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Effects of Prazosin Treatment on Ethanol- and Sucrose-Seeking and Intake in P Rats

For the degree of Master of Science

Is approved by the final examining committee:

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EFFECTS OF PRAZOSIN TREATMENT ON ETHANOL- AND SUCROSE-SEEKING AND INTAKE IN P RATS

A Thesis

Submitted to the Faculty

of

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by

Terril Lee Verplaetse

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For my family.

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ABSTRACT

Verplaetse, Terril L. M.S., Purdue University, December 2011. Effects of Prazosin Treatment on Ethanol- and Sucrose-Seeking and Intake in P Rats. Major Professor: Cristine Czachowski.

Background: Previous studies show that prazosin, an α_1-adrenergic receptor antagonist, decreases alcohol drinking in animal models of alcohol use and dependence and in alcohol-dependent men. These studies extended previous findings by using a paradigm that allows for separate assessment of prazosin on motivation to seek versus consume ethanol or sucrose in selectively bred rats given acute or chronic prazosin treatment. *Methods:* Alcohol-preferring P rats were trained to complete an operant response that resulted in access to either 2% (Exp. 1) or 1% (Exp.2) sucrose or 10% ethanol. In Experiment 1, a 4-week consummatory testing phase consisted of rats bar-pressing to "pay" a specified amount up front to gain access to unlimited ethanol (or sucrose) for a 20-minute period. A 4-week appetitive testing phase examined how much the rats would bar-press for ethanol in an extinction session when no reinforcer could be obtained. In Experiment 2, during testing, the response requirement was dropped to a 1 and daily session cycles of drug (3 weeks/ 14 sessions from Tues to Fri) or vehicle (2 weeks/ 9 sessions from Tues to Fri) treatment were alternated per drug dose for a total of 3 drug

doses (3 cycles) per rat. After each drug cycle, a single non-reinforced extinction session was conducted with no drug 'on board' and no reinforcer access. On test days, rats were given IP injections of either vehicle or one of three doses of prazosin (Exp 1: 0.5, 1.0, 1.5 mg/kg; Exp 2: 0.25, 0.5, 1.0 mg/kg; balanced design; -30 min). *Results:* In Experiment 1, prazosin significantly decreased ethanol-seeking at all doses tested. The highest dose decreased ethanol intake and increased the latency to first lever-press and first lick. Sucrose-seeking and intake were decreased by the same doses of prazosin. In Experiment 2, prazosin significantly decreased reinforcer-seeking at the lowest and highest doses while ethanol intake was not decreased by prazosin. Conversely, sucrose-seeking was decreased at the highest dose of prazosin tested while sucrose consumption was decreased by all doses. Latency to lever-press for sucrose was increased by the lowest dose of prazosin compared to vehicle. *Conclusions:* These findings extend previous research and indicate that prazosin decreases motivation to seek ethanol and sucrose. The specificity of prazosin on different behaviors and over different reinforcers suggests that these findings are not due to prazosin-induced motor-impairment or malaise. These data suggest that prazosin may work by decreasing the reinforcing properties of reinforcers in general.

INTRODUCTION

Alcohol is the most commonly used brain depressant affecting as many as 90% of adults in the United States with approximately 30-60% of those individuals having an adverse alcohol-related event at some point in their lives (American Psychiatric Association [APA], 1994). Furthermore, lifetime alcohol dependence amongst alcohol users is 20.1% (Grant et al., 1997) and the average volume of alcohol consumed has been found to increase the risk for alcohol use disorders, hypertension, cancer and a variety of other medical conditions (Rehm et al., 2003). Currently, there are only three major drugs on the market used to treat alcohol abuse (naltrexone, acamprosate, and disulfiram) and all three have limitations regarding their efficacy and use (Garbutt et al., 1999; Kranzler and Van Kirk, 2001). Clinical studies reveal that naltrexone attenuates the risk to relapse to heavy drinking and decreases the frequency of drinking days but does not affect abstinence (Garbutt et al., 1999; Kranzler and Van Kirk, 2001). Acamprosate also reduces the frequency of drinking in that cumulative abstinent days are decreased (Kranzler and Van Kirk, 2001). Finally, the use of disulfiram yields mixed outcomes when given as a treatment for alcohol dependence in that it occasionally decreases the frequency of drinking but has no effect on improving abstinence (Garbutt et al., 1999). These treatments are efficacious in reducing the frequency of alcohol consumption but their specific role in treating alcohol use disorders is unclear. New

pharmacotherapeutic treatments need to be studied in order to more clearly define how to treat and manage alcohol abuse.

Alcohol and Anxiety

Alcohol use disorders and anxiety disorders have consistently been found to be comorbid in clinical populations with different types of anxiety disorders relating more closely to alcohol use problems (Brady et al., 2000; Kushner et al., 1990). The literature remains mixed as to whether individuals start drinking to relieve anxiety (i.e. anxiety disorders precede the onset of alcohol use disorders) or whether the withdrawal symptoms associated with alcohol use disorders contributes to the development of an anxiety/ stress disorder. Alcohol-related problems tend to begin from attempts to self-medicate in individuals with social phobia but panic disorder and generalized anxiety disorder have been found to stem from alcohol abuse (Kushner et al., 1990). Consistent with these findings, there is a high comorbidity rate of alcohol abuse and post-traumatic stress disorder (PTSD) (Brady et al., 2000). Patients with comorbid PTSD and alcohol use disorders displayed more avoidance and arousal symptoms as well as an increase in sleep disturbances compared to patients with PTSD only (Saladin et al., 1995). Increased arousal symptoms in patients with alcohol use disorders and PTSD may be due to an increase in noradrenergic functioning. Specifically, patients with PTSD have been shown to have excessive noradrenergic firing (Southwick et al., 1993; 1999), and in rodent models, depletion of norepinephrine is associated with a decrease in ethanol self-administration (Davis et al., 1978). Taken together, the excessive noradrenergic signaling seen in stress disorders such as PTSD and the fact that norepinephrine depletion

reduces ethanol self-administration suggests that excessive noradrenergic functioning plays a key role in maintaining these two disorders.

Prazosin Background

Prazosin, an α_1-adrenergic receptor antagonist, works by reducing central adrenergic activity by means of blocking norepinephrine binding to postsynaptic receptors (Simpson et al., 2009) in several brain areas including but not limited to the locus coeruleus (Unnerstall, 1987), olfactory bulb, cerebral cortex, amygdala, dentate gyrus, and the thalamus (Pieribone et al., 1994). Prazosin, although primarily used as an α_1 antagonist and with significantly higher affinity for the α_1 receptor, also binds with modest affinity to α_2-adrenoceptors (Boyajian and Leslie, 1987). Prazosin is unique amongst α_1 antagonists in that it is active at central nervous system sites when administered peripherally (Menkes et al., 1981). The drug was originally marketed as "Minipress" by Pfizer Pharmaceuticals and is currently used as an antihypertensive drug (Constantine et al, 1973). Since prazosin works as a centrally active α_1-adrenergic antagonist, Raskind and colleagues (2003) hypothesized that prazosin should counteract excessive noradrenergic activity reported in PTSD patients and, therefore, should reduce symptoms seen in PTSD. Clinical studies provide evidence that prazosin decreases stressful symptoms related to PTSD, specifically distressing nightmares and night awakenings (Raskind et al., 2003; 2007). Interestingly, patients receiving prazosin treatment for PTSD also reported a decrease in motivation to drink alcohol and a decrease in alcohol intake (Raskind, unpublished observations). These findings, coupled with findings that chronic ethanol exposure and withdrawal in Spraque-Dawley rats caused

defects in HPA function, increases in sympthoadrenal activation during abstinence, and increases in anxiety suggesting alterations in the noradrenergic system related to alcohol use (Rasmussen et al., 2001; 2006), and the high comorbidity rate of alcohol use disorders and PTSD (Brady et al., 2000), led to the examination of prazosin as a treatment for alcohol abuse. Subsequently, prazosin has been found to decrease drug self-administration in animal models of drug abuse and to reduce alcohol drinking and craving in alcohol-dependent men (Bruijnzeel et al., 2010; Forget et al., 2010; Greenwell et al., 2009; Le et al., 2011; Rasmussen et al., 2009; Simpson et al., 2009; Walker et al., 2008).

Prazosin has been shown to decrease the reinforcing properties of nicotine and heroin self-administration (Bruijnzeel et al., 2010; Forget et al., 2010; Greenwell et al., 2009). Prazosin dose-dependently reduced heroin self-administration during the first hours of access in Wistar rats trained with extended 12 hr/day access to intravenous heroin self-administration (Greenwell et al., 2009). Likewise, prazosin dose-dependently decreased the self-administration of nicotine in Long Evans rats with the strongest decrease at the 1 mg/kg dose (Forget et al., 2010). This decrease in self-administration was maintained over consecutive daily sessions of nicotine self-administration (Forget et al., 2010). Forget and colleagues (2010) also found that prazosin dose-dependently decreased reinstatement of extinguished nicotine seeking induced by a nicotine prime or nicotine cues. Furthermore, Bruijnzeel et al. (2010) found that prazosin dose-dependently decreased the elevations in brain reward thresholds associated with nicotine withdrawal in Wistar rats.

Four studies to date have tested the hypothesis that prazosin would be effective in reducing the reinforcing properties of alcohol (Le et al., 2011; Rasmussen et al., 2009; Simpson et al., 2009; Walker et al., 2008). Prazosin has been found to block dependence-induced increases in operant responding for ethanol in Wistar rats at doses of 1.5 and 2.0 mg/kg (Walker et al., 2008). Interestingly, non-dependent animals increased responding for ethanol at the 0.25 mg/kg dose and decreased responding at the 2.0 mg/kg dose of prazosin (Walker et al., 2008). Similarly, prazosin has been found to decrease ethanol drinking in alcohol-preferring (P) rats in a 2-hour, 2-bottle choice (ethanol versus water) paradigm which tested the effects of prazosin in a 2-day treatment and in a subsequent "chronic" 5-day treatment (Rasmussen et al., 2009). It is interesting to note that the lowest dose of prazosin (0.5 mg/kg) did not reduce alcohol consumption in the 2-day treatment but exhibited an effect after three days of administration in the subsequent 5-day treatment (Rasmussen et al., 2009). Likewise, the highest doses of prazosin lost effectiveness after three days of treatment (Rasmussen et al., 2009). Further, prazosin has been found to block yohimbine-induced reinstatement of ethanol seeking in Wistar rats as well as footshock-induced reinstatement of ethanol seeking in Long Evans rats (Le et al., 2011). Yohimbine is an α_2-adrenoceptor antagonist that increase norepinephrine release in the brain. Finally, prazosin has been shown to decrease relapse alcohol drinking in treatment-seeking, alcohol-dependent men without PTSD (Simpson et al., 2009). Subjects receiving prazosin treatment reported fewer drinking days per week and fewer drinks per week than the placebo group during the last three weeks of the 6-week study (Simpson et al., 2009).

Prazosin, the α_1-adrenergic receptor antagonist, has therefore been shown to decrease the reinforcing properties of ethanol in animal models using three different paradigms and in a pilot clinical study (Le et al., 2011; Rasmussen et al., 2009; Simpson et al., 2009; Walker et al., 2008). Prazosin has also been effective in reducing the reinforcing properties of nicotine and heroin (Bruijnzeel et al., 2010; Forget et al., 2010; Greenwell et al., 2009). Since prazosin administration has been successful in decreasing the reinforcing value of alcohol and other drugs, I first investigated the effects of acute prazosin administration on the motivation to initiate ethanol-seeking and drinking (see Experiment 1).

Alcohol-Preferring (P) Rat

Animal models of alcohol abuse and alcoholism are necessary in order to study new pharmacotherapeutic treatments for alcohol use disorders. The alcohol-preferring (P) rat is one such model that was developed to study excessive ethanol drinking. P rats are less sensitive to the sedative effects of ethanol and are more susceptible to the stimulatory effects of ethanol as opposed to their non-preferring (NP) counterparts (Bell et al., 2002; Gatto et al., 1987; Stewart et al., 1991). Ethanol self-administration has been found to increase locomotor activity and heart rate in P rats (Bell et al., 2002) while P rats have also been found to develop tolerance to the motor impairing (Gatto et al., 1987) and aversive effects (Stewart et al., 1991) of ethanol. In fact, P rats will drink greater than 5 g/kg/day ethanol, achieving pharmacologically relevant blood ethanol concentrations, compared to 1 g/kg/day in their non-preferring counterpart (Li et al., 1987). Additionally, P rats will work for ethanol access in that they will operantly respond for

ethanol in concentrations up to 30% and show preference for ethanol over water whereas NP rats won't respond for ethanol above concentrations of 10% (Murphy et al., 1989). P rats have also been found to increase responding for ethanol after repeated deprivation periods (Rodd et al., 2003). Moreover, P rats have been found to self-administer ethanol for its pharmacological effects as opposed to its taste or caloric value (Gatto et al., 1994; Murphy et al., 1988). P rats will respond to self-administer ethanol intragastrically (Murphy et al., 1988) and will self-administer ethanol directly into the ventral tegmental area (Gatto et al., 1994). Finally, evidence suggests that P rats exhibit withdrawal symptoms after chronic ethanol consumption (Waller et al., 1982) and relapse-like behavior is exhibited in P rats in the form of the alcohol deprivation effect after chronic consumption (Rodd-Henricks et al., 2000). Interestingly, evidence also suggests that animal models genetically bred for high ethanol consumption may exhibit a down-regulation of norepinephrine transporters in the locus ceruleus (Murphy et al., 2002), a brain region involved in the stress response and a major site of CNS norepinephrine synthesis.

Important to the present experiments is the consideration that selecting rats for high alcohol preference might also inadvertently select for other traits. Again, clinical studies have consistently shown that alcohol use disorders and anxiety disorders are highly comorbid (Brady et al., 2000; Kushner et al., 1990) and findings suggest a relationship between noradrenergic activity, stress, and ethanol use (Davis et al., 1978; Raskind et al., 2003; Southwick et al., 1993;1999). Animal models of high alcohol preference have shown that P rats display an increase in footshock –induced suppression of operant responding as well as a decreased latency in the open arms of the elevated plus

maze and longer time in the passive avoidance test compared to their NP counterpart (Stewart et al., 1993), indicating that selective breeding for high alcohol drinking in rodent lines might also select for high stress and anxiety

The 'Sipper-Tube' Paradigm

Prazosin has been shown to decrease ethanol self-administration in rats in two different behavioral paradigms. The behavioral paradigms previously utilized focus on an exclusive consummatory response (i.e., home-cage drinking) (Rasmussen et al., 2009) or a combined seeking/drinking response (i.e. lever-press required for access to each 0.1ml of the reinforcer solution) (Walker et al., 2008). The acute prazosin administration study (Experiment 1) and the chronic prazosin treatment study (Experiment 2) further examined the effects of seeking and drinking in a paradigm that separately assesses the ethanol (or sucrose) seeking response (i.e. lever-presses) from the consummatory response (i.e. drinking) (Samson and Czachowski, 2002). Other models of ethanol-seeking and consumption often measure these behaviors while the animal is under the influence of the pharmacological effects of ethanol thus seeking of the drug cannot be accurately assessed (Samson and Czachowski, 2002). The 'sipper-tube' model utilizes a response requirement that the animal has to emit before gaining access to twenty minutes of free access to ethanol (consummatory behaviors) so operant behavior is not affected by the pharmacological properties of the ethanol. This paradigm also measures seeking (or craving) with no ethanol 'on board' thus accurately measuring if the animal will seek the reinforcing properties of ethanol when no reinforcer can be obtained. Thus, the 'sipper-

tube' paradigm examines seeking and intake within an operant session and in completely separate phases. This model has effectively demonstrated that a drug can exclusively affect reinforcer-seeking verses drinking (Czachowski et al., 2001a; Czachowski et al., 2001b; Czachowski et al., 2002) and that rats will self-administer ethanol to binge-like levels in 20-minute free access sessions (Samson and Czachowski, 2002).

EXPERIMENT 1

Introduction

The goal of Experiment 1 was to further examine the acute effects of prazosin administration on ethanol (or sucrose)-seeking and intake. As noted in the general introduction, P rats were used in the 'sipper-tube' paradigm which procedurally separates consummatory (intake) and appetitive (seeking) behaviors. This model allows for the measurement of start latencies, lever-presses (seeking), reinforcer intake (drinking), and number of licks. Prazosin dosing was similar to previous studies in which doses ranged from 0.25 mg/kg to 2.0 mg/kg and were injected intraperitoneally (IP) 30 minutes prior to the start of the operant session. Based on previous findings, it was hypothesized that prazosin, tested in the P rat, would dose-dependently decrease ethanol responding and intake while no effect of prazosin would be seen in the sucrose-reinforced group.

Materials and Methods

Subjects

Sixteen alcohol-naïve, P rats from the sixty-eighth generation of selective breeding served as subjects in one of two groups (ethanol group, n=8 and sucrose group, n=8). Rats were individually housed with food and water available ad libitum except as noted below. The animals were maintained on a 12-hour light/dark cycle (lights on from7 AM to 7 PM), and animal care was in accordance with NIH guidelines (Guide for

the Care and Use of Laboratory Animals; NIH Guide 1996) and approved by the Institutional Animal Care and Use Committee.

Apparatus

Daily sessions were conducted in operant chambers (Med-Associates; St. Albans, VT, USA; 30 x 30 x 24.5 cm) equipped with a house light, one retractable lever, and a retractable graduated cylinder tube with a rubber stopper and stainless steel spout with double ball bearings to prevent leakage. The lever was located on the opposite wall to the sipper tube drinking bottle. Solution became available upon completing the response requirement on the lever. Operant chambers were individually housed in ventilated, sound-attenuating enclosures to minimize disturbances. Electrical inputs and outputs were controlled using Med-Associates software (Med-Associates).

Drugs

Ethanol solutions were prepared volume/volume in water using 95% ethanol. Sucrose/ethanol solutions and sucrose solutions were prepared weight/volume and used as a solute. Prazosin hydrochloride (Sigma-Aldrich, Inc., St. Louis, MO) was dissolved in sterile water at 2 ml/kg BW. Prazosin was injected IP in a dose of 0.0, 0.5, 1.0, or 1.5 mg/kg BW, 30 minutes prior to operant sessions. Prazosin was given 30 minutes prior to the start of the operant session based on an onset of action between 5-40 minutes (Menkes et al., 1981).

Training and Ethanol Initiation

Upon arrival, subjects were weighed and handled for a minimum of three days. Daily sessions were conducted five days/week at the same time each day during the lights-on cycle. Subjects were initially trained to press the lever on a fixed-ratio (FR) one schedule that resulted in 15 seconds of access to the sipper tube with 10% sucrose in approximately 30 minute sessions. Subjects were water restricted for the initial sessions only, after which food and water were available ad libitum in the home cage. Operant training was carried out over a 3 week period using the sucrose-fading procedure (Samson, 1986). The sucrose fading procedure involves increasing the FR from 1 to 4 while decreasing the sucrose concentration to 2%, and in the ethanol group introducing ethanol and increasing the concentration from 2% to 10% while fading out sucrose completely with final solutions reaching 2% sucrose for the sucrose group and 10% ethanol for the ethanol group. The procedural separation between seeking (lever-pressing) and consumption was then established. Initially, following completion of a single response requirement (4 lever-presses) access to a sipper tube was provided for 20 uninterrupted minutes. Over two weeks, the response requirement was increased from 4 to 10, and then the response requirement of 10 was maintained for two additional weeks (Figure 1).

Treatment Schedule and Test Sessions

A 4-week consummatory testing phase was initiated in which all animals got one IP injection of either vehicle or one of three doses of prazosin (0.5, 1.0, 1.5 mg/kg) in a balanced/random design on one day each week (the other four days were normal, no

injection days). IP injections were given 30 minutes prior to the start of the operant session in which animals lever-pressed (i.e., the response requirement was reduced to 1 on testing days so that animals were more likely to obtain access to the reinforcer) to gain unlimited access to ethanol or sucrose for a 20 minute period. Following the consummatory phase, three weeks of no treatment began to ramp the animals up from a RR10 to an RR20. Next, a 4-week appetitive phase began in which the same type of drug treatments each preceded a single, weekly (Thursdays) extinction session such that the animals could still press the lever for the entire 20 minute session but never get reinforced. Animals were injected on Tuesday of the same week with vehicle (1 ml/kg BW) to control for the possibility of injections predicting an extinction session. These vehicle injections were accompanied by reinforced operant sessions. The other three days of the week were normal, injection-free reinforced sessions.

Blood Ethanol Concentration (BEC) Determination

Following all prazosin treatments and immediately following the final 20 minutes of ethanol access, blood samples were collected (100 µl) into heparinized capillary tubes from a nick to the tip of the tail while the rats were restrained briefly for a maximum of 2-4 minutes. Samples were stored on ice during collection and then immediately centrifuged and a 5 µl sample of plasma was analyzed using the AMI Analyzer (Analox Instruments, Lunenburg, MA, USA). Ethanol concentration was determined with an amperometric oxygen electrode that measures oxygen consumption during the enzymatic oxidation of alcohol to acetaldehyde.

Data Analyses and Statistics

Dependent measures for the consummatory testing phase were total intake of sucrose and ethanol determined from the change in fluid volume in the graduated cylinder sipper tube and g/kg intake were calculated from the intake volume and daily body weight measures. Total lever-presses, licks, and cumulative records of responding were recorded for each session. Latency to lick in seconds was also recorded for each daily session. Dependent measures for the appetitive testing phase were total number of lever-presses and latency in seconds to lever-press for each appetitive session.

Data were analyzed using two-way within-subject repeated measures analysis of variance (RM ANOVA; reinforcer and dose as the main variables) and post hoc comparisons were performed using Student-Newman-Keuls. In addition, t-tests were used to compare the alcohol and sucrose groups for all responses in the vehicle condition to further assess "baseline" responding. Percent change from baseline was calculated by subtracting intake/responding during prazosin administration from vehicle intake/responding and dividing this difference by vehicle intake/responding. Percent change data were analyzed using two-way repeated measures ANOVA and post hoc comparisons were performed using Student-Newman-Keuls. All analyses were conducted using the SigmaStat 3.5 program (Systat Software, Inc., Chicago, IL) with significance accepted at $p < 0.05$. Data are presented as mean ± SEM.

Results

For the Consummatory Phase only, one rat was dropped from the analyses (ethanol group) for failure to respond on the vehicle injection day (i.e. failure to provide a

reliable control response) due to difficulty with the injection procedure (broken toe nail).

On the final day of operant sessions, BECs ranged from 20.9 to 102.6 with an average BEC of 54.9 (± 10.3), as measured at the end of the 20 minute self-administration period. Vehicle-treated rats that were lever-pressing for ethanol averaged 69.7 responses and 0.90 g/kg of ethanol consumed. Vehicle-treated rats that were lever-pressing for sucrose averaged 104.7 responses and 1.10 g/kg sucrose consumed. Figure 2 shows mean (± SEM) reinforcer-seeking (lever-presses in 20 min) on the appetitive testing days for the sucrose-reinforced (white bars) and ethanol-reinforced (black bars) groups over one of three doses of prazosin (0.5, 1.0, 1.5 mg/kg) or vehicle (zero) treatments (-30 min). A repeated measures ANOVA indicated that there was a main effect of treatment [$F(3,39)=8.3$, $p \leq 0.001$] with post hoc analyses showing that all prazosin doses differed from control (*). There was no effect of reinforcer and no reinforcer by dose interaction. A follow up analysis of percent change from baseline on responding for ethanol- and sucrose-reinforced groups confirmed that there was no main effect of reinforcer ($p=0.180$) or a reinforcer by dose interaction ($p=0.492$). With regard to consumption, Figure 3 shows mean (± SEM) total reinforcer intake (g/kg over 20 min) on consummatory testing days for the sucrose-reinforced (white bars) and ethanol-reinforced (black bars) groups over one of three doses of prazosin (0.5, 1.0, 1.5 mg/kg) or vehicle (zero) treatments (-30 min). A repeated measures ANOVA indicated that there was a main effect of treatment [$F(3,36)=7.9$, $p \leq 0.001$] with post hoc analyses showing that only the high dose of prazosin differed from control (*).

Further, prazosin (1.5 mg/kg) increased the latency to lever-press and to lick for ethanol but not for sucrose. Figures 4 and 5 show mean (± SEM) latency (seconds) to

first lever-press and first lick on appetitive and consummatory testing days, respectively. Sucrose-reinforced (white bars) and ethanol-reinforced (black bars) groups over control and prazosin treatments (-30 min) are shown. For both measures, a repeated measures ANOVA indicated an interaction effect such that in the ethanol group only, the high dose of prazosin increased the time to first lever-press [$F(3,39)=4.6$, $p \leq 0.01$] and first lick [$F(3,39)=4.6$, $p \leq 0.01$]. Because of the high variability of these responses and the absence of a response in some cases (assigned 1200 sec), a Mann-Whitney Rank Sum test at the high dose confirmed these findings (*) (Median values - Appetitive: sucrose 24.4, ethanol 182.6; Consummatory: sucrose 2.9, ethanol 656.8).

Discussion

The purpose of experiment 1 was to extend previous findings and evaluate the role of prazosin, an α_1-adrenergic receptor antagonist, on distinct ethanol-seeking and intake behaviors in P rats consuming binge-like levels of ethanol. Overall, prazosin, administered acutely, decreased ethanol consumption at the highest dose, whereas ethanol-seeking was attenuated at all doses. These findings are consistent with and extend those of Walker et al. (2008) where the high dose of prazosin (2.0 mg/kg) was necessary to attenuate a combined seeking/drinking response in non-dependent Wistar rats. These findings also extend those of Rasmussen et al. (2009) where prazosin (1.0, 1.5, 2.0 mg/kg) decreased ethanol intake, with the 0.5 mg/kg dose becoming effective only after three consecutive days of treatment. Further, latencies to first lick (on consummatory test days) and lever-press (on appetitive test days) were increased at the

highest dose in the ethanol group indicating a decrease in motivation to seek and obtain ethanol.

Generally, prazosin decreased sucrose-seeking and consumption at the same doses required to decrease ethanol-seeking and intake. However, there are indications that the effects of prazosin were selective for ethanol. The highest dose of prazosin decreased ethanol-seeking and drinking by 76% and 67%, respectively, whereas sucrose-seeking and drinking was decreased by only 44% and 39%, respectively. Additionally, the highest dose of prazosin increased the latency to first lever-press and to first lick in the ethanol group only suggesting a decrease in motivation to obtain ethanol. The fact that latencies to first lever-press or lick for sucrose were not altered by prazosin indicates that the increased latencies for ethanol-seeking and drinking were not due to prazosin-induced motor-impairment or malaise. Also, latency measures were taken during extinction sessions when no ethanol was available (on appetitive test days) or up until the first lick (on consummatory test days), and therefore the increased latencies to lever-press and lick were not due to the pharmacological properties of ethanol. These findings suggest that prazosin may be an effective pharmacotherapeutic drug for treating alcohol use disorders, and possibly a treatment that targets the motivation to initiate episodes of heavy ethanol consumption. Since any drug given to treat alcohol abuse would not be given acutely, it is necessary to look at the effects of chronic prazosin treatment on reinforcer-seeking and intake.

EXPERIMENT 2

Introduction

Clinically, it is important to examine prazosin chronically as any treatment for alcohol abuse would be administered over multiple days. Additionally, chronic studies looking at the effects of other treatments for alcohol abuse (e.g. naltrexone) using repeated dosing should be examined in order to better understand a chronic dosing regimen. Chronic dosing of naltrexone has been found to decrease the palatability of ethanol (Hill et al., 2010) and repeated dosing of naltrexone, using identical procedures as Experiment 2, has been found to dose dependently decrease ethanol and sucrose intake and decrease seeking of ethanol only (Czachowski and DeLory, 2002). Here, repeated dosing of a drug could lead to reinforcer devaluation over time due to a decrease in motivation caused by repeated drug/reinforcer pairings. In fact, Czachowski and DeLory (2002) suggest that the decrement in responding for ethanol after chronic naltrexone treatment may be due to reinforcer devaluation. However, extinction of the reinforcing properties of a reinforcer and conditioned taste aversion could also account for a decrement in intake and responding with repeated dosing of any drug treatment. Important to Experiment 2 is the fact that repeated dosing of naltrexone over 14 days did not lead to tolerance or sensitivity to the drug (Czachowski and DeLory, 2002). Also, previous studies of acute naltrexone administration have shown non-reinforcer specific

(ethanol vs. sucrose) effects on consumption (Stromberg et al., 2002) while chronically, ethanol consumption was decreased to a slightly greater degree than for sucrose (Czachowski and DeLory, 2002). Similarly, acute administration of prazosin in Experiment 1 decreased both ethanol- and sucrose-seeking and intake, with a slightly greater decrement in responding and consumption in the ethanol-reinforced group. Therefore, chronic prazosin treatment, using the same paradigm and identical methods to Czachowski and DeLory (2002), should be tested for its specific effects on ethanol- and sucrose-seeking and intake in the P rat.

Thus, the aims of Experiment 2 were to expand on the data from Experiment 1 and assess the effects of chronic prazosin treatment on ethanol- and sucrose-seeking and consumption in the 'sipper-tube' paradigm. Although latency data in Experiment 1 showed that drug-induced motor impairment or malaise was not an issue, animals still appeared to be somewhat sedated by visual observation. Therefore, the chronic study utilized a lower dose range to minimize any sedative effect on the subjects while ideally maintaining the pharmacotherapeutic effect of the drug. This lower dose range has been used in previous studies of prazosin effects on ethanol drinking (Rasmussen et al., 2009; Walker et al., 2008). Moreover, while acute treatment of prazosin yielded non-reinforcer specific results, Experiment 2 aimed to determine if chronic treatment of prazosin yields reinforcer specific effects for ethanol and if these effects work by decreasing consumption versus seeking of ethanol or both. Experiment 2 also aimed to determine whether prazosin is an 'anti-craving' drug since acute administration of prazosin (1.5 mg/kg) increases the latencies to lever-press and to lick indicating a decrease in the motivation to initiate ethanol drinking (reduction in craving), or whether chronic prazosin

treatment works by blocking the reinforcing properties of ethanol when the animal has been drinking during treatment.

Furthermore, this study aimed to determine if an animal would seek (in lever-presses) ethanol once the animal has been without treatment (i.e., the prazosin/ethanol pairings) for three days. Essentially, the paradigm seeks to find if chronic prazosin treatment attenuates seeking once drug treatment stops. Finally, Experiment 2 aimed to determine if there is tolerance to prazosin when administered chronically and at what time-point during administration tolerance occurred or if sensitivity to the drug is seen after chronic treatment.

Based on previous literature and findings from Experiment 1, it was hypothesized that chronic prazosin treatment would be effective as a pharamacotherapeutic agent for the treatment of alcohol use disorders. Specifically, it was hypothesized that chronic prazosin would decrease ethanol-seeking and intake to a greater degree than for sucrose and that lower doses would become more effective with further days of treatment. Again, this is based on findings that during a 5- day prazosin treatment regimen the lowest dose of prazosin became effective after the second day and the highest doses of prazosin became less effective after the third day of treatment (Rasmussen et al., 2009). Additionally, it was hypothesized that latency to lever-press and lick would be increased for ethanol based on findings from Experiment 1, indicating a decrease in motivation to respond for ethanol. Furthermore, based on the non-reinforcer specific findings of Experiment 1, it was hypothesized that if prazosin were to decrease sucrose-seeking and intake it would be at the highest doses of prazosin and would not affect latencies to lick or lever-press.

Materials and Methods

Subjects

Twenty-four adult male P rats from the sixty-ninth generation of selective breeding served as subjects and were randomly divided into two groups; an ethanol reinforced group and a sucrose reinforced group (n=12/group). Their daily handling and weighing, housing, and feeding conditions were identical to Experiment 1.

Apparatus

Daily sessions were conducted in operant chambers identical to Experiment 1 and solutions became available in a manner identical to Experiment 1.

Drugs

Ethanol, sucrose, and sucrose/ethanol solutions were prepared identically to Experiment 1. Prazosin hydrochloride (Sigma-Aldrich, Inc., St. Louis, MO; Tocris Bioscience, Ellisville, MO) was dissolved in sterile water at 1 ml/kg BW. Prazosin was injected IP in a dose of 0.0, 0.25, 0.5, or 1.0 mg/kg BW, 30 minutes prior to the start of the operant sessions.

Training and Ethanol Initiation

Training and ethanol initiation were identical to Experiment 1 except final solutions reached 1% sucrose for the sucrose group and 10% ethanol for the ethanol group. 1% sucrose was used in this study as opposed to 2% sucrose (used in

Experiment 1) to best equate intake volumes with 10% ethanol. After establishing stable responding for the reinforcer, the procedural separation between seeking (lever-pressing) and consumption was established. Initially, following completion of a single response requirement (4 lever-presses) access to a sipper tube was provided for 20 minutes. The response requirement was then increased from an RR 4 to an RR20, and this training phase was completed by the end of week 6 (see Figure 6).

Treatment Schedule and Test Sessions

Daily session cycles of drug (3 weeks/ 14 sessions from a Tuesday to Friday) or vehicle (2 weeks/ 9 sessions from a Tuesday to Friday) treatment were alternated per drug dose for a total of three drug doses per rat. Like Experiment 1, during operant sessions the response requirement was decreased to an RR1 to ensure that all subjects gained access to the reinforcing solution (10% ethanol or 1% sucrose). At the end of each cycle, a single, non-reinforced extinction session was conducted on a Monday following two days of no injections or reinforcer access. Extinction sessions consisted of twenty minutes of access to the lever with no presentation of the sipper tube. Solutions were present in the sipper tube to control for scent cues. Unlike Experiment 1, prazosin was not 'on-board' during each extinction session. The response requirement was then gradually increased again to an RR20 for the remainder of that week (no injections) to ensure that subjects continued to respond at an RR20 (i.e. that the prolonged exposure to the RR1 schedule did not cause a decrement in responding) and then the next drug dose cycle was initiated. That is, a 14 day drug cycle was followed by an extinction session (Monday after cycle ends), with the remainder of the week consisting of increasing the

RR to 20. Then, a 9-day sterile water cycle began followed by an extinction session (Monday after cycle ends) with the remainder of the week consisting of increasing the RR to 20. This process then repeated itself until all three drug doses were administered to each rat. The extinction session at the end of each cycle is used to assess ethanol or sucrose seeking after chronic administration of the drug or vehicle. This is the appetitive portion of the experiment and, again, was conducted with no drug on board and when no reinforcer could be obtained. Once more, three doses of prazosin, 0.25, 0.5, and 1.0 mg/kg, were used in a balanced/random order design by subject across the three dosing cycles with a corresponding sterile water cycle paired to each dose (a total of three drug cycles, one for each dose, and two intervening sterile water cycles) (See Figure 6 for a timeline of each drug cycle and vehicle cycle and Table 1 for daily within session response requirements during each cycle).

BEC Determination

BEC determination was similar to Experiment 1. BECs were collected at the end of the last drug dose cycle at the end of a 20 minute drinking session when no drug was on board.

Data Analyses and Statistics

Dependent measures for the consummatory and appetitive testing were identical to Experiment 1. The ethanol group and the sucrose group were analyzed separately using repeated measures ANOVAs and post hoc comparisons were performed using the Student-Newman-Keuls method when appropriate. Sterile water treatment data were

collapsed for each subject when possible as determined by a repeated measures ANOVA. Dose (0.0, 0.25, 0.5, 1.0 mg/kg prazosin) and Day were the main factors over the first nine days. In addition, mean intake over the nine sterile water or 14- drug day treatments were analyzed with dose as the main factor. For appetitive data, dose was the main factor. Percent change from baseline was calculated and analyzed identically to Experiment 1.

Results

All groups started with twelve subjects, and a total of one rat was dropped from the initial 24 animals. One animal was dropped from the analyses (sucrose group) for failure to respond on the first two days of testing due to difficulty with the injection procedure (broken toe nails). There were no significant differences of intake between the two vehicle control periods (9 days of vehicle injections after each drug cycle) for ethanol- and sucrose-reinforced groups ($p=0.08$ and $p=0.29$, respectively). Likewise, there were no significant differences in responding for ethanol or sucrose between vehicle cycles 1 and 2 ($p=.915$), therefore, vehicle control periods were collapsed and a third vehicle cycle was not initiated.

On the final day of operant sessions, BECs ranged from 28.1 to 97.5 with an average BEC of 56 (± 6), as measured at the end of the 20 minute self-administration period. In the ethanol-reinforced group, analysis of ethanol intake (g/kg) following vehicle or prazosin (Figure 7) over days 1-9 showed that there was a main effect of day [$F(3, 264)=4.45$, $p<0.001$]. Post hoc analyses indicated that the effect of day was due to an increase in intake on day 5 (day 5 vs. days 2, 3, 6, 7, and 8). There was no main effect

of dose and no day by dose interaction. Analysis of total mean ethanol intake collapsed over all days (days 1-14) indicated that there was no main effect of dose (Figure 8).

In the sucrose-reinforced group, analysis of sucrose intake (g/kg) following vehicle or prazosin (Figure 9) over days 1-9 showed that there was a main effect of dose [$F(3,240)=5.1$, $p=0.006$], with post hoc analyses indicating that the low and medium dose decreased intake relative to both vehicle treatment and the high dose of prazosin. There was also a main effect of day [$F(8,240)=10.96$, $p<0.001$]. Post hoc analyses indicated that this was due to slightly higher intakes over day 1 (day 1 vs. days 2-9) and day 2 (day 2 vs. days 7, 8, and 9). There was no day by dose interaction. Analysis of total mean sucrose intake collapsed over all days showed a main effect of dose [$F(3,30)=5.71$, $p=0.003$], and post hoc analyses showed that all three doses of prazosin decreased intake as compared to vehicle (Figure 10). An analysis of percent change of baseline on intake for ethanol- and sucrose-reinforced groups confirmed that there was not a main effect of reinforcer ($p=0.600$), dose ($p=0.790$), and no reinforcer by dose interaction ($p=0.558$).

Ethanol-seeking and sucrose-seeking following sterile water and prazosin treatments were assessed using extinction responding. There was a main effect of dose [$F(3, 63)=5.302$, $p=0.003$], with post hoc analyses indicating that the low and high dose of prazosin decreased seeking relative to vehicle. There was no main effect of reinforcer and no reinforcer by dose interaction (Figure 11). An analysis of percent change of baseline on responding for ethanol- and sucrose-reinforced groups indicate that there was not a main effect of reinforcer ($p=0.556$), dose ($p=0.084$), and no reinforcer by dose interaction ($p=0.379$).

With regard to latency, in the ethanol-reinforced group, analyses of latency to lick (on consummatory testing days) following vehicle or prazosin (Figure 12) over days 1-9 and total mean latencies to lick collapsed over all days (1-14) indicated that there was no effect of dose, day, and no day by dose interaction. Likewise, analyses of latency to lever-press (on appetitive testing days) showed no main effect (Figure 13). In the sucrose-reinforced group, analysis of latency to lick (on consummatory testing days) following vehicle or prazosin (Figure 14) over days 1-9 showed that there was a main effect of day [$F(8,240)=2.29$, $p=0.029$], with post hoc analyses indicating that day 1 was significantly different from all other days. There was no main effect of dose and no day by dose interaction. Analysis of total mean latencies to lick collapsed over all days (1-14) indicated that there was no main effect of dose. Analysis of latency to lever-press (Figure 13) (on appetitive testing days) showed a main effect of dose [$F(3,30)=4.32$, $p=0.012$], with post hoc analyses indicating that the lowest dose of prazosin increased the latency to lever-press as compared to vehicle. Further, in the ethanol- and sucrose-reinforced groups, analysis of day 1 intakes following vehicle or prazosin showed a main effect of reinforcer [$F(1,63)=34.2$, $p<0.001$] with greater intake of sucrose overall, but no interaction between reinforcer and dose.

Discussion

The purpose of Experiment 2 was to expand on the findings from Experiment 1 and evaluate the role of chronic prazosin treatment on reinforcer-seeking and self-administration. Overall, chronic prazosin treatment decreased reinforcer seeking at the lowest and highest dose, whereas ethanol consumption was not affected by prazosin.

Conversely, in the sucrose-reinforced group, prazosin at the lowest doses, 0.25 mg/kg and 0.5 mg/kg, decreased intake when looking at the 9-day dosing regimen. However, prazosin attenuated sucrose intake at all doses tested (0.25, 0.5, 1.0 mg/kg) when looking at mean intakes over 14 days. Latency to first lick was only significant on the first day of treatment and the lowest dose of prazosin increased the latency to lever-press during appetitive extinction sessions for the sucrose group only. There was no indication of tolerance to treatment effects over the 9 or 14-day dosing regimens and no indication of prazosin-induced motor impairment or malaise as evidenced by the lack of effects on latency to lick or respond in the ethanol group.

GENERAL DISCUSSION

Again, the 'sipper-tube' paradigm used in the present study made it possible to determine whether prazosin treatment had any differential effects on reinforcer-seeking and drinking behaviors in the P rat. This was ensured by the use of a low response requirement for intake assessment as well as single extinction sessions to assess seeking. Because the present investigations included a sucrose-reinforced control group, a comparison between prazosin treatment effects, specific behaviors (appetitive versus consummatory) targeted by prazosin treatment, and reinforcer selectivity of prazosin could be determined.

Important to the present findings is the fact that these are the first two experiments to use a control reinforcer (sucrose) when examining the effects of prazosin on seeking and consumption behaviors. Water intake has been assessed in non-thirsty animals being treating with prazosin (Rasmussen et al., 2009; Walker et al., 2008). Water intake was examined in a 2-hour, 2-bottle choice (ethanol versus water) paradigm looking at the effects of prazosin on free-access drinking (Rasmussen et al., 1009). This study found no evidence of a decrease in water intake in a 2-day and subsequent 5-day treatment of prazosin (Rasmussen et al., 2009). This is also consistent with findings reporting no effect on water intake during acute withdrawal of alcohol-dependent rats being treated with prazosin (Walker et al., 2008). Due to the non-reinforcer specific effects of prazosin

on seeking and drinking in Experiment 1 and because prazosin attenuated reinforcer-seeking, sucrose intake, and latency to lever-press for sucrose in Experiment 2, the drug may work to decrease the reinforcing properties of oral reinforcers in general. Additionally, prazosin has been shown to reduce the reinforcing properties of nicotine and heroin self-administration (Bruijnzeel et al., 2010; Forget et al., 2010; Greenwell et al., 2009) and prazosin has been found to decrease yohimbine-induced reinstatement of food seeking (Le et al., 2011). These findings, together with results from Experiment 1 and Experiment 2, suggest that prazosin may work by suppressing the reinforcing properties of oral reinforcers (ethanol, sucrose, food) and other drugs (nicotine, heroin). Therefore, inhibition of central adrenergic activity by prazosin may, in fact, attenuate seeking and intake behaviors universally.

Experiment 2 tested seeking three days after prazosin treatment and three days after ethanol- or sucrose-reinforced sessions. This is unlike Experiment 1 in that Experiment 1 examined reinforcer-seeking with prazosin on board. Overall, there was a decreased tendency to seek the reinforcers during appetitive sessions at the lowest dose of prazosin (0.25 mg/kg) and highest dose of prazosin (1.0 mg/kg) compared to vehicle. Since subjects had no prazosin on board at the time of these extinction sessions, the decreases in responding indicate that the rats must have learned something about the prazosin experience that attenuated their reinforcer-seeking response three days post-treatment. Specifically, it is likely that rats learned to devalue ethanol and sucrose caused by repeated and chronic prazosin/reinforcer pairings during reinforced sessions. This indicates that prazosin may work by decreasing motivation to seek ethanol and sucrose which is in agreement with Experiment 1 in that all doses of prazosin in Experiment 1

attenuated ethanol- and sucrose-seeking. It is unlikely that the decrement in responding is due to a conditioned taste aversion of the reinforcer when prazosin is on board because intakes were not affected in the ethanol-reinforced group. Likewise, it is improbable that prazosin/reinforcer pairings extinguished the rewarding properties of each reinforcer because, again, ethanol intake was not affected by prazosin. It is unlikely that prazosin produced a general malaise over treatment days that interfered with seeking because latencies to respond were not affected in the ethanol-reinforced group. It is also unlikely that prazosin had general effects on arousal because prazosin's effects on latency to respond were specific to sucrose.

With regard to intake in the ethanol-reinforced group, although a decrease in intake by prazosin was not observed, an increase in ethanol consumption was observed on day 5 (Monday following three days of no treatment). Figure 7 suggests that, even though there was no day by dose interaction and no day 10 vehicle treatment for comparison, there was a rebound effect on ethanol intake after the weekends (72 hours without ethanol access and no prazosin treatment). These findings indicate that individuals taking prazosin to treat alcohol abuse must take this medication consecutively in order for a decrease in alcohol consumption to occur. This should be addressed in future projects by testing chronic prazosin treatment seven days per week for three consecutive weeks instead of five days per week for three consecutive weeks as seen in the Experiment 2. Notable is the observation that ethanol intake was variable for vehicle treatment and all three doses of prazosin. Figure 7 and Figure 8 show that ethanol intake barely exceeded 0.7 g/kg during vehicle treatment. This is in sharp contrast to Experiment 1 where P rats were drinking approximately 0.9 g/kg of ethanol at vehicle

and with previous findings suggesting that the P rat drink excessive amounts of ethanol (Bell et al., 2006; Li et al., 1987). Figure 7 shows ethanol intake 14 days prior to the start of drug testing. During these 14 days (normal reinforced operant sessions without prazosin injections), ethanol intake only exceeded 0.8 g/kg three times with an average intake over all 14 days of 0.77 g/kg. If ethanol intake were higher in Experiment 2, then the effects of chronic prazosin treatment may have been much greater.

Additionally, chronic administration of prazosin decreased sucrose consumption. This is consistent with Experiment 1, which utilized a slightly higher dosing range (0.5 -1.5 mg/kg), in that the highest dose of prazosin was able to decrease sucrose consumption. However, lower doses decreased sucrose intake in Experiment 2 than required to decrease consumption in Experiment 1 indicating that prazosin is effective at decreasing the reinforcing properties of sucrose at lower doses when administered chronically. Further, the highest dose of prazosin was not effective in decreasing sucrose intake when examining intake over nine days. However, sucrose consumption decreased at the highest dose of prazosin when assessing intake over a 14 day period. This indicates that animals become more sensitive to the highest dose of prazosin with further days of treatment. Also, day 1 and day 2 of treatment resulted in higher sucrose intakes overall as compared to the other 12 days of treatment. This could suggest, again, that prazosin becomes more effective at decreasing sucrose consumption with further days of treatment. However, Figure 9 suggests that this is highly unlikely because intake during vehicle treatment was high on day 1 and, on day 2, intake decreased below the medium and high doses of prazosin before becoming stable. The fact that sucrose intake was

decreased by all doses of prazosin does not indicate that any neuroadaptation was caused by prazosin in subjects drinking sucrose.

Chronic prazosin treatment also increased the latency to lick on the first day of treatment in the sucrose group only. The highest dose and medium dose of prazosin increased the latency to first lick in consummatory sessions over day 1-9. One animal (sucrose group) was dropped from Experiment 2 for failure to respond for multiple days due to multiple broken toenails. However, two other animals in the sucrose group failed to respond and took longer to respond on the first day of injections only due to difficulty with the injection procedure (i.e. one animal bit the plastic carrier cart and another animal broke one toenail). Therefore, the increased latency to lick in the sucrose group on the first day may be more of a reflection of first day difficulties with the injection procedure or injuries associated with the first day. These findings are inconsistent with Experiment 1 in which latency to lick was increased in the ethanol group only at the highest dose tested (1.5 mg/kg) where only one animal (ethanol group) was dropped for failure to respond on the vehicle injection day (i.e. failure to provide a reliable control response). Perhaps latency to first lick was not increased in the ethanol group in Experiment 2 because the dose range used was slightly lower than Experiment 1. The dose that increased the latency to lick and lever-press in the ethanol group (1.5 mg/kg) in Experiment 1was not used in Experiment 2 (0.25 - 1.0 mg/kg).

Similarly, chronic prazosin treatment increased the latency to first lever-press in the sucrose group only. The lowest dose of prazosin increased the latency to lever-press as compared to vehicle during appetitive testing sessions when no prazosin was on-board and no sucrose could be obtained (three days after consecutive prazosin treatment). The

present findings indicate that prazosin, when administered chronically, decreases the motivation to initiate sucrose-seeking behaviors. Again, this is inconsistent with findings from Experiment 1 in which latency to lever-press was increased by the highest dose (1.5 mg/kg) in the ethanol group only. Perhaps, this discrepancy could, again, be attributed to the lower dose range utilized in Experiment 2. Lower doses (0.25 mg/kg) than used in Experiment 1 increased latency to lever-press in the sucrose group in Experiment 2. Also, perhaps, subjects drinking ethanol need a higher dose than those utilized in Experiment 2 in order to decrease their motivation to initiate and engage in ethanol consumption such as the 1.5 mg/kg dose used in Experiment 1. Again, it is unlikely that the increased latency to lick and lever-press were due to prazosin-induced motor impairment or malaise because latencies were not increased in the ethanol-reinforced group.

Overall, the results of Experiment 1 and Experiment 2 are consistent with and extend previous literature on the effects of prazosin, at similar doses, route and timing of administration, on ethanol seeking and self-administration. Experiment 1 is consistent with that of Walker et al. (2008) where prazosin blocked ethanol dependence-induced operant responding in Wistar rats and that of Rasmussen et al. (2009) where prazosin decreased voluntary ethanol consumption in the P rat. It also extends findings that alcohol-dependent men reported fewer drinks per week and fewer drinking days per week while being treated with prazosin (Simpson et al., 2009). Experiment 2 is consistent with Experiment 1 in that seeking was attenuated at the lowest dose and highest doses of prazosin. Experiment 1 and Experiment 2 are consistent with each other in that neither study found prazosin's effects to be exclusive to one reinforcer. It is problematic that

prazosin was not reinforcer-specific and could be an indication that prazosin works by devaluing oral reinforcers overall. However, the present findings are limited to male, P rats, and other rat strains as well as females should be examined in order to assess prazosin treatment effects on different rodent populations.

The current results suggest that the noradrenergic system plays a key role in maintaining ethanol- and sucrose-seeking and drinking in animals selectively bred for high alcohol drinking. Previous findings suggest that noradrenergic signaling may modulate ethanol self-administration in that depletion of brain levels of norepinephrine results in an attenuation of ethanol self-administration in Sprague-Dawley rats (Davis et al., 1978) which is consistent with the central actions of prazosin. Similarly, lesions of noradrenergic neurons were found to decrease ethanol intake (Brown and Amit, 1977). Important to the present studies, excessive noradrenergic signaling is characteristic of patients with PTSD and comorbid alcohol abuse (Raskind et al., 2003). These patients show signs of heightened arousal (Saladin et al., 1995) or hyperexcitability, which is also seen in P rats exhibiting a greater startle response relative to their non-preferring counterparts (Chester et al., 2004). This suggests that rodents genetically bred for alcohol-preference may display excessive noradrenergic signaling and prazosin, an α_1-adrenergic antagonist, can suppress this noradrenergic activation thereby decreasing the reinforcing properties of ethanol. However, prazosin also decreases the reinforcing properties of nicotine and heroin, and decreases yohimbine-induced reinstatement of food seeking (Bruijnzeel et al., 2010; Forget et al., 2010; Greenwell et al., 2009; Le et al., 2011) and may work on attenuating seeking and intake behaviors in general.

Future Directions and Conclusions

In the future, these findings could be extended to different rat strains to see if prazosin has an effect in other rodents genetically bred for alcohol drinking such as the HADs as well as rat strains that are not bred for alcohol preference such as the Long Evans. This could lead to treatment approaches in alcoholics seeking treatment and in alcohol abusers that seek to control their intake. It would also be beneficial to look at dosing strategies. Higher doses of prazosin appear to decrease the intake of oral reinforcers as evidenced by Experiment 1 and Experiment 2, whereas lower doses decreased reinforcer-seeking. Further, enriched environments have been shown to decrease ethanol drinking, preference, and motivation to obtain ethanol in P rats compared to those in an impoverished environment (Deehan et al., 2011). Since prazosin has been observed to decrease alcohol drinking in patients with PTSD as well as to alleviate their distressing nightmares, it would be interesting to look at ethanol-seeking and intake in rats treated with prazosin when the environment of the subject is manipulated i.e. enriched, normal, or impoverished (stressful context).

Additionally, prazosin was found to decrease elevations in brain reward thresholds associated with nicotine withdrawal while the α_2-adrenergic receptor agonist clonidine and the β-adrenergic receptor antagonist propranolol attenuated somatic signs associated with nicotine withdrawal (Bruijnzeel et al., 2010). Future studies should look at the combination treatment of prazosin and clonidine or prazosin and propranolol on their combined efficacy in attenuating ethanol-seeking and consumption as well as the withdrawal symptoms of alcohol abuse. Similarly, a combined prazosin and clozapine, an atypical antipsychotic, treatment could be effective in reducing ethanol-seeking and

consumption while not devaluing other oral reinforcers, such as sucrose. Clozapine has been found to increase reward evaluation of sucrose (Galistu et al., 2011), and attenuate ethanol withdrawal symptoms in Wistar rats (Kayir and Uzbay, 2008). Further, it has been shown that chronic treatment with clozapine in rodents decreases ethanol drinking in the continuous access paradigm (Chau et al., 2010; Green et al., 2004). Future studies could also examine the effects of injecting prazosin directly into the brain. $Alpha_1$-adrenergic receptors are located ubiquitously throughout the brain (Pieribone et al., 1994) thus central administration of prazosin may further extend the effects on ethanol-seeking and intake observed when the drug is administered peripherally. Finally, doxazosin, an α_1-adrenergic receptor antagonist, is used to treat hypertension, like prazosin, but has a longer half life (11 hours) (Elliott et al., 1982). Future studies could examine the effects of doxazosin on ethanol-seeking and consumption in the same operant paradigm used in the present studies.

In summary, acute prazosin administration was able to decrease reinforcer-seeking and intake and decrease the motivation to initiate ethanol-seeking. Chronic treatment of prazosin was able to decrease reinforcer-seeking. However, sucrose intake was attenuated by chronic prazosin treatment and, perhaps, the motivation to initiate sucrose-seeking was decreased. The operant model utilized, one that separately assesses seeking and drinking behaviors, revealed that prazosin, an α_1-adrenergic receptor antagonist, may not be reinforcer specific. If prazosin is given as treatment for alcohol use disorders then patients should be advised that prazosin may affect their seeking and intake of other reinforcers.

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TABLES

TABLES

Table 1. Daily within session descriptions for Experiment 2. An ethanol-reinforced and a sucrose-reinforced group underwent the following conditions for the indicated number of days (more detailed descriptions are given in 'Materials and Methods' of Experiment 2.

		M	T	W	Th	F	M	T	W	Th	F	M	T	W	Th	F
Training Week 6		RR20	RR20	RR20	RR20	RR20										
Drug Cycle 1 (14 days)		EXT (baseline)	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1
DC1 EXT & ↑RR		EXT (DC1)	RR5	RR10	RR15	RR20										
Vehicle Cycle 1 (9 days)		RR20	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1					
VC1 EXT & ↑RR		EXT (VC1)	RR5	RR10	RR15	RR20										
Drug Cycle 2 (14 days)		RR20	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1
DC2 EXT & ↑RR		EXT (DC2)	RR5	RR10	RR15	RR20										
Vehicle Cycle 2 (9 days)		RR20	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1					
VC2 EXT & ↑RR		EXT (VC2)	RR5	RR10	RR15	RR20										
Drug Cycle 3 (14 days)		RR20	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1	RR1
DC3 EXT		EXT (DC3)	RR5	RR1 (Bloods)												

FIGURES

FIGURES

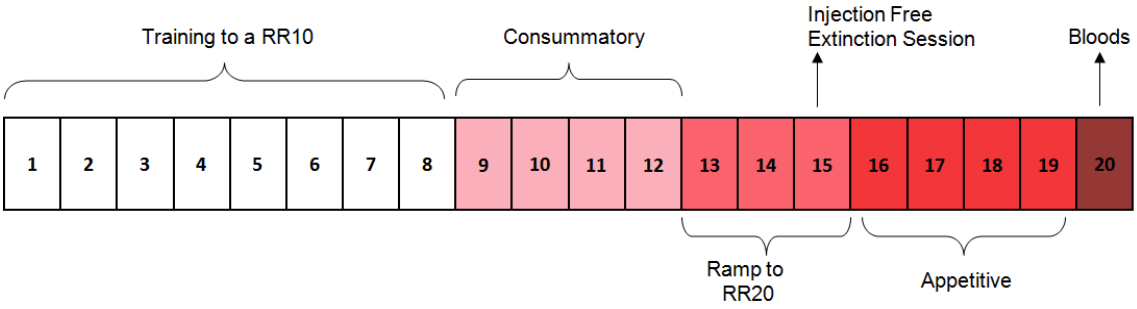


Figure 1. Timeline for acute prazosin administration.

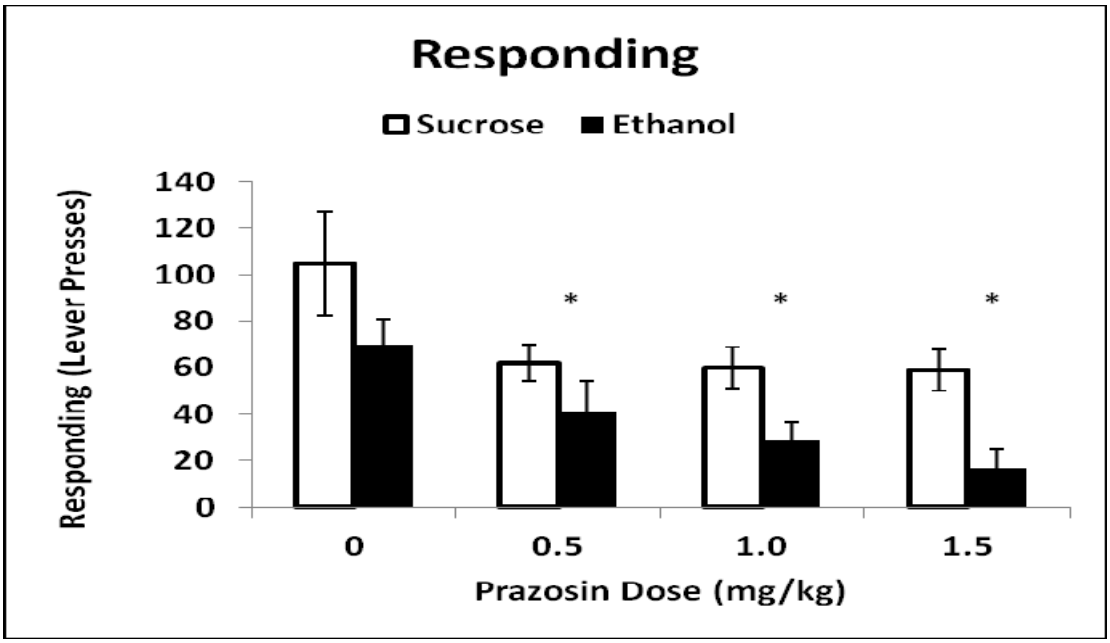


Figure 2. Reinforcer seeking. Mean (± SEM) reinforcer-seeking (bar presses in 20 min) on the appetitive testing days for the sucrose-reinforced (white) and ethanol-reinforced (black) groups over one of four doses of prazosin (0, 0.5, 1.0, 1.5 mg/kg) treatments (-30 min). There was a main effect of Treatment [$F(3,39)=8.3$, $p \leq 0.001$] with post hoc analysis showing that all prazosin doses differed from control (*).

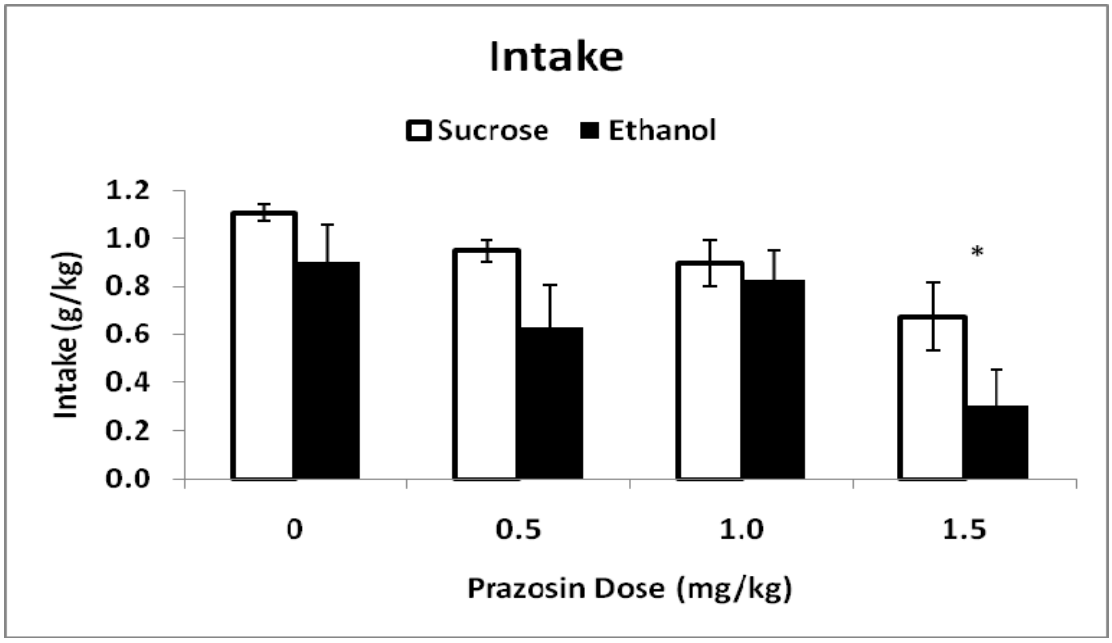


Figure 3. Reinforcer intake. Mean (± SEM) total reinforcer intake (g/kg over 20 min) on consummatory testing days for the sucrose-reinforced (white) and ethanol-reinforced (black) groups over one of four doses of prazosin (0, 0.5, 1.0, 1.5 mg/kg) treatments (-30 min). There was a main effect of Treatment [$F(3,36)=7.9$, $p \leq 0.001$] with post hoc analysis showing that only the high dose of prazosin differed from control (*).

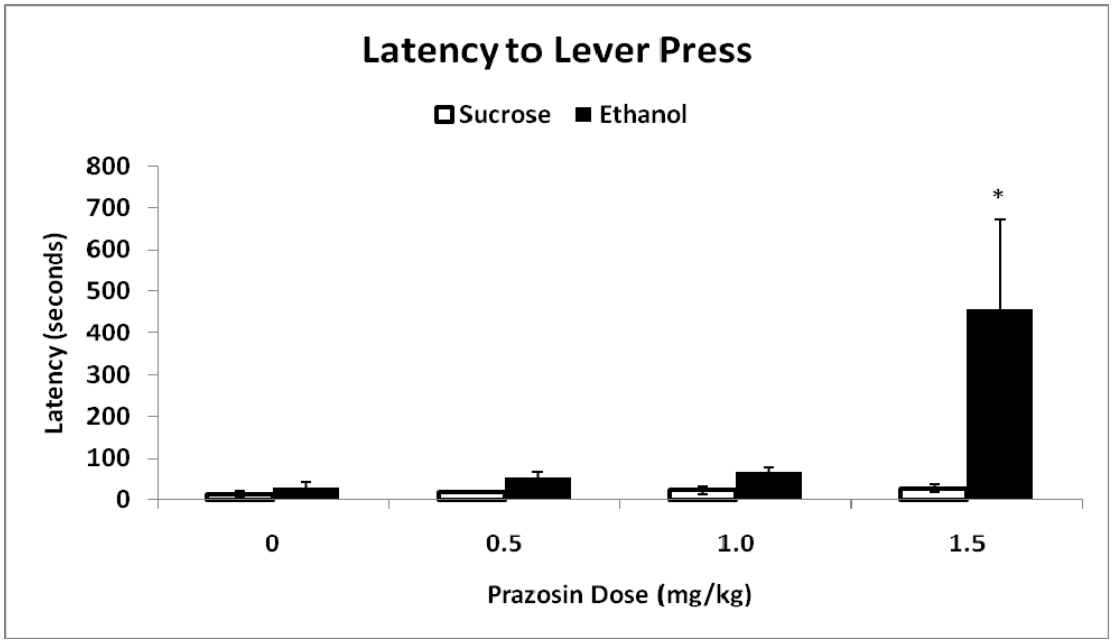


Figure 4. Latency to lever-press. Mean (± SEM) latency (sec) to first lever-press on appetitive testing days. Sucrose-reinforced (white) and ethanol-reinforced (black) groups over control and prazosin treatments (-30 min) are shown. RMANOVA indicated an interaction effect such that in the ethanol group only, the high dose of prazosin increased the time to first lever-press [$F(3,39)=4.6$, $p \leq 0.01$]. Because of the high variability of these responses and the absence of a response in some cases (assigned 1200 sec), a Mann-Whitney Rank Sum test at the high dose confirmed these findings (*) (Median values-Appetitive: sucrose 24.4, ethanol 182.6).

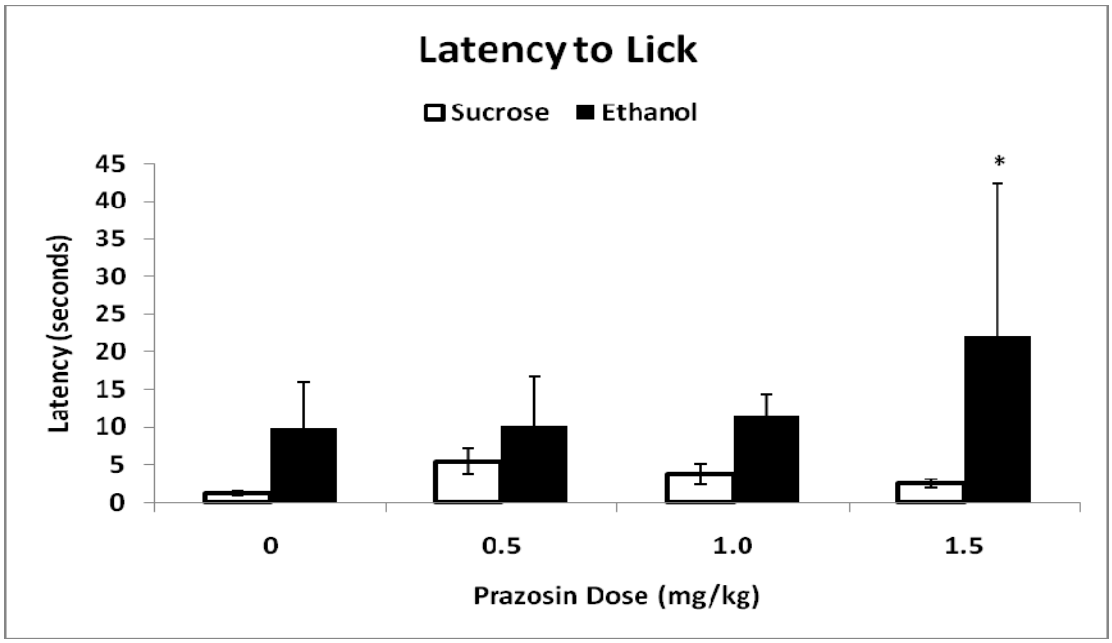


Figure 5. Latency to lick. Mean (± SEM) latency (sec) to first lick on consummatory testing days. Sucrose-reinforced (white) and ethanol-reinforced (black) groups over control and prazosin treatments (-30 min) are shown. RMANOVA indicated an interaction effect such that in the ethanol group only, the high dose of prazosin increased the time to first lick [$F(3,39)=4.6$, $p \leq 0.01$]. Because of the high variability of these responses and the absence of a response in some cases (assigned 1200 sec), a Mann-Whitney Rank Sum test at the high dose confirmed these findings (*) (Median values-Consummatory: sucrose 2.9, ethanol 656.8).

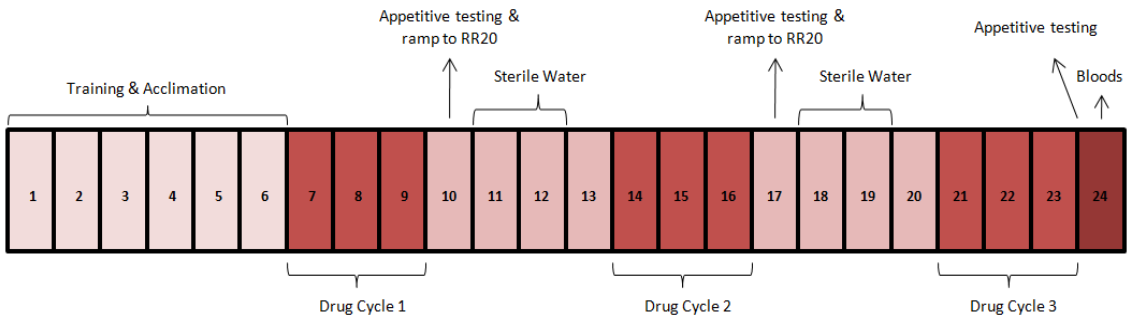
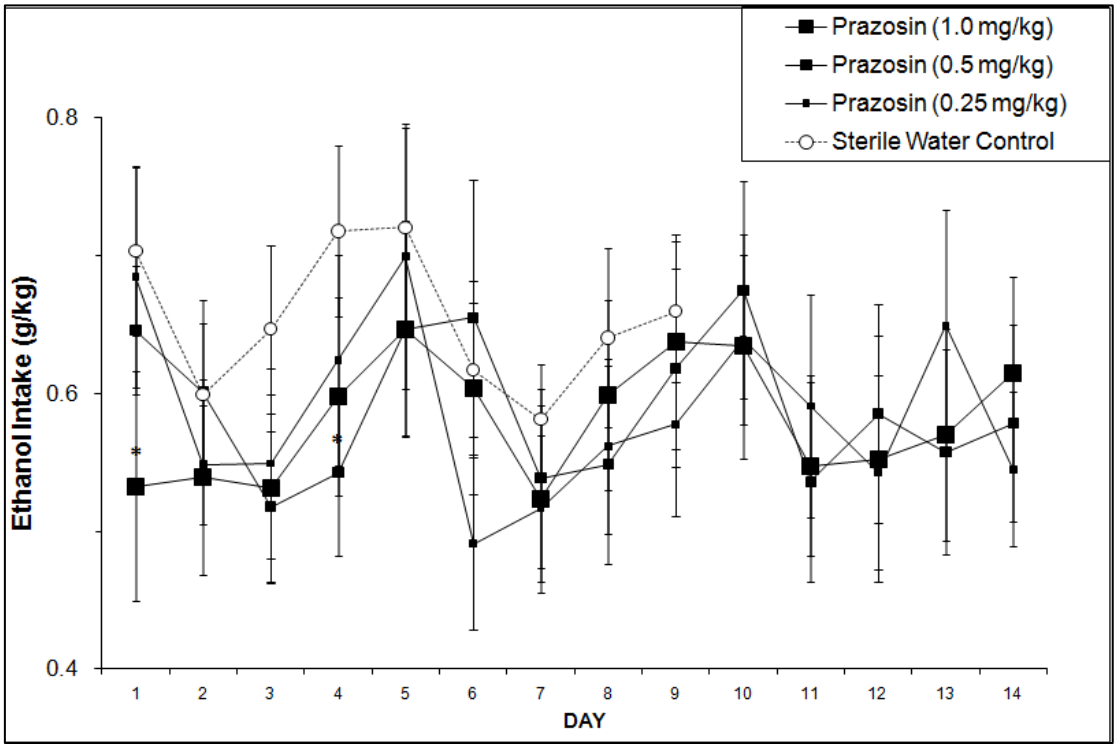


Figure 6. Timeline for chronic prazosin administration.



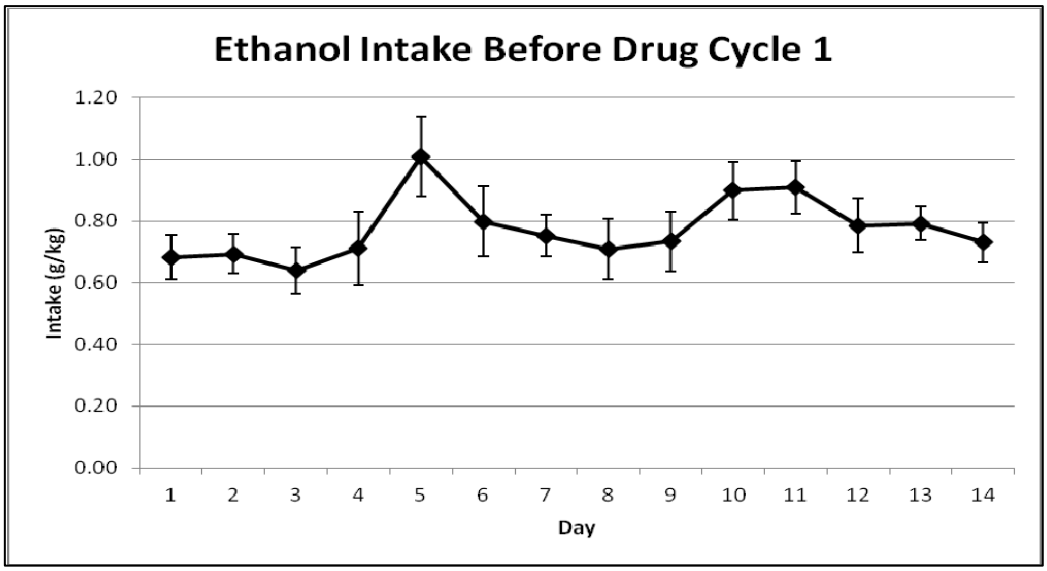


Figure 7. Chronic ethanol intake. *Top*: In the ethanol-reinforced group (n=12), mean (± SEM) ethanol intake (g/kg) over days following either vehicle or prazosin treatment. *Bottom*: Mean ethanol intake before the start of drug manipulation.

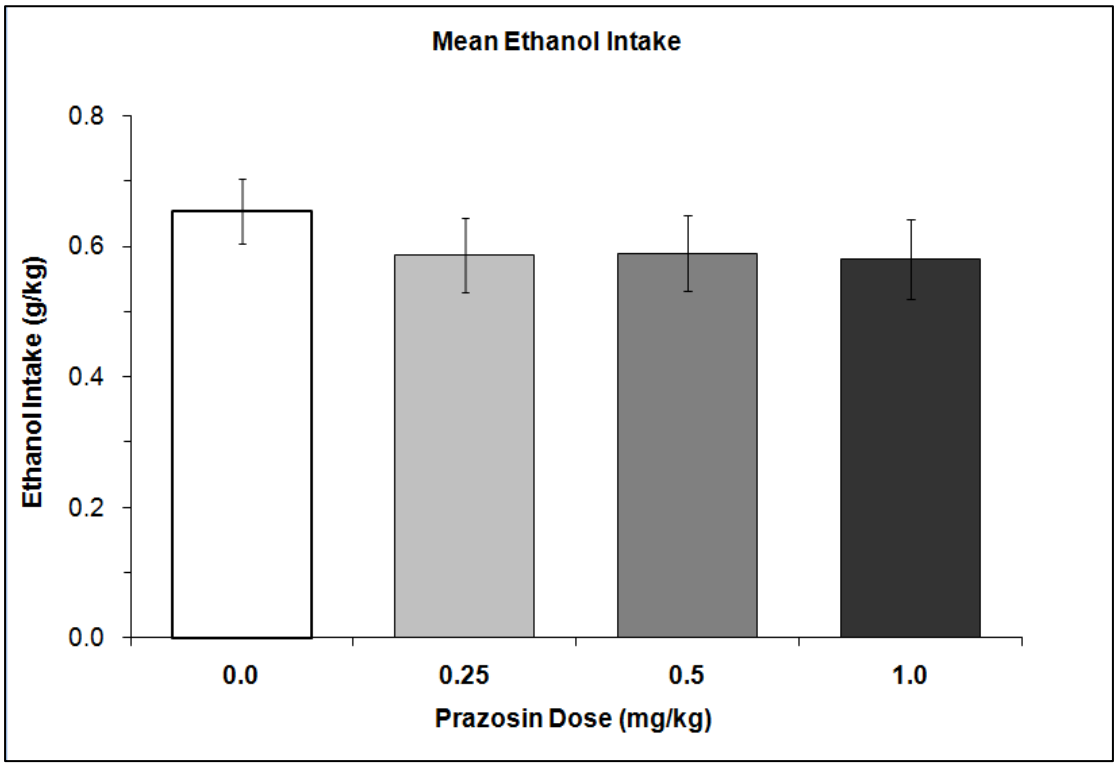


Figure 8. Chronic mean ethanol intake. Total mean (± SEM) ethanol intake collapsed over all days.

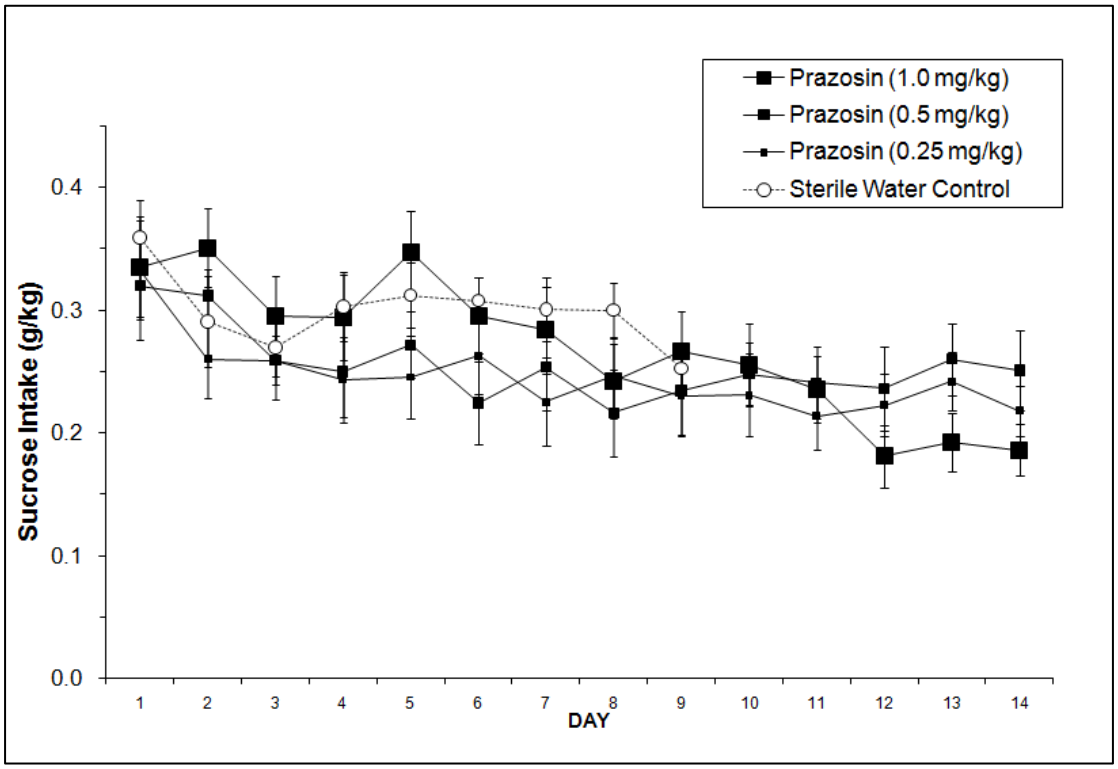


Figure 9. Chronic sucrose intake. In the sucrose-reinforced group (n=11), mean (± SEM) sucrose intake (g/kg) over days following either vehicle or prazosin treatment.

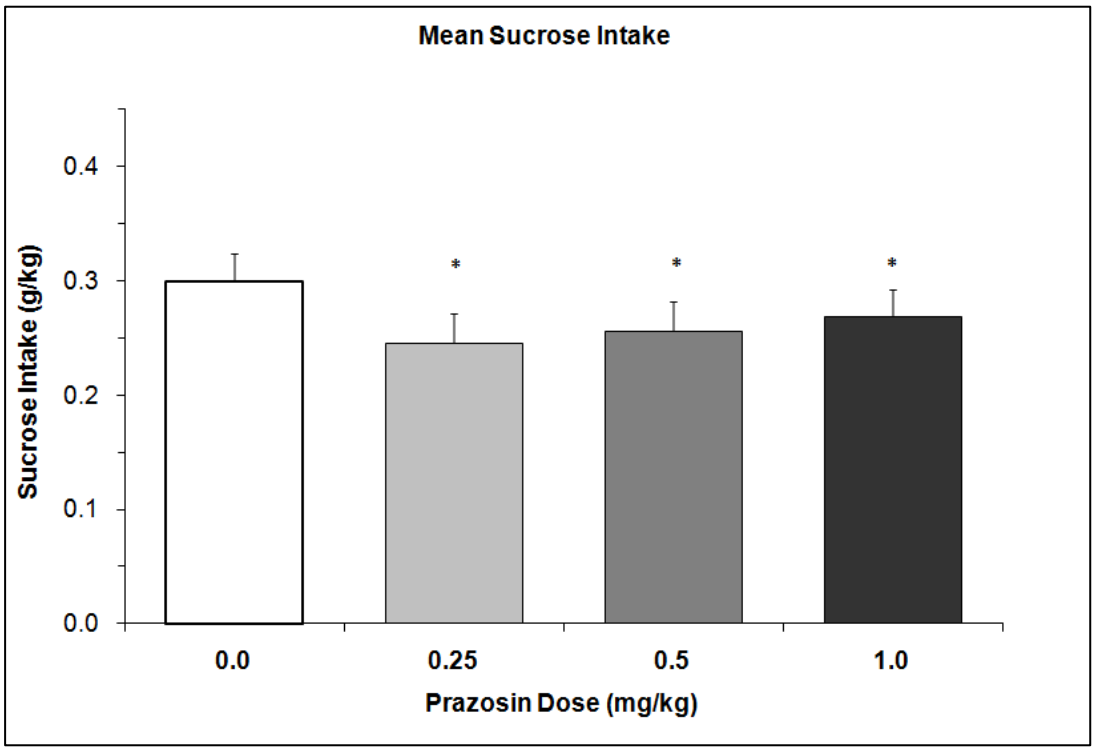


Figure 10. Chronic mean sucrose intake. Total mean (± SEM) sucrose intake collapsed over all days. *Asterisk* indicates a main effect of dose, with all doses of prazosin differing from vehicle.

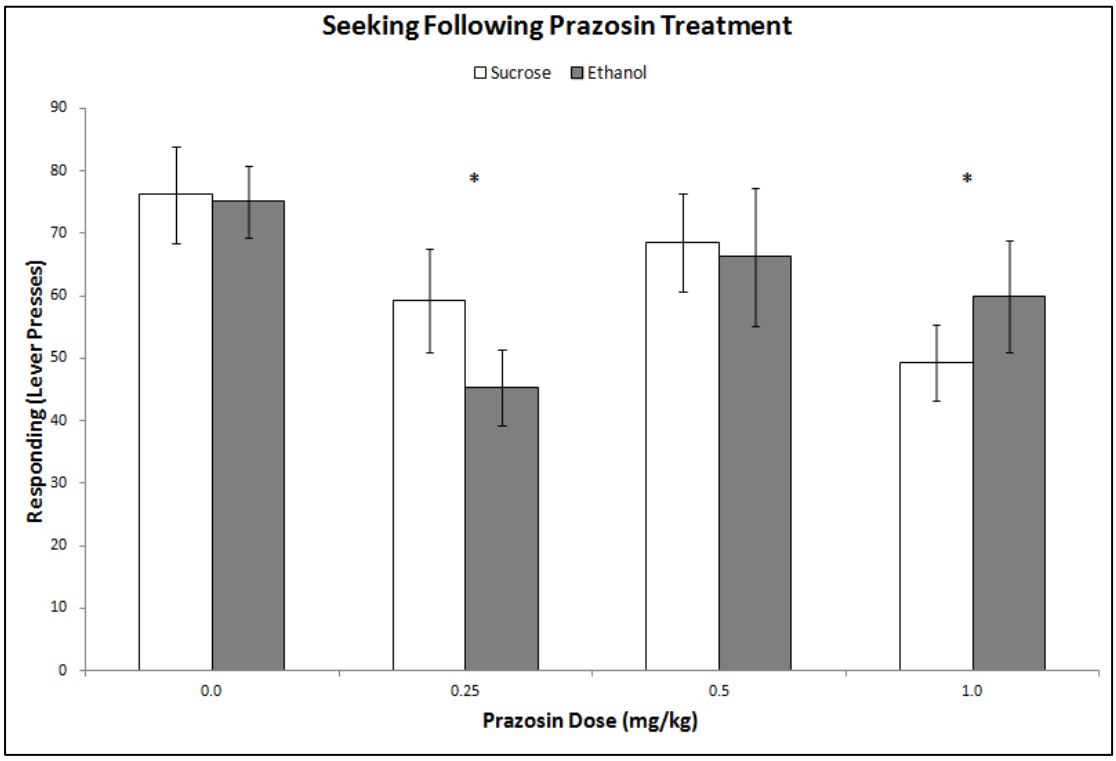


Figure 11. Seeking following prazosin treatment. Mean (± SEM) lever-press responses during single, non-reinforced sessions in the ethanol and sucrose groups in prazosin-treated rats. *Asterisk* indicates a main effect of dose, differing from vehicle.

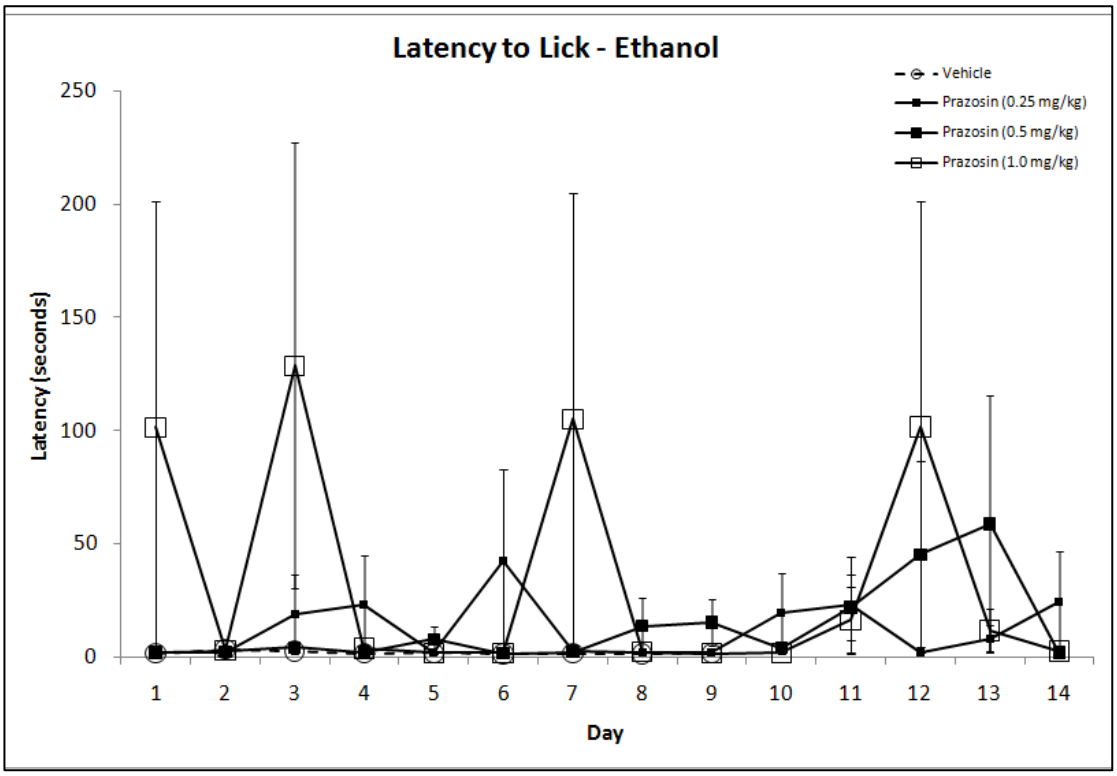


Figure 12. Latency to lick for ethanol in Experiment 2. In the ethanol-reinforced group (n=12), mean (± SEM) latency (sec) to first lick in consummatory sessions.

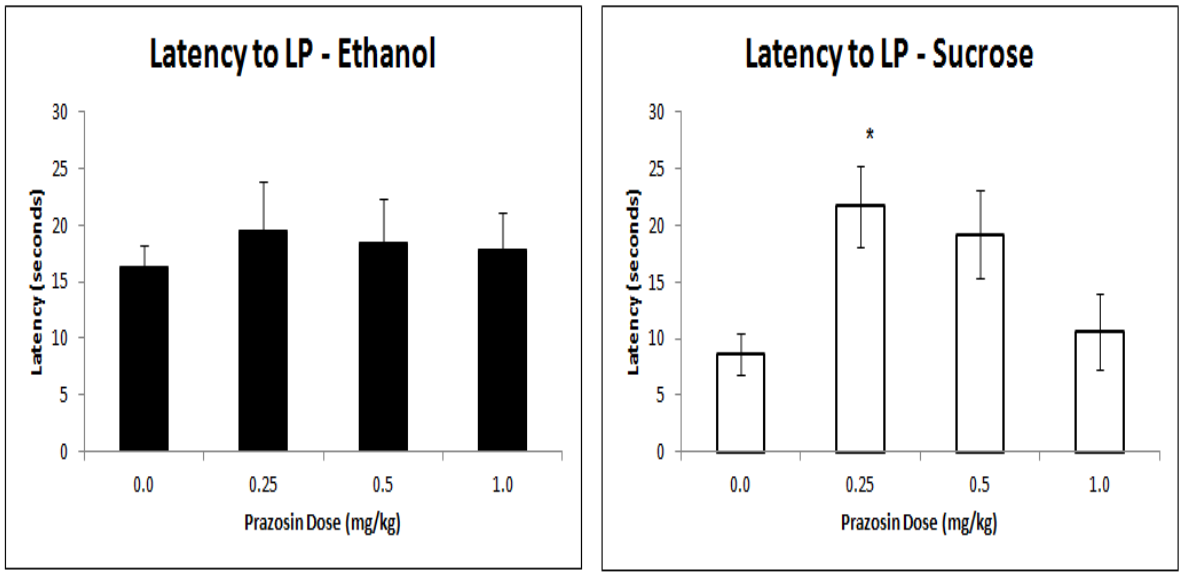


Figure 13. Latency to lever-press in Experiment 2. In ethanol- and sucrose-reinforced groups (n=12/n=11, respectively), mean (± SEM) latency (sec) to lever-press on appetitive testing days. *Asterisk* indicates a main effect of dose, with the lowest dose of prazosin increasing latency to lever-press in appetitive sessions.

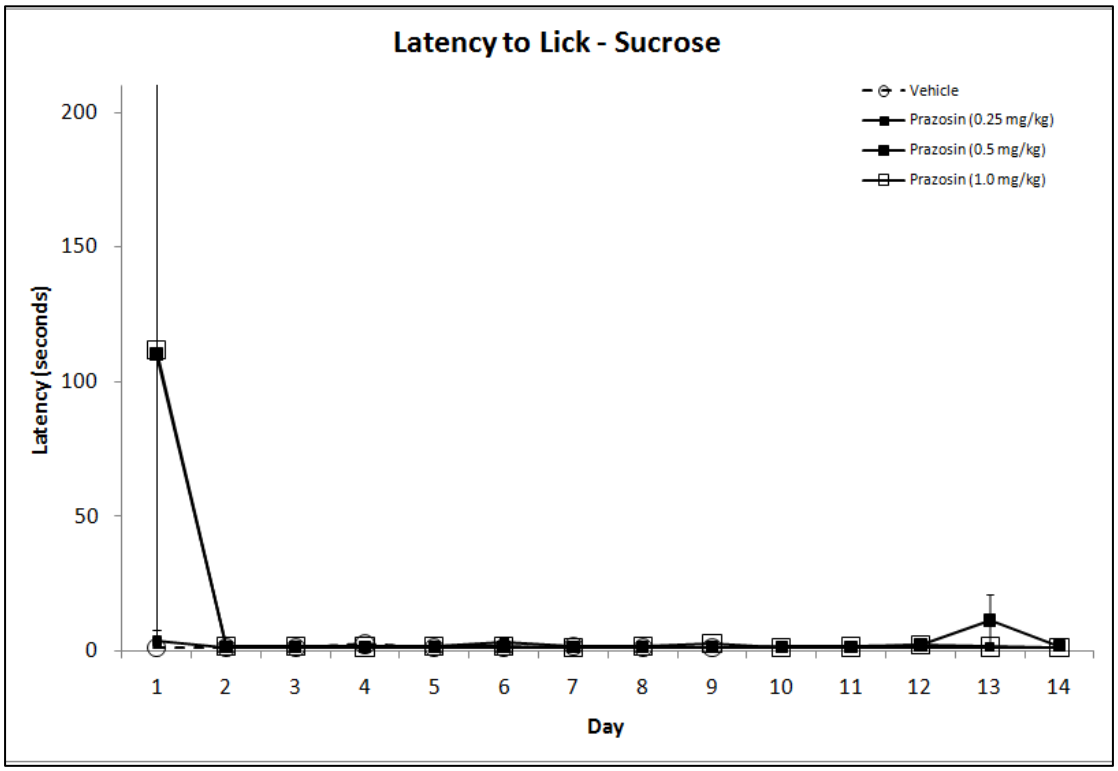


Figure 14. Latency to lick for sucrose in Experiment 2. In the sucrose-reinforced group (n=11), mean (± SEM) latency (sec) to first lick in consummatory sessions.