Pharmacology, Biochemistry and Behavior 102 (2012) 357–365

Contents lists available at SciVerse ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Antidepressant-like effects of Δ^9-tetrahydrocannabinol and rimonabant in the olfactory bulbectomised rat model of depression

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ARTICLE INFO

Article history:
Received 21 November 2011
Received in revised form 8 May 2012
Accepted 19 May 2012
Available online 24 May 2012

Keywords:
Cannabinoids
Δ^9-tetrahydrocannabinol
Rimonabant
Olfactory bulbectomy

ABSTRACT

The endocannabinoid signalling system is widely accepted to play a role in controlling the affective state. Plant cannabinoids are well known to have behavioural effects in animals and humans and the cannabinoid CB_1 receptor antagonist rimonabant has recently been shown to precipitate depression-like symptoms in clinical trial subjects. The aim of the present study was to investigate the behavioural and neurochemical effects of chronic administration of Δ^9-tetrahydrocannabinol (THC) and rimonabant on intact and olfactory bulbectomised (OB) rats used as a model of depression.

As expected, OB rats were hyperactive in the open field. Repeated THC (2 mg/kg, i.p. once every 48 h for 21 days) and rimonabant (5 mg/kg, i.p. once every 48 h for 21 days) reduced this hyperactivity, which is typical of clinically effective antidepressant drugs. In intact animals, chronic THC increased brain derived neurotrophic factor (BDNF) expression levels in the hippocampus and frontal cortex but rimonabant had no effect. Rimonabant increased the levels of phosphorylated extracellular signal regulated kinases (p-$ERKs_{1/2}$) in the hippocampus and prefrontal cortex and THC also increased expression in frontal cortex. OB did not affect BDNF or p-$ERK_{1/2}$ expression in the hippocampus or frontal cortex and in, contrast to the intact animals, neither THC nor rimonabant altered expression in the OB rats.

These findings indicate antidepressant-like behavioural properties of both THC and rimonabant in OB rats although additional studies are required to clarify the relationship between the chronic effects of cannabinoids in other pre-clinical models and in human depression.

1. Introduction

It is estimated by the WHO that depression will be the most important cause of disability in the world by the year 2020 (Murray and Lopez, 1997). The current treatments for depression are only partially effective (Post et al., 2010), necessitating the development of alternative pharmacotherapies. Retrospective studies in cannabis users and small clinical trials have suggested possible therapeutic benefit of cannabinoid use in depression (Gruber et al., 1996). However, other previous studies have suggested that cannabis use may be a contributory cause of depression and suicidal behaviours (Bovasso, 2001). These human findings have their counterpart in animal studies, but the situation is complicated by reports of cannabinoid receptor agonists and antagonists displaying both anxiolytic- and anxiogenic-like effects in rodent models of anxiety and depression (Bambico et al., 2007; Berrendero and Maldonado, 2002; Griebel et al., 2005; Jiang et al., 2005; Viveros et al., 2005). Rimonabant can be classified as a CB1 antagonist but its inverse agonist properties have been well documented by in vitro pharmacological experiments (Howlett et al., 2002). Thus, its biochemical or behavioural effects generally are opposite in direction to effects produced by Δ^9-THC or other CB1 agonists. Rimonabant has been investigated mainly for the treatment of obesity and associated metabolic dysregulation; however, clinical trials showed an increased incidence of psychiatric side effects, mainly anxiety and depression-like states, in obese patients which resulted in rimonabant being withdrawn from the market (Leite et al., 2009).

The involvement of the endocannabinoid system in depression is supported by pre-clinical studies such as that of Hill et al. (2008a) showing increased CB_1 receptor expression and decreased endocannabinoid content in different brain regions in the chronic mild unpredictable stress model; effects that were generally reversed by chronic antidepressant administration. In transgenic animals lacking the cannabinoid CB_1 receptor, there are enhanced behavioural signs of anxiety and depression and an amplified sensitivity to stressful stimuli (Aso et al., 2008). In clinical investigations, Hill et al. (2008b) demonstrated that circulating levels of endocannabinoids were significantly reduced in a population with major clinical depression. Together, these data are consistent with the hypothesis that an endogenous

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doi:10.1016/j.pbb.2012.05.009

endocannabinoid system operates to maintain an appropriate affective state. They are also consistent with the traditional mood-elevating properties of cannabinoids and, perhaps, the anxiety and depression experienced by some patients prescribed the CB_1 receptor antagonist rimonabant as an adjunct for weight reduction (Hill and Gorzalka, 2009).

Animal models of psychiatric disorders represent valuable tools for invasively studying molecular changes in brain tissue which cannot be done in patients (Licinio and Wong, 2004). Thus, olfactory bulbectomy (OB) in rodents has been proposed as a model with high predictive validity for chronic psychomotor agitated depression (Harkin et al., 2003; Kelly et al., 1997). The bilateral removal of the olfactory bulbs creates chronic behavioural, endocrine, neurotransmitter and immunological changes that are qualitatively similar to those occurring in depressed patients (Song et al., 1994a, 1994b; van Riezen and Leonard, 1990). Moreover, in the context of the neurogenesis hypothesis of depression (Duman and Monteggia, 2006), some studies have reported that impaired cell proliferation and/or neuronal degeneration observed following olfactory bulbectomy are reduced by some antidepressants (Jaako-Movits et al., 2006; Jarosik et al., 2007; Keilhoff et al., 2006).

The aim of the present study was to investigate the behavioural and neurochemical effects of chronic administration of Δ^9-tetrahydrocannabinol (Δ^9-THC), the principal psychoactive plant cannabinoid, and the CB1 receptor antagonist rimonabant on intact and OB rats (as a model of depression). Our working hypothesis was that THC would show antidepressant-like activity whilst rimonabant might reflect its clinical side effects and exacerbate the effects of bulbectomy.

2. Methods

2.1. Animals

Male Lister hooded rats (n = 8–10 per group; Charles River UK) weighing 180–300 g were housed four per cage and acclimatised to the laboratory conditions for one week before the experiment. Rats were kept in a temperature-regulated (22 ± 2 °C) room and artificial lighting was provided from 0700 h to 1900 h. Food and water were available ad libitum and each animal was handled daily through the first week. Experimental testing began seven days after the acclimatisation period and was performed during the light cycle.

Animals were assigned to surgical groups with regard to their basal open field activity to ensure a comparable inter-group average activity. After surgery, all animals were allowed two weeks to recover before performing a post-surgical (pre-injection) open field test to verify the effect of the lesion. Body weight was measured daily between 9:00 and 10:00 h.

Efforts were made to minimise animal suffering and to reduce number of animals used. All experiments were carried out in accordance with UK Animals Scientific Procedures Act 1986 and Local Ethical Committee Approval (Project licence 40/2715).

2.2. Bilateral olfactory bulbectomy (OB) surgery

Removal of olfactory bulbs was carried out according to the method of Redmond et al. (1999). Animals were anaesthetised using isofluorane gas (3–4%), mixed with oxygen and nitrous oxide. The top of the skull was shaved and swabbed with an antiseptic. The animals were placed in a stereotaxic instrument (Kopf) using atraumatic-blunt ear bars with local analgesic cream applied to their ends. Topical eye lubricant was used to avoid drying of the cornea during surgery. A midline sagittal incision was made in the skin overlying the skull and the skin was retracted bilaterally. Two burr holes, 2 mm in diameter, were drilled on either side of the skull, 5 mm anterior to bregma, and 2 mm from the midline of the frontal bone overlying the olfactory bulbs. The bulb tissue was removed using a blunt hypodermic needle connected to a water vacuum pump and care was taken not to damage the frontal cortex. The burr holes were packed with haemostatic sponge (Claudius Ash, UK) to prevent blood loss. Animals receiving sham surgery underwent a similar procedure with two burr holes drilled but no removal of olfactory bulbs. All incisions were closed using silk sutures. After surgery, all animals received 1 ml of saline (subcutaneously), and Rimadyl (5 mg/kg, subcutaneously) for post-operative analgesia. Rats were allowed to recover in a standard housing cage (but without sawdust bedding) placed on heated blanket, for 12 h with free access to water and a pot of mash. Rats were then moved to the housing room and were individually housed with twice daily handling (5–10 min each) to reduce the development of aggressive behaviour.

Animals were allowed 14 days to recover from surgery after which they were divided into 4 groups: one sham and three OB-groups (n = 9–10). After recovery, all animals were tested in the open field for lesion verification (hyperactivity) and for equal distribution of the hyperactive rats between treated groups. The OB rats were subdivided into 3 groups according to treatment: OB-vehicle treated, OB-THC treated and OB-SR treated. The sham-operated groups received the same volume of vehicle as OBs.

On the day of killing, rats were quietly taken out of the housing room by the person who handled the animals in the previous weeks, and rats were killed by a blow to the head by sufficient force to cause immediate loss of consciousness followed by decapitation to confirm death (Code of Practice for the Humane Killing of Animals under Schedule 1 to the Animals (Scientific Procedures) Act 1986). Mixed arterio-venous trunk blood was collected in heparinised ice-cold tubes and plasma was prepared by centrifugation (at 1600 *g* for 10 min) and frozen at −80 °C until estimation of corticosterone levels. The brains were rapidly removed from the skull and the anatomical success of the lesions verified visually. One animal was eliminated from the analysis because the surgical damage extended to the frontal cortex. The brains were immediately dissected on ice and brain regions were stored at −80 °C. Finally, both adrenal glands were removed from each rat and weighed.

2.3. Drug administration

Vehicle (Cremophor/ethanol/saline, 1:1:18, 1.0 mg/kg i.p.), THC (Sigma UK, 2.0 mg/kg i.p.) and/or rimonabant (formerly called SR141716-A; 5.0 mg/kg i.p.) were administered to intact, sham or OB animals every 48 h for 21 days (n = 9–10 per group).

The dose and frequency of drug administration was chosen after several preliminary studies by our research group. THC was chosen after several preliminary experiments using different doses (0.5, 1, and 2 mg/kg) on the locomotor activity and protein expression and the 2 mg/kg produced the most consistent effect (data not published). In addition, the used dose of rimonabant (5 mg/kg; every 48 h) was equivalent to human dose of rimonabant that previously used for treatment of obesity (20 mg/kg; daily). Moreover, in our previous study daily i.p. injection of rimonabant (2 mg/kg) did not affect locomotor activity of the same strain of rats (Assareh et al., 2012). The frequency of injection every 48 h was chosen to decrease the stress of daily injection. Rimonabant was generously provided by the NIMH compound synthesis programme. All other drugs and chemicals were purchased from Tocris Bioscience, Bristol, UK or Sigma-Aldrich Company Ltd. Gillingham, UK.

2.4. Locomotor activity

For intact animals, spontaneous locomotor and exploratory behaviour were measured for 65 min following drug or vehicle treatment 10 min after the final injection. The activity box (65 × 45 × 45 cm) was placed in a sound-isolated room with constant illumination of 40 lx and was fitted with two parallel horizontal and vertical infrared