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Effects of CRF₁-Receptor and Opioid-Receptor Antagonists on Dependence-Induced Increases in Alcohol Drinking by Alcohol-Preferring (P) Rats

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Abstract

Background—Selective breeding of rats over generations and induction of alcohol dependence via chronic vapor inhalation both enhance alcohol consumption in animal models. The purpose of this study was to determine whether dependence-induced increases in alcohol consumption by P rats is sensitive to naltrexone, a general opioid receptor antagonist (but with highest affinity at the μ -opioid receptor at low doses), and the recently characterized small molecule CRF₁-receptor antagonist MPZP (*N,N*-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo [1,5-*a*]pyrimidin-7-amine).

Methods—P rats ($n = 20$) were trained to respond for alcohol and water in a 2-lever operant situation during daily 30-minute sessions. P rats were then matched for alcohol intake and exposed to chronic intermittent alcohol vapor ($n = 10$) or ambient air ($n = 10$) for approximately 10 weeks. All rats were then administered MPZP and naltrexone in 2 separate and consecutive Latin-square designs.

Results—MPZP attenuated dependence-induced increases in alcohol intake by P rats while having no effect on alcohol consumption by nondependent controls. Conversely, operant alcohol responding was reduced similarly in dependent and nondependent P rats by naltrexone.

Conclusions—These results confirm a role for brain CRF₁-receptor systems in dependence-induced changes in the reinforcing properties of alcohol, and CRF₁-receptor blockade appears to suppress dependence-induced drinking at lower doses in P rats relative to other rat lines. Therefore, brain CRF₁-receptor systems are important in the regulation of dependence-induced alcohol consumption, whereas brain opioid systems are important in the regulation of basal alcohol consumption by rats.

Keywords

CRF; Opioids; Naltrexone; MPZP; Alcohol-Preferring P Rats; Dependence

Alcohol-preferring (P) rats have been bred over many generations to be highly prone to voluntary consumption of alcohol (Lumeng et al., 1977). P rats voluntarily drink amounts of alcohol sufficient to achieve pharmacologically significant blood-alcohol levels (BALs; Li et al., 1979; Lumeng and Li, 1986), and they also self-administer alcohol via nonoral routes (e.g., intracranially; Gatto et al., 1994). P rats do not consume alcohol simply for its taste, smell, or caloric properties (Bice and Kiefer, 1990; Lankford et al., 1991), and they work for alcohol in an operant situation even when water and food are concurrently available (Murphy et al.,

1989). Following free-choice alcohol drinking, P rats exhibit both functional (Gatto et al., 1987) and metabolic tolerance (Lumeng and Li, 1986) and eventually exhibit signs of physical dependence following long periods of alcohol access (Kampov-Polevoy et al., 2000; Waller et al., 1982). P rats also exhibit additional increases in alcohol intake when made dependent via chronic intermittent exposure to alcohol vapor (Gilpin et al., 2008, see previous article in this issue).

It is generally accepted that the relative contributions of the positive and negative reinforcing effects of alcohol to the motivation for alcohol-drinking behavior change significantly across the various stages of the addiction cycle. Alcohol-drinking behavior by organisms not dependent on alcohol is likely motivated in large part by the positive reinforcing (i.e. pleasant) effects of alcohol (Altshuler et al., 1980). Alcohol-preferring P rats provide an excellent animal model of these motivational aspects of alcohol self-administration behavior, mainly for the reasons described above. Conversely, chronic exposure to high doses of alcohol (e.g., via alcohol vapor exposure) produces somatic and affective symptoms indicative of an alcohol dependence syndrome, defined in large part by the aversive symptoms that manifest upon discontinuation of alcohol exposure. The negative affective state produced by the absence of alcohol in dependent animals is hypothesized to provide the unconditioned stimulus for negative reinforcing properties of alcohol, and it is further hypothesized that these negative reinforcing effects eventually become the primary motivational force driving alcohol consumption in the dependent organism (Koob, 2003).

Brain corticotropin-releasing factor (CRF) systems play a major role in the affective-like disturbances associated with alcohol dependence (Heilig and Koob, 2007; Valdez and Koob, 2004). Withdrawal from chronic alcohol exposure produces increases in anxiety-like behavior that are blocked by CRF antagonists and, more specifically, by antagonists of the CRF₁-receptor subtype (Baldwin et al., 1991; Overstreet et al., 2004). Increased alcohol consumption associated with acute withdrawal from chronic alcohol is also blocked by CRF₁ antagonists in dependent animals (Chu et al., 2007; Funk et al., 2007; Gehlert et al., 2007; Sabino et al., 2006). More specifically, the small molecule CRF₁-receptor antagonist used in the present study, MPZP (*N,N*-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethylpyrazolo[1,5a]pyrimidin-7-amine), attenuates dependence-induced increases in alcohol drinking in Wistar rats (Richardson et al., 2008).

Brain CRF systems are also involved in alcohol-related behaviors in rats selectively bred for high alcohol preference. Rats selectively bred for high and low alcohol preference exhibit innate differences in brain CRF levels (Hwang et al., 2004; Richter et al., 2000). Pharmacology studies have shown that CRF₁-receptor antagonists suppress alcohol drinking by rats selectively bred for high alcohol preference under a variety of experimental conditions (Hansson et al., 2006; Overstreet et al., 2007; Sabino et al., 2006).

Brain opioid systems are heavily implicated in alcohol-drinking behavior (for reviews, see Froehlich and Li, 1994; Gianoulakis, 2004). Naltrexone, classified as a general opioid receptor antagonist (but with highest affinity at the μ -opioid receptor at low doses), blocks craving and relapse drinking in humans (Volpicelli et al., 1992) and has been approved as a pharmacological treatment for alcoholism. Very low doses of naltrexone are effective in suppressing operant alcohol responding by nondependent outbred rats (Walker and Koob, 2007), and even lower doses are effective in Sardinian-preferring (sP) rats (Sabino et al., 2006). In direct contrast to CRF antagonists, naltrexone may actually be more effective in suppressing alcohol drinking by nondependent rats relative to dependent rats (Walker and Koob, 2007).

The primary purpose of the present investigation was to examine the contributions of brain CRF and opioid systems to dependence-induced changes in the reinforcing effects of alcohol

in genetically selected P rats. It was hypothesized that naltrexone would suppress alcohol drinking similarly in alcohol-dependent and nondependent rats, a finding that would indicate a role for brain opioid systems in the regulation of baseline alcohol-drinking behavior by P rats, but not in dependence-induced enhancement of alcohol consumption by those animals. Conversely, it was hypothesized that the CRF₁-receptor antagonist (MPZP) would suppress dependence-induced, but not baseline, alcohol drinking by P rats.

Method

Subjects

Twenty male alcohol-preferring (P) rats (Indiana University School of Medicine) were used in this experiment. Immediately prior to the start of these experiments, these rats were used in a parametric study of the effects of alcohol vapor exposure on alcohol drinking but were naïve to all drugs except alcohol prior to the start of this experiment (see Gilpin et al., 2008, in this issue). P rats were quarantined for 10 weeks upon arrival at The Scripps Research Institute (TSRI) and weighed between 427 and 607 g at the start of operant training. Rats were group-housed in standard plastic cages with wood chip bedding under a 12 hour light/12 hour dark cycle (lights on at 8 _{PM}). Animals were given ad libitum access to food and water throughout except during experimental drinking sessions. All procedures were conducted in the dark cycle and met the guidelines of the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996).

Drugs

N,N-bis(2-methoxyethyl)-3-(4-methoxy-2-methylphenyl)-2,5-dimethyl-pyrazolo[1,5a]pyrimidin-7-amine (MPZP) is an antagonist of CRF₁ receptors. MPZP was dissolved in 1 M HCl (10% of total volume), diluted with 20% (w/v) 2-HBC (2-hydroxypropyl- β -cyclodextrin) in distilled water (80% of total volume), then backtitrated with NaOH to pH 4.5. MPZP solution was administered s.c. in a volume of 2 ml/kg body weight. Naltrexone hydrochloride (Sigma-Aldrich, St. Louis, MO) is classified as a general opioid receptor antagonist, but has higher affinity for the μ -opioid receptor subtype than the δ and κ subtypes at low doses (i.e. the dose range used in the present study). Naltrexone HCl was dissolved in saline and administered s.c. in a volume of 1 ml/kg body weight.

Operant Chambers

The operant chambers (Coulbourn Instruments, Allentown, PA) utilized in the present study had 2 retractable levers located 4 cm above a grid floor and 4.5 cm to either side of a 2-well acrylic drinking cup. Operant responses and resultant fluid deliveries were recorded by custom software running on a PC computer. A single lever-press activated a 15 rpm Razel syringe pump (Stanford, CT) that delivered 0.1 ml of fluid to the appropriate well over a period of 0.5 second. Lever presses that occurred during the 0.5 second of pump activation were not recorded and did not result in fluid delivery. Operant chambers were individually housed in sound-attenuated ventilated cubicles to minimize environmental disturbances.

Operant Ethanol Self-Administration Training

The treatment timeline for all P rats is illustrated in Fig. 1. Upon arrival at TSRI, P rats were quarantined for 10 weeks. During that time, P rats were allowed 30-minute 2-bottle choice drinking sessions of 10% (w/v) ethanol versus water 3 to 4 d/wk to allow them to habituate to the ethanol solution (data not shown). P rats were then delivered to the research facility colony room and allowed several days to habituate to the new housing conditions before operant training began.

P rats were trained to orally self-administer 10% (w/v) ethanol or water in a concurrent, 2-lever, free-choice contingency. Lever-presses were reinforced on a continuous fixed ratio-1 (FR1) schedule such that each response resulted in delivery of 0.1 ml of fluid. P rats were initially allowed 4 extended sessions in operant chambers in order to learn the lever-pressing procedure. Then sessions were shortened to the standard 30-minute length, and P rats were allowed 11 sessions of operant responding for 10% (w/v) ethanol versus water. Operant responding was stable and reliable for these rats by the eleventh day of operant responding. P rats were divided into 2 groups based on mean intakes across the final 5 days of this baseline period, and these groups were then chronically exposed to either alcohol vapor or ambient air.

Ethanol Vapor Inhalation

P rats were exposed to chronic inhalation of either ethanol vapor (dependent group; $n = 10$) or ambient air (nondependent controls; $n = 10$). To induce ethanol dependence, standard rat cages were housed in separate, sealed, clear plastic chambers into which ethanol vapor was intermittently introduced. This procedure has been described in detail elsewhere (Funk et al., 2006). Briefly, 95% ethanol was evaporated, and vapor was delivered at rates between 22 and 27 mg/l. Ethanol vapor was turned on (6 PM) for 14 h/d and off (8 AM) for 10 h/d (O'Dell et al., 2004) for 10 consecutive weeks, and the target range for BALs during vapor exposure was 150 to 200 mg%. Nondependent control rats were treated in parallel except they were exposed to vapor that did not contain ethanol (for the duration of the present experiment *and* the one that preceded it). During that time, rats were tested periodically for dependence-induced operant responding in the absence of injections (see previous article, this issue). Tail blood samples were collected periodically at 8 AM for BAL determination and vapor adjustments during vapor exposure. This chronic intermittent vapor exposure produces somatic and affective disturbances associated with alcohol dependence during withdrawal from alcohol vapor (O'Dell et al., 2004).

Effects of MPZP on Dependence-Induced Operant Alcohol Responding by P Rats

Following approximately 10 weeks of vapor exposure, rats were tested for the effects of MPZP on dependence-induced operant alcohol responding. Rats were injected s.c. with MPZP (0, 5, 10, 20 mg/kg) 60 minutes prior to operant test sessions in a within-subjects Latin-square design (randomized and counterbalanced to prevent order effects). Rats were injected in an anteroom and then returned to the home cage for the 60-minute interim period. Rats were then tested for operant ethanol responding 6 hours into withdrawal and subsequently returned to vapor chambers. During the testing phase, rats were exposed to daily intermittent vapor but were injected and tested 2 d/wk. Doses and pretreatment times were selected based on previous work (Richardson et al., 2008).

Effects of Naltrexone on Dependence-Induced Operant Alcohol Responding by P Rats

Subsequently, rats were tested for the effects of naltrexone on dependence-induced operant alcohol responding. Rats were injected s.c. with naltrexone (0, 16, 50, 150, 450 μ g/kg) 30 minutes prior to operant test sessions in a within-subjects Latin-square design (randomized and counterbalanced to prevent order effects). Rats were injected in an anteroom and then returned to the home cage for the 30-minute interim period. Rats were then tested for operant ethanol responding 6 hours into withdrawal and subsequently returned to vapor chambers. During the testing phase, rats were exposed to daily intermittent vapor but were injected and tested 2 d/wk. Doses and pretreatment times were selected based on previous work (Sabino et al., 2006; Walker and Koob, 2007).

Blood-Alcohol Level Determinations

Tail blood was sampled at the end of 14-hour ethanol vapor exposure periods and also following representative operant ethanol self-administration sessions. Rats were gently restrained while the tip of the tail (2 mm) was cut off with a clean razor blade. Tail blood (0.2 ml) was collected and centrifuged. Plasma (5 μ l) was used for measurement of BALs using an Analox AM 1 analyzer (Analox Instruments LTD, Lunenburg, MA). The reaction is based on the oxidation of alcohol by alcohol oxidase in the presence of molecular oxygen (alcohol + O₂ → acetaldehyde + H₂O₂). The rate of oxygen consumption is directly proportional to the alcohol concentration. Single point calibrations are done for each set of samples with reagents provided by Analox Instruments (25 to 400 mg%).

Statistical Analysis

Ethanol and water responses and intakes are expressed as mean $\pm$ SEM, and ethanol intake is normalized for body weight (i.e., g ethanol/kg body weight). For each drug tested, self-administration data were analyzed using a series of 2-way repeated-measures analyses of variance (RM ANOVAs), with ethanol dependence history (dependent vs. nondependent) as the between-subjects factors and drug dose as the within-subjects factor. Post-hoc comparisons were conducted using the Student Newman-Keuls test. Statistical significance was set at $p < 0.05$. Rats in the dependent group that did not exhibit stable and elevated responding across noninjection and vehicle days during the various phases of testing were excluded from all data analyses: a single dependent rat was excluded from MPZP analyses and 2 dependent rats were excluded from naltrexone analyses for this reason. One additional dependent rat was excluded from naltrexone analyses because of health complications that arose prior to completion of the Latin-square. In these cases, *all data points* for excluded rats were removed from analyses.

Results

Effects of MPZP on Dependence-Induced Operant Ethanol Responding by P Rats

Figure 2 illustrates operant alcohol responding (Fig. 2A) and alcohol intake (g/kg; Fig. 2B) by dependent and nondependent P rats following i.p. injection with 1 of 4 doses (0, 5, 10, 20 mg/kg) of MPZP. Dependent rats exhibited higher alcohol responding, $F(1,51) = 6.88$, $p = 0.018$, and alcohol intake (g/kg), $F(1,51) = 10.57$, $p = 0.005$, than nondependent rats. There were significant interaction effects of alcohol dependence history and MPZP dose on operant alcohol responding, $F(3,51) = 2.84$, $p = 0.047$, and alcohol intake (g/kg), $F(3,51) = 3.02$, $p = 0.038$. Post-hoc analyses indicated that dependent rats exhibited higher operant alcohol responding than nondependent rats on vehicle injection day ($p < 0.001$), but that this elevated responding was abolished by all doses of MPZP ($p > 0.05$ in all cases). The 10 mg/kg and 20 mg/kg doses of MPZP also suppressed operant alcohol responding by dependent rats relative to vehicle injection day ($p < 0.05$); there was a tendency toward a suppression of operant alcohol responding by the 5 mg/kg dose ($p = 0.06$). Post-hoc analyses also indicated that dependent rats exhibited higher alcohol intake (g/kg) than nondependent rats on vehicle injection day ($p < 0.001$), and that this elevated responding was abolished by the 5 mg/kg and 10 mg/kg doses of MPZP ($p > 0.05$ in both cases). All doses of MPZP also suppressed alcohol intake (g/kg) by dependent rats relative to vehicle injection day ($p < 0.05$). There were no order effects of MPZP dose administration ($p > 0.05$). Rats generally did not respond for water during operant test sessions, and alcohol preference was very high ($> 95\%$) throughout the experiment (Table 1).

Effects of Naltrexone on Dependence-Induced Operant Ethanol Responding by P Rats

Figure 3 illustrates operant alcohol responding (Fig. 3A) and alcohol intake (g/kg; Fig. 3B) by dependent and nondependent P rats following s.c. injection with 1 of 5 doses (0, 16, 50, 150,

450 $\mu\text{g/kg}$) of naltrexone. There was no interaction effect of dependence history and naltrexone dose on alcohol responding or alcohol intake (g/kg). Dependent rats exhibited higher alcohol responding, $F(1,60) = 9.19$, $p = 0.008$, and alcohol intake (g/kg), $F(1,60) = 12.46$, $p = 0.003$, than nondependent rats. There were also significant main effects of naltrexone dose on operant alcohol responding, $F(4,60) = 4.83$, $p = 0.002$, and alcohol intake (g/kg), $F(4,60) = 4.83$, $p = 0.002$. Post-hoc analyses indicated that the 2 highest naltrexone doses (150 and 450 μg) suppressed operant alcohol responding and alcohol intake (g/kg) relative to vehicle condition across all rats ($p < 0.05$ in all cases). There were no order effects of naltrexone dose administration ($p > 0.05$). Rats generally did not respond for water during operant test sessions, and alcohol preference was very high ($> 95\%$) throughout the experiment (Table 1).

Discussion

Alcohol dependence reliably produced increases in alcohol intake by P rats relative to nondependent controls. The highly selective CRF₁-receptor antagonist, MPZP, selectively suppressed alcohol intake by alcohol-dependent P rats while having no effect on alcohol consumption by nondependent controls. This result suggests that brain CRF systems mediate dependence-induced increases in alcohol intake by selectively bred P rats but are not involved in baseline alcohol-drinking behavior by this line of rats. Conversely, operant alcohol responding was reduced similarly in dependent and nondependent rats by naltrexone. This result suggests that brain opioid systems contribute to high innate alcohol drinking by P rats but that this contribution is not exaggerated during alcohol dependence.

In the present investigation, MPZP, a CRF₁-receptor antagonist, blocked dependence-induced increases in alcohol drinking by P rats without affecting operant alcohol responding by nondependent controls. This result is consistent with past findings that dependence-induced increases in operant alcohol responding by Wistar rats are blocked by antagonists specific for CRF₁ receptors (Funk et al., 2007; Gehlert et al., 2007). Recently, MPZP was characterized in vitro and in vivo and was shown to reduce anxiety-like behavior and dependence-induced drinking in Wistar rats (Richardson et al., 2008). A systemically administered CRF₁-receptor antagonist also attenuates increases in operant alcohol responding induced by dependence and acute stressors in alcohol-preferring sP rats (Sabino et al., 2006). The ability of CRF blockade to suppress dependence-induced elevations in alcohol drinking has been localized to the central nucleus of the amygdala (CeA), and withdrawal produces a decrease in CRF immunoreactivity within the CeA, suggesting an increased extracellular release of CRF during withdrawal (Funk et al., 2006). Microdialysis experiments also indicate that alcohol withdrawal and acute stressors produce increases in extracellular CRF content in rat amygdala (Merlo-Pich et al., 1995; Richter et al., 2000), and alcohol-dependent animals exhibit increased CRF₁-receptor transcript in amygdala (Sommer et al., 2008). Together, these findings suggest that alcohol dependence is defined by a salient role for brain CRF₁ receptors in regulating the negative reinforcing properties of alcohol, and that increases in anxiety-like behavior and alcohol consumption during dependence are partly mediated by CRF systems in the CeA.

In the present study, all 3 doses of MPZP (5, 10, 20 mg/kg i.p.) suppressed alcohol intake (g/kg) in dependent P rats relative to vehicle condition, and the 2 lower doses of MPZP (5 & 10 mg/kg i.p.) abolished dependence-induced increases in alcohol responding and intake relative to nondependent controls (20 mg/kg dose abolished the difference in alcohol responding, but not intake, between groups). In a study of the same MPZP doses in Wistar rats, only the highest dose (20 mg/kg i.p.) suppressed alcohol intake (g/kg) in dependent Wistar rats relative to vehicle condition (Richardson et al., 2008). Together, these results suggest heightened sensitivity to MPZP in dependent P rats relative to dependent Wistar rats. The effective dose range of MPZP in P rats in the current study was identical to the doses of LWH-63, another

CRF₁-receptor antagonist, effective in suppressing dependence-induced increases in alcohol drinking by sP rats (Sabino et al., 2006).

MPZP and established high-affinity highly selective CRF₁-receptor antagonists have been directly compared (Zorrilla et al., 2007): the binding affinities of MPZP (IC₅₀ = 6.1 nM) and DMP904 (IC₅₀ = 2.1 nM) for CRF receptors in rat brain are similar; also, the anatomical pattern of MPZP binding in rat brain mimics that of R121919 and indicates high specificity for CRF₁, but not CRF₂, receptors (Richardson et al., 2008). Limbic CRF₁-receptor systems are hypothesized to play a major role in the negative affective state that drives dependence-induced drinking (Heilig and Koob, 2007), and brain CRF₁-receptor systems of alcohol-preferring rats may be more sensitive to the effects of chronic high-dose alcohol exposure than those of Wistar rats.

Evidence from past studies suggests that the role of CRF in alcohol dependence may have a genetic component. Relative to their nonpreferring (NP) counterparts, alcohol-naïve P rats exhibit increased EEG responses to CRF (Ehlers et al., 1992). Alcohol-naïve P rats have lower levels of CRF-immunoreactivity and CRF mRNA in the CeA relative to NP rats (Hwang et al., 2004), indicative of increased CRF release in P rats. Alcohol-preferring sP rats exhibit increases in extracellular CRF content in the CeA relative to Sardinian NP rats (Richter et al., 2000). Relative to genetically heterogeneous Wistar rats, Marchigian-Sardinian-preferring (msP) rats exhibit an upregulation of CRF₁-receptor transcript that is accompanied by increases in CRF₁-receptor density in multiple limbic brain regions (Hansson et al., 2006). P rats and sP rat lines both exhibit higher basal anxiety-like behavior than their nonpreferring counterparts (Colombo et al., 1995; Richter et al., 2000; Stewart et al., 1993). These results are consistent with the finding that CRF₁-receptor antagonists suppress alcohol drinking in dependent, but not in nondependent sP rats, and rescue novelty stress-induced decreases in alcohol intake by nondependent sP rats (Sabino et al., 2006). Similarly, CRF₁-receptor antagonists block stress-induced increases in alcohol drinking and anxiety-like behavior in P rats (Overstreet et al., 2007) and also suppress stress-induced reinstatement of responding in msP rats (Hansson et al., 2006). Although P rats and outbred rats have not been systematically compared, CRF₁-receptor antagonists suppress elevations in anxiety-like behavior produced by repeated withdrawals/stress exposures in both groups of rats (Breese et al., 2004; Overstreet et al., 2004, 2005). Therefore, genetic selection for high alcohol preference may make animals more susceptible to the effects of alcohol dependence and/or stressors on brain CRF systems, particularly in the CeA. This hypothesis suggests that behavior of alcohol-dependent alcohol-preferring animals would be more sensitive than behavior of dependent outbred animals to manipulations (e.g., pharmacological) of brain CRF systems.

In the present investigation, naltrexone suppressed alcohol intake (g/kg) in dependent and nondependent P rats. Naltrexone reliably suppresses alcohol intake in other rat models of excessive alcohol intake and relapse. For example, naltrexone blocks alcohol deprivation-induced increases in alcohol drinking in Wistar rats (Heyser et al., 1997, 2003; Holter and Spanagel, 1999). In P rats, naltrexone reduces expression of Pavlovian spontaneous recovery responding on a lever previously associated with ethanol deliveries (Rodd et al., 2004). In Warsaw High Preferring rats, chronic naltrexone treatment suppresses continuous free-choice alcohol drinking (Zalewska-Kazubska et al., 2008). Therefore, the ability of naltrexone to reduce alcohol intake extends to multiple rat models of components of alcoholism.

Naltrexone suppressed alcohol intake (g/kg) similarly and at the same doses in dependent and nondependent P rats. This finding is consistent with past findings in which naltrexone suppressed alcohol intake (g/kg) similarly (i.e. magnitude of effect) in dependent and nondependent Wistar rats, and the same or even lower doses of naltrexone were effective in nondependent Wistars relative to dependent rats (Walker and Koob, 2007). Although the

naltrexone dose range used in the present study was very low (microgram doses) compared to some studies (e.g., Krishnan-Sarin et al., 1998; Williams, 2007), the effective dose range in P rats in the current study is comparable to the low doses of naltrexone effective in suppressing alcohol consumption by dependent and nondependent Wistar rats (Stromberg et al., 1998; Walker and Koob, 2007). These findings suggest that brain mu-opioid-receptor systems are involved in baseline alcohol-drinking behavior, but that the role of these systems is not altered following the transition to alcohol dependence.

At low doses such as those used in the present study, naltrexone has higher affinity for mu-opioid receptors than for delta- or kappa-opioid receptors (Abbott et al., 1986; Millan, 1989; Millan et al., 1989; Stromberg et al., 1998; Walker et al., 1994). Alcohol consumption is suppressed by markedly lower doses of naltrexone in alcohol-preferring sP rats (Sabino et al., 2006) relative to P rats in the current study, and possibly also relative to Wistar rats (Walker and Koob, 2007). The differential sensitivities of P (current study) and sP (Sabino et al., 2006) rats to naltrexone may indicate that selective breeding for high alcohol preference does not consistently alter the function of brain mu-opioid-receptor systems, which could be attributable to the lack of a consistent genetic correlation between alcohol preference and mu-opioid-receptor levels across the P/NP (P > NP) and sP/sNP (sNP > sP) pairs of rat lines (Fadda et al., 1999; McBride et al., 1998). These results suggest that the difference in baseline alcohol consumption between P rats and Wistar rats is due to differences not in opioid levels, but rather in other neurotransmitter systems.

In summary, MPZP attenuates dependence-induced increases in alcohol intake by P rats while having no effect on alcohol consumption by nondependent controls. Conversely, operant alcohol responding was reduced similarly in dependent and nondependent P rats by naltrexone. These results, together with past findings, suggest that brain CRF₁-receptor systems of P rats likely play an important role in dependence-induced changes in the reinforcing properties of alcohol and that CRF systems in P rats are likely more sensitive to the effects of chronic high-dose alcohol exposure relative to Wistar rats. However, brain CRF₁-receptor systems do not appear to regulate baseline alcohol consumption by P rats. Conversely, opioid systems appear important in the regulation of basal alcohol consumption in both P rats and outbred Wistar rats.

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2-Bottle Choice During Quarantine	Operant Training	Chronic Intermittent Vapor	MPZP Latin Square				Naltrexone Latin Square				
			Test 1	Test 2	Test 3	Test 4	Test 1	Test 2	Test 3	Test 4	Test 5
10 Weeks	11-16 Days	10 Weeks	2 Tests per Week for 2 weeks				2 Tests per Week for 2.5 weeks				

Fig. 1.

Timeline of operant training, alcohol vapor exposure, and pharmacological testing for P rats. For 4 days of each of the 10 weeks spent in quarantine, rats underwent limited-access 2-bottle choice sessions in which they were allowed to consume either 10% (w/v) ethanol or water in the presence of food. Rats were then transferred to the colony room and trained to respond for 10% (w/v) ethanol and water in a 2-lever operant situation for 11 days. Rats were divided into groups based on baseline intake levels and exposed to either alcohol vapor or ambient air on a chronic intermittent schedule (14 hours ON/10 hours OFF) for 10 weeks. Dependent and nondependent P rats were tested first for the effects of MPZP, then for the effects of naltrexone on operant alcohol responding. Tests always occurred 6 hours following the termination of alcohol vapor exposure (i.e. at 6 hours withdrawal) and were administered twice per week.

Fig. 2.

Operant lever presses for ethanol (A) and ethanol intake (g/kg; B) by alcohol-dependent (black bars) and -nondependent (white bars) P rats following injection of 1 of 4 MPZP doses (0, 5, 10, 20 mg/kg). Operant tests occurred 6 hours following termination of vapor exposure (i.e. 6 hours withdrawal). * $p < 0.05$ significant difference from nondependent control rats; # $p < 0.05$ significant difference from vehicle condition in dependent rats.

Fig. 3.

Operant lever presses for ethanol (A) and ethanol intake (g/kg; B) by alcohol-dependent (black bars) and -nondependent (white bars) P rats following injection of 1 of 5 naltrexone doses (0, 16, 50, 150, 450 μg/kg). Operant tests occurred 6 hours following termination of vapor exposure (i.e. 6 hours withdrawal). # $p < 0.05$ significant difference from vehicle condition across dependent and nondependent rats.

Table 1

Mean $\pm$ SEM Water Responses, Alcohol Preference (Alcohol/Total), and Total Fluid Intake (ml) for Alcohol-Dependent and -Nondependent Rats Following i.p. Injection With 4 Doses (0, 5, 10, 20 mg/kg) of MPZP and 5 Doses (0, 16, 50, 150, 450 μ g/kg) of Naltrexone

	Water lever presses	Preference (alcohol/total)	Total fluid (ml)
<i>MPZP dose (mg/kg)</i>			
Alcohol-dependent			
0	1.89 $\pm$ 1.01	0.98 $\pm$ 0.01	8.57 $\pm$ 0.72
5	3.00 $\pm$ 1.92	0.96 $\pm$ 0.02	6.88 $\pm$ 0.87
10	2.11 $\pm$ 0.91	0.97 $\pm$ 0.01	6.34 $\pm$ 0.92
20	2.67 $\pm$ 1.15	0.96 $\pm$ 0.02	6.98 $\pm$ 0.67
Alcohol-nondependent			
0	0.90 $\pm$ 0.43	0.98 $\pm$ 0.01	4.53 $\pm$ 0.50
5	1.20 $\pm$ 0.54	0.96 $\pm$ 0.03	5.55 $\pm$ 0.99
10	1.60 $\pm$ 0.88	0.97 $\pm$ 0.02	4.95 $\pm$ 0.77
20	1.40 $\pm$ 0.69	0.97 $\pm$ 0.02	4.70 $\pm$ 0.75
<i>Naltrexone dose (μg/kg)</i>			
Alcohol-dependent			
0	3.14 $\pm$ 1.67 *	0.95 $\pm$ 0.02	6.77 $\pm$ 0.98
16	1.00 $\pm$ 0.62	0.98 $\pm$ 0.01	4.97 $\pm$ 0.90
50	1.00 $\pm$ 0.62 *	0.99 $\pm$ 0.01	6.63 $\pm$ 0.69
150	1.57 $\pm$ 0.52	0.96 $\pm$ 0.01	4.54 $\pm$ 0.58
450	0.71 $\pm$ 0.51	0.99 $\pm$ 0.01	4.59 $\pm$ 0.75
Alcohol-nondependent			
0	0.70 $\pm$ 0.45	0.97 $\pm$ 0.02	5.07 $\pm$ 0.68
16	1.40 $\pm$ 0.65	0.96 $\pm$ 0.02	4.03 $\pm$ 0.72
50	3.60 $\pm$ 1.14	0.92 $\pm$ 0.02	4.83 $\pm$ 0.70
150	2.20 $\pm$ 0.95	0.95 $\pm$ 0.02	3.82 $\pm$ 0.60
450	1.70 $\pm$ 0.80	0.94 $\pm$ 0.03	2.64 $\pm$ 0.26

* $p < 0.05$ significant difference from nondependent rats at the same doses.