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CB1 receptors regulate alcohol-seeking behavior and alcohol self-administration of alcohol-preferring (P) rats

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ABSTRACT

Rationale: The endogenous cannabinoid (CB) system mediates a number of behaviors associated with drug-seeking and drug self-administration. In this study the effects of CB1 receptor manipulations on operant ethanol (EtOH) responding during EtOH-seeking, EtOH-relapse as well as on-going EtOH self-administration were determined.
Methods: Alcohol-preferring (P) rats were trained in 2-lever operant chambers to self-administer 15% EtOH (v/v) and water on a concurrent fixed-ratio 5–fixed-ratio 1 (FR5–FR1) schedule of reinforcement in daily 1-h sessions. After 10 weeks, rats underwent 7 extinction sessions, followed by 2 weeks in their home cages without access to EtOH or operant chambers. Rats were then returned to the operant chambers for testing of EtOH-seeking behavior (no EtOH present) for 4 sessions. After a week in their home cages following the EtOH-seeking test, rats were returned to the operant chambers with access to EtOH and water (relapse). Rats were then maintained in the operant chambers for daily 1-h sessions with access to 15% EtOH and water for several weeks.
Results: The CB1 receptor antagonist (SR141716A), at doses of 1 and 2 mg/kg, i.p. reduced EtOH-seeking and transiently reduced EtOH self-administration during relapse and maintenance. Conversely, treatment with the CB1 receptor agonist CP 55, 940, at doses of 1 and 10 μg/kg i.p., increased EtOH-seeking and EtOH self-administration during relapse.
Conclusions: The results of this study demonstrate that activation of CB1 receptors are involved in regulating EtOH-seeking as well as the reinforcing effects of EtOH under relapse and on-going self-administration conditions.

1. Introduction

Epidemiological data indicates that 58% of subjects who abuse alcohol or are alcohol dependent also abuse marijuana (Martin et al., 1996). Alcohol and Δ^9 tetrahydrocannabinol (THC), the main psychoactive constituent of marijuana, activate similar reward pathways (Gessa et al., 1998). There also exists cross-tolerance between alcohol and THC suggestive of the involvement of possible common pathway(s) (Basavarajappa and Hungund, 2002). One of the systems that are activated by both alcohol and CBs/THC is the endogenous cannabiniod (CB) system. The CB system plays an important role in homeostatic control of emotions and regulation of motivated behavior (Navarro et al., 2001), and the pharmacological and behavioral effects of EtOH (Hungund & Basavarajappa, 2000; Gonzalez et al., 2002; Hungund et al., 2002; Colombo et al., 2005). For instance, chronic (Ortiz et al., 2004), as well as intermittent EtOH (Rimondini et al., 2002) results in alterations of the CB1receptor: i.e., gene expression, receptor binding (Basavarajappa et al., 1998), and function (Basavarajappa and Hungund, 1999).

CB1 agents manipulate different aspects of alcohol related behaviors, such as EtOH modulate CB system in different animal models and experimental designs. Microinjections of the CB1 antagonist, SR141716 (SR) into the nucleus accumbens (NAcc) and ventral tegmental area (VTA) attenuates EtOH-responding in Alko Alcohol (AA) rats (Malinen and Hyytiä, 2008). Systemic administration of SR, suppresses acquisition and maintenance as well as relapse drinking in selectively bred Sardinian EtOH-preferring (sP) rats (Colombo et al., 1998; Serra et al., 2001, 2002). Further, SR treatment of EtOH-consuming C57BL/6 mice (Arnone et al., 1997) and chronically EtOH-exposed Wistar rats (Lallemand et al., 2001) also reduces drinking. Similar results were reported in unselected Long Evans and Wistar rats (Freedland et al., 2001; Hungund et al., 2002; Cippitelli et al., 2005; Economidou et al., 2006). Microinjections of CB1 receptor antagonists into the posterior, but not anterior, VTA significantly reduced EtOH consumption in Wistar rats (Alvarez-Jaimes et al., 2009a). Chronic EtOH consumption (liquid diet) potentiates the increase in endocannabinoid levels in the nucleus accumbens produced by challenge administration of EtOH (Alvarez-Jaimes et al., 2009b). CB1 receptor knockout mice that lack CB1

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receptors display significantly lower levels of EtOH-preference and consumption compared to the wild-type mice (Hungund et al., 2003; Poncelet et al., 2003; Wang et al., 2003; Naassila et al., 2004). Conversely, AA rats have reduced fatty acid amidohydrolase (FAAH) function in the prefrontal cortex, the main endocannabinoid-degrading enzyme, and a compensatory reduction in CB1 receptor sensitivity (Hansson et al., 2007). Inhibiting FAAH activity in the prefrontal cortex increases EtOH consumption in Wistar rats (Hansson et al., 2007). Therefore, preclinical data indicate the possible genetic linkage with an altered endocannabinoid system and predisposition to consume EtOH.

Administration of the CB1 receptor agonist, CP 55, 940 (CP), promotes EtOH-intake (Gallate et al., 1999; Colombo et al., 2002); chronic exposure to a CB1 agonist potentiates operant self-administration of EtOH and relapse drinking (Lopez-Moreno et al., 2005). Further, CP stimulates the activity of mesolimbic dopaminergic (DA) neurons and enhances brain stimulation-induced reward (Gardner and Vorel, 1998). CB1 receptor knockout mice lack EtOH-induced DA release in the NAcc (Hungund et al., 2003). Taken together, the data from SR, CP as well as knockout mice studies suggest a role for CB system in EtOH-related behaviors.

Animal models of drug-seeking have been developed which attempt to parallel drug craving that occurs in humans. It has been hypothesized that drug craving is a critical precipitating factor to relapse (c.f., O'Brien et al., 1998). The reinstatement of responding model can be considered a drug-elicited craving model since priming injections of drugs of abuse are used to elicit drug-seeking (de Wit and Stewart, 1981, 1983; Shaham et al., 1997). Exposure to a physical stressor (e.g., footshock) or creating a physical state of extreme distress (yohimbine) can elicit behaviors which where previously associated with drug self-administration (stress-induced drug-seeking; Le et al., 1998; Richards et al., 2008). Cue-elicited drug-seeking is readily observed following presentation of discriminative stimuli previously paired with the availability of a drug (Katner et al., 1999; Katner and Weiss, 1999). Contextual drug-seeking models are unique in that the drug self-administration environment is used to elicit drug-seeking. The two models which examine contextual drug-seeking are the renewal and spontaneous recovery paradigms. The renewal model can be described as the recovery of an extinguished behavior that is dependent upon a change in context (Bouton and Bolles, 1979) Briefly, subject are trained to self-administer a drug in one environment (A), the behaviors are extinguished in a different context (B), and responding is returned when the animal is returned to the original context (A). The renewal model has been recently used to study drug-seeking behaviors (Hamlin et al., 2007, 2008).

Spontaneous recovery is defined as a recovery of responding, in the absence of the previously trained reward, which is observed following a period of rest after extinction (Domjan and Burkhard, 1982; Macintosh, 1977). In the alcohol field, the term spontaneous recovery has been used to define the phenomenon of human alcoholics terminating alcohol consumption without any outside intervention. Therefore, to avoid confusion we have used the term Pavlovian Spontaneous Recovery (PSR). Conceptually, PSR is a unique phenomenon in that it is time dependent, and the behavior appears to be dependent on the re-exposure of the organism to all the cues in the behavioral environment previously associated with the reinforcer. The expression of a PSR is directly correlated to reward saliency (Macintosh, 1977; Robbins, 1990), contextual cues associated with first-learned signals, and the amount of first- and second-learned associations (Brooks, 2000). In general, the PSR phenomenon has been asserted to be the result of an intrinsic shift away from the recent extinction (second-) learning to the initial reinforced learning responses, which reflects an intrinsic motivation to obtain the previously administered reward (Bouton, 2002, 2004; Rescorla, 2001). Therefore, the PSR model may represent a unique paradigm to study craving-like behaviors.

P rats readily express a PSR for EtOH (Rodd-Henricks et al., 2002a,b; Dhaher et al., 2010). Peri-adolescent EtOH drinking potentiates the expression of an EtOH PSR when tested during adulthood (Rodd-Henricks et al., 2002a). Additionally, the expression of an EtOH PSR can be enhanced by exposure to EtOH odor or EtOH priming (Rodd-Henricks et al., 2002a,b). Thus, responding in the PSR test has a high degree of face validity for an animal model of EtOH-seeking behavior (c.f., Rodd et al., 2004).

The alcohol deprivation effect (ADE) is defined as a temporary increase in the voluntary intake of EtOH when EtOH is reinstated following a period of alcohol deprivation (Sinclair and Senter, 1967, 1968). The ADE has been used to examine the efficacy of pharmacological agents to reduce or prevent EtOH-relapse (Heyser et al., 1998; Kornet et al., 1990; Spanagel and Zieglgansberger, 1997). Under operant or free-choice EtOH drinking conditions, P rats exhibit a robust ADE (Rodd et al., 2003).

Pharmacological studies suggest that different mechanisms may underlie relapse drinking and on-going EtOH drinking. For example, serotonin-3 receptor antagonists were less effective in reducing 24-h EtOH intakes of P rats during relapse conditions than in reducing EtOH intakes under on-going maintenance conditions (Rodd-Henricks et al., 2000). Moreover, the operant paradigm used in the current study has been used to examine the involvement of metabotropic glutamate 2/3 receptors (mGluR2/3) in EtOH-seeking and relapse behaviors (Rodd et al., 2006). The results of this study indicated that the mGluR2/3 agonist LY404039 effectively reduced both EtOH-seeking and EtOH-relapse responding but had little effect on on-going EtOH-responding.

The goal of the present study was to assess the effects of CB1 receptor antagonist (SR141716A) and agonist (CP 55, 940) on operant EtOH-responding of P rats under EtOH-seeking, relapse, and on-going self-administration conditions. The overall hypothesis to be tested is that CB1 receptors are involved in regulating of EtOH-seeking, relapse and on-going drinking. The CB1 antagonist would reduce EtOH-seeking, relapse and on-going drinking whereas the CB1agonist would enhance these behaviors.

2. Materials and methods

2.1. Animals

Adult female P rats weighing 250–325 g at the start of the experiment were used. Rats were maintained on a 12-h reversed light–dark cycle (lights off at 0900 h). Food and water were available *ad libitum* throughout the experiment, except during operant testing. The animals used in these experiments were maintained in facilities fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC). All research protocols were approved by the institutional animal care and use committee and are in accordance with the guidelines of the Institutional Care and Use Committee of the National Institute on Drug Abuse, National Institutes of Health, and the *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, 1996).

2.2.1. Operant apparatus

Experiments were conducted in standard two-lever operant chambers (Coulbourn Instruments, Whitehall, PA) contained within ventilated, sound-attenuated enclosures. Two operant levers were located on the same wall and were placed 15 cm above a grid floor and 13 cm apart. Directly beneath each lever was a trough through which a dipper cup (0.1 ml) was raised to deliver response-contingent fluid. Upon a reinforced response, a small light cue was illuminated in the drinking trough during the 4-s dipper cup access. A computer controlled all operant chamber functions and recorded lever responses and dipper presentations. Operant sessions were 60 min in duration and were conducted daily.

2.2.2. Operant training

Without any prior training, exposure to the experimental set-up, or access to EtOH, rats were placed in the operant chambers. Both the EtOH (15% v/v) and water levers were maintained on a fixed-ratio 1 (FR1) schedule of reinforcement for the first 5 weeks. Subsequently, the reinforcement schedule on the EtOH lever was increased to FR3 in weeks 6–7, and to FR5 in weeks 8–10. The FR requirement for EtOH was increased to ensure a high baseline level of responding. The FR1 schedule was maintained for water because increasing the requirement would result in a further reduction in the low level of responding on this lever. Responses on the water lever during the PSR and relapse test sessions served to evaluate non-specific effects of CB1 agents on motor systems. The number of responses on the EtOH and water lever and the number of EtOH and water reinforcements were recorded throughout each session. Levers associated with EtOH or water were counterbalanced among rats but remained constant for each animal.

2.2.3. Extinction

After 4 weeks of responding on the FR5 schedule for EtOH and FR1 for water, rats underwent extinction. The lever previously associated with the delivery of EtOH was maintained on a FR5 schedule, and the lever previously associated with the delivery of water was maintained on an FR1. With the exception of no fluid being presented, the delivery system operated exactly as the preceding EtOH self-administration sessions; rats still received the auditory stimulus of the dipper raising and the visual cue of the small light being illuminated above the dipper trough. Rats were exposed to 7 consecutive extinction sessions which has been previously been shown to extinguish the EtOH response (Rodd–Henricks et al., 2002a).

2.2.4. Pavlovian Spontaneous Recovery (PSR) testing

After extinction training, all rats were maintained in their home cages for 14 days, without access to EtOH or operant chambers. Following this home cage period, rats were returned to the operant chambers without EtOH or water. Lever contingencies and dipper functioning were maintained, as described for operant self-administration and extinction training. Rats were given 4 consecutive PSR test sessions, as previously described (Rodd–Henricks et al., 2002b).

2.2.5. Relapse

Following the PSR phase of the experiment, all rats were maintained in the home cages for 7 days. Rats were then transferred to the operant chambers with both 15% EtOH and water available for the 60-min sessions. Responses on the levers resulted in dipper delivery of EtOH or water (cue lights also present). The EtOH lever was maintained on a FR5 schedule and the water lever on a FR1 schedule.

2.2.6. Maintenance

Following the relapse phase, rats received daily EtOH operant sessions for 3–4 weeks on the concurrent FR5–FR1 schedule of reinforcement. During maintenance sessions both 15% EtOH and water were available.

2.3. Effects of CB1 antagonist SR141716A on PSR, relapse, and maintenance

SR141716A (SR, was provided by NIDA, Washington DC, USA). SR was suspended in 1 ml/kg saline with 0.1% Tween 80. The doses of SR were 0, 0.3, 1 and 2 mg/kg. The typical log dose of 3 mg/kg was not used because of uncertainty of a constant dispersion of SR in the suspension at this concentration. Following extinction training, adult female P rats (n = 36) were randomly assigned to one of four groups, which received a single i.p. injection of 0, 0.3, 1 or 2 mg/kg SR (n = 8–10/group) 15 min prior to the first PSR test session only. Rats were not injected prior to the subsequent 3 PSR test sessions.

These same rats were also used to test the effects of SR during relapse and maintenance responding, using a counterbalanced design (i.e., rats that were administered 1 mg/kg SR during the PSR test sessions were randomly assigned to separate groups that received one of the 4 doses of SR during the relapse phase, which were then counterbalanced for maintenance). The SR compound was administered immediately prior for 4 consecutive sessions to assess the consistency of the effects on relapse drinking and because other compounds have been shown to only delay the expression of an ADE in P rats ($5HT_3$ antagonists; Rodd-Henricks et al., 2000). Eight rats were removed prior to maintenance testing (thus n = 28 for maintenance testing) because of another planned study. For relapse testing, rats received 0, 0.3, 1 or 2 mg/kg SR (n = 8–10/group) 15 min prior to each of the first 4 relapse sessions. Following relapse testing , rats were maintained on the 1-h operant sessions with access to EtOH and water for 25 days; they were then assigned to groups to receive i. p. injection of 0, 0.3, 1 or 2 mg/kg SR (n = 6–8/group) 15 min prior to four consecutive operant sessions.

2.4. Effects CB1 agonist CP 55, 940 on PSR and relapse

CP 55, 940 (CP; Tocris, Bristol, UK) was suspended in 1 ml/kg saline with 0.1% Tween 80. Following extinction training, the effects of CP on lever responses in the PSR test was examined in drug-naïve adult female (n = 23) P rats. P rats received an i.p. injection of 0, 1, 10, or 30 μg/kg CP (n = 5–6/group) 15 min prior to the first PSR test session. Rats were not injected prior to the subsequent 3 PSR test sessions. The same P rats were used to test the effects of CP during relapse responding, using a counterbalanced design (i.e., rats that were administered 30 μg/kg CP during the PSR test sessions were randomly assigned to separate groups that received one of the 4 doses of CP during the relapse testing. For relapse testing, rats received 0, 1, 10, or 30 μg/kg CP (n = 5–6/group) 15 min prior to each of the first 4 reinstatement sessions.

2.5. Statistical analyses

Overall operant responding (60-min) data were analyzed with a mixed factorial ANOVA with a between subject factor of dose and a repeated measure of 'session'. For the PSR experiments, the baseline measure for the factor of 'session' was the average number of responses on the EtOH lever for the last 3 extinction sessions. For the relapse studies, the baseline measure for the factor of 'session' was the average number of responses on the EtOH lever for the 3 sessions immediately prior to extinction. Baseline values for the maintenance experiment were the 3 sessions immediately prior to testing the CB1 compounds. Post-hoc Tukey's b tests were performed to determine individual differences.

3. Results

3.1. Effects of the CB1 antagonist SR141716A on PSR, relapse, and maintenance

3.1.1. PSR

For the PSR test, the number of responses on the lever previously associated with the delivery of EtOH (Fig. 1) indicated a significant effect of 'session' ($F_{4,29} = 8.0$; $p < 0.001$) and 'session' by 'dose' interaction ($F_{12,93} = 2.8$; $p = 0.003$), but no significant effect of dose ($F_{3,32} = 2.8$; $p = 0.055$). Decomposing the significant interaction by examining the effect of 'session' within each 'dose' group indicated that, in all groups, there was significant alteration in responding during the initial PSR session compared to extinction baseline (p values < 0.016). Individual ANOVAs performed on each PSR test session indicated that only during

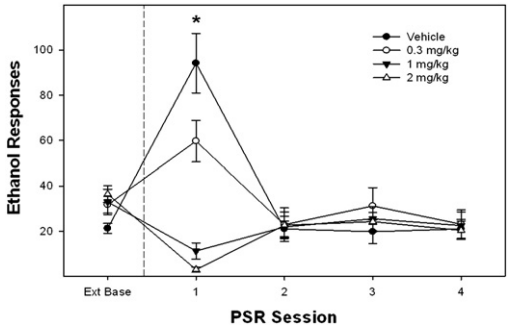

Fig. 1. Depicts the Mean (± SEM) responses/session on the lever previously associated with the delivery of EtOH in female P rats (n = 8–10/group) given 0, 0.3, 1, or 2 mg/kg SR 141716, 15 min prior to 1st PSR session. * Indicates that vehicle and 0.3 mg/kg groups responded significantly more ($p<0.05$) on the EtOH lever during the 1st Pavlovian Spontaneous Recovery (PSR) session compared to baseline levels, and all other groups were different compared to extinction baseline ($F_{3,32} = 22.4$, $p<0.001$).

the first PSR test session was there a significant effect of 'dose' ($F_{3,32} = 22.14$; $p<0.001$). Post-hoc comparisons (Tukey's b) indicated that responses by rats treated with vehicle were significantly higher than responding by all other groups, and responses by rats treated with 0.3 mg/kg SR were significantly higher than P rats treated with 1 or 2 mg/kg SR (which did not differ from each other). Performing t-tests within each group contrasting the average number of responses performed during the last three days of extinction with the number observed during the 1st PSR test session indicated that P rats treated with saline or 0.3 mg/kg SR had higher EtOH lever responses (p values <0.022). In contrast, administration of 1 or 2 mg/kg SR had lower EtOH responses during the 1st PSR test session compared to the level observed during the last three extinction sessions (p values <0.005). Water responding (data not shown) was generally low throughout the experiment, and did not alter from values observed prior to extinction (23.4 ± 2.4 responses/session), during extinction (19.6 ± 3.2 responses/session), or during the 1st PSR test session (16.8 ± 5.8 responses/session). Statistically, there was no effect on water responding; 'session' ($F_{4,29} = 0.2$; $p = 0.89$), dose ($F_{3,32} = 2.4$; $p = 0.13$), 'session' by 'dose' interaction ($F_{12,93} = 1.3$; $p = 0.23$).

3.1.2. Relapse

During relapse testing, injections of the 2 highest doses of the CB1 receptor antagonist reduced EtOH-responding (Fig. 2). There were no significant carry-over effects of treatment with SR during PSR testing (all p values >0.23). Therefore, PSR doses were not included as factors in the relapse statistical analysis. The overall analysis indicated a significant effect of 'session' ($F_{8,17} = 9.5$; $p<0.001$), 'dose' ($F_{3,24} = 3.15$; $p = 0.044$), and a 'session' by 'dose' interaction ($F_{24,57} = 2.8$; $p = 0.001$). There was a significant effect of 'dose' during the 1st and 4th relapse session ($F_{3,24}$ values >5.9; p values <0.004). During the 1st through 4th relapse session, post-hoc comparisons indicated that P rats treated with vehicle and 0.3 mg/kg SR were significantly higher than P rats treated with 1 or 2 mg/kg SR. In P rats treated with 1 mg/kg SR, responding for EtOH was reduced for the 1st and 2nd relapse sessions compared to baseline (p values <0.033). In P rats treated with 2 mg/kg SR, responding for EtOH was reduced during the 1st, 2nd, and 3rd relapse session ($p<0.017$). Water responding (data not shown) was not altered between pre-extinction levels and the amount of responding observed during the 1st–7th relapse sessions (average water responses/session 25.7 ± 3.8; all p values >0.36). The expected ADE was not observed in the current experiment. This is the first occasion that an ADE has not been observed in P rats in a number of experiments. The lack of an ADE in the current experiment could be the result of an anomalous finding or an artifact of vehicle treatment (Tween). The amount of EtOH self-administered prior to deprivation would result in an estimated EtOH consumption of 1.1 g/kg for a 280 g rat. The amount of EtOH self-administered during the first relapse session in P rats administered 2 mg/kg SR would be estimated at less than 0.3 g/kg.

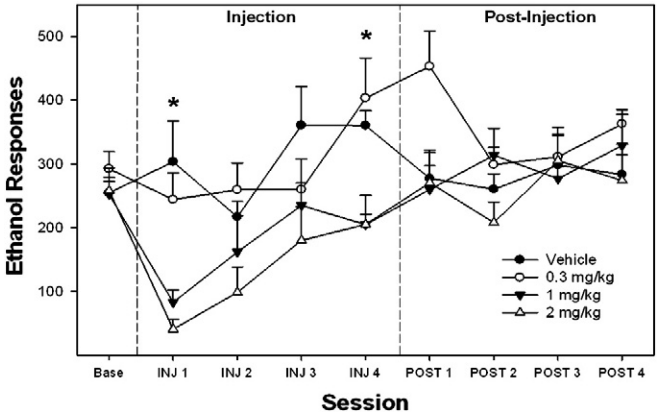

Fig. 2. Depicts the Mean (± SEM) responses/session on the EtOH lever in female P rats (n = 8–10/group) given 0, 0.3, 1, or 2 mg/kg SR141716 15 min prior to 4 operant relapse sessions (alcohol deprivation effect: ADE). * Indicates that vehicle and 0.3 mg/kg groups were significantly different from the 1 and 2 mg/kg groups.

3.1.3. Maintenance

During maintenance testing, injections of the 2 highest doses of CB1 antagonist reduced EtOH-responding (Fig. 3). There were no significant carry-over effects of treatment with on maintenance testing (all p values >0.49). The overall analysis indicated a significant effect of 'session' ($F_{8,25} = 32.5$; $p<0.001$) and a 'session' by 'dose' interaction ($F_{24,81} = 2.5$; $p = 0.002$). There was a significant effect of 'dose' for the 4 sessions that SR was administered prior to each test session ($F_{3,32}$ values >3.6; p values <0.023). During the initial maintenance session, post-hoc comparisons indicated that P rats treated with vehicle and 0.3 mg/kg SR responded significantly more than P rats treated with 1 or 2 mg/kg SR. During the subsequent injection sessions, P rats treated with the 1 and 2 mg/kg doses began to recover toward baseline. Vehicle treated rats had a small decrease in responding compared to baseline responding during the 1st maintenance session, but the decrease was not statistically significant. In P rats treated with 1 or 2 mg/kg SR, responding during the 1st maintenance session was reduced compared to baseline responding (p values <0.001). Responding during injection sessions 2–4 increased significantly compared to the 1st injection session (F values $_{3,21} >6.5$; p values <0.003). Similar to results for relapse responding, responding

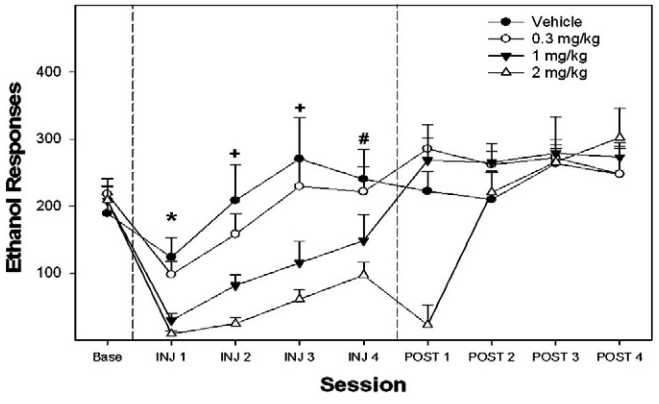

Fig. 3. Depicts the Mean (± SEM) responses/session on the EtOH lever by female P rats (n = 8–10/group) given 0, 0.3, 1, or 2 mg/kg SR141716, 15 min prior to the initial four sessions (maintenance). * Indicates that vehicle and 0.3 mg/kg groups were significantly different from the 1and 2 mg/kg groups. + Indicates that vehicle and 0.3 mg/kg rats were significantly different from the 1 and 2 mg/kg groups, which were different from each other. # Indicates that vehicle and 0.3 mg/kg groups were different from the 2 mg/kg group.

began to recover toward baseline in the 1 and 2 mg/kg group in sessions 2–4. Water responding (data not shown) was consistent during maintenance in all groups (average water responses/session 21.8 ± 5.3; all p values > 0.41). The amount of EtOH self-administered prior to maintenance testing would result in an estimated EtOH consumption of 1.1 g/kg for a 280 g rat. The amount of EtOH self-administered during the first drug test session in P rats administered 2 mg/kg SR would be estimated at less than 0.2 g/kg.

3.2. Effects of CB1 agonist CP 55, 940 (CP) on PSR and relapse

3.2.1. PSR

In PSR test, the CB1 receptor agonist had a biphasic effect on responding on the EtOH lever, with the 2 lowest doses increasing responding and highest dose reducing responding compared to vehicle control values (Fig. 4). Examining the effects of CP on EtOH lever responses by P rats (Fig. 4), indicated a significant effect of 'session' ($F_{4,16} = 5.9$; $p = 0.004$), 'dose' ($F_{3,19} = 4.4$; $p = 0.016$), and 'session' by 'dose' interaction ($F_{12, 54} = 4.1$; $p < 0.001$). Decomposing the interaction term by examining the effect of 'session' within each 'dose' group indicated that, in all groups, except the 30 μg/kg CP group ($p = 0.38$) there was significant increase in responding on the EtOH lever during the initial PSR session compared to extinction baseline (p values < 0.033). Individual ANOVAs performed on each PSR test session indicated that only during the first PSR test session was there a significant effect of 'dose' ($F_{3,19} = 4.8$; $p = 0.012$). Post-hoc comparisons (Tukey's b) indicated that there were significant differences between all groups in female P rats responding on the lever previously associated with the delivery of EtOH. P rats treated with the highest dose of CP (30 μg/kg) responded less than vehicle treated rats, whereas P rats treated with the low doses of CP (1 and 10 μg/kg) responded more than the vehicle group. P rats treated with vehicle or 1 and 10 μg/kg CP prior to the 1st PSR session, responded more on the lever previously associated with the delivery of EtOH than performed during extinction training (p values < 0.0001). The 30 μg/kg CP group 1st PSR session responding was not significantly different from extinction baseline responding. Examining the effects of CP treatment on water lever responding during PSR testing in P rats indicated a significant effect of 'session' ($F_{4,16} = 3.1$; $p = 0.046$), but no effect of 'dose' or 'dose' × 'session' interaction. The significant effect of session was the result of a small increase in water responding during the 3rd and 4th (22 ± 3) PSR test session compared to extinction responding (16 ± 2), whereas no effect on water lever responding was observed during PSR sessions 1 and 2.

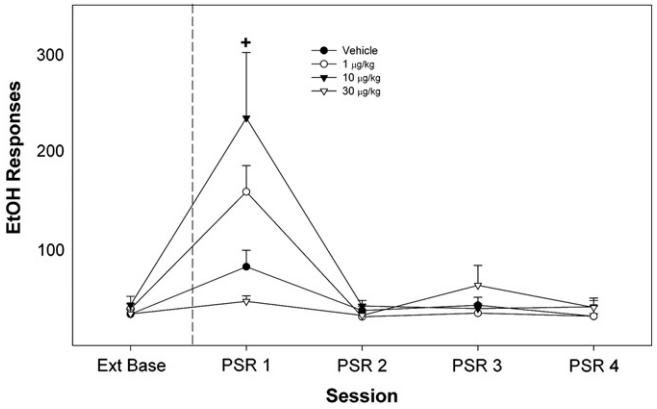

Fig. 4. Depicts the Mean (± SEM) responses/session on the lever previously associated with the delivery of EtOH in female P rats (n = 5–6 group) given 0, 1, 10, or 30 μg/kg CP 55, 940 15 min prior to the 1st Pavlovian Spontaneous Recovery (PSR) session. + Indicates that vehicle, 1 or 10 μg/kg CP groups responded significantly (p < 0.033) more on the EtOH lever during the 1st PSR session compared to baseline levels and 1 or 10 μg/kg CP groups responded more than vehicle treated group.

3.2.2. Relapse

Under relapse conditions, examining the effects of CP on EtOH-responding (Fig. 5) indicated a significant effect of 'session' ($F_{8, 12} = 12.2$; $p < 0.001$), 'dose' ($F_{3,19} = 7.2$; $p < 0.001$), and 'session' by 'dose' interaction ($F_{24, 42} = 3.9$; $p < 0.001$). Decomposing the significant interaction term by examining the effect of 'session' within each 'dose' group indicated that, in all groups, there was a significant alteration in responding on the EtOH lever during the initial relapse session compared to baseline (p values < 0.007). Within subjects t-tests indicated that P rats treated with saline increased EtOH-responding during the 1st relapse session compared to baseline levels (Fig. 5). Rats administered low doses of CP (1 and 10 μg/kg) responded more compared to baseline during the initial 2 relapse sessions. In contrast, the 30 μg/kg dose of CP reduced EtOH-responding during all 4 relapse sessions. Individual ANOVAs performed on each of the four relapse sessions indicated that only during the first two reinstatement sessions was a significant effect of 'dose' (p values = 0.008). Post-hoc comparisons (Tukey's b) indicated that there were significant differences between all groups of P rats responding on the EtOH lever during the 1st and 2nd reinstatement session. P rats treated with the highest dose of CP (30 μg/kg) responded less than vehicle treated rats, whereas P rats treated with the low doses of CP (1 and 10 μg/kg) responded more than vehicle group. During the 3rd and 4th relapse sessions, post-hoc comparisons indicated that the highest dose of CP reduced responding compared to all other groups, whereas the 1 and 10 μg/kg doses were no longer effective. Water responding was low (~15 responses/session) and was not significantly altered by any of the treatments (P values > 0.17). The amount of EtOH self-administered prior to deprivation would result in an estimated EtOH consumption of 1.1 g/kg for a 280 g rat. The amount of EtOH self-administered during the first relapse session in P rats administered 30 μg/kg would be estimated at less than 3.9 g/kg.

4. Discussion

The major findings of the current study are that 1 and 2 mg/kg of the CB1 antagonist, SR-141716A (SR), suppressed seeking and transiently reduced EtOH self-administration during relapse and maintenance; whereas, CB1 agonist, CP 55, 940 (CP) at doses of 1 and 10 μg/kg increased seeking and relapse of EtOH in female P rats. These results suggest that activation of CB1 receptors is involved in regulation of seeking, relapse and maintenance of EtOH self-administration. This is in agreement with previous reports (Gallate et al., 1999; Hungund & Basavarajappa, 2000; Colombo et al., 2002,

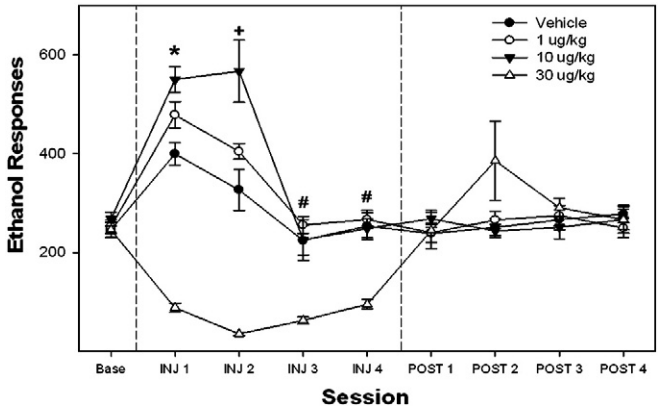

Fig. 5. Depicts Mean (± SEM) responses/session on the EtOH lever in female P rats (n = 5–6/group) given 0, 1, 10, or 30 μg/kg CP 55, 940 15 min prior to the initial 4 reinstatement (alcohol deprivation effect: ADE) sessions. * Indicates that vehicle, 1 or 10 μg/kg groups responded more compared to baseline levels, 30 μg/kg group responded less compared to baseline, and all groups were different from each other. + Indicates that 1 or 10 μg/kg groups responded more compared to baseline levels, 30 μg/kg group responded less compared to baseline, and all groups were different from each other. # Indicates that 30 μg/kg group responded less compared to baseline levels and were different from all other groups.

2004; Gonzalez et al., 2002; Hungund et al., 2002; Malinen and Hyytiä, 2008) that showed the CB1 receptor system plays a role in the regulation of EtOH-preference, consumption and mediation of EtOH reinforcing and motivational properties.

The high responding on the EtOH lever during the PSR test (Figs. 1 and 4) suggests that P rats are expressing robust EtOH-seeking behavior. These results are consistent with previously published findings (Rodd-Henricks et al., 2002; Dhaher et al., 2010). Systemic administration of the SR compound (Fig. 1) reduced responding on the EtOH lever at all 3 doses, whereas the two lowest doses of the CB1 agonist increased responding on the EtOH lever during the PSR test (Fig. 4). The reduction in responding by the SR compound does not appear to be due to a motor impairing effect since responses on the water lever were not altered at any dose. Likewise, the increased responding on the EtOH lever during the PSR test by the two lowest doses of the CP compound does not appear to be due to a general increase in motor activity since responding on the water lever was not significantly altered. Therefore, the results suggest that the CB1 receptor system may be activated during EtOH-seeking behavior. If EtOH-seeking responding reflects craving-like behavior, these results suggest that marijuana smoking could promote EtOH drinking. The results with the CB1 antagonist observed in the present study are in agreement with the findings of Cippitelli et al. (2005), which indicated that administration of SR141716 reduced cue-induced responding in a conditioned reinstatement of EtOH-seeking behavior in non-selected Wistar rats, as well as in Marchigian Sardinian alcohol-preferring (msP) rats. The ability of CB1 antagonists to reduce drug-seeking is not limited to EtOH since heroin-seeking is also reduced by CB1 antagonists infused directly into the nucleus accumbens core or prefrontal cortex (Alvarez-Jaimes et al., 2008).

In support of the interpretation that activation of the CB1 receptor system is involved in regulating EtOH-seeking behavior are the findings with the CB1 agonist (Fig. 4). The two lowest doses of the CB1 agonist markedly increased responding on the EtOH lever (without altering responses on the water lever) suggesting that further increasing the activation of CB1 receptors enhances EtOH-seeking behavior. On the other hand, the higher dose of the CB1 agonist (30 ug/kg) reduced responding on the EtOH lever in the PSR test (Fig. 4), suggesting that this dose may be having a secondary effect to inhibit EtOH-seeking behavior or prevent expression of EtOH-seeking behavior in the PSR test.

Similar to the effects observed in the PSR test, systemic administration of the SR compound reduced responding, whereas the CB1 agonist (at the two lowest doses) increased responding on the EtOH lever under relapse EtOH drinking conditions (Figs. 2 and 5). These results suggest that activation of the CB1 receptor is also involved in regulating EtOH drinking under relapse conditions. Furthermore, these results suggest that exposure to cannabinoids can promote relapse drinking during periods of abstinence, and support an argument that marijuana smoking could have a negative influence on individuals who are undergoing treatment to reduce their EtOH drinking behavior. The present results are in agreement with the findings of Gessa et al. (2005), who reported that administration of the CB1 antagonist reduced relapse drinking in sP rats, and the results of Lopez-Moreno et al. (2004), who demonstrated that a CB1 agonist increased EtOH drinking under relapse conditions.

The CB1 antagonist, at the two highest doses, reduced responding on the EtOH lever under maintenance conditions (Fig. 3). These results are compatible with the findings by Gallate et al. (1999) and Colombo et al. (2002), who reported that CB1 agonists increased EtOH intake of Wistar and sP rats, respectively. In addition, the present results (Fig. 3) are also in agreement with the findings that systemic administration of the SR compound reduced acquisition and maintenance of EtOH drinking in sP rats (Colombo et al., 1998; Serra et al., 2001, 2002).

With repeated administration, there was a progressive loss of the effectiveness of the SR compound to reduce responding on the EtOH lever during maintenance (Fig. 3) or relapse (Fig. 2). Similarly, the effectiveness of the two lowest doses of the CB1 agonist to increase responding was also diminished with repeated administrations (Fig. 5). The loss of effectiveness with repeated treatments could be due to a combination of factors, including increased metabolism or clearance of the SR or CP compound, alterations in the affinity or number of CB1 receptors, and/or internalization of the CB1 receptors.

At the highest dose of the CB1 agonist, there was decreased responding on the EtOH lever compared to control values (Figs. 4 and 5). At the higher dose, the CP compound may be acting at other receptors (Ross, 2003; Herkenham et al., 1991; Devane et al., 1988). The action at other receptors may counter the low-dose stimulating effects and/or produce motor impairment to prevent responding (Romero et al., 2002; Fan et al., 1996).

These results suggest that activation of the CB1 receptor is involved in regulating EtOH-seeking, relapse and maintenance behaviors, and further support the idea that marijuana smoking could have a significant impact on promoting EtOH drinking behavior. In conclusion, administration of the CB1 receptor antagonist, SR, reduced EtOH-seeking and transiently reduced EtOH self-administration during relapse and maintenance conditions. Conversely, treatment with the CB1 receptor agonist CP increased EtOH-seeking and EtOH self-administration during relapse and maintenance conditions. Therefore, compounds that modulate cannabinoid receptors are good targets for the development of drugs that could be useful in the treatment of alcoholism particularly in alcoholics that also smoke marijuana.

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