Suppressing Effect of the Cannabinoid CB_1 Receptor Antagonist, Rimonabant, on Alcohol Self-Administration in Alcohol-Preferring Rats

Paola Maccioni, Noemi Fantini, Mauro A.M. Carai, Gian Luigi Gessa and Giancarlo Colombo*

C.N.R. Institute of Neuroscience, Viale Diaz 182, I-09126 Cagliari (CA), Italy

Abstract: Administration of the cannabinoid CB_1 receptor antagonist, rimonabant (also known as SR 141716), has been reported to reduce alcohol intake (measured under the homecage 2-bottle "alcohol *vs* water" choice regimen) in selectively bred, Sardinian alcohol-preferring (sP) rats. The present study investigated whether rimonabant also had the capacity to decrease, in this rat line, alcohol's reinforcing properties. To this end, male sP rats were initially trained to lever-press (on a fixed ratio 4 schedule of reinforcement) to orally self-administer alcohol (15%, v/v) in daily 30-min sessions. Once lever-pressing and self-administration behaviors reached stable levels (150-200 responses/session and 0.8-1 g/kg alcohol per session, respectively), the effect of rimonabant (0, 0.3, 1, and 3 mg/kg, i.p.) on responding for alcohol and amount of self-administered alcohol was determined. Pretreatment with rimonabant resulted in a significant, dose-dependent reduction in both variables; specifically, the total number of lever responses for alcohol and amount of self-administered alcohol in the rat groups treated with 0.3, 1, and 3 mg/kg rimonabant were approximately 20%, 35%, and 60% lower than those recorded in vehicle-treated rats. Pretreatment with 3 mg/kg rimonabant also resulted in a significant increase in the latency to the first response on the "alcohol" lever. These results demonstrate the capacity of rimonabant to suppress alcohol's reinforcing properties in alcohol-preferring sP rats. These data constitute a further piece of experimental evidence in support of the hypothesized role for the CB_1 receptor in the control of alcohol drinking and reinforcement.

INTRODUCTION

Acute and repeated administration of the prototypic cannabinoid CB_1 receptor antagonist, rimonabant (also known as SR 141716), has been repeatedly reported to suppress alcohol seeking and drinking behaviors in rats and mice exposed to multiple experimental procedures. Specifically, treatment with rimonabant produced dose-dependent reductions in: (a) acquisition [1-5] and maintenance [5-15; see however also 13] of alcohol drinking under the homecage 2-bottle "alcohol *vs* water" choice regimen in rats and mice; (b) "alcohol deprivation effect", defined as the temporary increase in alcohol intake or alcohol self-administration occurring after a period of forced abstinence from alcohol and validated as experimental model of alcohol relapse episodes, in rats [5, 14, 15]; (c) alcohol's reinforcing properties, measured in rats exposed to conventional procedures of operant, oral alcohol self-administration [16-23; see however also 24]; (d) alcohol's motivational properties, measured by the progressive ratio schedule of responding [19, 25] and the extinction responding procedure [26] in rats; (e) reinstatement of alcohol seeking behavior, triggered by injection of nicotine or exposure to cues previously associated to alcohol availability (another validated experimental models of alcohol relapse episodes) in rats [15, 17, 19, 20]. Notably, most of these results have been replicated with other *in vivo* effective cannabinoid CB_1 receptor antagonists, including surinabant [27-29], AM251 [30], and LH-21 [21].

The present study has been designed to provide a further piece of evidence to the "anti-alcohol" profile of rimonabant: its capacity of suppressing operant, oral alcohol self-administration in Sardinian alcohol-preferring (sP) rats, one of the few rat lines selectively bred worldwide for excessive alcohol preference and consumption [see 31]. When tested under standard operant procedures of alcohol self-administration, sP rats display a robust lever-pressing behavior (*e.g.*, 150-200 responses under the fixed ratio (FR) 4 schedule of reinforcement in daily 30-min sessions) and self-administer pharmacologically revelant amounts of alcohol (≥0.8 g/kg/session) [*e.g.*: 32], suggesting that, in these rats, alcohol functions as a reinforcer and has strong incentive motivational capacities in directing lever-pressing behavior.

Extension of the above-mentioned operant studies with rimonabant to sP rats is of interest since alcohol-preferring rat lines – although selectively bred for the same phenotype (*i.e.*, high alcohol preference and consumption under the homecage 2-bottle "alcohol *vs* water" choice regimen) – differ in a number of alcohol-related traits, including sensitivity of their alcohol drinking and seeking behavior to pharmacological manipulations [see 31], making these rat lines potential animal models of the proposed different types, or typologies, of alcoholism (in which pharmacotherapies have often been observed to be differentially effective [*e.g.*: 33]).

MATERIALS AND METHODOLOGY

All experimental procedures employed in the present study were in accordance with the Italian Law on the "Protection of animals used for experimental and other

*Address correspondence to this author at the C.N.R. Institute of Neuroscience, Viale Diaz 182, I-09126 Cagliari (CA), Italy; Tel: +39 070 302227; Fax: +39 070 302076; E-mail: colomb@unica.it

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scientific reasons", which fully incorporates Directive 86/609/EEC of the European Union Legislation.

Animals

Male sP rats, from the 69th generation and 75-days-old at the start of the study, were used. Rat body weight averaged 290 ± 7 g and 530 ± 10 g at the start and at the end of the study, respectively. Rats were housed four per cage in standard plastic cages with wood chip bedding. The animal facility was under an inverted 12:12 hour light-dark cycle (lights on at 7:00 p.m.), at a constant temperature of 22±2°C and relative humidity of approximately 60%. Rats were extensively habituated to handling and intraperitoneal injections. Food pellets (Harlan, San Pietro al Natisone, Italy) and water were always available in the homecage, except as noted.

Apparatus

Self-administration sessions were conducted in modular chambers (Med Associates, St. Albans, VT, USA) located in sound-attenuated cubicles, with fans for ventilation and background white noise. The front panel of each chamber was equipped with (a) two retractable response levers, (b) one dual-cup liquid receptacle positioned between the two levers, and (c) two stimulus lights (one green and one white) mounted above each lever. The liquid receptacle was connected by polyethylene tubes to two syringe pumps located outside the chamber. A white house light was centered at the top of the back wall of each chamber. For half of the rats, the right lever was associated to alcohol, and achievement of response requirement (a) activated the "alcohol" pump, resulting in the delivery of 0.1 ml alcohol solution, and (b) switched on the green light for the 2-s period of alcohol delivery; for these rats, the left lever was associated to water, and achievement of the response requirement (a) activated the "water" pump, resulting in the delivery of 0.1 ml water, and (b) switched on the white light for the 2-s period of water delivery. For the other half of the rats, the opposite condition applied (left lever: alcohol solution; right lever: water).

Experimental Procedure

Rats (n=16) were initially exposed to the homecage 2-bottle "alcohol (10%, v/v) *vs* water" choice regimen with unlimited access for 24 hours/day for 10 consecutive days. This initial phase was conducted to allow the rats to become accustomed to the taste of alcohol and start to experience its pharmacological effects, in order to possibly shorten the subsequent auto-shaping phase of the operant procedure.

Immediately after the 2-bottle choice regimen, rats were introduced into the operant chambers and trained to lever-press for alcohol. Rats were deprived of food pellets and water during the 18 hours before the first session in the operant chamber. Self-administration sessions lasted 30 min and were conducted 5 days per week (Monday to Friday) during the dark phase of the light/dark cycle. Rats were initially exposed to an FR1 schedule of alcohol reinforcement with 10% alcohol (v/v) for 4 consecutive daily sessions. FR was then increased to FR2 and FR4 over 4 consecutive sessions. In sessions 9 and 10, the alcohol solution was presented at the final concentration of 15% (v/v). Rats were then exposed to 4 consecutive sessions during which the "water" lever or the "alcohol" lever alone was available every other day; water and alcohol were available on FR1 and FR4, respectively. From then onwards, both levers were concomitantly available (maintenance phase); these sessions were conducted with FR4 and FR1 on the "alcohol" and "water" lever, respectively.

After approximately 20 self-administration sessions of the maintenance phase, rats were selected for inclusion in the rimonabant experiment. Specifically, the 12 rats displaying the most stable responding behavior over the last 5 daily sessions (less than 10% daily difference) were selected. Test sessions were conducted on Fridays; four consecutive (Monday-Thursday) baseline (no drug treatment) sessions elapsed between test sessions. All doses of rimonabant were tested in each rat under a latin-square design. Notably, after each test session alcohol self-administration immediately recovered to baseline levels. Rimonabant (Sanofi-Aventis, Montpellier, France) was suspended in 2 ml/kg saline with a few drops of Tween 80, and administered intraperitoneally – at the doses of 0, 0.3, 1, and 3 mg/kg – 30 min before the start of the session.

Measured Variables and Data Analysis

Measured variables were: total number of responses on each lever; total amount of self-administered alcohol and water (expressed in g/kg pure alcohol and ml/kg water, respectively, and determined from the total number of reinforcers); latency (expressed in s) to the first response on the "alcohol" lever.

Data on the effect of rimonabant on each variable were statistically analyzed by separate 1-way ANOVAs for repeated measures, followed by the Newman-Keuls test for *post hoc* comparisons.

RESULTS

During the 20-day maintenance phase, the 12 rats subsequently selected for the rimonabant experiment displayed an average number of total responses for alcohol of 165 per session; the resulting average of the amount of self-administered alcohol was of 0.92 g/kg per session. Conversely, a negligible number of total responses for water (averaging <2 per session) was recorded during the entire maintenance phase as well as in vehicle-treated rats in the subsequent test sessions.

Pretreatment with rimonabant resulted in a dose-dependent reduction in the total number of responses for alcohol [$F(3,47)=16.43$, $P<0.0001$] (Fig. **1**, top panel). Specifically, lever-responses in 0.3, 1, and 3 mg/kg rimonabant-treated rats was approximately 20%, 35%, and 60% lower, respectively, than that recorded in vehicle-dosed rats. *Post hoc* analysis revealed that all three doses of rimonabant decreased – relative to control value – the total number of responses for alcohol (0.3 mg/kg: $P<0.05$; 1 mg/kg: $P<0.01$; 3 mg/kg: $P<0.001$). Rimonabant-induced reduction in the total number of responses for alcohol resulted in a proportional decrease in the amount of self-administered alcohol [$F(3,47)=15.72$, $P<0.0001$] (Fig. **1**, center panel).

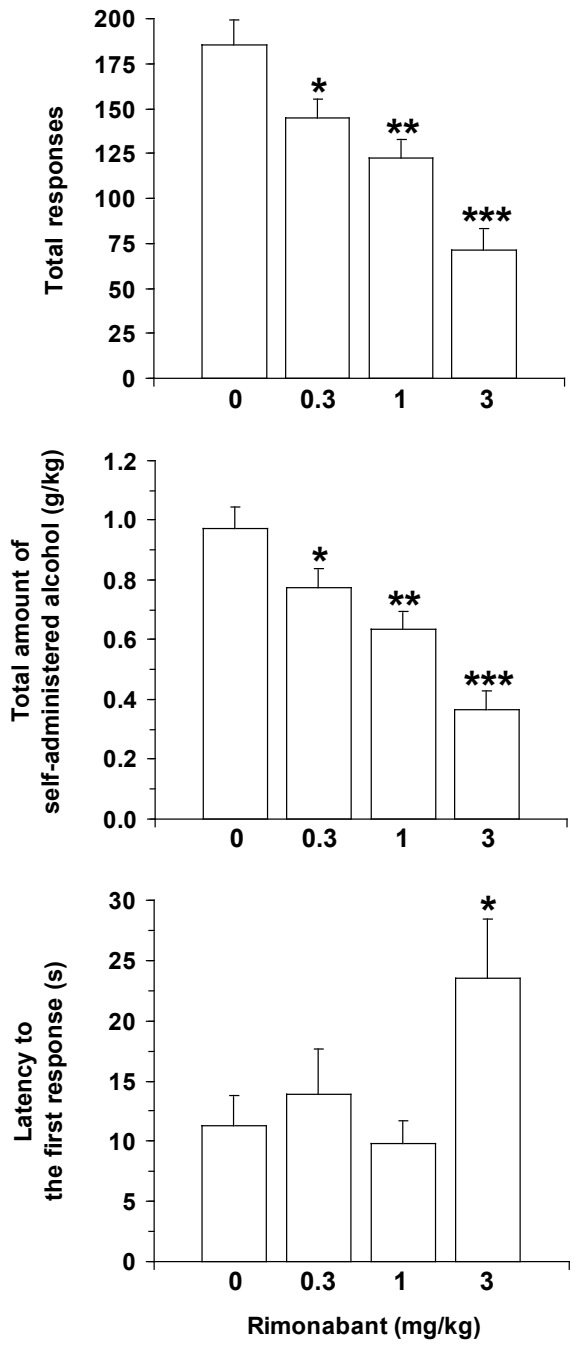

Fig. (1). Effect of pretreatment with different doses of the cannabinoid CB_1 receptor antagonist, rimonabant, on total number of responses for alcohol (top panel), total amount of self-administered alcohol (expressed in g/kg pure alcohol) (center panel), and latency to the first response (expressed in s) for alcohol (bottom panel) in selectively bred Sardinian alcohol-preferring (sP) rats trained to lever-press for oral alcohol (15%, v/v) (FR4) and water (FR1) in daily 30-min sessions. Each bar is the mean ± SEM of n=12 rats. ∗: P<0.05, ∗∗: P<0.01, and ∗∗∗: P<0.001 with respect to vehicle-treated rats (Newman-Keuls test).

Pretreatment with rimonabant significantly altered latency to the first response on the "alcohol" lever [F(3,47)=3.47, P<0.05] (Fig. **1**, bottom panel). Specifically, latency to the first response on the "alcohol" lever was more than double in the rat group treated with 3 mg/kg rimonabant than in the vehicle-treated rat group (P<0.05).

Fig. (**2**) depicts the cumulative response patterns of alcohol self-administration after pretreatment with vehicle or the three doses of rimonabant. Analysis of these data confirms that (a) only the highest dose of rimonabant (3 mg/kg) affected the very initial stages of responding for alcohol (see the inserted graph), resulting in a longer latency to the first response and a slower rate of responding over the first 1-2 min, and (b) pretreatment with rimonabant dose-dependently decreased the value at which the responding for alcohol reached its *plateau*.

Finally, responding for water was not altered by pretreatment with rimonabant [F(3,47)=1.34, P>0.05] (data not shown).

DISCUSSION

The results of the present study indicate that treatment with the prototypic cannabinoid CB_1 receptor antagonist, rimonabant, dose-dependently suppressed oral alcohol self-administration in selectively bred, alcohol-preferring sP rats. This effect occurred at low-to-moderate doses of rimonabant (0.3-3 mg/kg, i.p.), well below those that may produce changes in the rats' spontaneous locomotor activity and motor coordination [*e.g.*: 2, 34, 35], tending to exclude that the suppressing effect of rimonabant on alcohol self-administration was secondary to an impaired lever-pressing performance. These data extend to alcohol's reinforcing properties the ability of rimonabant to suppress alcohol intake, relapse-like drinking, and alcohol's motivational properties in sP rats [3, 6, 7, 14, 26].

The rimonabant-induced suppression of alcohol self-administration observed in the present study is also consonant with a number of previous lines of experimental evidence. Specifically, the results of the present study are in close agreement with data indicating the capacity of rimonabant to suppress different alcohol-related behaviors in other lines of selectively bred alcohol-preferring rats, including Alko Alcohol (AA) [22, 23], Indiana alcohol-preferring (P) [5, 18], and Warsaw High-Preferring (WHP) rats [11]. These results are also consistent with previous reports demonstrating that treatment with rimonabant dose-dependently suppressed the reinforcing properties of alcohol in rats exposed to operant procedures of oral alcohol self-administration similar to that used in the present study [16-23; see however also 24].

In the present study, pretreatment with the highest dose of rimonabant (3 mg/kg) increased the latency to the first response on the "alcohol" level and tended to decrease the response rate during the first 1-2 min of the session, suggesting that this dose of rimonabant reduced rats' motivation to start drinking alcohol. In agreement with this finding, 3 mg/kg rimonabant was found to (a) decrease the number of first-min responses on the "alcohol" lever in alcohol-preferring AA rats exposed to an operant procedure of oral alcohol self-administration [23], and (b) suppress extinction responding for alcohol (a valuable index of the motivational properties of alcohol) in sP rats [26]. Analysis of the graph depicting the cumulative response patterns of alcohol self-administration (Fig. **2**) also reveals that pretreatment with all doses of rimonabant resulted, in comparison to pretreatment with vehicle, in a lower *plateau* value of responding on the "alcohol" lever, indicating that

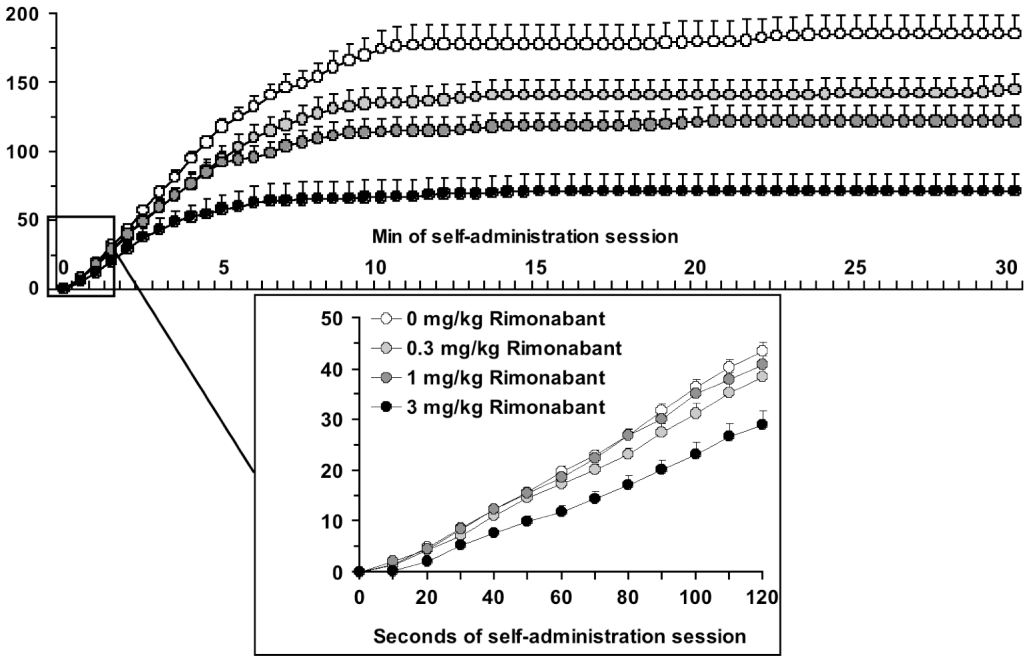

Fig. (2). Effect of pretreatment with different doses of the cannabinoid CB_1 receptor antagonist, rimonabant, on cumulative response patterns of self-administration for alcohol over the 30-min session (divided into 60 intervals of 30 s each) and the first 2 min of the session (divided into 12 intervals of 10 s each; inserted graph) in selectively bred Sardinian alcohol-preferring (sP) rats trained to lever-press for oral alcohol (15%, v/v) (FR4) and water (FR1) in daily 30-min sessions. Each point is the mean ± SEM of *n*=12 rats.

fewer ratios were completed before responding was ended. All together, these data confirm that the reinforcing properties of alcohol were attenuated in rimonabant-treated rats.

In terms of the mechanism of the suppressing action of rimonabant on alcohol self-administration, involvement of the cannabinoid CB_1 receptors located on the dopamine mesocorticolimbic neurons (the latter being the neural substrate mediating the positive reinforcing and rewarding properties of different natural stimuli and addicting drugs, including alcohol [see 36]) may be hypothesized. Accordingly, 3 mg/kg rimonabant (i.p.) suppressed alcohol-stimulated, but not basal, dopamine release in the nucleus accumbens of rats [37] and mice [38]; 1 mg/kg rimonabant (i.v.) fully prevented alcohol-stimulated firing rate of mesolimbic dopamine neurons in rats [39]. Finally, microinjections of rimonabant into the ventral tegmental area (the area where dopamine mesocorticolimbic neurons originate) or into the nucleus accumbens and the prefrontal cortex (the areas where dopamine mesocorticolimbic neurons project their axons) suppressed oral alcohol self-administration in alcohol-preferring AA rats [22, 23].

The recent discontinuation – due to the occurrence of some adverse effects – of all ongoing clinical trials on rimonabant and other cannabinoid CB_1 receptor antagonists apparently prevents the translating of the "anti-alcohol" effects of rimonabant observed in laboratory rodents to human alcoholics. The only clinical study conducted to date yielded inconclusive results, mostly because of a high response rate in the placebo group [40].

CONCLUSION

The results of the present study, demonstrating that the cannabinoid CB_1 receptor antagonist, rimonabant, dose-dependently suppressed operant, oral alcohol self-administration in alcohol-preferring sP rats, constitute a further piece of experimental evidence in support of the hypothesis that (a) the cannabinoid CB_1 receptor is part of the neural substrate mediating the reinforcing and motivational properties of alcohol, and (b) blockade of the cannabinoid CB_1 receptor is a powerful tool to suppress alcohol reinforcement and motivation.

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