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ORIGINAL INVESTIGATION

Effects of β-adrenoceptor antagonists on alcohol drinking by alcohol-dependent rats

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Abstract
Rationale Alcohol-dependent animals display enhanced stress responsivity, reward thresholds, and alcohol self-administration during alcohol withdrawal, and some of these aspects of alcohol dependence may be mediated by activation of brain norepinephrine (NE) systems.
Objectives This study examined the effects of propranolol, a β-adrenoceptor antagonist, on operant alcohol-reinforced responding by alcohol-dependent and non-dependent rats.
Methods Adult male Wistar rats were trained to respond for alcohol in an operant conditioning paradigm on fixed-ratio-1 (FR-1) and progressive ratio (PR) reinforcement schedules. Rats were either made dependent on alcohol via chronic intermittent (14 h ON/10 h OFF) alcohol vapor inhalation or were not exposed to alcohol vapor. Rats were tested for the effects of propranolol (0–10 mg/kg) or nadolol (0–20 mg/kg) on operant alcohol-reinforced responding at the time point corresponding to 6–8 h withdrawal in dependent animals.
Results All doses of propranolol suppressed FR-1 operant alcohol-reinforced responding in alcohol-dependent rats, but only the highest dose suppressed FR-1 responding by controls. No dose of propranolol affected water responding. Nadolol did not affect operant behavior. Propranolol suppressed PR operant alcohol-reinforced responding across groups, an effect attributable to significant suppression of alcohol responding at the highest dose.
Conclusions Following development of alcohol dependence, rats exhibit hypersensitivity to the suppressive effects of propranolol on operant alcohol-reinforced responding. This effect is mediated by central actions of the drug, is not attributable to motor effects, and may reflect activation of brain NE systems that contributes to withdrawal-induced negative emotional states and drives alcohol drinking in the dependent organism.

Keywords Alcohol · Dependence · Withdrawal · Propranolol · Norepinephrine · Beta-adrenoceptors

Compulsive alcohol drinking behavior is motivated by positive reinforcing effects of alcohol and the negative reinforcement associated with alcohol dependence, the latter of which is hypothesized to be prominent in drinkers with long-term high-dose alcohol histories (Koob and LeMoal 1997). The negative reinforcement motivational properties of alcohol are contingent on the negative affective state that defines alcohol withdrawal (Koob and LeMoal 1997). Alcohol-dependent animals display enhanced stress responsivity, increased reward thresholds, and increased alcohol self-administration during acute withdrawal and protracted abstinence from chronic high-dose alcohol exposure (Gilpin et al. 2008; Roberts et al. 2000; Schulteis et al. 1995; Sommer et al. 2008; Valdez et al. 2002, 2003). At least some of these aspects of alcohol dependence may be mediated by brain norepinephrine (NE) systems (Amit et al. 1977; Brown and Amit 1977).

Norepinephrine is a major neurotransmitter with widespread distribution in rat brain (see Koob 2008). Dysfunction of brain NE systems has been implicated in arousal, attention, stress, anxiety, and affective disorders. More specifically, projections from the locus coeruleus have been hypothesized to have a key role in maintaining attentional

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homeostasis (Aston-Jones and Cohen 2005), whereas projections from brainstem to limbic regions are linked to behavioral responses to stressors (Delfs et al. 2000; Forray and Gysling 2004; Koob 1999). For example, antagonism of NE receptors in limbic regions blocks stress-induced anxiety-like responses in rats (Cecchi et al. 2002a,b).

NE acts on three distinct families of receptors: α_1, α_2, and β adrenergic receptors (Rohrer and Kobilka 1998), the latter of which can be divided into β_1, β_2, and β_3 subtypes (Hall 2004). Propranolol is a non-selective β-adrenoceptor antagonist that has been used clinically to treat hypertension and migraine headaches, among other conditions (Chrysant et al. 2008; Evans et al. 2008). More recently, a clinical role has been indicated for propranolol in post-traumatic stress disorder because of its effects on memory reconsolidation (Strawn and Geriacoti 2008).

Early evidence for an interaction between brain NE and alcohol reinforcement came from a series of studies in which selective pharmacological and neurotoxin-specific disruption of NE function reduced voluntary alcohol consumption by rats (Amit et al. 1977; Brown and Amit 1977). Administration of selective dopamine β-hydroxylase inhibitors (Amit et al 1977) and 6-hydroxydopamine (Brown and Amit 1977; Davis et al. 1979) markedly reduced voluntary alcohol self-administration in rats via oral and non-oral routes. Selective depletion of NE in the medial prefrontal cortex of high alcohol-consuming C57BL/6J mice produces a decrease in alcohol consumption (Ventura et al. 2006), and mice unable to synthesize NE (due to lack of dopamine-beta hydroxylase) exhibit reduced preference for alcohol (Weinshenker et al. 2000).

More relevant to the current investigation, NE has been implicated in alcohol dependence and withdrawal in both humans and animals. Symptoms of alcohol withdrawal in humans are blocked by postsynaptic β-adrenergic antagonists, including propranolol (Horwitz et al. 1989; Kraus et al. 1985; Sellers et al. 1977; Zilm et al. 1975, 1980). In alcohol-dependent rats, withdrawal symptoms are blocked by propranolol as well as prazosin, an α_1-adrenoceptor antagonist (Trzaskowska and Kostowski 1983). Prazosin attenuates excessive alcohol consumption by alcohol-dependent rats and alcohol-preferring rats (Rasmussen et al. 2009; Walker et al. 2008), and also reduces number of drinking days and drinks per day in alcoholic humans (Simpson et al. 2009). Because β-adrenoceptors may contribute to the constellation of aversive symptoms associated with alcohol withdrawal, the purpose of the present investigation was to determine the effects of propranolol on dependence-induced increases in motivation to consume alcohol. This question was addressed by testing the dose–response effects of propranolol on two different schedules of alcohol-reinforced operant responding by alcohol-dependent and non-dependent rats.

Methods

Subjects

In Experiment 1, 21 adult male Wistar rats obtained from Charles River Laboratories (Kingston, NY, USA) were used. In Experiment 2, 14 adult male Wistar rats obtained from Charles River Laboratories (Kingston, NY, USA) were used. All animals were single-housed in standard plastic cages with wood chip bedding under a 12-h light/12-h dark cycle (lights off at 8 AM). 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.

Drugs

Propranolol hydrochloride and nadolol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Propranolol (0, 2.5, 5, and 10 mg/kg) was dissolved in saline at a concentration of 10 mg/ml vehicle and nadolol (0, 5, 10, and 20 mg/kg) was dissolved in saline at a concentration of 20 mg/3 ml vehicle before dilutions to lower doses. All propranolol injections were intraperitoneal at a total volume of 1.0 ml/kg body weight and all nadolol injections were intraperitoneal at a total volume of 3.0 ml/kg body weight. Drug injections always occurred 20 min prior to the start of behavioral testing.

Operant conditioning chambers

The operant conditioning chambers (Coulbourn Instruments, Allentown, PA, USA) utilized in the present study had two retractable levers located 4 cm above a grid floor and 4.5 cm to either side of a two-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, USA) that delivered 0.1 ml of fluid to the appropriate well over a period of 0.5 s. Lever presses that occurred during the 0.5 s of pump activation were not recorded and did not result in fluid delivery. Operant conditioning chambers were individually housed in sound-attenuated ventilated cubicles to minimize environmental disturbances.

Operant response training

Wistar rats were trained to respond for a “supersaccharin” solution (3% glucose and 0.125% saccharin; Valenstein et al. 1967) versus water in a concurrent, two-lever, free-

choice contingency as previously described (Walker et al. 2008). Briefly, lever presses were reinforced on a fixed-ratio-1 (FR-1) schedule such that each response resulted in delivery of 0.1 ml of fluid. Following the second session of operant response training with supersaccharin, 10% (*w*/*v*) ethanol was added and then sweeteners gradually removed from the experimental solution across eight operant training sessions. Upon completion of this fading procedure, Wistar 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 11th day of training. Rats in each experiment were divided into two groups matched for mean intakes across the final 6 days of this baseline period: rats to be exposed to chronic alcohol vapor and control rats to be exposed to ambient air.

Alcohol vapor inhalation

To induce alcohol dependence, standard rat cages were housed in separate, sealed, clear plastic chambers into which ethanol vapor was intermittently injected. This procedure has been described in detail elsewhere (Funk et al. 2006; Gilpin et al. 2008). Briefly, 95% ethanol was evaporated and vapor was delivered at rates between 22 and 27 mg/L. Alcohol vapor was turned on (4 PM) for 14 h per day and off (6 AM) for 10 h per day (O'Dell et al. 2004), and the target range for blood-alcohol levels (BALs) in dependent rats during vapor exposure was 150 to 200 mg%. Non-dependent rats were treated in parallel except they were exposed to vapor that did not contain ethanol. Tail blood samples were collected on multiple days at 6 AM for BAL determination and adjustment of vapor settings. This chronic intermittent vapor exposure reliably produces somatic and motivational aspects of alcohol dependence during alcohol withdrawal (Gilpin et al. 2009; O'Dell et al. 2004).

Operant responding during alcohol vapor exposure

In Experiments 1 and 2, rats were exposed to intermittent alcohol/air vapor for 4 weeks before operant response testing began, and intermittent vapor exposure continued for the remainder of the study (i.e., for the duration of FR and progressive ratio (PR) operant response testing). Wistar rats were tested twice weekly for operant alcohol self-administration. Once operant response behavior stabilized across sessions, rats were tested for the effects of propranolol on operant response behavior. All rats were injected intraperitoneally (i.p.) with four drug doses (0, 2.5, 5, and 10 mg/ml/kg propranolol in Experiment 1; 0, 5, 10, and 20 mg/ml/kg nadolol in Experiment 2) in a within-subjects Latin-square design, and also once with saline preceding and following the Latin-square. Immediately after injections, rats were placed in operant conditioning chambers in the absence of levers for a period of 2 min, following which levers were made available and rats were tested for FR-1 operant alcohol-reinforced responding behavior. These tests occurred twice weekly and always occurred at the time point corresponding to 6 h withdrawal for alcohol-dependent rats.

In Experiment 1 only, following the termination of the FR-1 Latin-square testing, and in order to confirm the effects of propranolol on the motivational aspects of operant alcohol-reinforced responding behavior, rats were re-trained to respond for alcohol in 90-min PR operant conditioning sessions. In these two-lever operant conditioning sessions, rats were allowed to respond for 10% (*w*/*v*) alcohol on a PR schedule of reinforcement according to the following progression: 1, 1, 2, 2, 3, 3, 4, 4, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15, 18, 18, 21, 21, 24, 24, etc., and rats were always allowed access to a second lever that produced water deliveries on an FR-1 schedule. The point at which rats stopped responding for ethanol (i.e., no responses within a 15-min time period) was defined as the breakpoint and reflected the number of reinforcers earned during the session. Between-session PR response rates stabilized following 12 operant conditioning sessions, at which point all rats were once again injected with four doses (0, 2.5, 5.0, and 10.0 mg/ml/kg) of propranolol in a within-subjects Latin-square design, and also once with saline preceding and following the Latin-square. These tests occurred twice weekly at the time point corresponding to 6 h withdrawal for alcohol-dependent rats, and drug pre-treatment time was always 20 min.

Blood-alcohol level determinations

Tail blood was sampled at several points during alcohol vapor exposure to ensure that blood-alcohol levels were being maintained in the appropriate range. Rats were gently restrained while the tip of the tail (2 mm) was cut with a clean razor blade. Tail blood (0.2 ml) was collected and centrifuged. Plasma (5 μl) was used for measurement of BALs using an Analox AM 1 analyzer (Analox Instruments LTD, Lunenberg, MA, USA). The reaction is based on the oxidation of alcohol by alcohol oxidase in the presence of molecular oxygen (alcohol + O_2→acetaldehyde + H_2O_2). 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 (0.025–0.400 g%). Blood-alcohol levels were maintained between 175 and 250 mg/dl for the duration of the experiment.

Statistical analysis

In Experiments 1 and 2, data from FR-1 tests were analyzed for responses on the ethanol and water levers, and ethanol

consumption (g ethanol/kg body weight) by alcohol-dependent and non-dependent rats using two-way repeated-measures analyses of variance (RM ANOVAs) where dose was the within-subjects factor, and dependence history was the between-subjects factor (dependent vs. non-dependent). In Experiment 1, operant alcohol-reinforced responding following propranolol treatment was also analyzed within 3-min bins. One rat was removed from the Experiment 1 between the FR-1 and PR test phases due to health complications. In Experiment 1, data from full 90-min PR sessions and also from the first 30 min of PR sessions were analyzed for responses on the ethanol and water levers, ethanol reinforcers, and ethanol break point (full 90-min session only) by alcohol-dependent and non-dependent rats using two-way repeated-measures analyses of variance (RM ANOVAs) where propranolol dose (0, 2.5, 5.0, and 10.0 mg/kg) was the within-subjects factor and dependence history (dependent vs. non-dependent) was the between-subjects factor. In some cases, trend analysis was also used to determine whether the linear (change in dependent variable as a function of change in independent variable) and/or quadratic (*rate of change* in dependent variable as a function of change in independent variable) components of responding were significantly affected by drug dose. Post-hoc comparisons were conducted using the Student Newman–Keuls test. Statistical significance was set at $p<0.05$.

Results

Experiment 1

Effects of propranolol on fixed-ratio operant responding

Figure 1 illustrates operant responding for ethanol and water on an FR-1 schedule by dependent and non-dependent Wistar rats during 30-min sessions following i.p. injection with four doses of propranolol (0, 2.5, 5.0, and 10.0 mg/kg). Dependent rats exhibited higher ethanol responding, $F(1,19)=25.44$, $p<0.001$, and ethanol intake (g/kg), $F(1,19)=29.71$, $p<0.001$, than non-dependent rats. There were also significant main effects of propranolol dose on ethanol responding, $F(3,57)=23.61$, $p<0.001$, and ethanol intake (g/kg), $F(3,57)=23.50$, $p<0.001$. Finally, there were significant history × dose interaction effects on ethanol responding, $F(3,57)=2.79$, $p=0.048$, and ethanol intake (g/kg), $F(3,57)=3.11$, $p=0.033$. Post-hoc analyses revealed that all three doses of propranolol suppressed ethanol responding and ethanol intake (g/kg) in dependent rats ($p<0.002$ in all cases), whereas only the highest propranolol dose suppressed ethanol responding and ethanol intake (g/kg) in non-dependent rats ($p<0.001$ in both cases). A separate two-way repeated-measures ANOVA indicated no effects of dependence or propranolol on operant responding for water.

Figure 2 illustrates operant responding for ethanol and water by dependent and non-dependent Wistar rats during 3-min bins of 30-min operant session. There were significant dose × bin interaction in both dependent, $F(27,243)=4.18$, $p<0.001$, and non-dependent, $F(27,270)=6.16$, $p<0.001$, rats. Post-hoc analyses revealed that, *within* bin 1, all doses of propranolol suppressed operant alcohol-reinforced responding by dependent and non-dependent rats relative to vehicle ($p<0.005$ in all cases). Also, within bin 1, 10 mg/kg propranolol suppressed operant alcohol-reinforced responding by dependent rats relative to the two lower doses ($p<0.001$ in both cases). The 10 and 2.5 mg/kg doses also suppressed operant alcohol-reinforced responding within bin 3 relative to vehicle ($p<0.05$ in both cases).

Effects of propranolol on progressive ratio operant responding

Figure 3 illustrates operant responding for ethanol on a PR schedule by dependent and non-dependent Wistar rats across the last 11 baseline sessions. Alcohol-dependent rats exhibited significantly higher ethanol responses, $F(1,19)=9.54$, $p=0.006$; ethanol rewards, $F(1,19)=10.79$, $p=0.004$; ethanol break point, $F(1,19)=10.32$, $p=0.005$; and water

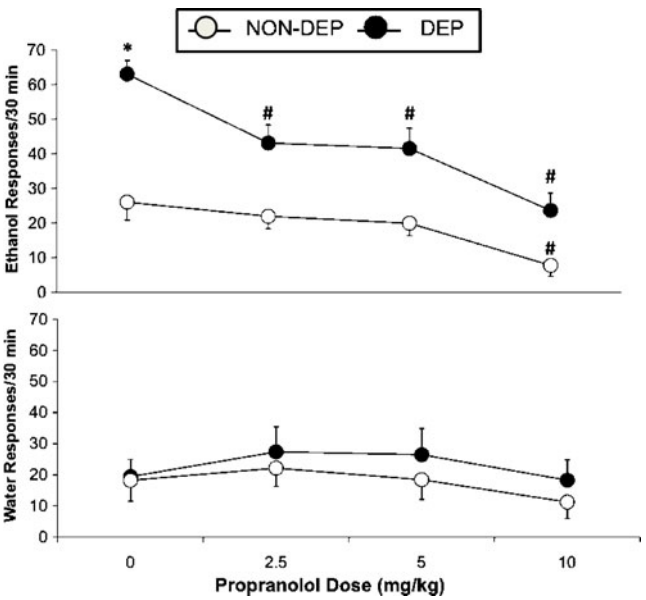

Fig. 1 Mean (±SEM) fixed-ratio (FR-1) operant lever presses for ethanol (*top panel*) and water (*bottom panel*) by alcohol-dependent (*black circles*) and non-dependent (*white circles*) Wistar rats following injection of one of four propranolol doses (0.0, 2.5, 5.0, and 10.0 mg/kg). Operant tests occurred 6–8 h following termination of vapor exposure (i.e., 6–8 h withdrawal). *indicates $p<0.05$ significant difference from non-dependent control rats; # indicates $p<0.05$ significant difference from vehicle condition

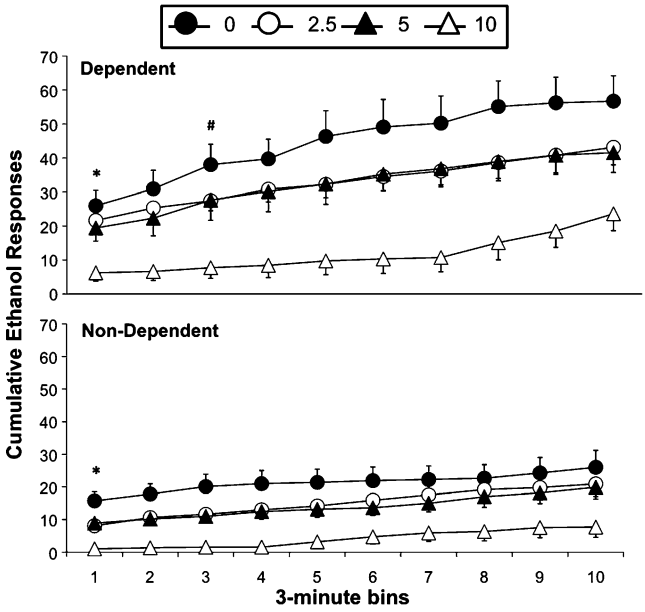

Fig. 2 Mean (±SEM) cumulative ethanol responses across the 30-min operant session divided into 3-min bins. Fixed-ratio (FR-1) operant lever presses for ethanol by dependent (*top panel*) and non-dependent (*bottom panel*) Wistar rats following injection of one of four propranolol doses (0.0, 2.5, 5.0, and 10.0 mg/kg). Operant tests occurred 6–8 h following termination of vapor exposure (i.e., 6–8 h withdrawal). *$p<0.005$, all doses significantly lower than vehicle *within* bin (non-cumulative); #$p<0.05$, 2.5 and 10.0 mg/kg doses significantly lower than vehicle *within* bin (non-cumulative)

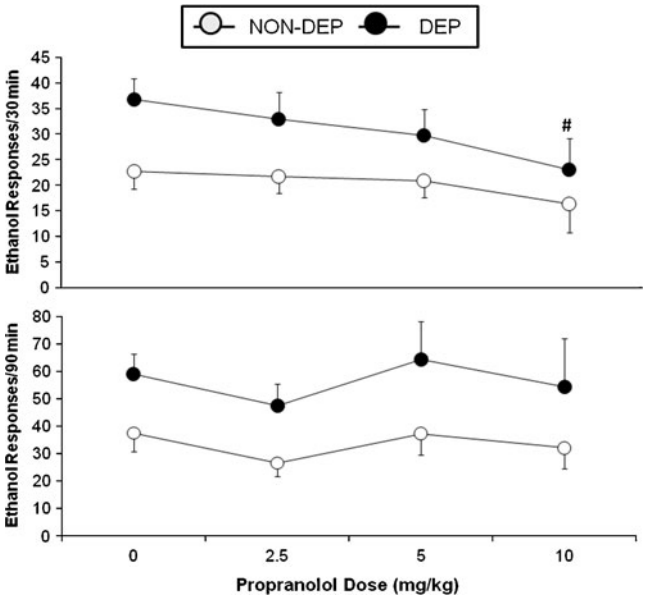

Fig. 4 Mean (±SEM) operant lever presses for ethanol during the first 30 min (*top panel*) and the entire 90 min (*bottom panel*) of progressive ratio sessions. Alcohol-dependent (*black circles*) and non-dependent (*white circles*) Wistar rats responded for ethanol at the 6–8 h withdrawal time point following injection of one of four propranolol doses (0.0, 2.5, 5.0, and 10.0 mg/kg). # indicates $p<0.05$ significant difference from vehicle condition across all rats

presses, $F(1,19)=5.55$, $p=0.029$, than non-dependent rats across the baseline period.

Figure 4 illustrates PR operant responding for ethanol by dependent and non-dependent Wistar rats during the first 30 min and full 90 min of operant conditioning sessions following i.p. injection with four doses of propranolol (0, 2.5, 5.0, and 10.0 mg/kg). Alcohol-dependent rats exhibited higher responding for ethanol, $F(1,18)=5.60$, $p=0.029$, and received more ethanol rewards, $F(1,18)=4.62$, $p=0.045$, than non-dependent rats during the first 30 min of PR operant response tests. There was also a significant main effect of propranolol dose on ethanol responding, $F(3,54)=2.93$, $p=0.042$, and ethanol rewards, $F(3,54)=5.10$, $p=0.004$, during the first 30 min of PR operant response tests. Post-hoc analyses revealed that the 10 mg/kg propranolol dose suppressed ethanol presses ($p<0.05$) and rewards ($p<0.05$) relative to the vehicle condition. There were no history × dose interaction effects on ethanol presses ($p=0.74$) or ethanol rewards ($p=0.84$). The linear component of a trend analysis for propranolol dose approached significance for presses ($p<0.08$) and rewards ($p<0.08$) in dependent animals, but not in non-dependent animals. A separate two-way RM ANOVA indicated no effects of dependence or propranolol on operant responding for water during the first 30 min of PR operant tests.

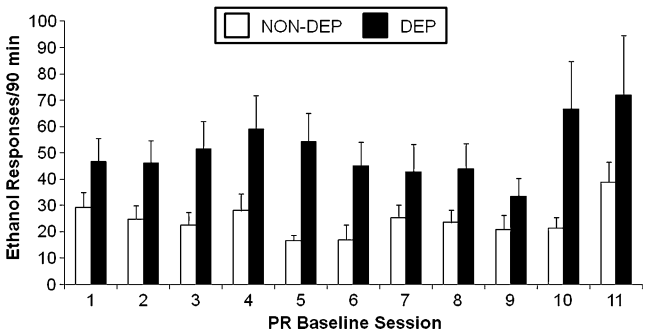

Fig. 3 Mean (±SEM) progressive ratio operant lever presses for ethanol during 11 baseline 90-min sessions by alcohol-dependent (*black bars*) and non-dependent (*white bars*) Wistar rats. Operant tests occurred 6–8 h following termination of vapor exposure (i.e. 6–8 h withdrawal)

Alcohol-dependent rats also achieved higher ethanol responding, $F(1,18)=7.01$, $p=0.016$; ethanol rewards, $F(1,18)=6.11$, $p=0.024$; and ethanol break points, $F(1,18)=7.50$, $p=0.014$, than non-dependent rats across full 90-min PR operant conditioning sessions. Propranolol produced a trend toward a suppression of ethanol rewards ($p=0.057$) across full 90-min PR sessions. A separate two-way RM ANOVA indicated no effects of dependence or propranolol on operant responding for water during full 90-min PR tests.

Experiment 2

Effects of nadolol on fixed-ratio operant responding

Nadolol did not affect operant alcohol-reinforced responding, operant water-reinforced responding, or alcohol consumption (g/kg), although alcohol-dependent and non-dependent rats injected with the highest (20 mg/kg) dose did exhibit somewhat lower alcohol-reinforced responding and alcohol consumption (g/kg) than rats injected with vehicle and the two lower doses (see Table 1). Alcohol-dependent rats consumed significantly more ethanol (g/kg) than non-dependent rats, $F(1,36)=6.11$, $p=0.029$, but there were no history × dose interaction effects on alcohol responding, water responding, or alcohol consumption (g/kg).

Discussion

In the present investigation, systemic administration of propranolol suppressed operant alcohol-reinforced responding in alcohol-dependent rats and non-dependent rats on a continuous reinforcement schedule. Alcohol-dependent rats exhibited heightened sensitivity to the suppressive effects of propranolol on operant alcohol-reinforced responding relative to non-dependent controls. All doses of propranolol suppressed alcohol drinking by alcohol-dependent rats, whereas only the highest dose of propranolol suppressed alcohol drinking by non-dependent rats. Conversely, operant alcohol-reinforced responding was not affected by nadolol, a peripherally acting beta-adrenoceptor antagonist that does not cross the blood–brain barrier. In progressive ratio operant response tests, when the work requirement for reinforcers is gradually increased and rewards are delivered intermittently, alcohol-dependent rats exhibited significantly higher responding on the ethanol lever than non-dependent rats, as previously shown (Walker and Koob 2008). Propranolol suppressed progressive ratio responding on the ethanol lever in alcohol-dependent and non-dependent rats. Taken together, these results suggest that propranolol suppresses both the appetitive and consummatory reinforcing properties of ethanol, and alcohol-dependent rats exhibit greater sensitivity to the suppressive effects of propranolol on alcohol consumption.

In the current study, propranolol suppressed FR alcohol-reinforced responding at low doses in alcohol-dependent animals but only at the highest dose in non-dependent controls. Propranolol suppressed PR alcohol-reinforced responding similarly in alcohol-dependent rats and non-dependent controls, indicative of a decrease in the reinforcement efficacy of alcohol. Both dependent and non-dependent rats received fewer alcohol reinforcers during PR operant response tests than did non-dependent rats in FR operant response tests, suggesting that the ability of propranolol to suppress PR operant alcohol-reinforced responding is not contingent on number of reinforcers received. These results argue against a simple rate-dependent effect of propranolol on operant alcohol-reinforced responding.

There were no effects of propranolol on operant water-reinforced responding. Although water responding was substantially lower than alcohol responding, water responding (~20 presses per session on average) was not low enough to produce a floor effect and preclude further reductions by drug. Also, systemic administration of a slightly higher range of doses of nadolol did not affect operant alcohol-reinforced responding nor did it affect operant water-reinforced responding. Rats injected with the highest nadolol dose (twice the highest propranolol dose) did appear to exhibit somewhat lower operant alcohol-reinforced responding but that trend did not extend to operant water-reinforced responding. Furthermore, rats injected with 10 mg/kg nadolol (equal to the highest dose of propranolol) did not exhibit a trend toward any change in operant behavior or alcohol consumption. Together, these data indicate that the effects of propranolol on operant alcohol-reinforced responding are mediated by the central effects of the drug, and are not due to non-specific changes in operant behavior or locomotor activity.

Table 1 Mean (±SEM) data for operant alcohol-reinforced responding, operant water-reinforced responding, and alcohol consumption (g/kg) by alcohol-dependent and non-dependent rats that injected with one of four nadolol doses 20 min prior to operant testing

	Dose (mg/kg)	Operant alcohol responses	Alcohol consumption (g/kg)	Operant water responses
Dependent	0	40.20 (8.13)	0.81 (0.16)	23.13 (6.66)
	5	39.20 (9.12)	0.79 (0.17)	28.20 (12.77)
	10	38.80 (9.11)	0.79 (0.18)	18.80 (7.43)
	20	28.50 (5.83)	0.57 (0.11)	26.60 (8.98)
Non-dependent	0	28.63 (3.27)	0.54 (0.06)	20.93 (9.29)
	5	24.00 (3.40)	0.45 (0.06)	27.22 (17.34)
	10	26.11 (5.49)	0.49 (0.10)	19.33 (7.24)
	20	21.22 (4.88)	0.39 (0.08)	15.44 (7.39)

Propranolol suppressed PR operant responding in all rats at the 30-min time point but not at the 90-min time point. This temporal effect differs from another study in which the ability of propranolol to suppress cocaine-reinforced responding in squirrel monkeys increased with time during a fixed-ratio operant conditioning session (Goldberg and Gonzalez 1976). This discrepancy may not be surprising because there are so many differences (species, drug of abuse, etc.) between the two studies. Furthermore, it is not clear whether the dissipation of propranolol effects across the 90-min PR operant conditioning session is due to the pharmacokinetic profile of the compound, but that might be a possibility. In rats, propranolol reaches peak plasma concentrations within 1 h of dosing and has an elimination half-life of 1–3 h (Qureshi and Buttar 1989). In humans, propranolol exhibits a similar pharmacokinetic profile with a distribution half-life of 10 min, peak plasma concentrations within 1–3 h following dosing, and an elimination half-life of 2–3 h (Johnsson and Regardh 1976).

Propranolol has long been examined in animal models of alcohol and drug self-administration for its potential ability to block the reinforcing effects of drugs of abuse. As stated above, propranolol doses similar to those used in the present study suppress fixed-ratio responding for cocaine in squirrel monkeys (Goldberg and Gonzalez 1976). Similar doses of propranolol also attenuate the somatic symptoms associated with spontaneous and precipitated withdrawal from morphine, and block the acquisition of morphine withdrawal-induced conditioned place aversion (Harris and Aston-Jones 1993a). Antagonism of beta-adrenergic receptors blocks anxiety-like behavior associated with abstinence from chronic cocaine and morphine (Harris and Aston-Jones 1993b; Rudoy and Van Bockstaele 2007) possibly via blockade of withdrawal-induced increases in corticotropin-releasing factor (CRF) gene expression in amygdala (Rudoy et al. 2008). Beta-adrenergic receptor antagonists also reverse cocaine withdrawal-induced increases in β_1-adrenergic receptors (Rudoy and Van Bockstaele 2007) and immunoreactivity for downstream PKA and CREB signaling elements of those receptors (Rudoy et al. 2008). Chronic administration of multiple drugs of abuse including alcohol, cocaine, and morphine produces hypersensitivity of NE systems to the stimulatory effects of another drug of abuse, namely amphetamine (Lanteri et al. 2008). Taken together, these studies suggest that NE neurotransmission is enhanced during drug withdrawal, and that antagonism of NE receptors blocks aspects of drug withdrawal that might otherwise drive the negative reinforcing effects of alcohol.

Prior data describing the effects of propranolol on alcohol drinking by non-dependent rats are somewhat inconsistent. An early study showed that a range of propranolol doses similar to that used in the present study does not suppress alcohol drinking by non-dependent rats in a two-bottle choice home cage drinking procedure (Begleiter 1974). A separate pair of studies showed that chronic injection with a dopamine-beta-hydroxylase inhibitor produces decreases in free-choice ethanol drinking by rats that may be mediated by blockade of norepinephrine synthesis in brain (Amit et al. 1977; Brown et al. 1977). Evidence from human studies indicates that propranolol affects the physical and motivational symptoms of alcoholism. For example, propranolol relieves morning withdrawal symptoms and stress in human alcoholics (Carlsson and Johansson 1971; Tyrer 1972). Propranolol also suppresses withdrawal tremor in human alcoholics (Koller et al. 1985), and the ability of propranolol to antagonize peripheral effects of alcohol withdrawal in alcoholic humans is at least partly predicted by prior daily alcohol consumption (Kähkönen et al. 2007). These effects of propranolol occur in the absence of effects on blood-alcohol elimination rates in humans (Korri 1990).

Norepinephrine appears to mediate alcohol consumption and withdrawal-related effects not only via β-adrenoceptors, but also via α-adrenoceptors. Prazosin, an antagonist with specificity for α_1-adrenoceptors, suppresses withdrawal-induced alcohol drinking in dependent rats at doses that do not affect operant alcohol-reinforced responding in non-dependent controls (Walker et al. 2008), in parallel with the results presented here for propranolol. Very low doses of prazosin also suppress alcohol drinking by alcohol-preferring (P) rats selectively bred for high alcohol consumption (Rasmussen et al. 2009). Likewise, in humans, prazosin reduces number of drinking days and number of drinks consumed by patients with alcohol dependence (Simpson et al. 2009).

It should be noted that, in the present investigation, the highest propranolol dose suppressed operant alcohol-reinforced responding similarly in dependent and non-dependent rats. Systemic administration of propranolol in the present study prevents identification of the discrete brain region that mediates drug effects on operant alcohol-reinforced responding, but it is possible that the highest propranolol dose (10 mg/kg) affected brain regions in which NE systems contribute to the positive reinforcing effects of alcohol (likely to be experienced by both dependent and non-dependent rats), whereas lower doses affect brain regions in which NE systems contribute to the negative reinforcing effects of alcohol (likely to be experienced only by alcohol-dependent alcohol-withdrawn rats). Such a scenario would account for the effectiveness of lower doses of propranolol (present study) and prazosin (Walker et al. 2008) in suppressing operant alcohol-reinforced responding by alcohol-dependent rats, whereas significantly higher doses are needed to affect operant behavior by non-dependent controls. Therefore, it seems possible that NE contributes to the negative affective symptoms of alcohol withdrawal via up-regulation in particular brain regions but not others, perhaps

accounting for the heightened sensitivity of alcohol-dependent rats to the suppressive effects of propranolol and prazosin on alcohol drinking.

It has been suggested that the role of norepinephrine in addiction may be related to its close interaction with corticotropin-releasing factor in brain (Smith and Aston-Jones 2008). This hypothesis is owed largely to the fact that NE projections from brainstem to limbic regions are regulated by feed-forward CRF projections from amygdala to brainstem (Curtis et al. 2002), and NE-CRF interactions have well-documented contributions to behavioral responses to stress (Dunn et al. 2004; Dunn and Swiergiel 2008; Koob 1999). This interaction is especially intriguing in light of the fact that amygdalar CRF systems are thought to be recruited during the transition to alcohol dependence and likely play an important role in the negative reinforcing properties of drugs of abuse (Koob 2008). Alcohol withdrawal produces increases in limbic CRF that are normalized by alcohol consumption (Olive et al. 2002), and CRF receptor antagonists suppress alcohol dependence-induced increases in anxiety-like behavior and alcohol drinking (Baldwin et al. 1991; Valdez et al. 2002), effects that have been localized to the amygdala (Funk et al. 2006, 2007; Rassnick et al. 1993).

The results of the present investigation suggest that central β-adrenoceptors modulate the reinforcing effects of alcohol, and that this role of β-adrenoceptors may be enhanced following the transition to alcohol dependence. Centrally acting propranolol, but not peripherally acting nadolol, suppresses operant alcohol-reinforced responding in alcohol-dependent rats at doses that do not affect water responding in dependent rats nor operant behavior in non-dependent rats. The increased behavioral efficacy of propranolol in alcohol-dependent rats parallels previous reports that CRF_1-receptor antagonists are more effective in suppressing anxiety-like behavior and alcohol drinking following the transition to alcohol dependence. These results are especially intriguing because the role of CRF in dependence-related behaviors has been localized to the amygdala, the same brain region that mediates CRF feed-forward projections to brainstem regions where NE is synthesized.

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