Becker, 2005; Roberts et al., 2000a; Valdez et al., 2004). Examples of binge-like drinking models without alcohol dependence are the Drinking In the Dark (DID) model in mice, the scheduled high alcohol consumption (SHAC) model in mice, the sweet solution ("supersac") model in rats, and the intermittent access 20% alcohol model ("Monday-Wednesday-Friday") in both rats and mice (Crabbe et al., 2009; Finn et al., 2005; Hwa et al., 2011; Ji et al., 2008; Rhodes et al., 2005; Simms et al., 2008; Szumlinski et al., 2007).

Among the different binge-like drinking models, the one that in rats has been shown to produce very high levels of drinking in a short period of time is the intermittent access to 20% alcohol. This model was initially proposed in 1973 by Wise, who showed that in outbred rats 24-hr access to 20% ethanol every other day produced high levels of drinking (9 g/kg/day). This procedure was recently readopted by several laboratories with some modifications. In these studies, however, only few animals were shown to reach BALs higher than 80 mg/dl, the average generally being lower in both outbred and in genetically selected rats (Cippitelli et al., 2012; Loi et al., 2010; Simms et al., 2008). More recently, Miczek's group has shown that C57Bl/6 mice under this protocol reach average BALs of approximately 100 mg/dl (Hwa et al., 2011). Furthermore, the neurotransmitter systems mediating alcohol drinking in this model are still unclear.

One approach to differentiating the behaviors and neural mechanisms associated with binge-like alcohol drinking and dependence-induced drinking is to examine the efficacy of a number of treatments with different pharmacological agents. The nonselective opioid antagonist naltrexone is more effective in suppressing excessive binge-like alcohol drinking than excessive drinking related to alcohol dependence (Ji et al., 2008; Sabino et al., 2006; Simms et al., 2008; Walker and Koob, 2008). On the other hand, antagonists of the

Corticotropin-Releasing Factor 1 receptor (CRF₁) are more effective in reducing alcohol drinking associated with withdrawal, compared to drinking of non-dependent animals (Koob, 2003).

The present investigation sought to pharmacologically characterize the intermittent (every other day) 24-hr access to 20% alcohol in Sardinian alcohol-preferring (sP) rats, while comparing it to the classical continuous 24-hr access to 10% alcohol, the sP rats selection phenotype. Specifically, we aimed at determining whether binge-like drinking was sensitive to the effects of drugs known to reduce drinking in non-dependent animals and/or by compounds that reduce drinking associated with withdrawal. For this purpose, we compared the effects of three compounds with different mechanisms of action: naltrexone, SCH 39166, and R121919. Naltrexone is a non-selective opioid antagonist used clinically as a treatment for alcoholism (Heilig and Egli, 2006); SCH 39166 is a dopamine D1 receptor antagonist, while R121919 is a CRF₁ receptor antagonist. These three compounds have all been shown to reduce alcohol intake in animal models, consistently with the hypothesis that the reinforcing properties of alcohol involve the recruitment of the mesocorticolimbic opioidergic and dopaminergic systems, as well as the antireward CRF system (Koob and Volkow, 2010).

MATERIALS AND METHODS

Animals

Adult male Sardinian alcohol-preferring rats, bred at the Department of Pharmacology of Boston University School of Medicine, were subjects of this study. Rats, 55–60-day old at study onset, were single-housed in a humidity- and temperature (22 °C)-controlled vivarium on a 12-h light–dark cycle (lights off at 10:00am) with water and regular rodent chow available *ad libitum* at all times. Experiments were conducted during the rats' dark cycle. Rats were $n=15$ – 16 /group in the escalation study; in the BAL study, $n=7$ in the 10%-7DAYS group and $n=11$ in the 20%-MWF group (a subset of the escalation experiment rats, since blood could not be drawn from a few rats); in the pharmacological studies, $n=8$ in the 10%-7DAYS and $n=11$ in the 20%-MWF group (separate group of rats). All experimental procedures adhered to the guidelines of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee of Boston University.

Drugs

Ethanol solutions (10% or 20% v/v) were prepared using 95% ethyl alcohol and tap water. Naltrexone (HCl) and SCH 39166 (HBr) were purchased from Tocris Cookson; R121919 (HCl); ((2,5-dimethyl-3-(6-dimethyl-4-methylpyridin-3-yl)-7-dipropylamino-pyrazolo[1,5-a]pyrimidine) or NBI 30775) was synthesized by Dr. Kenner C. Rice. Naltrexone (0, 0.01, 0.1, 1 mg/kg) and SCH 39166 (0, 0.03, 0.1, 0.3 mg/kg) were both dissolved in isotonic bacteriostatic saline and administered subcutaneously (s.c.) in a volume of 1 ml/kg of body weight, 20 minutes before the drinking sessions. R121919 (0, 5, 10, 20 mg/kg) was dissolved in 20% w/v hydroxypropyl- β -cyclodextrin (pH: 4.5) and administered subcutaneously (s.c.) in a volume of 2 ml/kg of body weight, 45 minutes before the drinking sessions. Doses of the three drugs, vehicles and pretreatment times were based on our previous reports (Cottone et al., 2009; Funk et al., 2007; Gilpin et al., 2009; 2011; Sabino et al., 2006) as well as on the available addiction literature in rats (Barrett et al., 2004; Czachowski and Delory, 2009; Ibba et al., 2009; Panocka et al., 1995; Simms et al., 2008; Williams and Broadbridge, 2009).

Two-bottle choice drinking paradigm

Sardinian alcohol-preferring rats were exposed to two different schedules of ethanol drinking. Half of the rats were allowed to drink 20% *v/v* alcohol or water for 24 hours on alternate days (Monday, Wednesday and Friday, 20%-MWF group) in their home cages, according to the method described by Wise (1973) and recently readopted by multiple laboratories in both rats and mice (Cippitelli et al., 2012; Hwa et al., 2011; Loi et al., 2010; Simms et al., 2008; 2010). On off days, rats were allowed to drink water from two bottles. The other half of the rats were instead allowed to drink 10% *v/v* alcohol and water for 24 hours every day (10%-7DAY group) in their home cages. The placement of the alcohol bottle was alternated at every session to control for side preferences. We used 50 ml conical tubes together with rubber stoppers and metal double-ball sipper tubes that produced very low spillage in repeated measurements. Possible fluid spillage was calculated by using bottles positioned in empty cages, interspersed in the cage rack; the volume lost was negligible and did not differ among the three solutions (water, 10% ethanol, and 20% ethanol): Mean $\pm$ SEM of ml/day 0.33 ± 0.04 , 0.29 ± 0.06 and 0.36 ± 0.05 , respectively; $F(2,11) = 0.88$, $p = 0.447$ (calculated by a one-way ANOVA). This procedure was continued for approximately 7 weeks (20 sessions for the 20%-MWF group). Rats were habituated to *s.c.* injections prior to pharmacological testing.

Procedure for blood alcohol levels determination

To determine blood alcohol levels that resulted from self-administration, blood samples (50 μ l) were collected from the tip of the tail of a subset of rats 1 hour after dark cycle (and session) onset. After centrifugation, plasma was assayed for alcohol content by injection into an oxygen-rate alcohol analyzer (Analox Instruments, Lunenburg, MA).

Pharmacological testing

To determine the effects of Naltrexone, SCH 39166, and R121919 on ethanol drinking, separate groups of rats were pretreated with each of the drugs in a within-subject Latin square design. Ethanol and water intake were determined by weighing bottles before session onset (corresponding to dark cycle onset) using a scale with 0.1 g precision and again 1, 6 and 24 hours later. Test days were spaced by 4–5 intervening treatment-free days. Due to the schedule of the 20% ethanol access group (Monday-Wednesday-Friday), rats were given at least one treatment-free drinking session; this resulted in either 4 (e.g. when injections were performed on Mon and Fri) or 5 (e.g. when injections were performed on Fri-Wed) treatment-free days.

Statistical analysis

Intake data were analyzed by analysis of variance (ANOVA) and expressed as Mean $\pm$ SEM, normalized for body weight (i.e., ethanol, g/kg; water and total fluid, ml/kg). The ethanol intake acquisition in the two groups (20%-MWF and 10%-7DAYS) was analyzed with a two-way mixed design ANOVA, with Group as a between-subjects factor and Day as a within-subject factor. The effects of the pharmacological treatments on intake were first analyzed using three-way ANOVAs, with Group as a between-subjects factor, and Dose and Time as within-subject factors. The effects of drugs on incremental 1-hr and 6-hr intake in each group (20%-MWF and 10%-7DAYS) were then analyzed using two-way repeated measure ANOVAs with Dose and Time as within-subject factors, while the effects of drugs on 24-hr intake were analyzed by one-way repeated measure ANOVA, with Dose as a within-subject factor. Pairwise post-hoc comparisons were made using Student's *t* test (to compare performance between two groups) or Dunnett's test for all other comparisons *vs.* vehicle conditions.

RESULTS

Experiment 1: Acquisition and maintenance of ethanol drinking in 20%-MWF vs. 10%-7DAYS groups

Adult male Sardinian alcohol-preferring (sP) rats were given either intermittent 20% *v/v* ethanol (20%-MWF, $n = 15$) or continuous 10% *v/v* ethanol (10%-7DAYS, $n = 16$) in a two-bottle choice situation. Figure 1 shows the first 10 drinking sessions during which both groups of sP rats quickly escalated their 24-hr ethanol intake, as demonstrated by a significant effect of the variable Session in the two-way ANOVA ($F(9,261) = 69.60$, $p = 0.000$). The 20%-MWF group consistently and dramatically drank more ethanol than the 10%-7DAYS group beginning from the second session of drinking, as demonstrated by a significant effect of the variable Group ($F(1,29) = 64.57$, $p = 0.000$) and a significant interaction Group x Session ($F(9,261) = 8.07$, $p = 0.000$). The intake reached a plateau after 8–9 sessions, attaining values of 10.05 ± 0.36 and 6.66 ± 0.17 g/kg for the 20%-MWF and the 10% -7 DAYS group, respectively (average of last 2 sessions).

Figure 2 shows the blood alcohol levels (BALs) measured in rats belonging to either the 20%-MWF group or the 10%-7DAYS group, 1-hr after dark cycle onset. BALs were 81.1 ± 6.81 mg/dl (the approximate equivalent of the blood alcohol level criterion for binge drinking) and 24.6 ± 7.59 mg/dl, which resulted from a consumption of 1.45 ± 0.11 g/kg and 1.05 ± 0.15 g/kg of ethanol, respectively. Blood alcohol levels correlated significantly with the 1-hr ethanol intake in both groups (20%-MWF: $p < 0.001$, $r = 0.919$; 10%-7DAYS: $p < 0.01$, $r = 0.887$).

Experiment 2: Effects of the Opioid Receptor Antagonist Naltrexone on ethanol intake in 20%-MWF vs. 10%-7DAYS groups

The Opioid Receptor Antagonist Naltrexone differentially affected intake in the 20%-MWF group and the 10%-7DAYS group, as demonstrated by a significant main effect of Dose ($F(3,51) = 16.75$, $p < 0.001$), as well as a significant interaction Dose x Group ($F(3,51) = 5.26$, $p < 0.01$) in the three-way ANOVA.

20%-MWF—As shown in the top panel of Figure 3, Naltrexone dose-dependently reduced ethanol intake in the 20%-MWF group of rats at both the 1-hr and 6-hr time points (Dose: $F(3,30) = 23.3$, $p < 0.001$; Dose x Time: $F(3,30) = 1.88$, n.s.). Post-hoc analysis indicated that the two highest doses of naltrexone significantly suppressed 20% ethanol intake at the 1-hr and 6-hr time points. The effect of naltrexone on 20% ethanol intake persisted at the 24-hr time point (Dose: $F(3,30) = 14.5$, $p < 0.001$), with only the highest dose being effective at this time point. Water intake was not affected by the treatment (Dose: $F(3,30) = 1.76$, n.s.; Dose x Time: $F(3,30) = 1.87$, n.s.), while consequently the total fluid intake was affected by the treatment (Dose: $F(3,30) = 11.6$, $p < 0.001$; Dose x Time: $F(3,30) = 1.59$, n.s.) (Table 1).

10%-7DAYS—As shown in the bottom panel of Fig. 3, naltrexone dose-dependently reduced ethanol intake in the 10%-7DAYS group, but it affected intake differentially at the 1-hr and 6-hr time points (Dose: $F(3,21) = 3.11$, $p < 0.05$; Dose x Time: $F(3,21) = 7.95$, $p < 0.001$); furthermore, the effect was no longer present at the 24-hr time point (Dose: $F(3,21) = 1.05$, n.s.). Post-hoc analysis indicated that naltrexone reduced 10% ethanol intake only in the first hour and only at the highest dose in this group. Water intake was not affected by the treatment (Dose: $F(3,21) = 0.20$, n.s.; Dose x Time: $F(3,21) = 0.51$, n.s.), while consequently the total fluid intake was affected by the treatment (Dose: $F(3,21) = 1.22$, n.s.; Dose x Time: $F(3,21) = 3.18$, $p < 0.05$) (Table 1).

Experiment 3: Effects of the Dopamine D1 Receptor Antagonist SCH 39166 on ethanol intake in 20%-MWF vs. 10%-7DAYS groups

The Dopamine D1 Receptor Antagonist affected ethanol intake in both the 20%-MWF and the 10%-7DAYS group, as demonstrated by a significant main effect of Dose ($F(3,51)=7.56$, $p<0.001$) and no significant interaction Dose x Group ($F(3,51)=0.11$, n.s.) in the three-way ANOVA.

20%-MWF—As shown in the top panel of Figure 4, SCH 39166 dose-dependently reduced ethanol intake in the 20%-MWF group of rats at both the 1-hr and 6-hr time points (Dose: $F(3,30)=5.27$, $p<0.01$; Dose x Time: $F(3,30)=3.88$, $p<0.05$). Post-hoc analysis indicated that the highest dose of SCH 39166 significantly suppressed 20% ethanol intake at the 1-hr and 6-hr time points. The effect of SCH 39166 persisted at the 24-hr time point only in the 20% ethanol intake group (Dose: $F(3,30)=2.85$, $p=0.054$; linear trend $F(1,30)=8.31$, $p<0.01$). Water intake was not affected by the treatment (Dose: $F(3,30)=0.43$, n.s.; Dose x Time: $F(3,30)=0.77$, n.s.), while consequently the total fluid intake was (Dose: $F(3,30)=6.82$, $p<0.001$; Dose x Time: $F(3,30)=2.86$, n.s.) (Table 1).

10%-7DAYS—As shown in the bottom panel of Fig. 4, SCH 39166 dose-dependently reduced ethanol intake in the 10%-7DAYS group (Dose: $F(3,21)=9.50$, $p<0.001$; Dose x Time: $F(3,21)=1.22$, n.s.); the effect was no longer present at the 24-hr time point (Dose: $F(3,21)=0.60$, n.s.). Post-hoc analysis indicated that the two highest doses of SCH 39166 reduced 10% ethanol intake at the 1-hr and 6-hr time points in this group. Water intake was not affected by the treatment (Dose: $F(3,21)=1.31$, n.s.; Dose x Time: $F(3,30)=1.73$, n.s.), while consequently the total fluid intake was (Dose: $F(3,21)=5.92$, $p<0.01$; Dose x Time: $F(3,21)=1.63$, n.s.) (Table 1).

Experiment 4: Effects of the CRF₁ Receptor Antagonist R121919 on ethanol intake in 20%-MWF vs. 10%-7DAYS groups

The CRF₁ Receptor Antagonist R121919 did not affect ethanol intake in either the 20%-MWF or the 10%-7DAYS group (Dose: $F(3,51)=0.32$, n.s.; Dose x Time: $F(3,51)=0.67$, n.s.).

20%-MWF—As shown in the top panel of Figure 5, R121919 had no effect on ethanol intake in the 20%-MWF group of rats (Dose: $F(3,30)=0.36$, n.s.; Dose x Time: $F(3,30)=1.80$, n.s.). Neither water intake (Dose: $F(3,30)=0.98$, n.s.; Dose x Time: $F(3,30)=0.49$, n.s.) nor total fluid intake (Dose: $F(3,30)=1.30$, n.s.; Dose x Time: $F(3,30)=0.05$, n.s.) was affected by the treatment (Table 1).

10%-7DAYS—As shown in the bottom panel of Fig. 5, R121919 did not affect ethanol intake in the 10%-7DAYS group (Dose: $F(3,21)=0.27$, n.s.; Dose x Time: $F(3,21)=0.93$, n.s.). Neither water intake (Dose: $F(3,21)=0.41$, n.s.; Dose x Time: $F(3,21)=0.32$, n.s.) nor total fluid intake (Dose: $F(3,21)=0.56$, n.s.; Dose x Time: $F(3,21)=1.04$, n.s.) was affected by the treatment (Table 1).

Discussion

The present series of studies was performed in Sardinian alcohol-preferring rats. Our line of sP rats descends from the original line that was selectively bred from a Wistar stock by Prof. G.L. Gessa (University of Cagliari, Italy) (Colombo, 1997). The selectively bred sP rats provide a model for identifying potential pharmacotherapies for alcoholism (Colombo et al., 2006; McBride and Li, 1998), as they voluntarily drink high quantities of ethanol, have a heritable component similar to human ethanol dependence (Bohman et al., 1981; Cloninger

et al., 1981), and the model has been shown to possess good predictive validity. However, the validity of the “standard” 10% alcohol continuous, two-bottle choice preference test in sP rats as a model for binge-like drinking has been questioned, since the pattern of alcohol consumption rarely results in BALs higher than 80 mg/dl (Becker, 2012; Crabbe, 2008); additionally, concerns have been raised over its use as a model for alcohol dependence, since the drinking pattern is mainly prandial and withdrawal signs are not usually observed (Colombo, 1997; Sabino et al., 2006).

The results of Experiment 1 in the present study show that the “Wise procedure”, i.e. the home cage intermittent (every other day) 24-hr exposure to 20% alcohol in sP rats (20%-MWF), results in intake of alcohol significantly higher than that obtained by exposing sP rats to the “classical” home cage continuous (7 days a week) access procedure to 10% alcohol (10%-7DAYS). Importantly, the intake of the 20%-MWF rats resulted in blood alcohol levels higher than 80 mg/dl in the first hour of access, therefore satisfying the criterion for “binge-like” drinking, according to the definition provided by the National Institute on Alcohol Abuse and Alcoholism (NIAAA, 2004). In Experiments 2-3-4, we then pharmacologically characterized the binge-like drinking model, testing the effects of several compounds with different mechanisms of action (an opioid receptor antagonist, a dopamine D1 receptor antagonist, and a CRF₁ receptor antagonist), in an effort to determine the neurotransmitter systems driving alcohol drinking in the two models. We found that both naltrexone and SCH 39166 are able to reduce ethanol intake in both the sP 20%-MWF and the sP 10%-7DAYS; however, in the binge drinking group naltrexone showed increased potency, and both naltrexone and SCH 39166 showed longer duration of action. On the other hand, both groups of sP rats were insensitive to the effects of R121919.

The intermittent access 20% alcohol model was originally proposed by Wise in 1973 (Wise, 1973); the procedure has recently been readopted by several laboratories, due to the high levels of alcohol intake that can be reached in outbred rats following a relatively short training period. In these studies, however, only few rats, if any, were shown to reach BALs higher than 80 mg/dl, with the average BALs generally being lower in both outbred and genetically-selected rats (Cippitelli et al., 2012; Loi et al., 2010; Melendez, 2011; Simms et al., 2008; 2010). Under this protocol, C57Bl/6 mice have recently been shown to reach average BALs of approximately 100 mg/dl (Hwa et al., 2011). Our studies confirm the results obtained by Loi et al. (2010), showing that the Wise procedure in sP rats results in increased ethanol intake as compared to continuous access. However, compared to that report, sP rats in the present paper reached higher blood alcohol levels following 1-hr of access to 20% alcohol (81.07 vs. 42.1 mg/dl); a possible explanation for this discrepancy is that sP rats at the beginning of our study were 55–60 days of age, compared to the 75 days of age of the Loi et al. study, which could potentially be responsible for the slightly higher levels of intake attained in this study (1.45 g/kg in 1-hr in our BALs study, compared to the 1.24 g/kg in the Loi et al.). On the other hand, rats exposed daily to 10% alcohol (the selection phenotype) reached BALs that were significantly lower (24.6 mg/dl), which is in agreement with previous literature showing that this procedure results in BALs in sP rats that rarely reach 80 mg/dl (Colombo, 1997).

Which neurotransmitter systems sustain alcohol drinking in the 20% alcohol intermittent model in sP rats is unknown. For this purpose, we compared the effects of pretreatment with compounds of different pharmacological mechanisms.

Naltrexone is a preferential mu-opioid receptor antagonist currently approved by the US Food and Drug Administration (FDA) to treat alcohol dependence. Naltrexone reduces ethanol intake in rodents, primates, and humans, (Koob, 2003; Mason, 2003; Srisurapanont and Jarusuraisin, 2005) likely via blockade of μ opioid receptors, an idea which is supported

by the results of pharmacological studies with more selective μ opioid subtype antagonists, as well as knockout studies (Hyytia, 1993; Roberts et al., 2000b; Stromberg et al., 1998). In our study, naltrexone reduced ethanol drinking in both the 20%-MWF and the 10%-7DAYS group, suggesting a clear involvement of the opioid receptor system. Interestingly, naltrexone was more potent in the 20%-MWF binge-like drinking group, and its effect lasted longer. This is an important finding, especially considering the fact that opioid receptor antagonists have been shown to be particularly effective in reducing episodes of binge drinking in patients with alcoholism (Mason, 2003; Mason et al., 1999). The heightened sensitivity of alcohol binge-like drinking to the reducing effects of naltrexone is consistent with previous results showing increased potency of naltrexone in the “supersac” binge-drinking model (Ji et al., 2008), along with the observation that very low doses of naltrexone in sP rats reduce self-administration (Sabino et al., 2006). Low doses of naltrexone also reduce self-administration in non-dependent Wistar rats, while higher doses are needed in alcohol-dependent rats (Walker and Koob, 2008). Together, these observations indicate that naltrexone is more effective in suppressing binge-like alcohol drinking, providing some predictive validity to this model.

SCH 39166 is a highly selective dopamine D1 receptor antagonist. SCH 39166, as well as other D1 antagonists, have been shown to reduce ethanol drinking when administered systemically and into the nucleus accumbens (Hodge et al., 1997; Panocka et al., 1995; Rassnick et al., 1992; Samson et al., 1993), which is consistent with the ample literature showing that the mesolimbic dopamine pathway is directly involved in the rewarding effects of alcohol. In the current investigation, the D1 antagonist SCH 39166 reduced ethanol intake in both the 20%-MWF and the 10%-7DAYS, confirming that D1 receptors play an important role in the control of alcohol intake. Importantly, the effect of SCH 39166 lasted longer (up to the 24-hr time point) in the 20%-MWF binge-like drinking group, suggesting that binge-like drinking may display increased sensitivity to blockade of the dopaminergic transmission.

Naltrexone and SCH 39166 also significantly decreased the total fluid intake, since they did not produce a compensatory increase of water intake, suggesting a more general suppression of ingestive behavior. However, the effect on total fluid intake was generally less pronounced and shorter (was never present beyond the 6-hr time point) compared to the specific effects on ethanol intake.

R121919 is a potent small-molecule CRF₁ receptor antagonist with a high affinity for the CRF₁ receptor and over 1000-fold selectivity compared to other targets (Heinrichs et al., 2002; Keck et al., 2001). R121919 was shown to possess anxiolytic-like activity in rodents (Zorrilla and Koob, 2004), and antidepressant and anxiolytic properties in humans (Zobel et al., 2000). In the present study, R121919 did not affect ethanol drinking in either group of sP rats. The extra-hypothalamic CRF systems have been proposed to be perturbed during the transition to alcohol dependence, and to not be involved in alcohol consumption in non-dependent animals. R121919, as well as other CRF₁ receptor antagonists such as MJL-1-109-2, efficiently decrease withdrawal-associated ethanol intake, with no effect in non-dependent rats (Funk et al., 2007). The newer heterocyclic CRF₁ receptor antagonists MPZP and MTIP also reduce withdrawal-associated drinking in dependent rats (Gehlert et al., 2007; Richardson et al., 2008). In non-dependent alcohol preferring rats, the pharmacological sensitivity seems very similar; we have previously shown that spontaneous drinking in sP rats is independent of the CRF₁ receptor system, unless sP rats are made dependent by the exposure to ethanol vapor (Sabino et al., 2006). We have also shown that the CRF₁ antagonists are still able to revert the reduction of drinking induced by an acute stress in sP rats, suggesting a dissociation between the anxiolytic and the anti-alcohol effects of R121919; furthermore, because of these past findings, we reputed that it was not

necessary to confirm again in this study the ability of R121919 to block stress-induced reduction of drinking in sP rats. Likewise, Indiana P rats' spontaneous drinking is sensitive to opioid antagonists but not to CRF₁ antagonists (Gilpin et al., 2008). In the present paper, we extend these observations, showing that although the 20% intermittent procedure leads to very high levels of drinking in sP rats, their drinking remains insensitive to the reducing effects of CRF₁ antagonists, or at least to the specific CRF₁ antagonist and doses used in the present study. Our data are consistent with the observations made by Ji et al. that in the "supersac" model of binge-like drinking, alcohol intake is reduced by naltrexone but not by the CRF₁ receptor antagonists MPZP (Ji et al., 2008), further confirming the hypothesis that binge drinking in this model is opioid- and dopamine-mediated and does not involve a recruitment of CRF stress systems. On the other hand, MTIP (Gehlert et al., 2007) and antalarmin (Hansson et al., 2006) reduce spontaneous drinking in non-dependent marchigian sardinian alcohol-preferring (msP) rats. This reduction is likely attributable to genetic differences between msP rats and other strains, as the msP line shows a high incidence of a CRF₁ receptor promoter polymorphism that yields elevated CRF₁ receptor expression (Gehlert et al., 2007; Hansson et al., 2007). Recent observations have shown that the CRF₁ receptor antagonist antalarmin attenuates 20% alcohol consumption in outbred wistar rats exposed to both intermittent and continuous access (Cippitelli et al., 2012), as well as 20% alcohol consumption in mice exposed to the drinking in the dark procedure (Lowery-Gionta et al., 2012). These results are an apparent discrepancy with the data we obtained in the present study; however, differences among the studies, such as the species or line of rats used (sP rats vs. outbred wistar or C57Bl/6 mice) and the type of CRF₁ receptor antagonist used (R121919 vs. antalarmin), make any direct comparison challenging.

In summary, in this series of studies, we have shown that the intermittent, 20% alcohol ("Wise") procedure in sP rats leads to binge-like drinking, and that this drinking is mediated by opioid- and dopamine- but not CRF₁ receptor-mediated mechanisms. Finally, we showed that the potency of naltrexone in suppressing alcohol intake, and the duration of action of both naltrexone and SCH 39166 is higher in binge-drinking rats.

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Fig. 1.

Acquisition of ethanol drinking in adult male Sardinian alcohol-preferring (sP) rats given either intermittent access to 20% v/v ethanol (20%-MWF, n = 15) or daily access to 10% v/v ethanol (10%-7DAYS, n = 16). Data are expressed as Mean $\pm$ SEM intake, normalized for body weight. *P < 0.05, **P < 0.01, ***P < 0.001 (Student's t test).

Fig. 2.

Correlation between individual blood alcohol levels and 1-hr ethanol intake in Sardinian alcohol-preferring (sP) rats given either intermittent access to 20% *v/v* ethanol (20%-MWF) or daily access to 10% *v/v* ethanol (10%-7DAYS) (left panel, $n = 7-11$). Blood samples were collected 1-hr after dark cycle onset. Right panel shows Mean + SEM blood alcohol levels for each of the two groups; *** $P < 0.001$ (Student's *t* test).

20% MWF, Effects of Opioid Antagonist Naltrexone

10% 7 Days, Effects of Opioid Antagonist Naltrexone

Fig. 3.

Effect of s.c. pretreatment (20 min) with the non-selective opioid receptor antagonist naltrexone on 1-hr, 6-hr and 24-hr alcohol intake, relative to dark cycle onset. Subjects were Sardinian alcohol-preferring (sP) rats, tested either under 20% intermittent access conditions (20%-MWF, top panel) or under 10% continuous access conditions (10%-7DAYS, bottom panel). Data represent Mean + SEM intake, normalized for body weight. ** $P < 0.01$ vs. vehicle-treated group (Dunnett's test); ### $P < 0.001$ linear trend for dose.

20% MWF, Effects of D1 Antagonist SCH-39166

10% 7 Days, Effects of D1 Antagonist SCH-39166

Fig. 4.

Effect of s.c. pretreatment (20 min) with the selective dopamine D1 receptor antagonist SCH 39166 on 1-hr, 6-hr and 24-hr alcohol intake, relative to dark cycle onset. Subjects were Sardinian alcohol-preferring (sP) rats, tested either under 20% intermittent access conditions (20%-MWF, top panel) or under 10% continuous access conditions (10%-7DAYS, bottom panel). Data represent Mean + SEM intake, normalized for body weight. * $P < 0.05$, ** $P < 0.01$ vs. vehicle-treated group (Dunnett's test); ## $P < 0.01$, ### $P < 0.001$ linear trend for dose.

20% MWF, Effects of CRF1 Antagonist R121919

10% 7 Days, Effects of CRF1 Antagonist R121919

Fig. 5.

Effect of s.c. pretreatment (45 min) with the selective CRF1 receptor antagonist R121919 on 1-hr, 6-hr and 24-hr alcohol intake, relative to dark cycle onset. Subjects were Sardinian alcohol-preferring (sP) rats, tested either under 20% intermittent access conditions (20%-MWF, top panel) or under 10% continuous access conditions (10%-7DAYS, bottom panel). Data represent Mean + SEM intake, normalized for body weight.

Table 1

Effect of pretreatment with naltrexone, SCH 39166 or R121919 on 1-hr, 6-hr and 24-hr cumulative water intake (top) and total fluid intake (bottom), relative to dark cycle onset. Subjects were Sardinian alcohol-preferring (sP) rats, tested either under 20% intermittent access conditions (20%-MWF, left side) or under 10% continuous access conditions (10%-7DAYS, right side). Data represent Mean $\pm$ SEM intake, normalized for body weight (ml/kg).

Water Intake, 20%-MWF				Water Intake, 10%-7DAYS			
		1 hr	6 hr	24 hr			
NTX				NTX			
Vehicle	1.60 ± 0.27	9.16 ± 1.91	18.32 ± 4.83		Vehicle	4.11 ± 0.92	9.50 ± 1.16
0.01 mg/kg	2.01 ± 0.53	8.08 ± 1.22	19.66 ± 2.88		0.01 mg/kg	3.23 ± 0.91	9.24 ± 2.90
0.1 mg/kg	1.86 ± 0.42	10.70 ± 1.38	21.67 ± 3.31		0.1 mg/kg	3.01 ± 0.60	8.81 ± 0.95
1 mg/kg	1.85 ± 0.35	9.36 ± 1.55	19.42 ± 2.59		1 mg/kg	2.53 ± 0.43	7.94 ± 1.41
SCH 39166				SCH 39166			
Vehicle	1.11 ± 0.25	5.46 ± 0.84	8.07 ± 1.16		Vehicle	2.77 ± 0.59	5.78 ± 0.93
0.03 mg/kg	1.06 ± 0.26	5.22 ± 0.48	8.15 ± 0.88		0.03 mg/kg	4.04 ± 0.64	7.92 ± 1.67
0.1 mg/kg	1.29 ± 0.36	4.80 ± 0.65	6.98 ± 0.78		0.1 mg/kg	2.94 ± 0.72	6.59 ± 1.49
0.3 mg/kg	0.72 ± 0.11	4.68 ± 0.57	7.07 ± 0.89		0.3 mg/kg	1.66 ± 0.35	5.88 ± 1.80
R121919				R121919			
Vehicle	4.04 ± 1.28	10.62 ± 2.26	16.49 ± 3.39		Vehicle	5.53 ± 1.05	12.08 ± 2.67
5 mg/kg	4.56 ± 1.97	13.26 ± 2.48	19.82 ± 3.68		5 mg/kg	5.17 ± 1.04	10.32 ± 1.76
10 mg/kg	5.39 ± 1.36	13.47 ± 2.21	17.55 ± 3.15		10 mg/kg	4.75 ± 0.91	10.18 ± 1.09
20 mg/kg	2.14 ± 0.52	10.14 ± 1.57	15.69 ± 2.57		20 mg/kg	4.20 ± 1.29	10.21 ± 1.67
Total Fluid Intake, 20%-MWF				Total Fluid Intake, 10%-7DAYS			
		1 hr	6 hr	24 hr			
NTX				NTX			
Vehicle	12.00 ± 0.65	40.56 ± 1.57	77.19 ± 2.85		Vehicle	13.43 ± 0.60	40.00 ± 1.63
0.01 mg/kg	11.68 ± 0.94	40.41 ± 1.56	80.14 ± 2.49		0.01 mg/kg	12.38 ± 0.82	36.91 ± 2.35
0.1 mg/kg	8.68 ± 0.41**	37.60 ± 1.12	77.65 ± 2.20		0.1 mg/kg	9.97 ± 1.12 *	37.57 ± 1.77
1 mg/kg	6.81 ± 0.50**	32.92 ± 1.43 **	71.19 ± 2.28		1 mg/kg	6.76 ± 0.97 **	33.29 ± 2.97
							76.80 ± 2.83

Water Intake, 20%-MWF				Water Intake, 10%-7DAYS			
	1 hr	6 hr	24 hr		1 hr	6 hr	24 hr
NTX				NTX			
SCH 39166				SCH 39166			
Vehicle	9.60 ± 0.42	31.29 ± 1.31	60.55 ± 2.22	Vehicle	10.82 ± 1.06	34.15 ± 2.23	75.10 ± 3.55
0.03 mg/kg	9.37 ± 0.65	29.35 ± 1.32	59.65 ± 2.09	0.03 mg/kg	10.44 ± 0.99	32.98 ± 1.63	76.55 ± 3.06
0.1 mg/kg	8.24 ± 0.59	28.91 ± 0.92	57.27 ± 1.91	0.1 mg/kg	7.03 ± 0.72 *	28.83 ± 0.72 *	74.87 ± 3.06
0.3 mg/kg	5.57 ± 0.55 **	26.62 ± 1.10 **	56.91 ± 1.77	0.3 mg/kg	4.92 ± 0.92 **	26.48 ± 2.03 **	73.80 ± 3.21
R121919				R121919			
Vehicle	11.84 ± 1.60	35.09 ± 2.49	69.09 ± 3.24	Vehicle	16.10 ± 1.55	39.63 ± 1.30	90.71 ± 2.84
5 mg/kg	14.00 ± 1.98	38.10 ± 2.01	72.96 ± 3.13	5 mg/kg	16.79 ± 1.18	37.17 ± 2.16	83.25 ± 3.42
10 mg/kg	13.47 ± 1.55	38.49 ± 2.17	68.09 ± 2.82	10 mg/kg	15.43 ± 1.63	38.79 ± 1.39	81.43 ± 3.62
20 mg/kg	11.23 ± 1.01	33.73 ± 1.75	64.24 ± 2.35	20 mg/kg	15.69 ± 1.34	40.28 ± 2.53	84.75 ± 5.48