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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Received June 15, 2004; revised September 27, 2004; accepted October 21, 2004.

0006-3223/05/$30.00
doi:10.1016/j.biopsych.2004.10.032

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