Effects of the Cannabinoid CB1 Receptor Antagonist Rimonabant in Models of Emotional Reactivity in Rodents

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Background: *The endocannabinoid system has been implicated in the modulation of emotional processes.*
Methods: *These experiments aimed to investigate the effects of the cannabinoid CB1 receptor antagonist rimonabant (SR141716) in animal models measuring aspects of emotional reactivity and depression.*
Results: *Rimonabant had weak anxiolytic-like activity in the elevated plus-maze and failed to affect flight and risk assessment activities in the mouse defense test battery (MDTB). It produced clear anxiolytic-like effects in the Vogel conflict test (.3–3 mg/kg intraperitoneal [i.p.]) and on defensive aggression in the MDTB (1 and 10 mg/kg, i.p.). The effects of rimonabant in the MDTB paralleled those observed with CB1 receptor knockout mice in this procedure. In the forced-swimming test in rats and the tonic immobility paradigm in gerbils, rimonabant (3 and 10 mg/kg per os [p.o.]) produced antidepressant-like effects that were comparable to those observed with the reference antidepressant, fluoxetine. In the chronic mild stress model in mice, repeated administration of rimonabant (10 mg/kg, p.o.) for 5 weeks improved the deleterious effects produced by stress.*
Conclusions: *These findings point further to a role for the endocannabinoid system in the modulation of emotional processes and suggest that it may be primarily involved in the adaptive responses to unavoidable stressful stimuli.*

Key Words: Animal models, anxiety, CB1 receptor, depression, rimonabant, rodents

The involvement of the endocannabinoid system in controlling emotional behavior and mood is poorly understood. The behavioral effects of endocannabinoids are currently believed to be mediated through the CB1 receptor (Chaperon and Thiébot 1999), which is densely expressed in brain areas controlling motor, cognitive, sensory, and emotional processes, such as the limbic system and the paraventricular nucleus of the hypothalamus (Tsou et al 1998). This has prompted speculation as to a potential role of endocannabinoids in the control of mood and emotional processes.

Acute administration of cannabinoids may cause anxiogenic responses in humans (e.g., Hall and Solowij 1998). Moreover, Δ9-THC, as well as endogenous cannabinoids and synthetic CB1 receptor agonists (e.g., Arévalo et al 2001; Onaivi et al 1990), have been widely reported to enhance anxiety-related behaviors in rodent models. There are, however, a few reports of the opposite effects of these compounds, and the picture is even less clear with compounds that block the CB1 receptor, as illustrated by findings with the potent and selective CB1 receptor antagonist rimonabant (SR141716) in anxiety and depression models. The drug was found to display anxiolytic- or antidepressant-like effects (Akinshola et al 1999; Haller et al 2002; Rodgers et al 2003; Tzavara et al 2003), whereas others have reported a lack of activity or even an anxiogenic-like profile of the compound (e.g., Arévalo et al 2001; McGregor et al 1996; Navarro et al 1997; Rodriguez de Fonseca et al 1996). Experiments with knockout mice deficient in the CB1 receptor have shown that they display anxiogenic- and depressive-like phenotypes (Haller et al 2002; Maccarrone et al 2002; Martin et al 2002). As pointed out by Rodgers and colleagues (2003), studies that have investigated the anxiety-modulating action of rimonabant or the phenotype of CB1 knockout mice have adopted a rather limited behavioral approach, using mainly exploration-based models (e.g., elevated plus-maze) and their standard spatiotemporal measures (e.g., time and entries into the open arms of the elevated plus-maze). These authors explained that by employing more ethologic-oriented techniques or tests based not only on exploration activity, it is possible to better characterize the effects of CB1 receptor blockade on emotional processes.

These experiments aimed to investigate the effects of rimonabant in several animal models of anxiety in rats, the Vogel punished drinking and elevated plus-maze tests, and in the mouse defense test battery (MDTB). The behavioral profile of rimonabant in the MDTB was compared with that displayed by CB1 receptor knockout mice in this procedure. In addition, the antidepressant-like potential of rimonabant was evaluated in the forced-swimming test in rats, the chronic mild stress procedure in mice, and the tonic immobility paradigm in gerbils.

Materials and Methods

Ethics

All experimental procedures described here were approved by the Animal Care and Use Committee of Sanofi-Aventis and fully complied with French legislation on research involving animal subjects.

Animals

Male Sprague–Dawley or Wistar rats (Iffa Credo, L'Arbresle, and Charles River, Saint-Aubin-lès-Elbeuf, France) were used. They were housed in groups of four (punished drinking and elevated plus-maze) or seven (forced-swimming). Male Long Evans rats (400–500 g; Iffa Credo) were used as the threat stimulus in the MDTB. Male Mongolian gerbils (*Meriones unguiculatus*; Janvier, Le Genest St-Isle, France) weighing 50–70 g were used in the tonic immobility paradigm. They were housed 5–6 per cage. Ten-week-old singly housed male OF1 mice (Iffa Credo), cannabinoid knockout mice (CB1–/–) and their wildtype littermates were used in the MDTB. The homozygous CB1–/– and CB1+/+ mice were from a

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C57BL/6x129/Ola F2 genetic background and generated as described previously (Robbe et al 2002). Singly housed male BALB/cByJIco mice (Iffa Credo) weighing 20–27 g at arrival were used in the chronic mild stress (CMS). The knockout animals could not be used to parallel pharmacologic findings with rimonabant in this model because preliminary experiments have shown that C57BL/6x129/Ola mice were not suitable in this test as they developed some resistance to chronic mild stress. All animals were maintained under standard laboratory conditions (21°–24°C) and kept on a 12-hr light–dark cycle with light onset at 6 AM.

Drugs

The drugs used were rimonabant, diazepam, and fluoxetine (synthesized by Sanofi-Aventis). Compounds were prepared as solutions (fluoxetine) or suspensions in physiologic saline or distilled water containing Tween 80 (.1%) (rimonabant and diazepam). Drugs administered intraperitoneally (i.p.) or per os (p.o.) were given in a constant volume of 5 (rats) or 20 (mice and gerbils) mL/kg.

Punished Drinking Test in Rats

The procedure was a modification of the technique described by Vogel et al (1971). At the beginning of the experiment, Sprague–Dawley rats (190–235 g), deprived of water but not of food for 48 hours prior to testing, were placed in cages (32 × 25 × 30 cm) with a stainless steel grid floor. Each cage was placed in sound-attenuated boxes that were well ventilated and contained a drinking tube connected to an external 50-mL burette filled with tap water. Trials were started only after the animal's tongue entered in contact with the drinking tube for the first time. An electric shock (.6 mA/500 msec) was delivered to the tongue after every 20 licks. The number of shocks was recorded automatically during a 5-min period. Data were modified using a square-root transformation, analyzed with one-way analysis of variance (ANOVA), and comparisons between treatment groups and control were carried out using the Dunnett *t* test. The transformation was required for analysis because of the nonhomogeneity of the variances. Experiments were performed 30 min after i.p. injection of the drugs.

Elevated Plus-Maze Test in Rats

The apparatus is based on that described by Pellow et al (1985). The maze was elevated to a height of 70 cm with two open (50 × 10 cm) and two enclosed arms (50 × 10 × 50 cm), arranged so that the arms of the same type were opposite each other, connected by an open central area (10 × 10 cm). Experiments were performed under dim light conditions. At the beginning of the experiment, maze-naïve nonhandled Sprague–Dawley rats (180–200 g) were placed in the center of the maze, facing one of the enclosed arms, and observed for 4 min. The apparatus was equipped with infrared beams and sensors capable of measuring time spent in open arms and number of open- and closed-arm entries (defined as entry of all four limbs into an arm of the maze). In addition, rats were observed via video-link by an observer located in an adjacent room. This allowed the recording of a more ethologically orientated measure, namely, attempt at entry into open arms followed by avoidance responses. Data were modified using a square-root transformation, then analyzed with one-way ANOVA. Subsequent comparisons between treatment groups and control were carried out using the Dunnett *t* test. Experiments were performed 60 min after p.o. administration of the drugs.

Mouse Defense Test Battery

The test was conducted in an oval runway as described previously (Griebel et al 1997). 1) Pretest: Subjects were placed into the runway for a 3-min. familiarization period, in which line crossings were recorded. 2) Rat avoidance test: After this period, a handheld dead rat (killed by CO_2 inhalation) was introduced at the opposite end of the apparatus and brought up to the subject. If the mouse fled, avoidance distance was recorded. 3) Chase–flight test: The handheld rat was brought up to the subject. Chase was initiated only when the subject was at a standstill and completed when the subject had traveled a distance of 15 m. During the chase, the number of stops (pause in movement) was recorded. 4) Straight alley: By the closing of two doors (60 cm from each other), the runway was then converted to a straight alley in which the subject was constrained. The rat was introduced in one end of the straight alley. For 30 sec, the number of approaches and withdrawals (subject had to move more than 20 cm forward from the closed door, then return to it) was recorded. 5) Forced contact: The experimenter brought the rat up to contact the subject in the straight alley. For each such contact, upright postures and bites by the subjects were noted. Data were analyzed by one-way ANOVA, followed by a Dunnett *t* test (drug experiment) or Student *t* test (CB1–/– experiment).

Forced-Swimming Test in Rats

The procedure was a modification of the technique described by Porsolt et al (1977). Wistar rats (260–300 g) were placed in individual glass cylinders (40 cm in height and 17 cm in diameter) containing water (water depth was 30 cm; 23 ± 1°C). Two swimming sessions were conducted (an initial 15-min pretest followed 24 hours later by a 6-min test). The total duration of immobility was scored continuously for a 5-min period manually by an experimenter unaware of the drug treatment. Rimonabant and fluoxetine were administered p.o. twice (15 min after the first session on day 1, and 60 min before session 2 on day 2). This administration schedule is optimal for revealing drug effects (Griebel et al 2002). Data were analyzed by one-way ANOVA followed by a Dunnett *t* test.

Chronic Mild Stress in Mice

This test is based on the procedure originally designed by Willner et al (1992) for rats and has been described in detail in a previous paper (Griebel et al 2002). The CMS protocol consists of the sequential application of a variety of mild stressors, including restraint, forced swimming, water deprivation, and pairing with another stressed animal in a schedule that lasts for 3 weeks and is repeated thereafter. Chronic mild stress produces a decrease in grooming, which leads to a degradation of the physical state of the coat, consisting of a loss of fur and dirty fur. Thus, we measured physical state once a week over the entire CMS period, which lasted 7 weeks. Results were expressed as an average of 2-week blocks and were analyzed by a 2-way ANOVA (treatment × week) with repeated measures followed by the Newman–Keuls post hoc test. At the end of the CMS procedure, mice were tested in the 1) the elevated plus-maze to assess the impact of CMS on anxiety-like behaviors (anxiety was evaluated because individuals with major depressive episodes frequently present with anxiety and phobias; Cloninger 1990) and 2) the forced-swimming test to measure despair and coping in an aversive situation. The administration of rimonabant (10 mg/kg, p.o., once a day) started 2 weeks after the beginning of the CMS and lasted until all tests were completed (week 7). The forced-swimming test and the elevated plus-maze were performed 29 and 30 days after the

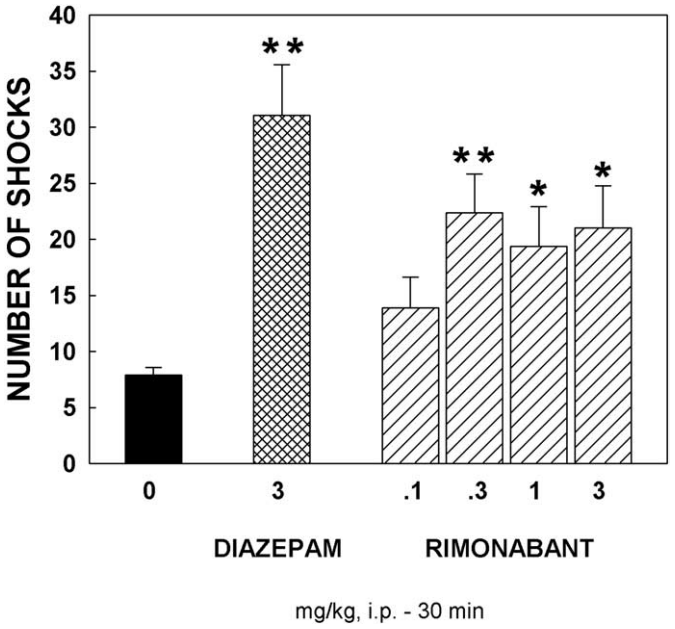

Figure 1. Anxiolytic-like effects of rimonabant and diazepam in the punished drinking conflict test in rats. Data represent mean ± SEM. *$p < .05$; **$p < .01$ (Dunnett *t* test). $n = 20$. i.p., intraperitoneal injection.

beginning of the treatments, respectively. Swim stress test experience did not alter subsequent behavior in the elevated plus-maze as shown in previous CMS experiments. Data from these latter experiments were analyzed by one-way ANOVA followed by a Dunnett *t* test.

Tonic Immobility in Gerbils

The test is based on that described by Simiand et al (2003). To induce tonic immobility, animals (6–9 per group) were held on a flat surface and were firmly pinched for 15 sec at the scruff of the neck using the thumb and the index finger. They were then placed on parallel bars (4 mm in diameter, 28 cm long, spaced 5 cm apart and having a 3 cm difference in height). The front paws were placed gently on the upper bar (situated at 43 cm above the base) and the hind paws on the lower bar. The duration of tonic immobility was measured in five successive trials with a 30-sec intertrial interval. Each trial ended when an animal started to move or after 90 sec of immobility. Data were analyzed by one-way ANOVA followed by a Dunnett *t* test. Experiments were performed 30 (fluoxetine) or 60 (rimonabant) min after i.p. or p.o. administration of the drugs.

Results

Punished Drinking Test in Rats

Rimonabant [$F(5,114) = 5.55$, $p < .001$] significantly increased punished responding from .3 mg/kg and diazepam produced maximal effects at 3 mg/kg (Figure 1).

Elevated Plus-Maze Test in Rats

Rimonabant increased significantly the percentage of time spent in open arms [$F(4,64) = 8.96$, $p < .001$] and decreased the number of aborted attempts [$F(4,64) = 8.86$, $p < .001$] at 10 mg/kg (Figure 2). Diazepam significantly increased the percentage of time spent and entries made into open arms and reduced attempts at 3 mg/kg. Finally, neither drug significantly affected the number of closed-arm entries, a reliable measure of motor activity [$F(4,64) = .46$, $p = .76$].

Mouse Defense Test Battery

Effects of Rimonabant. 1) Pretest: neither rimonabant [$F(4,40) = 2.06$, $p = .1$] nor diazepam [$F(3,40) = .77$, $p = .51$] significantly altered line crossings (Table 1). 2) Rat avoidance test: diazepam [$F(3,32) = 8.59$, $p < .001$], but not rimonabant [$F(4,36) = 2.49$, $p = .05$], significantly decreased avoidance

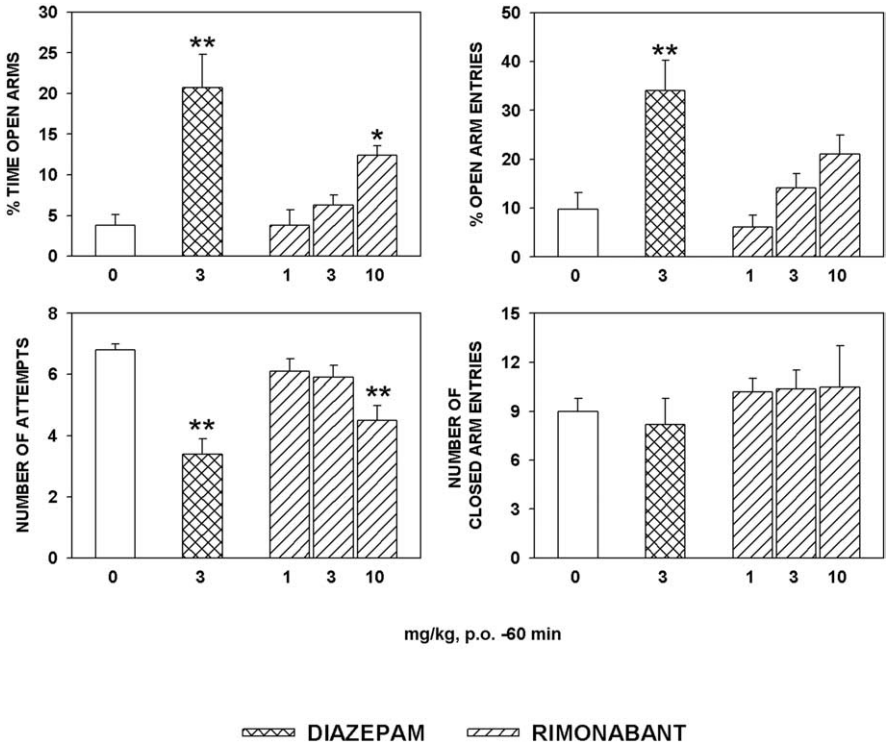

Figure 2. Anxiolytic-like effects of rimonabant and diazepam in the elevated plus-maze test in rats as measured by spatiotemporal (closed and open-arm activities) and risk assessement (attempts) parameters. Data represent mean ± SEM. *$p < .05$; **$p < .01$ (Dunnett *t* test). n = 13–14. p.o., oral administration.

Table 1. Effects of Rimonabant, Diazepam, and CB1 Receptor Deletion on Several Behavioral Responses Displayed by Mice Before (Line Crossings) and During (Avoidance Distance, Number of Stops, and A/W) Exposure to a Long Evans rat in the Mouse Defense Test Battery

	Dose (mg/kg)	Number of Line Crossings	Avoidance Distance (cm)	Number of Stops	Number of A/W
Diazepam	0	150.7 ± 5.7	102.9 ± 12.7	8.9 ± .3	.5 ± .3
	1	135.5 ± 9.3	107.9 ± 9.9	6.1 ± .7[b]	1 ± .3
	3	143 ± 9.9	59.7 ± 14.1[a]	2.7 ± .3[b]	2.8 ± .4[a]
	10	134.4 ± 8.7	21.7 ± 1.3[b]	2.8 ± .3[b]	3.2 ± .6
Rimonabant	0	121.3 ± 10.5	130.2 ± 14.4	6.8 ± .9	.3 ± .2
	.3	129 ± 6.1	114.3 ± 11.8	6.7 ± .9	.1 ± .1
	1	109.8 ± 15.6	132.3 ± 9.2	6.6 ± .7	.7 ± .4
	3	127.2 ± 13.4	144.9 ± 10.7	5.6 ± 1	.6 ± .4
	10	87.4 ± 11.8	92.7 ± 8.1	5.2 ± .3	.6 ± .3
$CB1^{+/+}$		103.5 ± 4.5	152.8 ± 17.9	8.5 ± .8	0 ± 0
$CB1^{-/-}$		121.3 ± 5[a]	143.2 ± 4.3	7.3 ± .4	0 ± 0

Data represent mean ± SEM. Rimonabant and diazepam were administered intraperitoneally or orally, 30 or 60 min before testing, respectively. $n = 6–11$.

A/W = approaches followed by avoidances responses.

[a] $p < .05$.

[b] $p < .01$ (Dunnett's t test).

behavior at 3 and 10 mg/kg (Table 1). 3) Chase–flight test: when mice were chased by the rat, only diazepam significantly decreased stops at all doses [diazepam: $F(3,40) = 5.9$, $p < .001$; rimonabant: $F(4,44) = .8$, $p = .52$] (Table 1). 4) Straight alley: when subjects were constrained in a straight alley, diazepam [$F(3,40) = 11.75$, $p < .001$], but not rimonabant [$F(4,40) = .57$, $p = .68$], increased the number of approaches towards the rat followed by avoidance responses at 3 mg/kg. 5) Forced contact: upon forced contact with the rat, both drugs significantly decreased defensive threat and attack reactions [rimonabant: upright postures, $F(4,40) = 7.43$, $p < .001$; bites, $F(4,40) = 7.71$, $p < .001$; diazepam: upright postures, $F(3,40) = 38.29$, $p < .001$; bites, $F(3,40) = 48.43$, $p < .001$; Figure 3).

Effects of CB1 Receptor Deletion

1) Pretest: CB1–/– mice showed increased locomotor activity compared with their wildtype littermates ($t = 7.01$, $p < .05$; Table 1). 2) Rat avoidance test: Flight behavior was not significantly affected by CB1 receptor deletion ($t = .27$, $p = .61$; Table 1). 3) Chase–flight test and 4) Straight alley: when CB1–/– and CB1+/+ mice were chased by the handheld rat or constrained in a straight alley, they displayed comparable risk assessment activities (stops: $t = 1.78$, $p = .21$; approaches and withdrawals: no such behaviors were observed in either genotype; Table 1). 5) Forced contact: defensive threat and attack behavior was reduced in CB1–/– mice compared with CB1+/+ mice, but only biting reached statistical significance (biting: $t = 5.16$, $p < .05$; upright postures: $t = 3.37$, $p = .09$; Figure 3).

Forced-Swimming Test in Rats

Analysis of variance indicated significant effects with rimonabant [$F(3,23) = 3.37$, $p < .05$] and fluoxetine [$F(3,23) = 3.43$, $p < .05$]. Dunnett analysis showed that rimonabant significantly decreased immobility time at 3 and 10 mg/kg, whereas fluoxetine produced such effects at 30 mg/kg (Figure 4).

Chronic Mild Stress in Mice

There was a significant degradation in the physical state of the coat of mice because of stress [$F(3,120) = 135.8$, $p < .0001$; Figure 5A], which was significantly improved by rimonabant (10 mg/kg) following 2 weeks of treatment, an effect that lasted until the CMS was completed. Moreover, the percentage of open-arm time in the elevated plus-maze was reduced in chronically stressed mice compared with nonstressed control animals [$F(2,39) = 6.79$, $p < .003$; Figure 5C]. Rimonabant reversed these anxiogenic-like effects of stress. Furthermore, chronically stressed control mice showed greater immobility in the forced-swimming test than nonstressed control animals [$F(2,40) = 4.3$, $p < .02$]. This behavior was not seen after the administration of

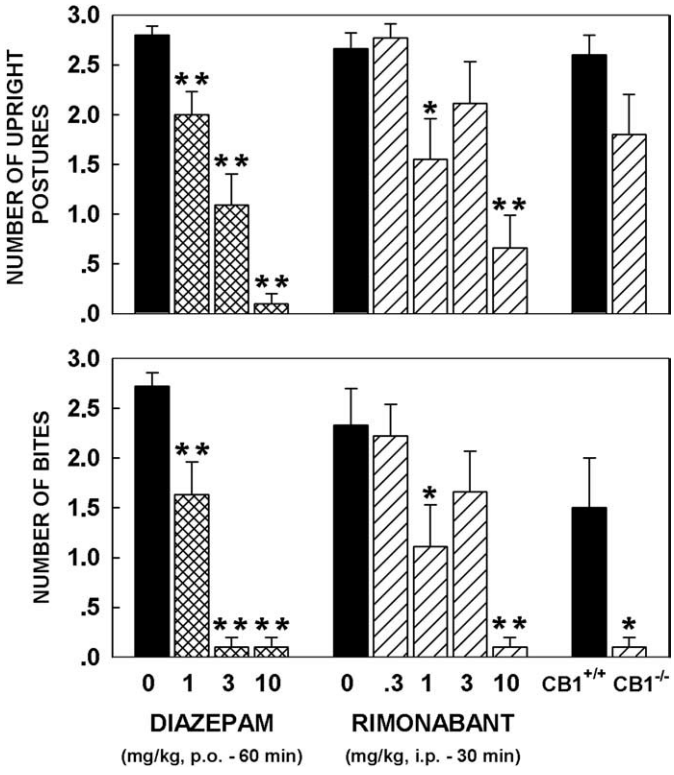

Figure 3. Anxiolytic-like effects of rimonabant, diazepam, and CB1 receptor deletion on defensive threat and attack reactions upon forced contact with a handheld Long Evans rat in the mouse defense test battery. Data represent mean ± SEM. * $p < .05$; ** $p < .01$ (Dunnett t test). $n = 6–11$. i.p., intraperitoneal injection; p.o., oral administration.

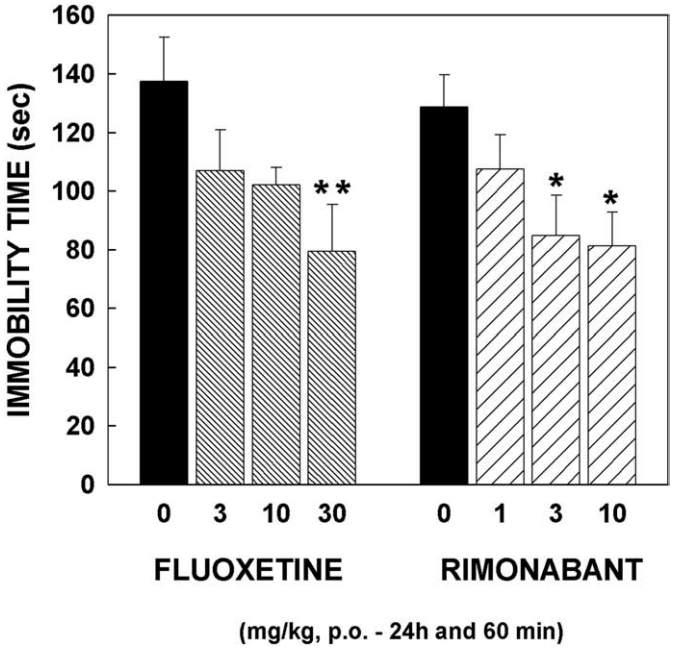

Figure 4. Antidepressant-like effects of rimonabant and fluoxetine in the forced-swimming test in rats. Data represent mean ± SEM. *$p < .05$; **$p < .01$ (Dunnett *t* test). n = 7.

rimonabant where levels of immobility reached those of nonstressed control subjects (Figure 5B).

Tonic Immobility in Gerbils

Rimonabant [$F(3,28) = 10.67$, $p < .001$] significantly decreased the duration of tonic immobility at 10 mg/kg, whereas fluoxetine [$F(3,27) = 21.58$, $p < .001$] produced similar effects at 7.5 and 15 mg/kg (Figure 6).

Discussion

The results of this study reveal that the potent and selective CB1 receptor antagonist rimonabant displayed a behavioral profile in rodents, which is consistent with an anxiolytic- and antidepressant-like action.

This is the first report on the behavioral action of a CB1 receptor antagonist in a traditional conflict procedure. Rimonabant produced an increase in rates of responding suppressed by punishment. It is unlikely that the positive effects of rimonabant in the punished drinking test are due to decreased sensitivity to electric shocks because compounds that are endowed with analgesic properties are inactive in conflict tests (Griebel et al 2002; Treit 1985). Moreover, a number of studies have clearly shown that rimonabant had no effect on the baseline sensitivity to pain stimuli in a variety of animal models, such as the hotplate and tail-flick paradigms or using chemical irritants (for review, see Iversen 1999).

Most previous studies with rimonabant in models of anxiety using exploratory-based procedures showed that the drug produced anxiogenic-like activity in these tests (Arévalo et al 2001; Navarro et al 1997). These findings were strengthened by studies using CB1–/– mice, which showed that the deletion of the CB1 receptor gene leads to anxiety-like behaviors as measured in the elevated plus-maze and open-field tests (Haller et al 2004; Maccarrone et al 2002; Martin et al 2002). A few reports exist, however, of an opposite (i.e., anxiolytic-like) action of rimonabant in exploration models of anxiety (Akinshola et al 1999; Haller et al 2002; Rodgers et al 2003). Our experiment with the elevated plus-maze showed that rimonabant elicited positive effects on some but not all the behavioral measures of anxiety. Thus, it increased percentage of time spent in open arms, but failed to modify significantly the percentage of open-arm entries, unlike diazepam, which affected clearly both parameters. On the ethologically derived measure, rimonabant decreased risk assessment, but the magnitude of the effects of rimonabant was less than that of diazepam, suggesting weaker anxiolytic-like activity of the CB1 receptor antagonist. The discrepant results between the earlier and the more recent studies on the effects of rimonabant in exploration-based anxiety models cannot be attributed to general test conditions or inappropriate dose range of the CB1 receptor antagonist because the doses used in all the studies overlap. Rodgers and colleagues (2003) found that rimonabant

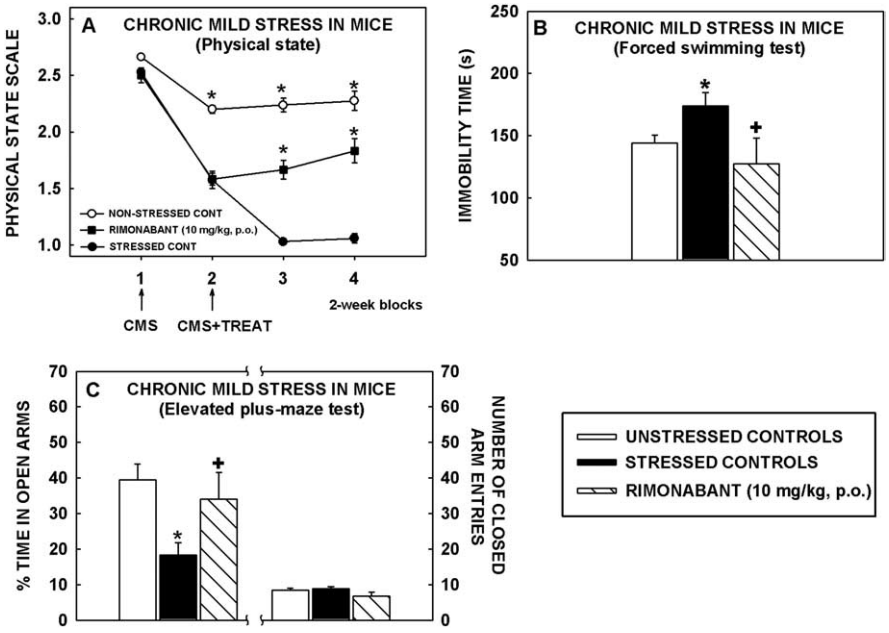

Figure 5. Effects of repeated administration of rimonabant for 5 weeks on chronic mild stress-induced **(A)** degradation of the physical state of the coat of animals; **(B)** despair behavior in the forced-swimming test; and **(C)** anxiogenic-like behavior in the elevated plus-maze test. Data represent mean ± SEM. + $p < .05$ (vs. stressed mice); *$p < .05$ (vs. nonstressed mice, Newman–Keuls or Dunnett). $n = 6–20$. p.o., oral administration.

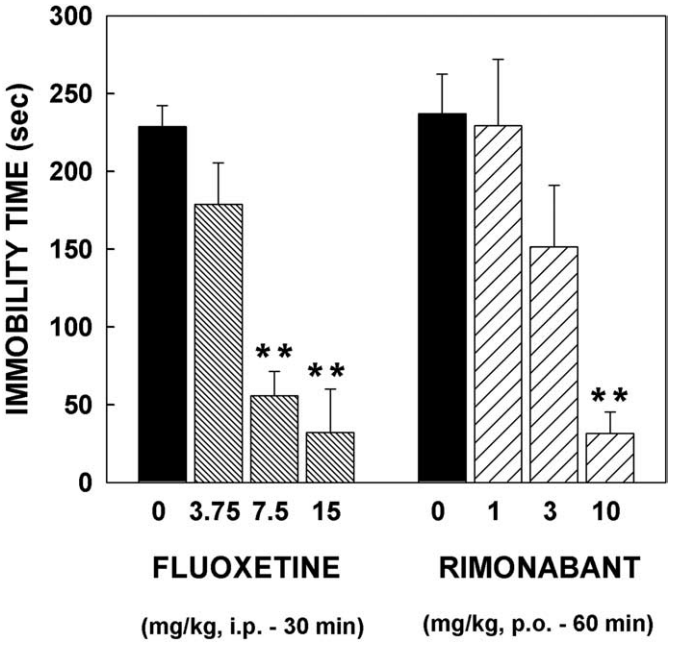

Figure 6. Antidepressant-like effects of rimonabant and fluoxetine in the tonic immobility paradigm in gerbils. To induce tonic immobility, animals were held on a flat surface and were firmly pinched for 15 sec at the scruff of the neck using the thumb and the index finger. Data represent mean ± SEM. $^{**}p < .01$ (Dunnett *t* test). $n = 6–9$. i.p., intraperitoneal injection; p.o., oral administration.

produced anxiolytic-like activity only in elevated plus-maze-experienced animals, underlining the importance of basal emotional reactivity when investigating the effects of the CB1 receptor antagonist. Prior exposure to the plus-maze has been shown to further enhance open-arm avoidance, suggesting an increase in basal anxiety levels (e.g., Griebel et al 1993; Holmes and Rodgers 1998, 2001; Holmes et al 2003). In our plus-maze experiment, baseline levels of anxiety were higher than in the studies in which rimonabant was inactive or displayed anxiogenic-like effects. The reason for these differences in baseline levels is unclear, but it is possible that the use of different rat strains or illumination conditions may account for this variability, because both aspects have been shown to have a major impact in the behavioral performances of rodents in the elevated plus-maze (Hogg 1996). It is possible that rimonabant is active only in strains that exhibit a clear anxiogenic-like behavior in the elevated plus-maze, as is the case here. The context dependency of the behavioral effects of rimonabant is indirectly supported by findings showing that disruption of the CB1 receptor affected elevated plus-maze behavior only under high light (i.e., anxiogenic) conditions (Haller et al 2004).

The findings obtained in the MDTB further support the idea that baseline levels of stress are important when investigating the behavioral actions of rimonabant. The drug had no effect on flight and risk assessment behaviors, which occurred in the phases during which escape from the oncoming rat was possible. In contrast, the CB1 antagonist displayed significant effects on contact with the rat as was shown by the action on defensive upright posture and biting. The forced contact test has been suggested to be particularly stressful for animals since they have no possibility to escape and confrontation with the threat stimulus is unavoidable. Although the behavioral profile displayed by rimonabant in the MDTB is consistent with an anxiolytic-like action, the drug was inactive on responses that include cognitive aspects of defensive behaviors (e.g., risk assessment). In contrast, it appeared to be as effective as diazepam on defensive aggression, a more affective-orientated defense behavior. Findings obtained with CB1–/– mice in the MDTB fit well with the effects of rimonabant in this procedure. Similar to the acute blockade of CB1 receptors by rimonabant, the permanent deletion of the CB1 receptor gene led to a profile of reduced defensiveness, which was limited to terminal defense reactions, suggesting that the CB1 receptor may play an important role in the expression of this particular set of behaviors.

The profile of rimonabant on emotionality was confirmed in the forced-swimming test in rats. Here, the CB1 receptor antagonist produced antidepressant-like activity, a result that agrees with the antidepressant-like activity reported recently with rimonabant in murine models of depression (Tzavara et al 2003). The potential of rimonabant on depressive-like behaviors was confirmed in the tonic immobility paradigm in gerbils and in the CMS model in mice. In the former, the drug counteracted a state of temporary motor inhibition observed when animals were grasped by the scruff of the neck and placed on two horizontal elevated parallel bars. Tonic immobility has been suggested to mimic freezing behavior observed in natural situations, representing an adaptive reaction to danger that is selectively reversed by antidepressants (Simiand et al 2003). In the CMS, repeated administration of rimonabant improved the degradation of the physical state of the coat of stressed animals. This finding suggests that the CB1 receptor antagonist normalized grooming, an activity impaired by repeated stress. CMS caused the appearance of an "anxious" profile as was evidenced by the findings from the elevated plus-maze. This behavioral change was not seen in animals treated with rimonabant, indicating that the drug was able to counteract the stress-induced increase in anxiety levels. In the forced-swimming test, stressed mice displayed a greater tendency toward despair behavior than nonstressed animals and those treated by rimonabant. The drug is therefore able to restore a normal coping response when animals are exposed to inescapable aversive stimuli.

The mechanisms underlying the anxiolytic- and antidepressant-like effects of rimonabant remain to be determined. A key component in the action of clinically effective antidepressants is their ability to increase levels of 5-HT, dopamine, and norepinephrine in the prefrontal cortex, an effect that has been related to their beneficial therapeutic action (Tanda et al 1994). In experiments using brain microdialysis, rimonabant was reported to produce elevations in dopamine and norepinephrine levels in the prefrontal cortex, effects that were similar in terms of magnitude and time course of the effects to those of the aforementioned compounds (Tzavara et al 2003). Moreover, in this latter study the CB1 receptor antagonist increased 5-HT efflux in the prefrontal cortex. It can therefore be hypothesized that rimonabant exerts its effects on emotional processes via the blockade of CB1 receptors localized on presynaptic axon terminals, thereby leading to a stimulation of major monoamine neurotransmitter systems, in particular, in the prefrontal cortex. Alternatively, the effects of rimonabant in stress models may involve CB1 receptors in the central amygdala and the paraventricular nucleus. In these structures, CB1 receptors have been shown to influence the HPA axis, through stimulation of neurons containing corticotropin releasing factor (Rodriguez de Fonseca et al 1991, 1995). In line with this are findings showing that rimonabant was able to prevent Δ9-THC-induced elevations in corticotropin and corticosterone in rats (Manzanares et al 1999;

Murphy et al 1998). Finally, evidence indicates the existence of a novel cannabinoid receptor in the brain (Wilson and Nicoll 2002). This receptor was suggested to be the target site of the anxiolytic-like action of rimonabant because the drug was reported to reduce anxiety responses in the elevated plus-maze in CB1–/– mice (Haller et al 2002).

In conclusion, our findings suggest that rimonabant may a have beneficial effect on emotional behavior, strengthening further the therapeutic value of this CB1 receptor antagonist, which was shown to have a strong potential for the treatment of obesity (Ravinet-Trillou et al 2003) and as an aid for smoking cessation within the same range of doses (Cohen et al 2002).

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Akinshola BE, Chakrabarti A, Onaivi ES (1999): In-vitro and in-vivo action of cannabinoids. *Neurochem Res* 24:1233–1240.

Arévalo C, De Miguel R, Hernandez-Tristan R (2001): Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters. *Pharmacol Biochem Behav* 70:123–131.

Chaperon F, Thiébot MH (1999): Behavioral effects of cannabinoid agents in animals. *Crit Rev Neurobiol* 13:243–281.

Cloninger CR (1990): Comorbidity of anxiety and depression. *J Clin Psychopharmacol* 10(suppl 3):43S–46S.

Cohen C, Perrault G, Voltz C, Steinberg R, Soubrié P (2002): SR141716, a central cannabinoid (CB1) receptor antagonist, blocks the motivational and dopamine-releasing effects of nicotine in rats. *Behav Pharmacol* 13:451–463.

Griebel G, Moreau J-L, Jenck F, Martin JR, Misslin R (1993): Some critical determinants of the behaviour of rats in the elevated plus-maze. *Behav Process* 29:37–47.

Griebel G, Sanger DJ, Perrault G (1997): Genetic differences in the mouse defense test battery. *Aggress Behav* 23:19–31.

Griebel G, Simiand J, Steinberg R, Jung M, Gully D, Roger P, et al (2002): 4-(2-Chloro-4-methoxy-5-methylphenyl)-N-[(1S)-2-cyclopropyl-1-(3-fluoro-4-me thylphenyl)ethyl]5-methyl-N-(2-propynyl)-1,3-thiazol-2-amine hydrochloride (SSR125543A), a potent and selective corticotrophin-releasing factor1 receptor antagonist. II. Characterization in rodent models of stress-related disorders. *J Pharmacol Exp Ther* 301:333–345.

Hall W, Solowij N (1998): Adverse effects of cannabis. *Lancet* 352:1611–1616.

Haller J, Bakos N, Szirmay M, Ledent C, Freund TF (2002): The effects of genetic and pharmacological blockade of the CB1 cannabinoid receptor on anxiety. *Eur J Neurosci* 16:1395–1398.

Haller J, Varga B, Ledent C, Barna I, Freund TF (2004): Context-dependent effects of CB1 cannabinoid gene disruption on anxiety-like and social behaviour in mice. *Eur J Neurosci* 19:1906–1912.

Hogg S (1996): A review of the validity and variability of the elevated plus-maze as an animal model of anxiety. *Pharmacol Biochem Behav* 54:21–30.

Holmes A, Iles JP, Mayell SJ, Rodgers RJ (2001): Prior test experience compromises the anxiolytic efficacy of chlordiazepoxide in the mouse light/dark exploration test. *Behav Brain Res* 122:159–167.

Holmes A, Rodgers RJ (1998): Responses of Swiss-Webster mice to repeated plus-maze experience: Further evidence for a qualitative shift in emotional state? *Pharmacol Biochem Behav* 60:473–488.

Holmes A, Rodgers RJ (2003): Prior exposure to the elevated plus-maze sensitizes mice to the acute behavioral effects of fluoxetine and phenelzine. *Eur J Pharmacol* 459:221–230.

Iversen L (1999): High times for cannabis research. *Proc Natl Acad Sci U S A* 96:5338–5339.

Maccarrone M, Valverde O, Barbaccia ML, Castane A, Maldonado R, Ledent C, et al (2002): Age-related changes of anandamide metabolism in CB1 cannabinoid receptor knockout mice: Correlation with behaviour. *Eur J Neurosci* 15:1178–1186.

Manzanares J, Corchero J, Fuentes JA (1999): Opioid and cannabinoid receptor-mediated regulation of the increase in adrenocorticotropin hormone and corticosterone plasma concentrations induced by central administration of Δ9-tetrahydrocannabinol in rats. *Brain Res* 839:173–179.

Martin M, Ledent C, Parmentier M, Maldonado R, Valverde O (2002): Involvement of CB1 cannabinoid receptors in emotional behaviour. *Psychopharmacology* 159:379–387.

McGregor IS, Dastur FN, McLellan RA, Brown RE (1996): Cannabinoid modulation of rat pup ultrasonic vocalizations. *Eur J Pharmacol* 313:43–49.

Murphy LL, Munoz RM, Adrian BA, Villanua MA (1998): Function of cannabinoid receptors in the neuroendocrine regulation of hormone secretion. *Neurobiol Dis* 5:432–446.

Navarro M, Hernandez E, Munoz RM, delArco I, Villanua MA, Carrera MRA, et al (1997): Acute administration of the CB1 cannabinoid receptor antagonist SR 141716A induces anxiety-like responses in the rat. *Neuroreport* 8:491–496.

Onaivi ES, Green MR, Martin BR (1990): Pharmacological characterization of cannabinoids in the elevated plus maze. *J Pharmacol Exp Ther* 253:1002–1009.

Pellow S, Chopin P, File SE, Briley M (1985): Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. *J Neurosci Methods* 14:149–167.

Porsolt RD, Le Pichon M, Jalfre M (1977): Depression: A new animal model sensitive to antidepressant treatments. *Nature* 266:730–732.

Ravinet-Trillou C, Arnone M, Delgorge C, Gonalons N, Keane P, Maffrand JP, et al (2003): Anti-obesity effect of SR141716, a CB1 receptor antagonist, in diet-induced obese mice. *Am J Physiol Regul Integr Comp Physiol* 284: R345–R353.

Robbe D, Kopf M, Remaury A, Bockaert J, Manzoni OJ (2002): Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proc Natl Acad Sci U S A* 99:8384–8388.

Rodgers RJ, Haller J, Halasz J, Mikics E (2003): "One-trial sensitization" to the anxiolytic-like effects of cannabinoid receptor antagonist SR141716A in the mouse elevated plus-maze. *Eur J Neurosci* 17:1279–1286.

Rodriguez de Fonseca F, Fernandez Ruiz JJ, Murphy L, Eldridge JC, Steger RW, Bartke A (1991): Effects of delta-9-tetrahydrocannabinol exposure on adrenal medullary function: Evidence of an acute effect and development of tolerance in chronic treatments. *Pharmacol Biochem Behav* 40: 593–598.

Rodriguez de Fonseca F, Rubio P, Menzaghi F, Merlo Pich E, Rivier J, Koob GF, et al (1996): Corticotropin-releasing factor (CRF) antagonist [D-Phe12, Nle21,38, CaMeLeu37]CRF attenuates the acute actions of the highly potent cannabinoid receptor agonist HU-210 on defensive-withdrawal behavior in rats. *J Pharmacol Exp Ther* 276:56–64.

Rodriguez de Fonseca F, Villanua MA, Munoz RM, San-Martin-Clark O, Navarro M (1995): Differential effects of chronic treatment with either dopamine D1 or D2 receptor agonists on the acute neuroendocrine actions of the highly potent synthetic cannabinoid HU-210 in male rats. *Neuroendocrinology* 61:714–721.

Simiand J, Guitard J, Griebel G, Soubrié P (2003): Tonic immobility in gerbils: A new model for detecting antidepressant-like effects. *Behav Pharmacol* 14(suppl 1):S40.

Tanda G, Carboni E, Frau R, Di Chiara G (1994): Increase of extracellular dopamine in the prefrontal cortex: A trait of drugs with antidepressant potential. *Psychopharmacology* 115:285–288.

Treit D (1985): Animal models for the study of anti-anxiety agents: A review. *Neurosci Biobehav Rev* 9:203–222.

Tsou K, Brown S, Sanudo-Pena MC, Mackie K, Walker JM (1998): Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. *Neuroscience* 83:393–411.

Tzavara ET, Davis RJ, Perry KW, Li X, Salhoff C, Bymaster FP, et al (2003): The CB1 receptor antagonist SR141716A selectively increases monoaminergic neurotransmission in the medial prefrontal cortex: Implications for therapeutic actions. *Br J Pharmacol* 138:544–553.

Vogel JR, Beer B, Clody DE (1971): A simple and reliable conflict procedure for testing anti-anxiety agents. *Psychopharmacologia* 21:1–7.

Willner P, Muscat R, Papp M (1992): Chronic mild stress-induced anhedonia: A realistic animal model of depression. *Neurosci Biobehav Rev* 16:525–534.

Wilson RI, Nicoll RA (2002): Endocannabinoid signaling in the brain. *Science* 296:678–682.