SUPPRESSING EFFECT OF THE CANNABINOID CB₁ RECEPTOR ANTAGONIST, SR147778, ON ALCOHOL INTAKE AND MOTIVATIONAL PROPERTIES OF ALCOHOL IN ALCOHOL-PREFERRING sP RATS

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(Received 30 July 2004; first review notified 1 September 2004; in revised form 14 September 2004; accepted 1 October 2004)

Abstract — Aims: The present study investigated the effect of the newly synthesized cannabinoid CB₁ receptor antagonist, SR147778, on alcohol intake and the motivational properties of alcohol in selectively bred Sardinian alcohol-preferring (sP) rats. Methods and Results: In Experiment 1, the repeated administration of SR147778 (0.3–3 mg/kg twice daily, i.p.) specifically suppressed the acquisition of alcohol drinking behaviour in alcohol-naive rats exposed to the two-bottle ‘alcohol vs water’ choice regimen for 24 h/day. In Experiment 2, an acute administration of SR147778 (2.5–10 mg/kg, i.p.) specifically reduced alcohol intake in alcohol-experienced rats that were given alcohol and water under the two-bottle choice regimen in daily sessions of 4 h. In Experiment 3, an acute administration of SR147778 (0.3–3 mg/kg, i.p.) suppressed the ‘alcohol deprivation effect’, i.e. the extra-intake of alcohol occurring after a period of alcohol abstinence. In Experiment 4, an acute administration of SR147778 (0.3–3 mg/kg, i.p.) specifically suppressed the extinction responding for alcohol, i.e. the maximal number of lever responses reached in the absence of alcohol in rats trained to lever-press for alcohol (measure of the motivational properties of alcohol). In Experiment 5, the combination of 3 mg/kg of SR147778 (i.p.) and 0.5 g/kg of alcohol (i.p.), a dose comparable with those usually consumed by sP rats in each drinking binge, failed to induce any conditioned taste aversion. Conclusion: Taken together, these results extend to SR147778 the anti-alcohol profile of the prototype cannabinoid CB₁ receptor antagonist, rimonabant (SR141716), and strengthen the hypothesis that the cannabinoid CB₁ receptor is part of the neural substrate mediating alcohol intake and the motivational properties of alcohol.

INTRODUCTION

Recent experimental studies have suggested the possible involvement of the cannabinoid CB₁ receptor system in the neural circuitry of the brain, which controls alcohol intake and alcohol reinforcing properties. As an example, the acute administration of the cannabinoid CB₁ receptor agonists, CP-55,940 and WIN-55,212-2, has been found to stimulate alcohol intake in selectively bred Sardinian alcohol-preferring (sP) rats (Colombo et al., 2002b) and breakpoint for alcohol in unselected rats (Gallate and McGregor, 1999; Freedland et al., 1999). More recently, the acute administration of SR147778 (0.3–3 mg/kg, i.p.) suppressed the ‘alcohol deprivation effect’, i.e. the extra-intake of alcohol occurring after a period of alcohol abstinence (Gallate and McGregor, 1999; Freedland et al., 1999). These effects were blocked by pretreatment with the cannabinoid CB₁ receptor antagonist, rimonabant (SR 141716) (Gallate et al., 1999; Colombo et al., 2002b; Wang et al., 2003).

Conversely, rimonabant, when given alone, has been repeatedly reported to produce effects on alcohol-related behaviours, which were opposite to those exerted by cannabinoid CB₁ receptor agonists. Specifically, rimonabant has been found to: (i) suppress the acquisition of alcohol drinking behaviour in alcohol-naive sP rats when offered alcohol under the standard, homecage two-bottle ‘alcohol vs water’ choice regimen (Serra et al., 2001); (ii) reduce voluntary alcohol intake under the two-bottle choice regimen in C57BL/6J mice (Arnone et al., 1997), sP rats (Colombo et al., 1998) and Wistar rats (Lallemand et al., 2001) that were alcohol-experienced at the time of rimonabant administration (model of the ‘maintenance’ or ‘active drinking’ phase of human alcoholism); (iii) suppress the temporary increase in voluntary alcohol intake occurring in sP rats after a period of deprivation from alcohol (a phenomenon termed ‘alcohol deprivation effect’, which has been proposed to model the relapse episodes occurring in human alcoholics) (Serra et al., 2002); (iv) decrease the oral self-administration of alcohol in unselected rats tested under operant procedures (Freedland et al., 2001); (v) attenuate the appetitive properties of alcohol, as revealed by a decrease in the probability of completion of response requirement for alcohol in operant procedures in unselected rats (Gallate and McGregor, 1999; Freedland et al., 2001) and in extinction responding for alcohol in sP rats (Colombo et al., 2004).

CB₁ receptor knockout mice tested under the two-bottle choice paradigm consistently displayed significantly lower levels of alcohol preference and consumption than the wild-type mice (Hungund et al., 2003; Poncet et al., 2003; Wang et al., 2003; Naassila et al., 2004; Lallemand and De Witte, 2004; see however, Racz et al., 2003). In summary, the pharmacological blockade and the genetic deletion of the cannabinoid CB₁ receptor resulted in a marked reduction in alcohol intake, alcohol preference and motivational properties of alcohol.

The recent availability of the newly synthesized cannabinoid CB₁ receptor antagonist, SR147778 (Rinaldi-Carmona et al., 2001)
2004), allows to possibly confirm and extend the above findings observed with rimonabant to a second CB1 receptor antagonist. SR147778 is a structural analogue of rimonabant (Rinaldi-Carmona et al., 2004); it possesses high affinity (in the nanomolar range) and specificity for the rat brain cannabino- noid CB1 receptor (Rinaldi-Carmona et al., 2004), representing a valid tool for the investigation of the cannabinoid CB1 receptor system.

Accordingly, the present study investigated whether treatment with SR147778 replicated the results of the studies, which have characterized the anti-alcohol properties of rimonabant in sP rats. Specifically, we investigated the effect of SR147778 on: (i) acquisition of alcohol drinking behaviour in alcohol-naive rats (Experiment 1); (ii) alcohol intake in alcohol-experienced rats (Experiment 2); (iii) the extra-intake of alcohol that characterizes the alcohol deprivation effect (Experiment 3); and (iv) extinction responding for alcohol (Experiment 4). A final portion of the study was devoted to investigate whether the combination of SR147778 with a dose of alcohol comparable with those usually consumed by sP rats in each drinking binge would result in the development of a conditioned taste aversion in sP rats (Experiment 5).

MATERIALS AND METHODS

All the experimental procedures employed in the present study were in accordance with the Italian Law on the ‘Protection of animals used for experimental and other scientific reasons’.

Animals

Male sP rats, from the 56–57th generation and 75-days-old at the start of the study, were used. The rats were derived from a population of sP rats that underwent caesarian derivation at Charles River (Lyon, France) for production of specific pathogen free individuals. The rats were individually housed in standard plastic cages (size: 425 × 266 × 180 mm) with wood chip bedding. The animal facility was under an inverted 12 h light–dark cycle (lights on at 23:00 h), at a constant temperature of 22 ± 2°C and relative humidity of ~60%. The rats were extensively habituated to handling and i.p. injections. Food pellets (Mucedola, Settimo Milanese, MI, Italy) were always available, except when noted.

Experiment 1: Effect of SR147778 on acquisition of alcohol drinking behaviour

The rats were alcohol-naive at the start of the study. They were divided into four groups of n = 10, matched for body weight. The rats received the first injection of SR147778 (0, 0.3, 1 and 3 mg/kg, i.p.) (Sanofi-Synthelabo, Montpellier, France) 20 min before alcohol presentation. SR147778 was suspended in 1 ml/kg of saline with 0.1% Tween 80. Alcohol presentation was initiated at the start of the dark phase of Day 1. Alcohol was offered under the homecage, two-bottle choice regimen between alcohol (10% v/v, in water) and water with unlimited access for 24 h/day. The bottles were refilled every day with fresh solution and their left–right positions interchanged daily to avoid development of position preference. Treatment with SR147778 was given twice daily, 6 h apart during the dark phase, for 10 consecutive days (treatment phase). Daily alcohol, water and food intake was monitored by weighing the bottles and food pellets (0.1 g accuracy) every day immediately before the start of the dark phase. Daily recording of alcohol, water and food intake was performed throughout the 10 days of treatment as well as for an additional 7 days after termination of treatment (post-treatment phase).

The data on daily alcohol, water, total fluid (i.e. the sum of alcohol solution and water consumed) and food intake during the 10 day treatment period and the 7 day post-treatment period were expressed in g/kg, ml/kg, ml/kg and g/kg, respectively, and evaluated by separate two-way (treatment; day) analyses of variance (ANOVAs) with repeated measures on the ‘day’ factor.

Experiment 2: Effect of SR147778 on maintenance of alcohol drinking behaviour

The study testing the effect of rimonabant on alcohol intake in alcohol-experienced rats (Colombo et al., 1998) indicated that the reducing effect of rimonabant on alcohol intake was separable from that on food intake only when alcohol and food availability was temporarily restricted, whereas this dissociation was not evident if alcohol and food were always available. It was concluded that the temporarily limited availability of alcohol and food possibly augmented the appetitive value of food, allowing the disclosure of the effect of rimonabant on alcohol intake. The present study employed the same procedure. Specifically, the rats were exposed to the homecage, two-bottle choice regimen between alcohol (10% v/v, in water) and water for eight consecutive weeks before the start of the experiment with SR147778. During the first 7 weeks, alcohol, water and food pellets were offered with unlimited access for 24 h/day. During the 8th week, alcohol and food pellets were offered during the 4 h drinking session daily (11:00–15:00 h; i.e. the first 4 h of the dark phase of the light–dark cycle). Water was also available during the remaining 20 h. The bottles were refilled every day with fresh solution and their left–right positions interchanged daily.

The rats were divided into four groups (n = 8), matched for body weight as well as alcohol and water intake during the last three drinking sessions preceding the experiment. SR147778 was suspended in 1 ml/kg of saline with 0.1% Tween 80 and an acute administration at doses of 0, 2.5, 5 and 10 mg/kg (i.p.) was given 20 min prior to the start of the session. On the test day, alcohol, water and food intake was monitored by weighing the bottles and food pellets at 60 min intervals from the start of the session.

Data on alcohol, water and food intake were expressed in g/kg, ml/kg and g/kg, respectively, and analysed by separate two-way (treatment; time interval) ANOVAs with repeated measures on time interval factor, followed by the Newman–Keuls test for multiple comparisons.

Experiment 3: Effect of SR147778 on alcohol deprivation effect

The rats were continuously offered alcohol (10% v/v, in water) and water under the homecage, two-bottle choice regimen for eight consecutive weeks. Alcohol and water intake was monitored once daily by weighing the bottle immediately before the onset of the dark phase. Bottles were refilled every day with fresh solution and their left–right positions interchanged daily.
At the end of the 8 week period of access to alcohol and water, the rats were divided into two groups (n = 40) and matched for body weight as well as alcohol and water intake over the last 7 days. One group of rats was deprived of alcohol for 15 consecutive days, wherein water was the sole fluid available (alcohol-deprived rats). The second group of rats continued to have unlimited access to alcohol and water (alcohol-nondeprived rats), with the exception of the last 6 h prior to the injection of SR147778, when the alcohol bottle was removed to ensure that blood alcohol levels were equal to zero at the time of the test.

At the end of the 15th day of the deprivation phase, the rats of both groups were divided into four subgroups (n = 10) and matched for body weight. An acute administration of 0, 0.3, 1 and 3 mg/kg of SR147778 (i.p.) was given. SR147778 was suspended in 1 ml/kg of saline with 0.1% Tween 80 and administered 20 min before lights off. Alcohol was presented at lights off. Alcohol, water and food intake was recorded 60 min later [i.e. the time interval wherein sP rats display the most pronounced alcohol deprivation effect (Agabio et al., 2000; Serra et al., 2003)].

The data on alcohol, water and food intake during the 60 min observation period were expressed in g/kg, ml/kg and g/kg, respectively, and analysed by separate two-way (deprivation; treatment) ANOVAs, followed by the Newman–Keuls test for multiple comparisons.

Experiment 4: Effect of SR147778 on extinction responding for alcohol
Self-administration and extinction sessions were conducted in modular chambers (Med Associates, Georgia, VT, USA) that were located in sound-attenuated cubicles. Each chamber contained one response lever and one liquid dipper (0.1 ml cup). Dipper presentation was associated with flashing of a green light positioned above the lever. Experimental sessions lasted for 30 min and were conducted for 6 days in a week (Monday to Saturday) during the dark phase of the light–dark cycle.

The rats were divided into two groups. One group of rats (n = 7) was initiated to lever-press for alcohol using the sucrose fading procedure (Samson, 1986). Initially, the rats of this group were trained to lever-press for sucrose (20% w/w, in water) for four consecutive days. Subsequently, over 22 consecutive sessions, sucrose concentration was progressively diminished to 0% while alcohol concentration was progressively increased to 15% (v/v). A fixed ratio (FR) schedule of 1 (FR1) was maintained throughout the initiation phase. After completion of the initiation phase, the FR schedule was progressively increased to FR4 over four consecutive sessions. FR4 and 15% alcohol concentration were maintained from then onwards (maintenance phase).

The second group of rats (n = 8) was trained to lever-press for sucrose. They were initially trained under FR1 and 3% (w/v) sucrose. Over 14 consecutive sessions, the FR schedule was progressively increased to FR4. FR4 and 3% sucrose concentration were maintained from then onwards (maintenance phase).

Alcohol and sucrose intake was measured by weighing the fluid reservoir before and after each self-administration session (0.1 g accuracy). Alcohol intake was expressed in g/kg alcohol; sucrose intake was expressed in ml/kg sucrose solution. After ~20 self-administration sessions of the maintenance phase (when number of lever-presses and intake of alcohol and sucrose were constant among sessions), extinction responding for alcohol or sucrose, defined as the maximal number of lever responses reached by each rat in the absence of alcohol or sucrose reinforcement, was determined. Extinction sessions were conducted once a week (they replaced the Saturday sessions of self-administration), following five consecutive self-administration sessions. Each rat was exposed to four extinction sessions. During extinction sessions, the rats were exposed to the operant chamber for 30 min but lever-pressing did not result in any dipper presentation. The fluid reservoir was, however, filled and located inside the chamber, to enable the rat to smell the fluid. After each extinction session, alcohol and sucrose self-administration rapidly recovered to baseline levels.

SR147778 was suspended in 1 ml/kg of saline with 0.1% Tween 80 and injected intraperitoneally at the doses of 0, 0.3, 1 and 3 mg/kg 20 min before the start of the extinction session. All doses of SR147778 were tested in each rat of both groups under a Latin square design.

Data on the number of responses and the amount of alcohol or sucrose (expressed in g/kg and ml/kg, respectively) consumed over the last self-administration sessions preceding the extinction sessions, among the rat subgroups subsequently assigned to SR147778 treatment, were analysed by separate one-way ANOVAs with repeated measures. Data on the effect of SR147778 on extinction responding for alcohol or sucrose were analysed by separate one-way ANOVAs with repeated measures, followed by the Newman–Keuls test for multiple comparisons.

Experiment 5: Development of conditioned taste aversion to the combination of SR147778 and alcohol
Initially, the rats were habituated to consume water in daily 20 min sessions for six consecutive days daily (preconditioning phase). The drinking session coincided with the first 20 min of the dark phase of the light–dark cycle. A second, empty bottle was also presented. Water intake was monitored by weighing the bottle immediately after the end of the session.

The conditioning phase started the day after the end of the preconditioning phase. The rats were divided into six groups (n = 8), matched for body weight and water intake during the last three sessions of the preconditioning phase; the groups were randomly assigned to one of the six treatments. Each conditioning session consisted of exposure for 20 min/day to a bottle containing a saccharin solution (0.1% w/v, in water) and a second, empty bottle; within 1 min of removal of the bottle, the rats were injected with SR147778 (0, 1 and 3 mg/kg, i.p.). SR147778 was suspended in 1 ml/kg of saline with 0.1% Tween 80. Twenty minutes after the SR147778 injection, the rats were treated with 0 and 0.5 g/kg alcohol (20% w/v, in saline; i.p.). The alcohol dose was chosen on the basis of previous results, indicating that it gives rise to blood alcohol levels comparable with those observed in sP rats after a single episode of alcohol drinking, and it did not elicit any conditioned taste aversion in sP rats when given alone (Brunetti et al., 2002). After injections, the rats were returned to their homecage and then left undisturbed. Five ‘saccharin–SR147778 plus alcohol’ pairings were given, one every other day. On intervening days, a bottle containing water alone and a second, empty bottle were presented for 20 min (development of conditioned aversion to saccharin is associated with a progressive decrease in the intake
of saccharin solution; access to water on alternate days would prevent possible dehydration of the rat). No injection followed the water session. The left–right position of the two bottles was alternated daily. The drinking session (either with the saccharin solution or water) coincided with the first 20 min of the dark phase. Intake of saccharin solution (in conditioning sessions) or water (on intervening days) was monitored immediately after the end of the drinking session.

The postconditioning phase started the day after completion of the conditioning phase. Rats were offered a free choice between the two bottles containing the saccharin solution (0.1% w/v) and water, respectively, in 20 min sessions daily and in the absence of any injection. Postconditioning sessions were repeated for seven consecutive days. The left–right position of the two bottles was rotated daily. The drinking session coincided with the first 20 min of the dark phase. Fluid consumption was monitored immediately after the end of the drinking session.

Data on daily intake of saccharin solution (expressed in ml/kg) during the postconditioning phase were analysed by two-way (treatment; day) ANOVA with repeated measures on the ‘day’ factor.

RESULTS

Experiment 1: Effect of SR147778 on acquisition of alcohol drinking behaviour
Mean daily alcohol intake in vehicle-treated rats rose to 5–6 g/kg/day [i.e. the amount of alcohol usually consumed daily by sP rats (Serra et al., 2001)] within a few days from the start of the exposure to the two-bottle choice (Fig. 1A). The repeated administration of SR147778 significantly [\(F_{\text{treatment}}(3,324) = 16.73, P < 0.0001\)] and dose-dependently reduced the acquisition of alcohol drinking in sP rats (Fig. 1A); in the group of rats treated with 3 mg/kg of SR147778, mean daily alcohol intake was steadily lower than 2 g/kg throughout the 10 day treatment period. Reduction in alcohol intake in SR147778-treated rats was associated with a compensatory, higher daily consumption of water [\(F_{\text{treatment}}(3,324) = 10.40, P < 0.0001\)] (Fig. 1B), such that the daily total fluid intake was minimally affected by drug treatment [\(F_{\text{treatment}}(3,324) = 6.13, P < 0.005\)] (Fig. 1C). Importantly, in terms of the specificity of the drug action on alcohol intake, daily food intake was not altered by treatment with SR147778 [\(F_{\text{treatment}}(3,324) = 0.17, P > 0.05\)] (Fig. 1D).

After completion of the treatment, daily alcohol intake in the 1 and 3 mg/kg SR147778-treated rat groups increased progressively, reaching control values on Days 2 and 4, respectively, of the post-treatment period [\(F_{\text{treatment}(3,216)} = 2.95, P < 0.05\)] (Fig. 1A). Daily water intake was inversely related to alcohol intake [\(F_{\text{treatment}(3,216)} = 4.80, P < 0.01\)] (Fig. 1B) resulting in no group differences in daily total fluid intake [\(F_{\text{treatment}(3,216)} = 0.13, P > 0.05\)] (Fig. 1C). Daily food intake during the post-treatment period tended to be higher in the 1 and 3 mg/kg SR147778-treated rats than in the vehicle-doses rats [\(F_{\text{treatment}(3,216)} = 9.90, P < 0.0001\)] (Fig. 1D).

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![Graph A](image1.png)
![Graph B](image2.png)
![Graph C](image3.png)
![Graph D](image4.png)

Fig. 1. Suppressing effect of the repeated administration of the cannabinoid CB1 receptor antagonist, SR147778, on the acquisition of alcohol drinking behaviour in Sardinian alcohol-preferring (sP) rats. SR147778 (0, 0.3, 1 and 3 mg/kg, i.p.) was injected twice daily (20 min before lights off and half-way through the dark phase) for 10 consecutive days. Alcohol (10% v/v in water) and water were offered under the two-bottle, free choice regimen with unlimited access for 24 h/day, starting after the first treatment with SR147778. Food pellets were always available. Alcohol, water and food intake was monitored once a day immediately before lights off. The dashed line indicates the completion of the 10 day treatment period. Each point is the mean ± SEM for \(n = 10\) subjects.
Experiment 2: Effect of SR147778 on maintenance of alcohol drinking behaviour

Acute administration of SR147778 produced a significant reduction of alcohol intake in alcohol-experienced SP rats \( F_{\text{treatment}(3,84)} = 4.89, P < 0.01 \) (Fig. 2A). The reducing effect of SR147778 on alcohol intake was already apparent at the first hour interval, when alcohol intake was lower by ~25, 40 and 55% in 2.5, 5 and 10 mg/kg SR147778-treated rats, respectively, when compared with vehicle-dosed rats. The attenuating effect of all doses of SR147778 persisted throughout the 4 h session. In contrast, treatment with SR147778 failed to significantly affect water \( F_{\text{treatment}(3,84)} = 1.85, P > 0.05 \) (Fig. 2B) and food \( F_{\text{treatment}(3,84)} = 0.90, P > 0.05 \) (Fig. 2C) intake.

Experiment 3: Effect of SR147778 on alcohol deprivation effect

ANOVA revealed significant effects of both deprivation \( F(1,72) = 10.66, P < 0.005 \) and treatment with SR147778 \( F(3,72) = 6.11, P < 0.001 \) on voluntary alcohol intake in the first hour of the post-deprivation phase. Alcohol intake was higher, by ~75%, in vehicle-treated alcohol-deprived rats than in vehicle-treated alcohol-nondeprived rats (Fig. 3), indicative of the development of the alcohol deprivation effect. Post hoc analyses showed that all doses of SR147778 suppressed the alcohol deprivation effect. Indeed, alcohol intake in all SR147778-treated alcohol-deprived rat groups was significantly lower than that recorded in vehicle-treated alcohol-deprived rats (Fig. 3). Furthermore, alcohol intake was not significantly different between alcohol-deprived and -nondeprived rats at each SR147778 dose.

Neither water nor food intake appeared to be affected by deprivation or treatment with SR147778 [water intake: \( F_{\text{deprivation}(1,55)} = 0.08, P > 0.05; F_{\text{treatment}(3,55)} = 1.01, P > 0.05 \); food intake: \( F_{\text{deprivation}(1,55)} = 0.93, P > 0.05; F_{\text{treatment}(3,55)} = 1.50, P > 0.05 \) (data not shown).
Experiment 4: Effect of SR147778 on extinction responding for alcohol

All rats acquired and maintained alcohol or sucrose self-administration. No differences in the number of lever presses and amount of alcohol or sucrose consumed over the Friday self-administration sessions (which preceded extinction sessions) were recorded among the subgroups subsequently treated with the different doses of SR147778 (data not shown).

Pretreatment with SR147778 dose-dependently suppressed the extinction responding for alcohol \(F(3,18) = 7.31, P < 0.005\) (Fig. 4A). Specifically, lever responses in 0.3, 1 and 3 mg/kg SR147778-treated rats were 11, 43 and 82% lower, respectively, than those recorded in vehicle-dosed rats. In contrast, extinction responding for sucrose was not significantly altered by treatment with SR147778 \(F(3,21) = 1.59, P > 0.05\) (Fig. 4B).

Experiment 5: Development of conditioned taste aversion to the combination of SR147778 and alcohol

In conditioned taste aversion procedures, the possible development of aversive responses to the tested drug (or combination of drugs) is usually unravelled during the postconditioning phase, when the animal is offered a free choice between the tastant with which the drug has been paired and water. With respect to the present study, ANOVA showed a lack of significant differences in saccharin intake among rat groups during the postconditioning phase \(F_{\text{treat.}}(5,252) = 2.32, P > 0.05\) (Table 1).

DISCUSSION

The results of Experiments 1–3 demonstrate the ability of the recently synthesized, cannabinoid CB\(_1\) receptor antagonist, SR147778 (Rinaldi-Carmona et al., 2004), to block the acquisition of alcohol drinking behaviour, reduce the maintenance of alcohol drinking behaviour and suppress a relapse-like alcohol drinking behaviour in selectively bred Sardinian alcohol-preferring (sP) rats trained to lever-press for oral alcohol (15% v/v, in water) or sucrose (3% w/v, in water) under a fixed ratio (FR) schedule of FR4. Extinction responding was defined as the maximal number of lever responses performed by each rat in the absence of alcohol or sucrose reinforcement. Extinction sessions were performed once self-administration behaviour stabilized. SR147778 (0, 0.3, 1 and 3 mg/kg, i.p.) was injected 20 min before the start of the extinction session. Each bar is the mean ± SEM of \(n = 7\) in the alcohol rat group and \(n = 8\) in the sucrose rat group. *\(P < 0.005\) with respect to vehicle-treated rats (Newman–Keuls test).

Table 1. Lack of development of conditioned taste aversion to the combination of the cannabinoid CB\(_1\) receptor antagonist, SR147778, and alcohol in Sardinian alcohol-preferring (sP) rats

<table>
<thead>
<tr>
<th>Rat groups</th>
<th>Sessions of the postconditioning phase</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>0 mg/kg SR147778 + 0 g/kg alcohol</td>
<td>44.9 ± 2.4</td>
</tr>
<tr>
<td>1 mg/kg SR147778 + 0 g/kg alcohol</td>
<td>20.4 ± 4.6</td>
</tr>
<tr>
<td>3 mg/kg SR147778 + 0 g/kg alcohol</td>
<td>29.1 ± 5.2</td>
</tr>
<tr>
<td>0 mg/kg SR147778 + 0.5 g/kg alcohol</td>
<td>41.2 ± 3.8</td>
</tr>
<tr>
<td>1 mg/kg SR147778 + 0.5 g/kg alcohol</td>
<td>32.7 ± 3.3</td>
</tr>
<tr>
<td>3 mg/kg SR147778 + 0.5 g/kg alcohol</td>
<td>36.2 ± 3.0</td>
</tr>
</tbody>
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In five of the 10 daily sessions of the conditioning phase, treatment with SR147778 (0, 1 and 3 mg/kg, i.p.) plus alcohol (0 and 0.5 g/kg, i.p.) was paired with saccharin solution (which was the only fluid available). On intervening days, a bottle containing water alone and a second, empty bottle were presented; no injection followed the water session. During the seven sessions of the postconditioning phase, rats were offered a free choice between saccharin solution and water. Data refer to intake of saccharin solution (expressed as ml/kg) during the daily 20 min sessions of the postconditioning phase. Each point is the mean ± SEM of \(n = 8\).
alcohol-preferring sP rats. These results closely replicate the data on the reducing effect of the prototype cannabinoid CB₁ receptor antagonist, rimonabant, on alcohol intake in sP rats (Colombo et al., 1998; Serra et al., 2001, 2002). In agreement with the binding data showing comparable affinity for the CB₁ receptor between the two drugs (Rinaldi-Carmona et al., 1994, 2004), potency and efficacy of SR147778 in reducing alcohol intake in sP rats were similar to those of rimonabant.

In close agreement with the results of the present study, SR147778 has been reported recently to reduce alcohol intake in C57BL/6J mice tested under the two-bottle free choice regimen as well as in Wistar rats tested under a schedule-induced polydipsia paradigm (Rinaldi-Carmona et al., 2004).

When the results of Experiments 1 and 2 were compared, SR147778 was shown to be more potent and effective in reducing acquisition than maintenance of the alcohol drinking behaviour of sP rats. Differences in drug potency and efficacy have been repeatedly observed when acquisition and maintenance of alcohol drinking behaviour in sP rats have been pharmacologically manipulated. For instance, rimonabant (Colombo et al., 1998; Serra et al., 2001), baclofen (Colombo et al., 2000, 2002a) and naltrexone (unpublished results) have been found to be 2–3-fold more potent and effective in reducing alcohol intake in the acquisition phase than when alcohol intake was already established. Similar results have been collected when the serotonin 5-HT₁ receptor antagonists, MDL 72222 and ICS 205–930, were tested on acquisition and maintenance of alcohol drinking behaviour in selectively bred Indiana alcohol-preferring P rats (Rodd-Henricks et al., 2000).

A possible explanation for this difference may reside in the likely alterations, induced by chronically consumed alcohol, in the functionality of the receptor systems mediating the central effects of alcohol that sustain alcohol drinking behaviour in sP rats; these neuroadaptation processes might result in a decreased potency and efficacy of the ligands. Alternatively, if the tested drug reduces the perception of the effects of alcohol that promote alcohol drinking (which might be also the case for cannabinoid CB₁ receptor antagonists), an alcohol-experienced rat is expected to initially consume some alcohol before the behaviour comes to extinction; in contrast, the same drug would preclude an alcohol-naïve rat from discovering alcohol effects, with the likely result of minimal intake of alcohol. Finally, alcohol drinking in alcohol-experienced but not naïve rats, may occur in part as a conditioned response to some external stimuli, such as the start of the dark phase of the light–dark cycle, and this response might be modestly sensitive to pharmacological treatment.

The results of Experiment 3 demonstrate that the acute administration of SR147778 suppressed the alcohol deprivation effect, that is, the extra amount of alcohol (~75% of baseline in the present experiment) consumed by rats after a period of alcohol deprivation. These results add further support to the hypothesis that the cannabinoid CB₁ receptor is part of the substrate mediating the alcohol deprivation effect in sP rats (Serra et al., 2002). Furthermore, these results apparently bear some clinical relevance because of the proposed validity of the alcohol deprivation effect as a model of alcohol relapse episodes and loss of control over alcohol occurring in human alcoholics (see Boening et al., 2001), and because the latter phenomena are considered to be the core features of alcohol dependence in humans (see Morse and Flavin, 1992).

The results of Experiment 4 extend to SR147778 the ability of rimonabant (Colombo et al., 2004) to suppress extinction responding for alcohol, a measure of the strength of the appetitive or motivational properties of alcohol (Samson et al., 2001, 2003) in sP rats. These results also suggest that the reducing effect of SR147778 on the consummatory aspects of alcohol drinking behaviour in sP rats is possibly secondary to a reduction of the motivation of the rats to consume alcohol.

Recently, Gallate and McGregor (1999) and Freedland et al. (2001) reported that the acute administration of rimonabant decreased the probability of response requirement completion for access to alcohol (another reliable procedure for measuring the appetitive strength of alcohol) in unselected rats trained to orally self-administer alcohol under an operant procedure. These results, together with those from this laboratory with rimonabant (Colombo et al., 2004) and SR147778 (present study, Experiment 4) suggest that the cannabinoid CB₁ receptor is involved in the mediation of the motivational attributes of alcohol.

A possible mechanism by which a given drug may reduce alcohol intake is the exacerbation of the aversive properties of alcohol. For instance, studies of conditioned taste aversion demonstrated that combination of low to moderate doses of alcohol with doses of naloxone or naltrexone that are effective in reducing alcohol intake produced aversive effects in rodents and monkeys (Broadbent et al., 1996; Williams and Woods, 1999), suggesting that this aversion might contribute to the decrement in alcohol intake. Experiment 5 of the present study used the conditioned taste aversion paradigm to investigate whether this ‘aversion’ mechanism could also contribute to the reducing effect of SR147778 on alcohol intake in sP rats. In the conditioned taste aversion paradigm, a specific dose of a given drug (or combination of drugs) is repeatedly paired with the availability of a taster (e.g. saccharin solutions). Once animals are given a choice between the taster and water, the possible avoidance of the former is indicative of the aversive, postabsorptive properties of the tested drug (or combination of drugs).

The results of Experiment 5 indicate that combination of 3 mg/kg of SR147778, a dose in the range of those that reduced alcohol intake in sP rats under all the procedures used in the present study (Experiments 1–3), with 0.5 g/kg of alcohol (i.p.), a dose that produced blood alcohol levels comparable with those reached by sP rats after a single drinking episode and which was devoid of aversive properties in this rat line (Brunetti et al., 2002), failed to induce any conditioned taste aversion in sP rats. These results suggest that SR147778, similar to rimonabant (unpublished data), does not render alcohol aversive in sP rats.

Overall, the results of the present study indicate that the cannabinoid CB₁ receptor antagonist, SR147778, similar to its structural analogue, rimonabant, suppressed acquisition and maintenance of alcohol drinking behaviour, relapse-like drinking and motivation to consume alcohol in alcohol-preferring sP rats. Because of the predictive validity of the above experimental models for different aspects of human alcoholism, these results add further strength to the hypothesis that pharmacological blockade of the CB₁ receptor may constitute a novel and potentially effective approach in the treatment of alcoholism.

Acknowledgements — The authors are grateful to Mrs Maria Elena Vincis for animal care. The present study was partially supported by a grant from Sanofi-Synthelabo, Milan, Italy.
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