Mol Neurobiol (2007) 36:102–112
DOI 10.1007/s12035-007-0017-y

The Cannabinoid CB_1 Receptor Antagonist, Rimonabant, as a Promising Pharmacotherapy for Alcohol Dependence: Preclinical Evidence

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Received: 6 November 2006 / Accepted: 24 January 2007 / Published online: 3 July 2007

Abstract Several lines of preclinical evidence indicate the ability of the prototypic cannabinoid CB_1 receptor antagonist, rimonabant, to suppress various alcohol-related behaviors, including alcohol drinking and seeking behavior and alcohol self-administration in rats and mice. Together, these data—synthetically reviewed in the present paper—suggest (a) the involvement of the cannabinoid CB_1 receptor in the neural substrate controlling alcohol intake, alcohol reinforcement, and the motivational properties of alcohol and (b) that rimonabant may constitute a new and potentially effective medication for the treatment of alcohol dependence.

Keywords Cannabinoid CB_1 receptor antagonist, rimonabant · Alcohol · Alcohol dependence (alcoholism) · Alcohol drinking and seeking behavior · Animal models of alcoholism

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Introduction

A growing body of biochemical and pharmacological evidence has established a role for the endocannabinoid system in the neurobiology of alcohol, as several alcohol effects and alcohol-related phenomena and behaviors have been found to be mediated by the brain cannabinoid CB_1 receptor [see 1–3]. For instance, pharmacological studies have invariably demonstrated that cannabinoid receptor agonists and antagonists stimulate and inhibit, respectively, alcohol intake, alcohol self-administration, and alcohol's motivational properties in rats and mice, suggesting that the cannabinoid CB_1 receptor is part of the neural circuit regulating alcohol consumption and motivation to consume alcohol.

The present paper is intended to review the data on the suppressing effects of the prototypic cannabinoid CB_1 receptor antagonist, rimonabant [4], on alcohol consumption, alcohol reinforcement, and alcohol's motivational properties in validated animal models of alcoholism. The outcome of these data features rimonabant as a potentially interesting, novel pharmacotherapy for alcohol dependence.

Effect of Rimonabant on Alcohol Intake

Alcohol intake in rodents is usually measured under the so-called two-bottle "alcohol vs water" choice regimen where a singly housed animal is exposed to two bottles containing a relatively low concentrated alcohol solution (alcohol in water, without addition of any tastant) and water, respectively. When exposed to this procedure, selectively bred alcohol-preferring rats, mice with an innate predisposition to high alcohol consumption, or rodents made dependent

upon alcohol by a previous forced exposure to alcohol (i.e., via inhalation of alcohol vapors) consume pharmacologically relevant amounts of alcohol and display preference for the alcohol solution over water.

Acquisition of Alcohol Drinking Behavior

The repeated administration of rimonabant has been reported to block the acquisition of alcohol drinking behavior in selectively bred, alcohol-naive Sardinian alcohol-preferring (sP) and Indiana alcohol-preferring (P) rats exposed to the two-bottle choice procedure. sP and P rats constitute two of the few rat lines selectively bred worldwide for high alcohol preference and consumption [see 5, 6].

In the study testing sP rats [7], rimonabant was administered twice a day for ten consecutive days at the doses of 0, 0.3, 1, and 3 mg/kg (i.p.). Alcohol (10% *v/v*, in water) was offered immediately after the first injection of rimonabant. In vehicle-treated rats, mean alcohol intake was higher than 4 g kg^{-1} day^{-1} from the first day of alcohol exposure and rose progressively to approximately 6 g/kg; over the 10-day treatment period, daily alcohol intake in vehicle-treated rats averaged approximately 4.8 g/kg. These data are indicative of a rapid disclosure and experience of the central effects of alcohol that sustain alcohol drinking behavior in sP rats. Treatment with rimonabant resulted in a dose-dependent suppression of daily alcohol intake, which lasted throughout the 10-day treatment period. Daily alcohol intake averaged 1.7, 1.8, and 0.7 g/kg in the rat groups treated with 0.3, 1, and 3 mg/kg rimonabant, respectively. Notably, in terms of specificity of the drug action on alcohol intake, a higher daily intake of water in rimonabant-treated rats fully compensated for the low alcohol intake, leaving daily total fluid intake virtually unchanged. Food intake was reduced only on the very first day of treatment with rimonabant.

Similar results have been collected in the study testing P rats [8]. Rimonabant was administered once a day for seven consecutive days at the doses of 0, 0.3, 1, and 2 mg/kg (i.p.). Alcohol (15% *v/v*, in water) was offered immediately after the first injection of rimonabant. Over the 7-day treatment period, daily alcohol intake averaged 6.4, 4.5, 2.5, and 2.2 g/kg in the rat groups treated with 0, 0.3, 1, and 2 mg/kg rimonabant, respectively.

These data suggest that the repeated blockade of the cannabinoid CB_1 receptor resulted in the virtually complete blockade of disclosure and experience of the psychopharmacological effects of alcohol on which alcohol drinking in alcohol-preferring sP and P rats is based. These data also suggest that functioning of the cannabinoid CB_1 receptor is essential for the development of high alcohol preference and consumption in these rat lines.

Additional lines of experimental evidence have supported these conclusions. Rimonabant administration reduced alcohol intake in alcohol-naive C57BL/6 mice exposed to four 6-h sessions of the two-bottle "alcohol vs water" choice regimen [9, 10] and alcohol-naive Wistar rats continuously exposed to the two-bottle "alcohol vs water" choice regimen for 30 consecutive days [11]. The repeated administration of surinabant (known also as SR147778), a cannabinoid CB_1 receptor antagonist structural analogue to rimonabant [12], suppressed the acquisition of alcohol drinking behavior in sP rats [13] and C57BL/6 mice [12]. Finally, CB_1 receptor knockout mice, tested under the two-bottle choice regimen, displayed a significantly slower rate of acquisition of alcohol drinking behavior and lower levels of daily alcohol preference and consumption in comparison to wild-type mice [14–18; see, however, 19]. Together, these data suggest that blockade of the cannabinoid CB_1 receptor—either pharmacologically or by genetic inactivation—resulted in the prevention of acquisition of high alcohol drinking behavior.

Maintenance of Alcohol Drinking Behavior

Different studies investigated the effect of rimonabant administration on alcohol intake in alcohol-experienced rats and mice, i.e., animals in which alcohol preference and consumption were already consolidated before the start of the treatment with rimonabant. These rats may represent a model of the "active" phase of human alcohol dependence. When administered acutely, rimonabant dose-dependently reduced alcohol intake in sP rats [20], P rats [8], C57BL/6J mice [15], and CD1 mice [18] exposed to the two-bottle choice regimen. In the study with sP rats [20], the well-documented anorectic effect of rimonabant [see 21–23] was limited, restricting the availability of food pellets to the 4 h of the drinking session; this restriction augmented the appetitive value of food and revealed the specificity of the rimonabant action on alcohol intake.

A decrease in alcohol intake was also recorded in the studies testing the repeated, rather than single, administration of rimonabant in alcohol-experienced C57BL/6J mice exposed to the two-bottle choice regimen [24]. Conversely, when administered chronically to Wistar rats during a series of forced alcohol exposures (via inhalation of alcohol vapors) and alcohol withdrawals, 10 mg/kg rimonabant (i.p.) increased, rather than decreased, daily alcohol intake and preference in a subsequent rimonabant-free two-bottle choice phase [25], suggesting that the previous history of alcohol exposure may have some impact on the effect of rimonabant.

This laboratory recently investigated the effect of the repeated administration of rimonabant on alcohol intake in sP rats. In this experiment, rats were given continuous

access to alcohol (10% *v/v*, in water) and water under the two-bottle choice regimen, with unlimited access to food pellets. Rimonabant was administered intraperitoneally, once a day and for 21 consecutive days, at the doses of 0, 1, 3, and 10 mg/kg. Alcohol, water, and food intake were recorded daily. Treatment with rimonabant produced a dose-dependent decrease in daily alcohol intake (Fig. 1, top panel); over the first 4 days, daily alcohol intake in the rat groups treated with 1, 3, and 10 mg/kg rimonabant was approximately 20, 35, and 60% lower, respectively, than that recorded in the control, vehicle-treated rat group. On continuing treatment, tolerance to the reducing effect of rimonabant on daily alcohol intake tended to develop: Post hoc analysis revealed that the reducing effect of 1, 3, and 10 mg/kg rimonabant on daily alcohol intake vanished after 5, 7, and 19 days of treatment, respectively. Water intake was unaffected by treatment with rimonabant (Fig. 1, center panel). Conversely, and in close agreement with a number of previous data [see 23], treatment with rimonabant resulted in a dose-dependent reduction in daily food intake (Fig. 1, bottom panel); this effect was, however, limited to the very first days of treatment, undergoing a rather rapid development of tolerance.

Together, the above results are suggestive of the ability of rimonabant, administered acutely or repeatedly, to reduce alcohol intake in different animal models of excessive alcohol drinking in which alcohol intake is already consolidated. A recent study [26] reported that chronic treatment with surinabant suppressed alcohol intake and preference in Wistar rats made dependent upon alcohol via inhalation of alcohol vapors. Together, these data provide further support for the involvement of the cannabinoid CB_1 receptor in the neural substrate controlling alcohol preference and consumption in rats and mice.

Effect of Rimonabant on Alcohol Self-Administration

Although widely employed in studies where alcohol intake is pharmacologically manipulated, the two-bottle choice procedure does not allow the motivation of the experimental animal to consume alcohol to be quantified; indeed, this procedure does not require the animal to do any "work" or make an effort to gain access to alcohol, as it merely has to lick the alcohol solution from the bottle spout. Alternative experimental procedures have been conceived to collect a more detailed analysis of alcohol ingestive behaviors, not limited to its *consummatory* aspects but examining also the *motivational* aspects. These procedures may also provide a better understanding of the possible anti-alcohol properties of a given pharmacological agent. As an example, rats and mice can be initiated, in an operant procedure, to press a lever—for a given number of times—to consume alcohol; besides information on the effect of the tested drug on the amount of alcohol consumed, this procedure allows investigation of its effect on the reinforcing properties of alcohol, i.e., the capability of alcohol to direct and maintain that specific behavior.

Different studies have investigated the effect of rimonabant on oral alcohol self-administration in rats. Apart from very few exceptions, the results of these studies were consonant in suggesting that the acute intraperitoneal administration of doses of rimonabant in the 1- to 10-mg/kg range dose-dependently suppressed alcohol self-administration in rats.

The first study addressing the effect of rimonabant on alcohol self-administration used Long Evans rats tested under the recently developed sipper-tube procedure [27]. Specifically, rats were required to perform a given number of responses on the lever (namely, 16) to gain access—for 20 consecutive minutes—to a sipper tube containing the alcohol solution. This procedure was theoretically expected to separate the appetitive and consummatory phases of alcohol self-administration. Acute treatment with 1 or 3 mg/kg rimonabant resulted in a decrease in the probability of completion of the response requirement (i.e., the 16 lever presses to gain access to alcohol) as well as a decrease in the amount of alcohol consumed when it was finally available. When tested in an independent group of rats self-administering a sucrose solution (included in the experimental design as an alternative reinforcer for evaluation of the specificity of rimonabant action on alcohol self-administration), rimonabant did not affect the probability of response requirement completion (appetitive phase), whereas it reduced the amount of self-administered sucrose (consummatory aspects). The relatively low value of the response requirement for access to alcohol rendered somewhat questionable the evaluation of the effect of rimonabant on the appetitive, or motivational, properties of alcohol that have been perhaps more properly investigated in other studies (see below).

Subsequent studies investigated the effect of rimonabant on alcohol self-administration in rats exposed to the more conventional procedure where a fixed number of responses on the lever (varying between one and five, depending upon the different studies) was needed to gain access to a limited amount of alcohol solution (0.1 ml of a 10–15% alcohol solution in water); rats were allowed to repeat this behavior several times in daily sessions of 30–60 min. These studies unanimously reported that the acute administration of rimonabant dose-dependently suppressed lever responding for alcohol and the corresponding amount of self-administered alcohol in selectively bred alcohol-preferring AA [28] and P [29] rats as well as in unselected Wistar rats [30–33]. As an example, administration of 1, 3, and 10 mg/kg rimonabant (i.p.) to AA rats was associated

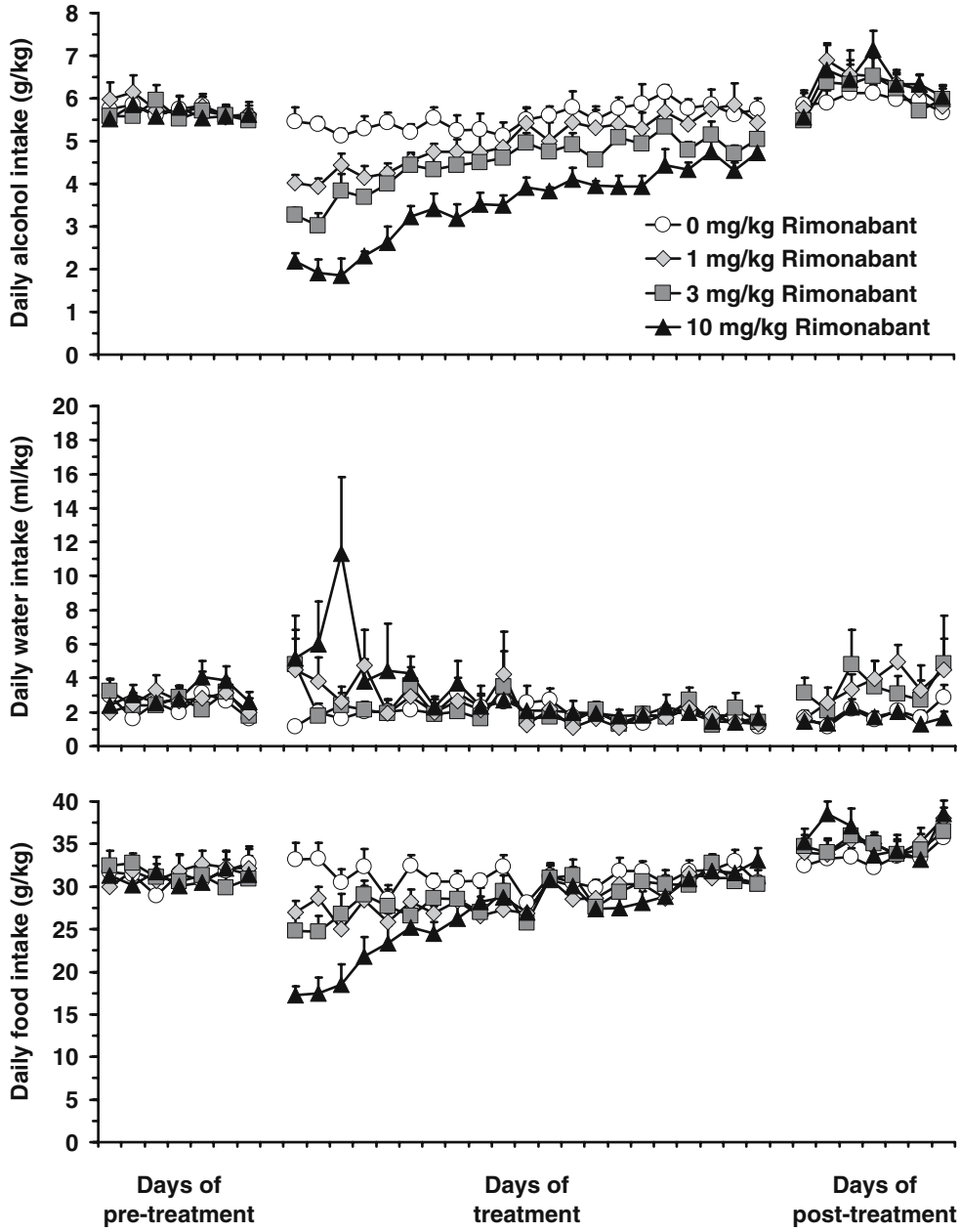

Fig. 1 Effect of the cannabinoid CB_1 receptor antagonist, rimonabant, on daily alcohol (*top panel*), water (*center panel*), and food (*bottom panel*) intake in Sardinian alcohol-preferring (sP) rats. Rats were males belonging to the 64th generation of the sP line. Rats were 9–12 months old, and their body weight averaged approximately 600 g. Rats were individually housed under standard environmental conditions. Rats were extensively habituated to handling and i.p. injection. Starting from the age of 75 days, rats were exposed to the standard, home cage two-bottle "alcohol (10% *v*/*v*, in water) vs water" choice regimen, with unlimited access for 24 h/day (alcohol-experienced rats). The left–right position of the alcohol and water bottle was interchanged at random to avoid development of position preference. Standard food pellets were available ad lib. Alcohol, water, and food intake was monitored by weighing the bottles and food pellets once daily (30–60 min before the start of the dark phase). Rats were divided into four groups (n=7–8), matched for alcohol, water, and food intake, as well as body weight, on the last 7 days preceding the start of rimonabant administration (pre-treatment period). Rimonabant (Sanofi-Aventis, Montpellier, France) was suspended in 1 ml/kg saline with 0.1% Tween 80 and administered intraperitoneally, 15 min before the start of the dark phase, once a day and for 21 consecutive days (treatment period). Recording of daily alcohol, water, and food intake was performed for an additional 7 days after termination of treatment with rimonabant (post-treatment period). Each point is the mean±SEM of n=7–8. Results of the two-way analysis of variance (ANOVA) on alcohol intake data during the treatment period: [$F_{dose}(3,27)=12.10$, $P<0.0001$; $F_{day}(20,540)=28.47$, $P<0.0001$; $F_{interaction}(60,540)=2.46$, $P<0.0001$]; results of the two-way ANOVA on water intake data during the treatment period: [$F_{dose}(3,27)=1.06$, $P>0.05$; $F_{day}(20,540)=3.61$, $P<0.0001$; $F_{interaction}(60,540)=1.69$, $P<0.01$]; results of the two-way ANOVA on food intake data during the treatment period: [$F_{dose}(3,27)=3.01$, $P<0.05$; $F_{day}(20,540)=14.22$, $P<0.0001$; $F_{interaction}(60,540)=4.37$, $P<0.0001$]

with a reduction in the number of responses on the "alcohol" lever of approximately 35, 75, and 90%, respectively, in comparison to vehicle-dosed rats (Fig. 2) [28].

When rimonabant (2 mg/kg, i.p.) was given repeatedly (for four consecutive daily self-administration sessions) to P rats, some degree of tolerance developed to its suppressing effect on responding for alcohol, as rimonabant proved to be no longer effective in the third and fourth sessions [29]. The latter results replicate those collected in the experiment testing the effect of the repeated administration of rimonabant on alcohol intake in sP rats exposed to the two-bottle choice regimen where a clear development of tolerance to rimonabant-induced reduction in daily alcohol intake was observed (Fig. 1).

At variance with the above results, a recent study by Ginsburg and Lamb [34] found that acutely administered rimonabant (0.3–5.6 mg/kg, i.p.) did not significantly reduce responding for alcohol in Lewis rats. In addition to several procedural differences, it has been proposed that the discrepancy between these results [34] and those of the previous reports [28–33] could be secondary to factors such as rat age and the length of the period of alcohol exposure, as (a) the study by Ginsburg and Lamb [34] apparently used rats that were older and exposed to alcohol at greater length than those in previous studies and (b) both aging and lengthy exposure to alcohol have been reported to desensitize cannabinoid receptors [15, 35, 36], reducing the intrinsic activity of rimonabant and its behavioral effects. However, in partial disagreement with this hypothesis, it should be noted that the above study demonstrating the efficacy of rimonabant in reducing daily alcohol intake in sP rats, depicted in Fig. 1, was conducted with relatively old rats (approximately 9–12 months) and exposed to alcohol for a relatively long period (approximately 6–9 months) before the start of the experiment.

When investigated, the specificity of the rimonabant action on alcohol self-administration was relatively limited, as rimonabant administration reduced, with comparable efficacy, the responding for an alternative reinforcer (sucrose or saccharin solutions) in a series of parallel experiments [30, 32]. These results are in agreement with the hypothesis that the cannabinoid CB_1 receptor may mediate the reinforcing properties of alcohol as well as those of other hedonic ingesta, including palatable solutions (e.g., sucrose and saccharin solutions, chocolate-flavored beverages) [27, 37].

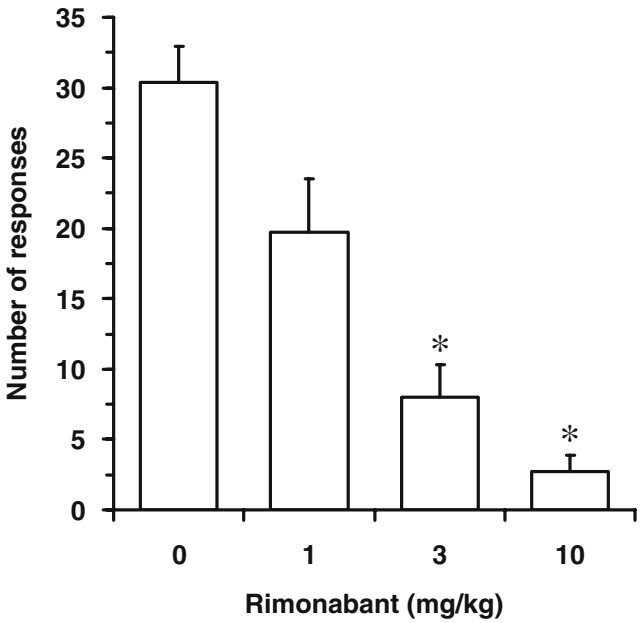

Fig. 2 Effect of the cannabinoid CB_1 receptor antagonist, rimonabant, on alcohol self-administration in alcohol-preferring AA rats. Rats were trained to lever-press for oral 10% (*w*/*v*, in water) alcohol under a fixed ratio (FR) schedule of FR1 in daily 30-min sessions. Rimonabant was injected i.p. 30 min before the start of the self-administration session. All doses of rimonabant were tested in each rat under a Latin-square design. Each bar is the means±SEM of $n=12$. *$P<0.001$ with respect to vehicle-treated rats. Reprinted from [28], with permission by Macmillan Publisher Ltd

Effect of Rimonabant on Alcohol's Motivational Properties

The effect of rimonabant, and more recently those of surinabant, on alcohol's motivational properties (or appetitive strength) have been investigated using two validated experimental procedures: the breakpoint and the extinction responding [38]. Both procedures require initial training of the rat to self-administer alcohol under an operant paradigm. In the breakpoint procedure, the response requirement needed to gain access to alcohol is increased progressively during the session (or over successive sessions) until the rat fails to complete the required number of responses; breakpoint is defined as the highest response requirement achieved by the rat. In the extinction responding procedure, responding is never reinforced during the test session irrespective of the number of responses made by the rat; extinction responding is defined as the total number of responses emitted by the rat.

Administration of rimonabant (0.3–3 mg/kg, i.p.) dose-dependently suppressed breakpoint for an alcoholic beer (4.5% alcohol, *v*/*v*) in Wistar rats trained on a lick-based progressive ratio paradigm (specifically, rats were required to make a progressively increasing number of licks on the bottle spout for a fixed amount of beer) [39]. Reduction in breakpoint for alcoholic beer averaged approximately 30, 75, and 90% after administration of 0.3, 1, and 3 mg/kg rimonabant, respectively (Fig. 3). This effect was relatively specific, as rimonabant reduced to a much lower extent the breakpoint of a nonalcoholic beer in a separate group of rats

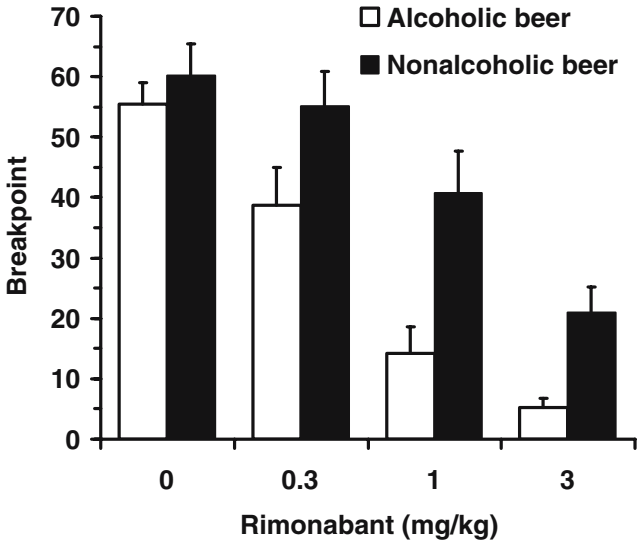

Fig. 3 Effect of the cannabinoid CB_1 receptor antagonist, rimonabant, on breakpoint for an alcoholic beer and a nonalcoholic beer in Wistar rats. Rats were trained on a lick-based progressive ratio paradigm (specifically, rats were required to make a progressively increasing number of licks on the bottle spout for a fixed amount of fluid) in daily 60-min sessions. Breakpoint was defined as the highest response requirement completed by each rat. Alcohol content in alcoholic beer and nonalcoholic beer was equal to 4.5% (*v*/*v*) and <0.5% (*v*/*v*), respectively. Rimonabant was injected i.p. 15 min before the start of the breakpoint session. All doses of rimonabant were tested in each rat under a Latin-square design. Each bar is the mean±SEM of $n=12$. Reprinted from [39], with permission by Springer

(Fig. 3). A subsequent experiment tested the effect of rimonabant on breakpoint for a plain alcohol solution (10% *v*/*v*, in water) in Wistar rats [32]. Doses of rimonabant, 0.3 and 3 mg/kg, dose-dependently, although not specifically, reduced the breakpoint for alcohol up to 40% at the dose of 3 mg/kg in comparison to vehicle-treated rats.

When tested in the extinction responding procedure, acutely administered rimonabant (0.3–3 mg/kg, i.p.) dose-dependently suppressed extinction responding for alcohol (15% *v*/*v*, in water) in alcohol-preferring sP rats [40]: The total number of responses on the lever in the rat groups treated with 0.3, 1, and 3 mg/kg rimonabant was reduced approximately 30, 60, and 90%, respectively, than that recorded in vehicle-treated rats (Fig. 4, top panel). Notably, in the rat group treated with the highest dose of rimonabant (3 mg/kg), 4/7 rats completely avoided performing any response on the lever (0/7 in the vehicle-treated group). Conversely, rimonabant did not significantly affect extinction responding for a 3% (*w*/*v*, in water) sucrose solution in a separate group of rats (Fig. 4, bottom panel), suggesting that the reducing effect of rimonabant was specific for alcohol's motivational properties. Similar results have been collected in a subsequent experiment testing surinabant [13] such that the acute administration of surinabant (0.3–3 mg/kg, i.p.) dose-dependently suppressed extinction responding for alcohol without altering extinction responding for sucrose.

These results suggest that blockade of the cannabinoid CB_1 receptor may decrease the motivational attributes of alcohol. Considering the role of craving in the development of alcohol dependence [see 41], the above results—featuring the suppressing effect of rimonabant on alcohol's

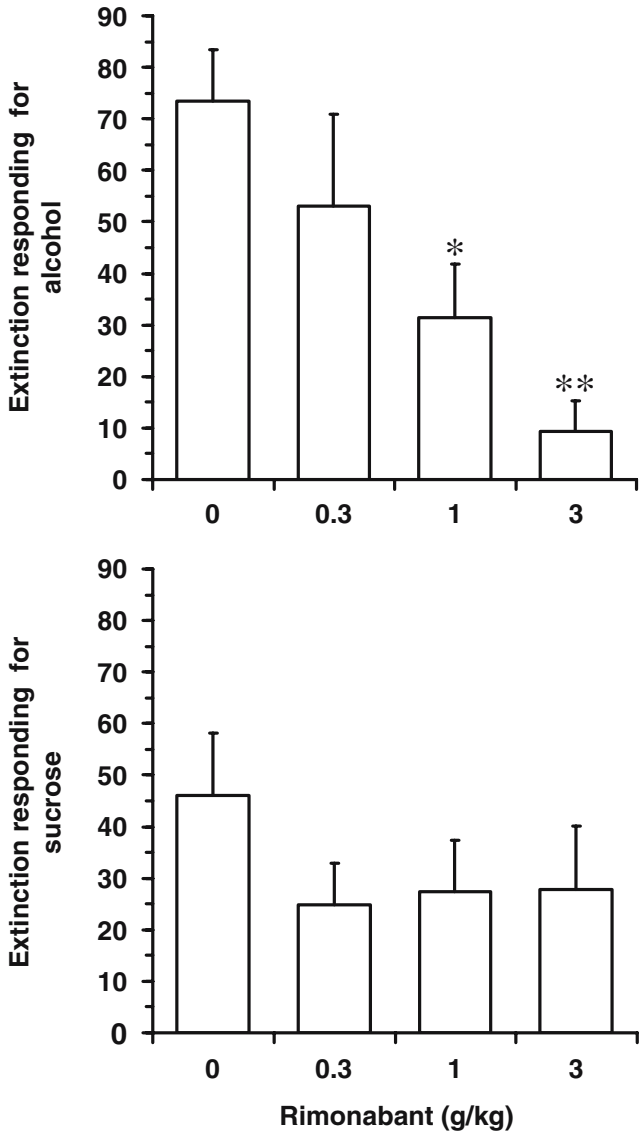

Fig. 4 Effect of the cannabinoid CB_1 receptor antagonist, rimonabant, on extinction responding for alcohol (*top panel*) or sucrose (*bottom panel*) in Sardinian alcohol-preferring (sP) rats. Rats were trained to lever-press for oral 15% (*v*/*v*, in water) alcohol or 3% (*w*/*v*, in water) sucrose under a fixed ratio (FR) schedule of FR4 in daily 30-min sessions. Extinction responding was defined as the total number of lever responses performed by each rat in the absence of alcohol or sucrose reinforcement. Extinction sessions were performed once self-administration behavior stabilized. Rimonabant was injected i.p. 20 min before the start of the extinction session. All doses of rimonabant were tested in each rat under a Latin-square design. Each bar is the mean±SEM of $n=7$ in the "alcohol" group and $n=8$ in the "sucrose" group. *$P<0.05$ and **$P<0.005$ with respect to vehicle-treated rats. Reprinted from [40], with permission by Elsevier

motivational properties—constitute a promising aspect for the clinical usefulness of the drug.

Effect of Rimonabant in Models of Alcohol Relapse

Two major experimental procedures have been proposed and validated for modeling relapse episodes that often occur in human alcoholics: the alcohol deprivation effect and the reinstatement of alcohol seeking behavior [see 42, 43]. The alcohol deprivation effect is defined as the temporary increase in voluntary alcohol intake occurring in different animal species after a period of forced abstinence, or deprivation, from alcohol. In rodents, the alcohol deprivation effect can be observed in individuals consuming alcohol under the home cage two-bottle choice regimen or exposed to operant self-administration conditions.

In the reinstatement model, rats or mice are first trained to lever-press for alcohol under standard operant procedures. Once the self-administration behavior is stable, animals are exposed to (a) a number of non-reinforced, extinction sessions until operant responding is virtually extinguished (between-session procedure) or (b) a single non-reinforced extinction session (within-session procedure). In both cases, once the animal reaches given criteria for unresponsiveness (e.g., less than 12 lever responses/session in the between-session procedure [44]; less than five lever responses in the last 10 min of the session in the within-session procedure [45]), specific stimuli (including a small drop of alcohol, an olfactory or visual stimulus previously associated to alcohol availability, or exposure to stress) are presented. The latter are considered to reinstate alcohol-seeking behavior if they promote some renewed degree of lever responding (still non-reinforced).

Notably, both the alcohol deprivation effect and the reinstatement of alcohol seeking behavior have been found to be suppressed in rats by drugs (e.g., naltrexone [46–49]) known to be effective in reducing the likelihood of relapses in alcoholics [see 50], providing evidence of the predictive value of these two procedures for the human disease.

Acute administration of rimonabant suppressed the alcohol deprivation effect in both sP [51] and P [8] rats. In the first study, sP rats were initially allowed to consume alcohol, under the two-bottle choice regimen with unlimited access, for four consecutive weeks; subsequently, rats underwent a 2-week alcohol deprivation period. Rimonabant (0.3, 1, and 3 mg/kg, i.p.) was administered 30 min before the subsequent alcohol representation. Over the first hour of re-access to alcohol (the choice of this time limit was based on the observation that it corresponds to the period during which the alcohol deprivation effect is maximal in sP rats [see 6]), vehicle-treated rats displayed an increase in alcohol intake of approximately 100% with respect to vehicle-treated alcohol-non-deprived rats, indicative of the development of the alcohol deprivation effect. Rimonabant, at the highest dose (3 mg/kg), resulted in a virtually complete suppression of this extra consumption of alcohol, with alcohol intake in rimonabant-treated rats approaching that of alcohol-non-deprived rats (Fig. 5). These results have been closely replicated in a subsequent experiment testing surinabant (0.3–3 mg/kg, i.p.) under an identical procedure in sP rats [13].

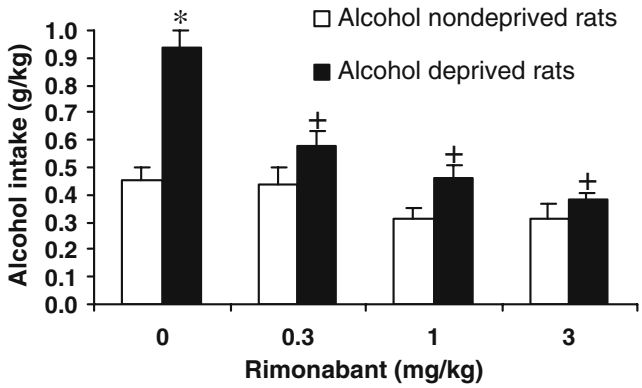

Fig. 5 Effect of the cannabinoid CB_1 receptor antagonist, rimonabant, on alcohol deprivation effect in Sardinian alcohol-preferring (sP) rats. Alcohol-deprived rats were initially allowed to consume alcohol (10% *v*/*v*, in water) and water under the two-bottle choice regimen with unlimited access for four consecutive weeks and then deprived of alcohol for 15 consecutive days; conversely, alcohol-non-deprived rats had continuous access to alcohol and water. Thirty minutes before the subsequent alcohol representation (coinciding with lights off), rats of both groups were injected intraperitoneally with rimonabant. Alcohol intake was recorded 60 min after lights off. Each bar is the mean± SEM of n=12–13. *P<0.05 with respect to vehicle-treated alcohol-non-deprived rats; +P<0.05 with respect to vehicle-treated alcohol-deprived rats. Reprinted from [51], with permission by Elsevier

P rats display a longer lasting alcohol deprivation effect than that observed in sP rats, as alcohol intake in alcohol-deprived P rats is still significantly elevated 24 or 48 h after re-access to alcohol [see 5]. In the study testing rimonabant [8], rats were initially exposed to a prolonged, continuous access to alcohol under the two-bottle choice regimen; subsequently, the alcohol bottle was removed for 2 weeks. Rimonabant was administered immediately before the subsequent alcohol representation. Over the first 24 h, alcohol intake in alcohol-deprived rats increased from 5.7 g/kg (baseline value) to 12.6 g/kg; administration of rimonabant suppressed this increase in daily alcohol intake, as rats treated with 0.3, 1, and 2 mg/kg rimonabant (i.p.) consumed 9.7, 5.7, and 5.5 g/kg alcohol, respectively.

In the "reinstatement" studies [30, 32], rimonabant, acutely administered at the doses of 0.3, 1, and 3 mg/kg (i.p.) to Wistar rats, dose-dependently reduced reinstatement of alcohol-seeking behavior elicited by presentation of olfactory and visual stimuli (namely, orange odor and flashing of the chamber houselight) previously associated

with alcohol availability, but not by exposure to foot-shock stress.

Because of the aforementioned predictive validity of the alcohol deprivation effect and the reinstatement of alcohol-seeking behavior as experimental models of alcohol relapses, the results of these studies suggest that rimonabant might possess some efficacy in preventing relapses in human alcoholics. These data also extend to alcohol the ability of rimonabant to prevent relapse-like behaviors in rats exposed to different drugs of abuse, including cocaine and heroin [see 52].

Finally, a recent study [29] investigated the effect of rimonabant on Pavlovian spontaneous recovery, an additional paradigm of relapse-like drinking. Specifically, P rats self-administered alcohol, under a standard operant procedure, in daily 1-h sessions for 10 weeks. Subsequently, rats underwent extinction (lever pressing was not reinforced) for seven sessions, followed by a 2-week stay in their home cages. When returned to the operant chamber, vehicle-treated rats displayed robust responding on the alcohol-associated lever, indicative of renewed seeking for alcohol; contrarily, pretreatment with rimonabant (0.3, 1, and 2 mg/kg, i.p.) dose-dependently suppressed—down to baseline extinction values—lever responding.

Effect of the Combination of Rimonabant and Opioid Receptor Antagonists on Alcohol-Related Behaviors

Several research data suggest the existence of functional links between the actions of opioids and cannabinoids [see 53, 54]; this interaction apparently extends also to the control of different alcohol-related behaviors [see 55]. As an example, the stimulatory effect of the CB_1 receptor agonists, Δ^9-tetrahydrocannabinol, WIN 55,212-2, and CP 55,940, on alcohol intake and breakpoint for alcohol were blocked by pre-administration of rimonabant or naloxone [56, 57]; equally, the boosting effect of morphine on alcohol intake was blocked not only by naloxone but also by rimonabant [58]. Therefore, the action of morphine was permitted by the simultaneous activation of the CB_1 receptor by endocannabinoids, and, conversely, activation of opioid receptors by endogenous opioids was required for the effects of the cannabinoids.

Our laboratory recently conducted a series of experiments evaluating the effect of a rimonabant and opioid receptor antagonist combination on alcohol drinking and alcohol's motivational properties in rats. Specifically, combination of subeffective doses of rimonabant and either naloxone or naltrexone resulted in (a) delayed acquisition of alcohol drinking behavior by alcohol-naive sP rats [3], (b) reduced alcohol intake in alcohol-experienced sP rats [3], (c) a reduction of the alcohol deprivation effect in sP rats [59]. Accordingly, Gallate et al. [60] reported that a rimonabant and opioid receptor antagonist combination reduced breakpoint for an alcoholic beer in Wistar rats trained to lick from the bottle spout under an operant procedure. These results indicate that combination of rimonabant *plus* an opioid receptor antagonist resulted in a synergistic reduction of different aspects of alcohol drinking and alcohol's motivational properties in rats. The possible generalization of these results to human alcoholics would suggest a novel therapeutic strategy, theoretically characterized by a higher efficacy and fewer and less pronounced side effects than each drug therapy alone.

Possible Mechanism of Action

The mesolimbic dopamine neurons, originating in the ventral tegmental area and projecting their axons to the nucleus accumbens, have been repeatedly proposed as the neural substrate mediating the positively reinforcing and rewarding properties of different natural stimuli and addicting drugs, including alcohol [see 61]. Specifically, low to moderate doses of alcohol stimulate the firing rate of dopamine neurons in the ventral tegmental area and dopamine release in the nucleus accumbens in rats (the latter effect is produced also by voluntarily consumed or self-administered alcohol) [see 61]. Cannabinoid CB_1 receptors expressed on excitatory glutamatergic and inhibitory GABA terminals impinging on ventral tegmental area dopamine neurons have been hypothesized to be one of the primary sites of the anti-alcohol actions of rimonabant. These afferents control mesolimbic dopamine neurons via a balance of excitatory and inhibitory presynaptic inputs [see 62, 63].

Recent microdialysis experiments demonstrated that 3 mg/kg rimonabant (i.p.) [a dose repeatedly reported to inhibit alcohol intake, alcohol reinforcement, and alcohol's motivational properties (see above)] suppressed alcohol-stimulated, but not basal, dopamine release in the nucleus accumbens of rats [64] and mice [14]; further, alcohol-induced stimulation of dopamine release in the nucleus accumbens was completely absent in CB_1 receptor knockout mice [14]. Accordingly, rimonabant (1 mg/kg, i.v.) fully prevented alcohol-stimulated firing rate of mesolimbic dopamine neurons in rats [65]. Additionally, rimonabant abolished alcohol-induced inhibition of GABAergic medium spiny neurons in the nucleus accumbens, one of the major GABAergic afferents to dopamine neurons [63]. These results led to the hypothesis that rimonabant would antagonize alcohol-induced, endocannabinoid-mediated alterations in the GABAergic vs glutamatergic balance controlling mesolimbic dopamine neurons. Blocking the alcohol-induced inhibition of GABAergic neuron firing rate or removing the inhibitory cannabinoidergic tone on their

terminals would result in the observed suppression of alcohol-stimulated dopamine release and, in turn, dopamine-mediated alcohol-reinforced and -motivated behaviors [62–65].

It has been proposed that the suppressing effect of rimonabant on alcohol relapse-like drinking (see above) might be secondary to the blockade of cannabinoid CB_1 receptors located on glutamatergic neurons in brain areas (e.g., hippocampus and basolateral amygdala) involved in memory processes related to drug reward effects [see 63].

The mechanisms responsible for the synergistic action of rimonabant and naloxone (or naltrexone) on alcohol intake and alcohol's motivational properties are not fully known. However, one mechanism, at least in part, includes the mesolimbic dopamine system, as administration of opioid receptor antagonists, similar to cannabinoid receptor antagonists (see above), blunted alcohol-stimulated dopamine release in the rat nucleus accumbens [see 61].

Conclusions

The results of the studies conducted to date with rimonabant and, when tested, surinabant convincingly suggest the involvement of the cannabinoid CB_1 receptor in the neural substrate controlling different aspects of alcohol drinking behavior, alcohol reinforcement, and alcohol's motivational properties in validated animal models of alcoholism. With very few exceptions, acute or repeated administration of rimonabant suppressed voluntary alcohol intake, relapse-like drinking, alcohol self-administration, and motivation to consume alcohol in rats and mice.

Rimonabant is currently under clinical investigation to understand whether the promising data collected in rodents may be translated to humans. Specifically, a phase II study funded by the US National Institute on Alcohol Abuse and Alcoholism is presently assessing the effect of rimonabant on alcohol consumption in non-treatment seeking individuals who drink heavily (20–40 alcohol drinks per week) (http://www.clinicaltrials.gov). According to a double-blind design, individuals receive either placebo or rimonabant for 2 weeks before being tested in an alcohol self-administration experiment where they receive a priming dose of alcohol (designed to raise the breath alcohol levels to 0.03 g/dl), and then have the opportunity to consume up to eight alcohol drinks or to receive a monetary reward for each drink not consumed over a 2-h period. The working hypothesis of this study is that participants receiving rimonabant will decrease alcohol consumption when compared to those receiving placebo.

Should the results of this study be positive, and extended by the subsequent phase III surveys, rimonabant might constitute a new strategy for treating alcohol dependence.

Acknowledgements The authors are grateful to Dr. Marco Pistis for helpful discussions and Ms. Anne Farmer for language editing of the manuscript.

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