HHS Public Access
Author manuscript
Pharmacol Biochem Behav. Author manuscript; available in PMC 2015 April 11.

Published in final edited form as:
Pharmacol Biochem Behav. 2011 February ; 97(4): 669–675. doi:10.1016/j.pbb.2010.11.006.

CB1 Receptors Regulate Alcohol-Seeking Behavior and Alcohol Self-administration of Female Alcohol-Preferring (P) Rats

Bruk Getachew[1], **Sheketha R. Hauser**[1], **Ronnie Dhaher**[1], **Richard L. Bell**[1], **Scott M. Oster**[2], **William J. McBride**[1], and **Zachary A. Rodd**[1,*]

[1]Department of Psychiatry, Institute of Psychiatric Research, Indiana University School of Medicine, Indianapolis, IN 46202

[2]Department of Psychology, Purdue School of Science, Indiana University-Purdue University at Indianapolis, IN 46202

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-hr 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-hr 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.

Keywords

ethanol self-administration; ethanol relapse; ethanol seeking; Pavlovian Spontaneous Recovery; operant; CB1 agonist; CB1 antagonist; SR141716A; CP, 55-940

*Address Correspondence to: Dr. Zachary A. Rodd, Indiana University School of Medicine, Institute of Psychiatric Research, 791 Union Drive, Indianapolis, IN 46202-4887 USA, Phone: 317-278-3003; Fax: 317-274-1365; zrodd@iupui.edu.

1. INTRODUCTION

Epidemiological data indicates that 58% of subjects who abuse ethanol (EtOH) or are alcohol dependent also abuse marijuana (Martin et al., 1996). EtOH 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 EtOH and THC suggestive of the involvement of possible common pathway(s) (Basavarajappa and Hungund, 2002). One of the systems that is activated by both EtOH 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 alcohol (Hugund & Basavarajappa, 2000; Gonzalez et al., 2002; Hungund et al., 2002 and 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 EtOH 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). The modulation of alcohol reward by the CB system previously has been shown to be via the NAcc (Caille et al., 2007) and the prefrontal cortex (Hansson et al., 2007). Recently, it was reported significant dose-dependent reduction in EtOH intake following SR141716A administration into the posterior, but not anterior VTA, consistent with evidence of a specific involvement of the posterior VTA in the regulation of EtOH intake (Alvarez-Jaimes, et al 2009).

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). CB1 receptor knockout mice that lack CB1 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 and Naassila et al., 2004).

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.

However, to date, few studies have been carried out in animals that display robust EtOH-seeking and- relapse drinking. Alcohol-preferring (P) rats do display long and robust relapse drinking; the temporary increase in EtOH intake observed with P rats under relapse conditions is indicative of alcohol deprivation effect (ADE). Although, AA rats display increased EtOH intake during the first hour after a few hours of EtOH deprivation (Sinclair and Li, 2003), however, longer deprivation produces progressively smaller first-hour intakes in the AAs (Sinclair and. Tiihonen, 1989). In the case of sP rats, the ADE is limited to the first hour of each repeated access period and magnitude of this ADE did not increase with repeated deprivation (Serra et al., 2003). By comparison the P rats exhibit both “long and short ADE” (Sinclair and Li, 1989, Vengeliene et al., 2003).

In addition, P rats exhibit significant Pavlovian Spontaneous Recovery (PSR), which is an index of seeking behavior. PSR is reinstatement of responding (goal seeking) or a conditional response, in the absence of the previously trained reward following a period of rest after extinction (Pavlov, 1927). The application of the PSR phenomenon to animal studies of alcohol abuse has a number of beneficial aspects. First, spontaneous responding procedures assess operant behavior in the absence of passive drug administration within the environment previously associated with drug availability. Therefore, all responses are thought to be intrinsically motivated (Pavlov, 1927) and are not the result of drug-induced actions. Thus, spontaneous responding can be conceived as a suitable paradigm to assess ‘drug-craving’ or ‘drug-seeking’ in animals. This persistence of responding in the absence of reward parallels the compulsive nature of drug abuse in humans (Anton, 1999). Thus, P rat are unique in its predisposition to expressing pronounced EtOH craving/relapse, and that the PSR procedure may be a potentially valid and important measure for studying EtOH-craving behavior. Therefore, use of P rats, implementation of PSR and ADE paradigms to examine seeking relapse and relapse drinking provides a unique experimental model to study pharmacological effects on these EtOH-related behaviors in animals.

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 female 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 250–325g at the start of the experiment were used. Rats were maintained on a 12-hr reversed light-dark cycle (lights off at 0900 hr). 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-sec 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., 2002).

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., 2002).

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. 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 minutes 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). 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-hr 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 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 minutes 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—In all groups, there was significant alteration in responding during the initial PSR session compared to extinction baseline (p values < 0.05). Individual ANOVAs performed on each PSR test session indicated that only during the first PSR test session was there a significant effect of 'dose' ($F_{3,32} = 22.14$; $p < 0.001$). 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 < 0.05). 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 < 0.01). 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 > 0.05). Therefore, PSR doses were not included as factors in the relapse statistical analysis. 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 < 0.05). In P rats treated with 2 mg/kg SR, responding for EtOH was reduced during the 1st, 2nd, and 3rd relapse session (p < 0.05). 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 > 0.05).

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 > 0.05). The overall analysis indicated a significant effect of 'session' ($F_{8,25} = 32.5$; $p < 0.001$) and a 'session' by 'dose' interaction ($F_{24,81} = 2.5$; $p = 0.01$). 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 < 0.01). Similar to results for relapse responding, responding 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 > 0.05).

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). 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 < 0.05). 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. 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.

3.2.2. Relapse—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 30μg/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 >0.05).

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 alcohol self-administration. This is in agreement with previous reports (Gallate et al., 1999; Hugund & Basavarajappa, 2000; Colombo et al., 2002, 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 alcohol preference, consumption and mediation of alcohol 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). 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 alcohol 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.

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 alcohol drinking conditions (Figs. 2 and 5). These results suggest that activation of the CB1 receptor is also involved in regulating alcohol 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 alcohol 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 & 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 alcohol 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.

Acknowledgments

This work was supported in part by research grants AA 07611, AA 11261, AA 13522, and AA 10721. The authors would like to thank Tylene Pommer-Oster for her technical assistance.

References

Anton RF. What is craving? Models and implications for treatment, Alcohol Res Health. 1999; 23:165–173.

Arnone M, Maruani J, Chaperon F, Thiebot M, Poncelet M, Soubrie P, Le Fur G. Selective inhibition of sucrose and ethanol intake by SR141716, an antagonist of central cannabinoid (CB1) receptors. Psychopharmacology. 1997; 132:104–106. [PubMed: 9272766]

Basavarajappa BS, Hungund BL. Neuromodulatory role of the endocannabinoid signaling system in alcoholism: an overview. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2002; 66:287–299.

Basavarajappa BS, Hungund BL. Chronic ethanol increases the cannabinoid receptor agonist anandamide and its precursor *N*-arachidonoylphosphatidyl-ethanolamine in SK-N-SH cells. Journal of Neurochemistry. 1999; 72:522–528. [PubMed: 9930723]

Basavarajappa BS, Cooper TB, Hungund BL. Chronic ethanol administration down-regulates cannabinoid receptors in mouse brain synaptic plasma membrane. Brain Res. 1998:212–218. [PubMed: 9630633]

Cippitelli A, Bilbao A, Hansson AC, del Arco I, Sommer W, Heilig M, Massi M, Bermudez-Silva FJ, Navarro M, Ciccocioppo R, de Fonseca FR. Cannabinoid CB1 receptor antagonism reduces conditioned reinstatement of ethanol-seeking behavior in rats. Eur J Neurosci. 2005; 21:2243–51. [PubMed: 15869521]

Colombo G, Serra S, Vacca G, Carai MA, Gessa GL. Endocanabinoid system and alcohol addiction: pharmacological studies. Pharmacol Biochem Behav. 2005; 81:369–380. [PubMed: 15939463]

Colombo G, Vacca G, Serra S, Carai MA, Gessa GL. Suppressing effect of the cannabinoid CB1 receptor antagonist, SR 141716, on alcohol’s motivational properties in alcohol-preferring rats. Eur J Pharmacol. 2004; 498:119–23. [PubMed: 15363985]

Colombo G, Serra S, Brunetti G, Gómez R, Melis S, Vacca G, Carai MM, Gessa GL. Stimulation of voluntary ethanol intake by cannabinoid receptor agonists in ethanol-preferring sP rats. Psychopharmacology. 2002; 159:181–187. [PubMed: 11862347]

Colombo G, Agabio R, FM, Guano L, Lobina C, Loche A, Reali R, Gessa G. Reduction of voluntary ethanol intake in ethanol preferring sP by the cannabinoid antagonist SR141716. Alcohol and Alcohol. 1998; 33:126–130.

Devane WA, Dysarz FA 3rd, Johnson M, Melvin LS, Howlett AC. Determination and characterization of a cannabinoid receptor in rat brain. Mol Pharmacology. 1988; 34:605–613.

Economidou D, Mattioli L, Cifani C, Perfumi M, Massi M, Cuomo V, Trabace L, Ciccocioppo R. Effect of the cannabinoid CB1 receptor antagonist SR-141716A on ethanol self-administration and ethanol-seeking behaviour in rats. Psychopharmacology (Berl). 2006; 183:394–403. [PubMed: 16261315]

Fan F, Tao Q, Abood M, Martin BR. Cannabinoid receptor down-regulation without alteration of the inhibitory effect of CP 55,940 on adenylyl cyclase in the cerebellum of CP 55,940-tolerant mice. Brain Res. 1996; 706:13–20. [PubMed: 8720487]

Freedland CS, Sharpe AL, Samson HH, Porrino LJ. Effects of SR 141716A on ethanol and sucrose self-administration. Alcohol Clin Exp Res. 2001; 25:277–282. [PubMed: 11236843]

Gallate JE, Saharov T, Mallet PE, McGregor IS. Increased motivation for beer in rats following administration of a cannabinoid CB1 receptor agonist. Eur J Pharmacol. 1999; 370:233–240. [PubMed: 10334497]

Gardner EL, Vorel SR. Cannabinoid transmission and reward-related events. Neurobiol Dis. 1998; 5:502–33. [PubMed: 9974181]

Gessa GL, Serra S, Vacca G, Carai MA, Colombo G. Suppressing effect of the cannabinoid CB1 receptor antagonist, SR147778, on alcohol intake and motivational properties of alcohol in alcohol-preferring sP rats. Alcohol Alcohol. 2005; 40:46–53. [PubMed: 15582988]

Gessa GL, Casu MA, Carta G, Mascia MS. Cannabinoids activate mesolimbic dopamine neurons by an action on cannabinoid CB_1 receptors. Eur J Pharmacol. 1998; 341:39–44. [PubMed: 9489854]

Gonzalez S, Grazia Cascio M, Fernandez-Ruiz J, Fezza F, Di Marzo V, Ramos JA. Changes in endocannabinoid contents in the brain of rats chronically exposed to nicotine, ethanol or cocaine. Brain Research. 2002; 954:73–81. [PubMed: 12393235]

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC. Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. J Neuroscience. 1991; 11:563–583.

Hungund BL, Szakall I, Adam A, Basavarajappa BS, Vadasz C. Cannabinoid CB1 receptor knockout mice exhibit markedly reduced voluntary alcohol consumption and lack alcohol-induced dopamine release in the nucleus accumbens. J Neurochemistry. 2003; 84:698–704.

Hungund BL, Basavarajappa BS, Vadasz C, Kunos G, Rodriguez de Fonseca F, Colombo G, Serra S, Parsons L, Koob GF. Ethanol, endocannabinoids, and the cannabinoidergic signaling system. Alcohol Clin Exp Res. 2002; 26:565–574. [PubMed: 11981134]

Hungund BL, Basavarajappa BS. Are anandamide and cannabinoid receptors involved in ethanol tolerance? A review of the evidence. Alcohol and Alcohol. 2000; 35:126–133.

Lallemand F, Soubrie PH, De Witte PH. Effects of CB1 cannabinoid receptor blockade on ethanol preference after chronic ethanol administration. Alcohol Clin Exp Res. 2001; 25:1317–1323. [PubMed: 11584151]

Lopez-Moreno JA, Rodríguez de Fonseca F, Navarro M. Behavioural effects of quinpirole following withdrawal of chronic treatment with the CB1 agonist, HU-210, in rats. Behav Pharmacol. 2005; 16:441–6. [PubMed: 16148449]

Malinen H, Hyytiä P. Ethanol self-administration is regulated by CB1 receptors in the nucleus accumbens and ventral tegmental area in alcohol-preferring AA rats. Alcohol Clin Exp Res. 2008; 32:1976–83. [PubMed: 18782338]

Martin CS, Kaczynski NA, Maisto SA, Tarter RE. Polydrug use in adolescent drinkers with and without DSM-IV alcohol abuse and dependence. Alcohol Clin Exp Res. 1996; 20:1099–1108. [PubMed: 8892534]

Naassila M, Pierrefiche O, Ledent C, Daoust M. Decreased alcohol self-administration and increased alcohol sensitivity and withdrawal in CB1 receptor knockout mice. Neuropharmacology. 2004; 46:243–253. [PubMed: 14680762]

National Reseach Council. Guide for the Care and Use of Laboratory Animals. National Acadamy Pres; Washington, DC: 1996.

Navarro M, Carrera MR, Fratta W, Valiverde O, Cossu G, Fattore L, Chowen JA, Gomez R, Del Arco I, Villanua MA, Maldonado R, Koob GF, Rodriguez de Fonseca F. Functional interaction between opioid and cannabinoid receptors in drug self-administration. J Neuroscience. 2001; 21:5344–5350.

Ortiz S, Olivia JM, Perez-Rial S, Palomo T, Manzanare J. Chronic Ethanol Consumption Regulate Cannabinoid CB1 Receptor Gene Expression in Selected Regions of Rat Brain. Alcohol Alcohol. 2004; 39:88–92. [PubMed: 14998822]

Pavlov, IP. Conditioned reflexes. Anrep, GV., translator. London: Oxford University Press; 1927.

Poncelet M, Maruani J, Calassi R, Soubrie P. Overeating, alcohol and sucrose consumption decrease in CB1 receptor deleted mice. Neurosci Lett. 2003; 343:216–218. [PubMed: 12770700]

Racz I, Bilkei-Gorzo A, Toth ZE, Michel K, Palkovits M, Zimmer A. A critical role for the cannabinoid CB_1 receptors in alcohol dependence and stress-stimulated ethanol drinking. J Neurosci. 2003; 23:2453–2458. [PubMed: 12657705]

Rimondini R, Arlinde C, Sommer W, Heilig M. Long-lasting increase in voluntary ethanol consumption and transcriptional regulation in the rat brain after intermittent exposure to alcohol. The FASEB Journal. 2002; 16:27–35.

Rodd-Henricks ZA, Bell RL, Kuc KA, Murphy JM, McBride WJ, Lumeng L, Li TK. Effects of concurrent access to multiple ethanol concentrations and repeated deprivations on alcohol intake of alcoholpreferring rats. Alcohol Clin Exp Res. 2002; 25:1140–1150. [PubMed: 11505045]

Romero EM, Fernández B, Sagredo O, Gomez N, Urigüen L, Guaza C, De Miguel R, Ramos JA, Viveros MP. Antinociceptive, behavioural and neuroendocrine effects of CP 55,940 in young rats. Brain Res Dev Brain Res. 2002; 136:85–92.

Ross RA. Anandamide and vanilloid TRPV1 receptors. Br J Pharmacol. 2003; 140:790–801. [PubMed: 14517174]

Serra S, Brunetti G, Vacca G, Lobina C, Carai MA, Gessa GL, Colombo G. Stable preference for high ethanol concentrations after ethanol deprivation in Sardinian alcohol-preferring (sP) rats. Alcohol. 2003; 29:101–8. [PubMed: 12782251]

Serra S, Carai MA, Brunetti G, Gomez R, Melis S, Vacca G, Colombo G, Gessa GL. The cannabinoid receptor antagonist SR 141716 prevents acquisition of drinking behavior in alcohol-preferring rats. Eur J Pharmacol. 2001; 430:369–371. [PubMed: 11711056]

Serra S, Brunetti G, Pani M, Vacca G, Carai MA, Gessa GL, Colombo G. Blockade by the cannabinoid CB(1) receptor antagonist, SR 141716, of alcohol deprivation effect in alcohol-preferring rats. Eur J Pharmacol. 2002; 443:95–97. [PubMed: 12044797]

Sinclair JD, Li TK. Long and short alcohol deprivation: effects on AA and P alcohol-preferring rats. Alcohol. 1989; 6:505–9. [PubMed: 2597353]

Vengeliene V, Siegmund S, Singer MV, Sinclair JD, Li TK, Spanagel R. A comparative study on alcohol-preferring rat lines: effects of deprivation and stress phases on voluntary alcohol intake. Alcohol Clin Exp Res. 2003; 27:1048–54. [PubMed: 12878910]

Wang L, Liu J, Harvey-white J, Zimmer A, Kunos G. Endocannabinoid signaling via CB1 receptors is involved in ethanol preference and its age-dependent decline in mice, Proc. Natl Acad Sci. 2003; 100:1393–8.

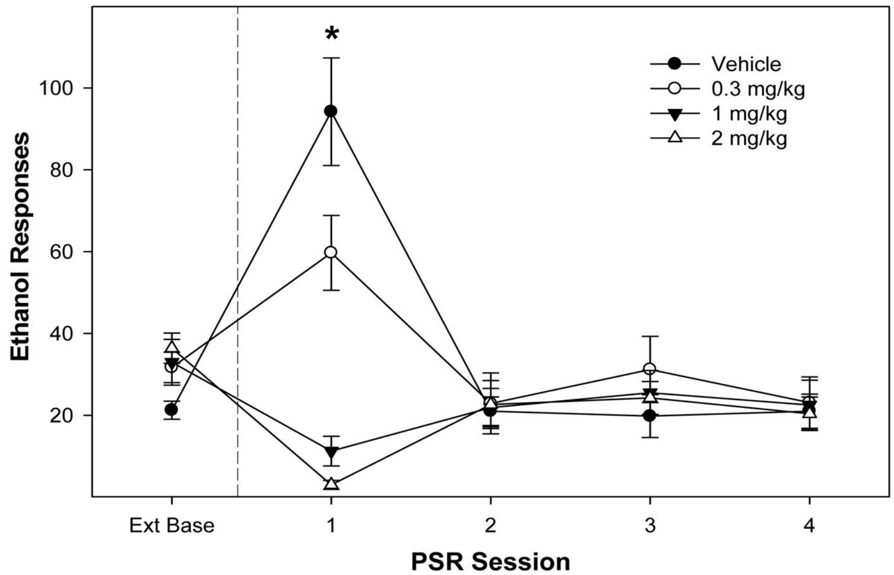

Fig. 1.

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 PSR session compared to baseline levels, and all other groups were different compared to extinction baseline ($F_{3,32}$)=22.4, $p<0.001$).

Author Manuscript
Author Manuscript
Author Manuscript

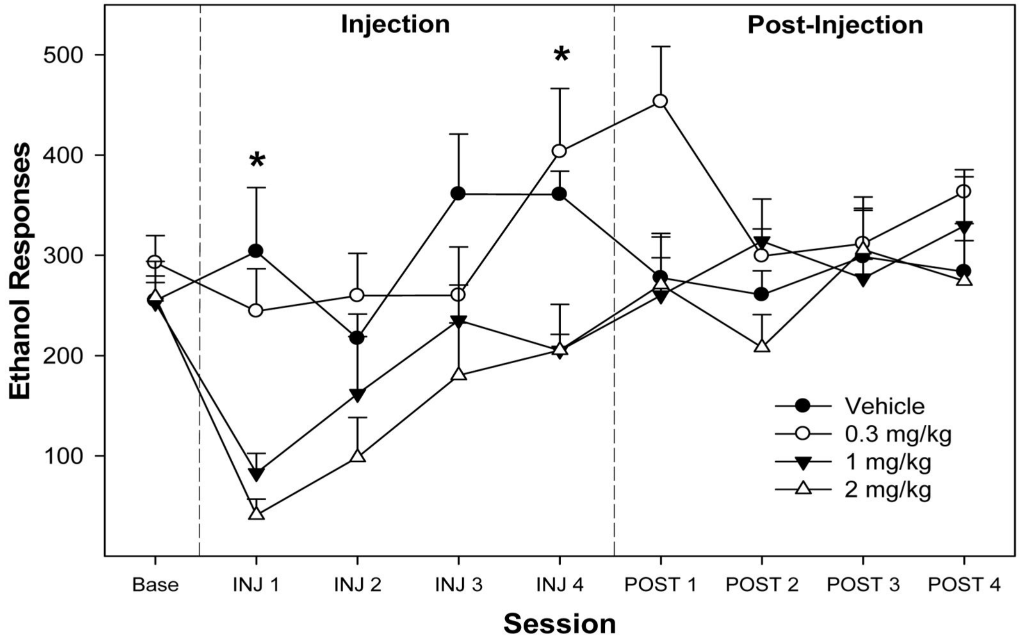

Fig. 2.

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 reinstatement sessions (ADE). * Indicates that vehicle and 0.3 mg/kg groups were significantly different from the 1 and 2 mg/kg groups.

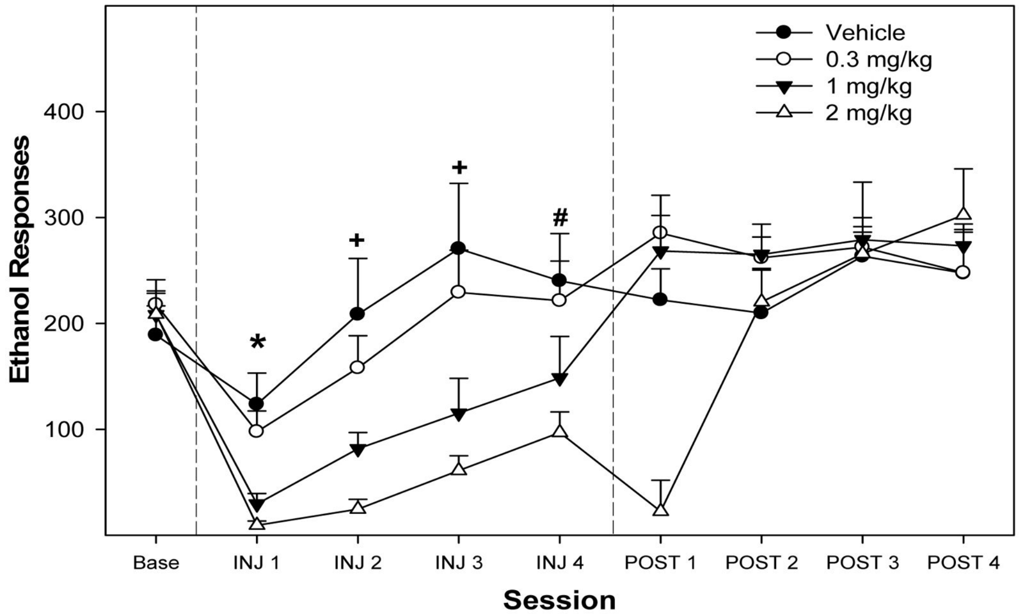

Fig. 3.

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.

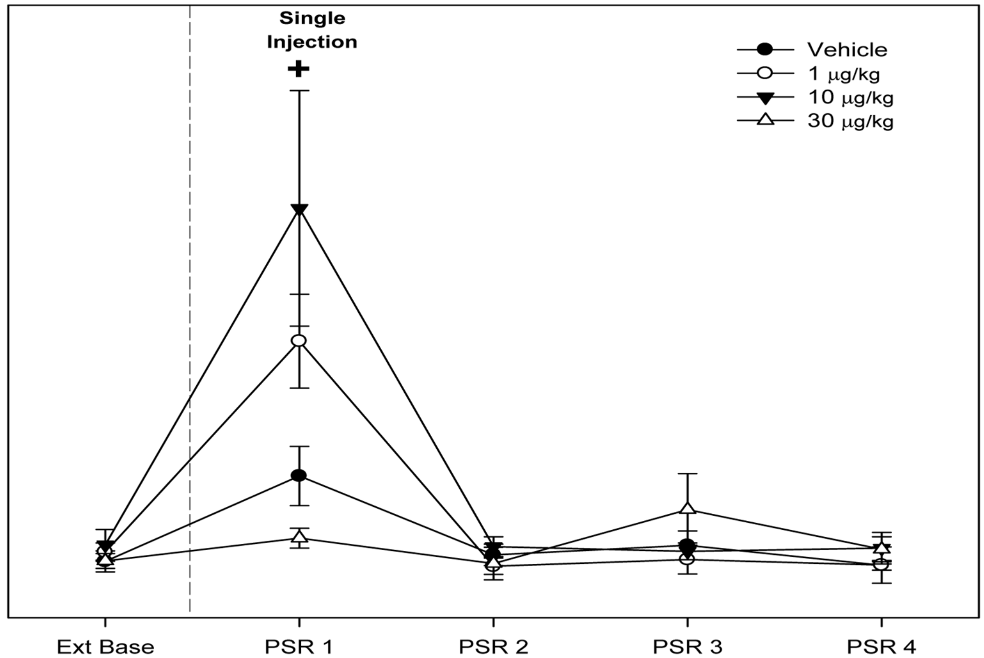

Fig. 4.

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 PSR session. + Indicates that vehicle, 1 or 10 μg/kg CP groups responded significantly ($p < 0.05$) 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.

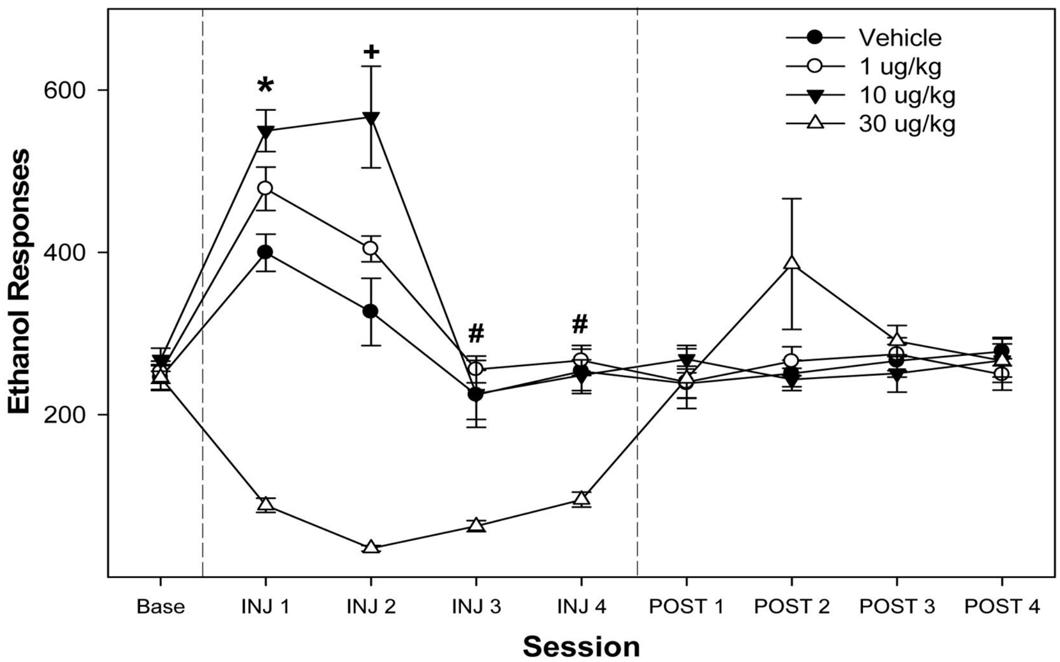

Fig. 5.

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 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.