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Long-term CB_1 receptor blockade enhances vulnerability to anxiogenic-like effects of cannabinoids

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ABSTRACT

Compelling evidence has documented the anxiolytic and mood-enhancing properties of cannabis. In susceptible users, however, consumption of this drug is conducive to panic, paranoia and dysphoria. We hypothesized that the up-regulation of CB_1 receptors (CB_1Rs) in select brain regions may enhance the vulnerability to cannabinoid-induced anxiety. To test this possibility, we assessed the behavioral impact of a potent cannabinoid agonist (CP55,940; 0.05–0.1 mg/kg, IP) on C57BL/6 male mice, respectively subjected to a prolonged pre-treatment of either the selective CB_1R antagonist/inverse agonist AM251 (1 mg/kg/day IP, for 21 days, followed by a 3-day clearance period before testing) or its vehicle (VEH1). Anxiety-like responses were studied in the novel open field, elevated plus maze (EPM) and social interaction assays. While CP55,940 induced anxiolytic-like effects in the EPM in VEH1-exposed animals, it elicited opposite actions in AM251-exposed mice. In this last group, CP55,940 also reduced rearing and social interaction in comparison to its vehicle (VEH2). The divergent effects of CP55,940 in AM251- and VEH1-pretreated animals were confirmed in 129SvEv mice. Immunoblotting analyses on brain samples of C57BL/6 mice revealed that AM251 pre-treatment caused a significant up-regulation of CB_1R expression in the prefrontal cortex and striatum, but also a down-regulation of these receptors in the hippocampus and midbrain. Notably, CB_1R levels in the prefrontal cortex were negatively correlated with anxiolysis-related indices in the EPM; furthermore, midbrain CB_1R expression was positively correlated with the total duration of social interaction. These results suggest that regional variations in brain CB_1R expression may differentially condition the behavioral effects of cannabinoids with respect to anxiety-related responses.

1. Introduction

The widespread popularity of cannabis as a recreational substance is generally regarded as a consequence of its anxiolytic, mood-enhancing and euphorigenic properties (Green et al., 2003; SAMHSA, 2009); nevertheless, multiple anecdotal reports indicate that the psychological effects experienced by occasional marijuana smokers range from relaxation and heightened sociability to panic, paranoid ideation and dysphoria (Tambaro and Bortolato, 2012). This high variability is confirmed by several preclinical studies, which have shown that anxiety-like behaviors in rodents can be either attenuated or exacerbated by Δ^9-tetrahydrocannabinol (Δ^9-THC), the key psychoactive ingredient of hemp, or other cannabinoids (Bortolato and Piomelli, 2008; Bortolato et al., 2010).

The ability of natural and synthetic cannabinoids to influence anxiety responses is mostly mediated by the cannabinoid CB_1 receptor (CB_1R), a G-protein coupled receptor abundantly expressed in all the major brain regions implicated in emotional regulation, including the prefrontal cortex (PFC), amygdaloid complex, septo-hippocampal system and periaqueductal gray in the midbrain (Hajos and Freund, 2002; Herkenham et al., 1990, 1991; Katona et al., 2001). Differences in brain CB_1R expression and/or sensitivity reflect the influence of multiple genetic and environmental factors (Kendler et al., 2003; Manzanares et al., 2004; Lazary et al., 2009) and may account for the polymorphous effects of cannabinoids on behavioral regulation. The role of CB_1Rs in the modulation of anxiety, however, remains incompletely understood.

Prior evidence has shown that low doses of cannabinoids have anxiolytic-like properties in mice and rats (Berrendero and

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Maldonado, 2002; Braida et al., 2007; Haller et al., 2004; Patel and Hillard, 2006; Valjent et al., 2002), whereas higher concentrations of the same compounds elicit the opposite outcome (Celerier et al., 2006; Crippa et al., 2009; Genn et al., 2004; Marco et al., 2004; McGregor et al., 1996; Onaivi et al., 1990; Rodriguez de Fonseca et al., 1996). Building on these premises, we hypothesized that the behavioral response to the same dose of cannabinoids may depend on the expression of CB_1Rs and that, specifically the up-regulation of these targets in specific brain regions may either abrogate or reverse the anxiolytic properties of low cannabinoid doses. To test this hypothesis, we endeavored to increase the expression of brain CB_1Rs in C57BL/6 mice with a 3-week administration of AM251, a highly selective antagonist/inverse agonist of these targets (Lan et al., 1999). Following a 3-day washout period to allow for a full clearance of AM251, the behavioral effects of CP55,940 – a highly potent, synthetic analog of Δ^9-THC with an analogous spectrum of pharmacological action – were studied across three complementary paradigms to test anxiety-related responses, namely the novel open field, elevated plus-maze and social interaction tests. Behavioral indices were then correlated with the regional expression of CB_1Rs. Furthermore, in consideration of the role of the genetic background on anxiety responses and cannabinoid-mediated effects (Chakrabarti et al., 1998; Onaivi et al., 1995), all behavioral tests were repeated in 129SvEv mice, another murine line commonly used in preclinical experimentation, in consideration of the differences of these two genotypes with respect to anxiety-related behaviors (Pratte and Jamon, 2009).

2. Materials and methods

2.1. Animals

Adult male C57BL/6 and 129SvEvTac (129S6) mice, weighing 25–30 g at the beginning of the study, were used. Animals were group-housed (3–4 for cage) with food and water available *ad libitum*. The room was maintained at 22 °C, on a 12-h light/dark cycle (with lights on at 06:00 AM). Experimental procedures were in compliance with the National Institute of Health guidelines and approved by the local Animal Use Committees of the Universities of Southern California and Cagliari. Each experimental group included 10–12 mice.

2.2. Drugs

AM251 [*N*-(Piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide] and CP55,940 [(−)-*cis*-3-[2-Hydroxy-4-(1,1-dimethylheptyl) phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol] were purchased from Tocris Cookson (Bristol, UK). AM251 was dissolved in a vehicle (VEH1) of polyethylene glycol and saline solution (1:9, vol:vol). CP55,940 was dissolved in a vehicle (VEH2) of Tween 80, polyethylene glycol and saline (1:1:18). Both compounds were administrated intraperitoneally (i.p.) in an injection volume of 10 ml/kg.

2.3. Behavioral analyses

2.3.1. Experimental procedure

The experimental procedure is schematized in Fig. 1. C57BL/6 mice were treated with daily injections of either AM251 (1 mg/kg/day) or its vehicle (VEH1) for 21 days. The dose of AM251 was selected in view of previous studies in mice (Chen et al., 2004; Zhou and Shearman, 2004), which showed its lack of significant effects on food intake and body weight. Injections were performed by expert personnel, so as to minimize pain or stress. Body weight was measured daily before the injections. At the end of the regimen, animals were left undisturbed in their cages for a period of 72 h (Fig. 1), to ensure full clearance of AM251. The duration of this clearance period was based on preliminary studies in our laboratory, as well as previous data showing a normalization of food intake in rodents previously treated with chronic AM251 (Chambers et al., 2006). Subsequently, each group of mice was further subdivided into 3 subgroups and treated with either CP55,940 (0.05–0.1 mg/kg, i.p.) or its vehicle (VEH2). To avoid possible sources of bias due to cage-related effects, all cage mates received different treatment combinations. Thirty minutes after injection of CP55,940 or VEH2, mice were tested in a battery of paradigms for testing of anxiety-like behaviors (open field; elevated plus maze; social interaction) and cataleptic responses (bar test) (see below). The overall duration of the battery was 30 min, with 7-min intervals between subsequent tests (with the exception of the bar test, which was performed immediately after social interaction testing for 1 min). The experimental schedule for 129SvEv mice was identical to that used for C57BL/6 mice, but only one dose of CP55,940 (0.1 mg/kg, i.p.) was used. All behavioral testing took place between 10:00 AM and 3:00 PM.

2.3.2. Open field

Testing was conducted as previously indicated (Bortolato et al., 2011). The open field consisted of a Plexiglas square grey arena (40 × 40 cm) surrounded by 4 black walls (40 cm high). On the floor, two zones of equivalent areas were defined: a central square quadrant of 28.28 cm per side, and a concentric peripheral frame including the area within 11.72 cm from the walls. Light and sound were maintained at 20 lux and 70 dB, respectively. Mice were placed in the central zone and their behavior was monitored for 5 min. The distance travelled in the whole arena and the time spent in the center, as well as the number of rears, were measured with Ethovision software (Noldus Instruments, Wageningen, The Netherlands) for locomotor pattern analyses.

2.3.3. Elevated plus-maze

Testing was performed as previously described (Bortolato et al., 2009), in a black Plexiglas apparatus consisting of two open (25 × 5 cm) and two closed arms (25 × 5 × 5 cm), which extended from a central platform (5 × 5 cm) at 60 cm from the ground. Mice were individually placed on the central platform facing an open arm, and their behavior was recorded for 5 min. An arm entry was counted when all four paws were inside the arm. Behavioral measures included: number of entries and duration of time spent in each partition of the elevated plus-maze, number of stretch-attend postures and head dips, defined as described by Rodgers et al. (1992).

2.3.4. Social interaction test

Mice were tested as previously described (Bortolato et al., 2011). Animals were introduced into a neutral, unfamiliar Makrolon cage (20 × 10 cm), with foreign strain-, age- and weight-matched male counterparts from separate litters and cages. To differentiate between the test mouse and the foreign conspecifics, the tail of the latter was colored with an odorless yellow acrylic paint marker. Testing sessions lasted 5 min and were video-recorded and later scored by observers blinded to the

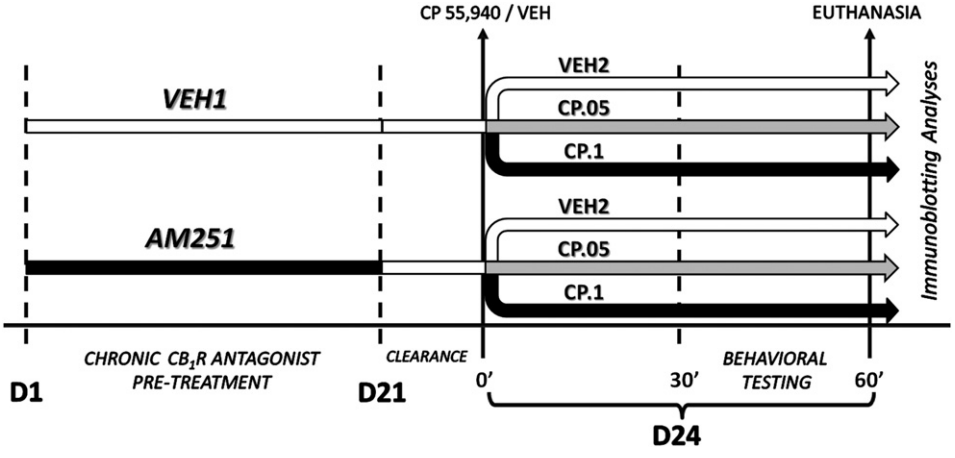

Fig. 1. Synopsis of the experimental design, including treatment schedule, behavioral tests and immunoblotting assays. For more details, see text.