BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2010), 159, 122–128

0007-1188/09
www.brjpharmacol.org

RESEARCH PAPER

Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT_{1A} receptors

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Background and purpose: Cannabidiol (CBD) is a non-psychotomimetic compound from *Cannabis sativa* that induces anxiolytic- and antipsychotic-like effects in animal models. Effects of CBD may be mediated by the activation of 5-HT_{1A} receptors. As 5-HT_{1A} receptor activation may induce antidepressant-like effects, the aim of this work was to test the hypothesis that CBD would have antidepressant-like activity in mice as assessed by the forced swimming test. We also investigated if these responses depended on the activation of 5-HT_{1A} receptors and on hippocampal expression of brain-derived neurotrophic factor (BDNF).

Experimental approach: Male Swiss mice were given (i.p.) CBD (3, 10, 30, 100 mg·kg^{-1}), imipramine (30 mg·kg^{-1}) or vehicle and were submitted to the forced swimming test or to an open field arena, 30 min later. An additional group received WAY100635 (0.1 mg·kg^{-1}, i.p.), a 5-HT_{1A} receptor antagonist, before CBD (30 mg·kg^{-1}) and assessment by the forced swimming test. BDNF protein levels were measured in the hippocampus of another group of mice treated with CBD (30 mg·kg^{-1}) and submitted to the forced swimming test.

Key results: CBD (30 mg·kg^{-1}) treatment reduced immobility time in the forced swimming test, as did the prototype antidepressant imipramine, without changing exploratory behaviour in the open field arena. WAY100635 pretreatment blocked CBD-induced effect in the forced swimming test. CBD (30 mg·kg^{-1}) treatment did not change hippocampal BDNF levels.

Conclusion and implications: CBD induces antidepressant-like effects comparable to those of imipramine. These effects of CBD were probably mediated by activation of 5-HT_{1A} receptors.

British Journal of Pharmacology (2010) **159,** 122–128; doi:10.1111/j.1476-5381.2009.00521.x; published online 4 December 2009

Keywords: cannabinoids; cannabidiol; imipramine; antidepressant; forced swimming; 5-HT_{1A} receptor; BDNF

Abbreviations: CBD, cannabidiol; BDNF, brain derived neurotrophic factor; Δ^9-THC, Δ^9-tetrahydrocannabinol

Introduction

Extracts of the *Cannabis sativa* plant elicit in humans a complex subjective experience that includes euphoria, heightened sensitivity to external stimuli and relaxation (Johns, 2001). This plant contains more than 400 different compounds, of which 66 are termed cannabinoids. Δ^9-tetrahydrocannabinol (Δ^9-THC), one of the major constituents of *C. sativa* extracts (Mechoulam, 1970), is thought to account for most of the effects of cannabis through the activation of cannabinoid CB_1 receptors in the brain (Huestis *et al.*, 2001; nomenclature follows Alexander *et al.*, 2008). The major endogenous agonists of the CB_1 receptor are anandamide and 2-arachidonoyl glycerol, referred to as endocannabinoids (Piomelli, 2003). Anandamide is removed from the synaptic space by a putative neuronal uptake mechanism (Alger, 2004) and is inactivated intracellularly by the enzyme fatty acid amide hydrolase (Di Marzo *et al.*, 1999).

It has recently been suggested that the endocannabinoid system may be involved in the pathophysiology of depression (Hill and Gorzalka, 2005). This is supported by several pieces of evidence showing that endocannabinoids and CB_1 receptors are widely distributed in brain areas that are often related to affective disorders (Devane, 1988) and that their expression is regulated by antidepressant drugs (Hill *et al.*, 2008). Moreover, administration of inhibitors of anandamide uptake or metabolism, as well as CB_1 receptor agonists induces

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Received 5 June 2009; revised 7 August 2009; accepted 12 August 2009

antidepressant-like effects in different animal models (Hill and Gorzalka, 2005; Adamczyk *et al.*, 2008). In accordance with these preclinical results, many patients report benefits from cannabis use in depressive syndromes (Gruber *et al.*, 1996; Ware *et al.*, 2005), although clinical trials of its use in affective disorders have yielded mixed results (Robson, 2001; Degenhardt *et al.*, 2003).

Cannabidiol (CBD) is another major component of *C. sativa* that exhibits a somewhat different pharmacology compared with that of Δ^9-THC (Mechoulam *et al.*, 2007). CBD is usually described as a non-psychoactive compound that inhibits some behavioural effects of Δ^9-THC, such as catalepsy in rats (Formukong *et al.*, 1988) and psychotomimetic and anxiogenic effects in humans (Zuardi *et al.*, 1982). CBD, however, has been shown to induce antipsychotic- and anxiolytic-like activity in preclinical and clinical studies (Zuardi *et al.*, 1982; 2006; Guimarães *et al.*, 1990; Resstel *et al.*, 2006). More recently, our group showed that systemic administration of CBD was able to attenuate the development of stress-induced behavioural consequences (Resstel *et al.*, 2009), raising the possibility that CBD could also be useful for treating psychiatric disorders thought to involve impairment of stress-coping mechanisms, such as depression.

The mechanism of action of CBD is not fully understood. This compound has a low affinity for CB receptors (Petitet *et al.*, 1998; Thomas *et al.*, 1998), although it may block the reuptake of anandamide (Bisogno *et al.*, 2001) and inhibit fatty acid amide hydrolase (Watanabe *et al.*, 1998). Moreover, Russo and colleagues (2005) reported that CBD may exhibit agonist properties at 5-HT_{1A} receptors. In fact, recent work has shown that several CBD effects can be blocked by pretreatment with 5-HT_{1A} receptor antagonists (Hayakawa *et al.*, 2007; Campos and Guimarães, 2008; Resstel *et al.*, 2009).

Although activation of 5-HT_{1A} receptors has been consistently related to the therapeutic effect of antidepressant drugs (Savitz *et al.*, 2009), a link between these receptors and antidepressant-like effect of CBD has not yet been investigated. Therefore, the aim of this work was to test the hypothesis that CBD would induce antidepressant-like effects in mice submitted to the forced swimming test and that this effect would involve the activation of 5-HT_{1A} receptors. In addition, considering the recent pieces of evidence relating the effects of antidepressant drugs with increased hippocampal expression of brain derived neurotrophic factor (BDNF) (see Saaralainen *et al.*, 2003; Duman and Monteggia, 2006) and that activation of 5-HT_{1A} receptors regulates antidepressant-induced hippocampal BDNF expression (Ivy *et al.*, 2003), we also investigated if CBD-induced behavioural effects in the forced swimming test would be associated with changes in BDNF expression in the hippocampus.

Methods

Animals

All animal care was in conformity with the Brazilian Society of Neuroscience and Behavior guidelines for the care and use of laboratory animals, which are in compliance with international laws. The experimental protocols were approved by the local Ethical Committee of the School of Medicine of Ribeirão Preto, University of Sao Paulo. Male Swiss mice (20–25 g) were provided by our local animal farm facility. After arriving at the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of Sao Paulo, the animals were housed in groups of 6–10 animals per cage (570 cm^2), in a temperature-controlled room (24 ± 1°C) under standard laboratory conditions with free access to food and water and a 12 h light/12 h dark cycle (lights on at 06:30h).

Forced swimming test

The forced swimming test was performed as described by Porsolt *et al.* (1977) with minor modifications. Mice were placed individually into glass cylinders (height 25 cm, diameter 17 cm) containing 10 cm of water maintained at 23–25°C. The animals were left in the cylinder for 6 min and the total duration of immobility was measured during the last 4 min period. Mice were considered to be immobile when they remained floating passively, performing only slow movements to keep their head above the water. The water was changed after each trial to avoid the influence of alarm substances (Abel and Bilitzke 1990). All experiments were videotaped and the immobility time was subsequently scored by an observer unaware of the treatments.

Exploratory activity

The test was performed according to Moreira and Guimarães (2005). Briefly, the animals were placed in a circular open field arena (40 cm in diameter with a 50 cm high Plexiglas wall) where the exploratory activity was videotaped during 6 min. The behaviour was analysed with the help of the Ethovision software (version 1.9; Noldus, the Netherlands). This software detects the position of the animal in the open field arena and calculates the distance moved.

Protein extraction and BDNF measurements

Immediately after the forced swimming test, mice were deeply anaesthetized (urethane 25%, 5 $mL \cdot kg^{-1}$), killed by decapitation and their hippocampi removed. The left and right hippocampus were homogenized in lysis buffer (NaCl 137 mM; Tris-HCl 20 mM pH 7.6; glycerol 10%) containing protease inhibitor cocktail (Sigma, St. Louis, MO, USA) and, after centrifugation (5600× *g*, 15 min), the supernatant was stored at –80°C. Hippocampal BDNF was measured by ELISA (BDNF Emax® ImmunoAssay System kit, Promega, Madison, WI, USA) according to the manufacture's instructions. Total proteins levels were measured by the Bradford method (Bradford 1976; Sapan *et al.* 1999) and used to normalize the samples.

Experimental design

Experiment 1. Mice received i.p. injections of CBD (3, 10, 30, 100 $mg \cdot kg^{-1}$), imipramine or vehicles and were submitted to the forced swimming test. Independent groups of mice received CBD at the same doses or its vehicle and were submitted to the open field arena.

Experiment 2. Mice received i.p. injections of WAY100635 or saline followed, 30 min later, by a second injection of CBD (30 $mg \cdot kg^{-1}$) or vehicle and they were exposed to the forced swimming test, 30 min later.

Experiment 3. Independent groups of mice received i.p. injections of CBD (30 mg·kg^{-1}), imipramine (30 mg·kg^{-1}) or vehicle and were submitted to the forced swimming test as described above. Immediately afterwards, the animals were anaesthetized and killed. Their hippocampi were removed and processed for ELISA measurements of BDNF content. This time point was chosen for BDNF measurements as an attempt to correlate BDNF levels at the moment of the test with the behavioural effects induced by the drug treatments (Takeda *et al.*, 2006; Rantamäki *et al.*, 2007; Shieh *et al.*, 2008).

In all experiments the animals were submitted to the behavioural test 30 min after the last drug injection.

Data analysis

The Kolmogorov-Smirnov and Levene tests were initially employed to ensure that the data satisfied the criteria for carrying out ANOVA. The behavioural data were expressed as means ± SEM. The immobility time in the first and third experiments was analysed using one-way ANOVA followed by Duncan's *post hoc* test. The distance moved in the open field arena was analysed by repeated measure analysis of variance with time (1–6 min) as the within-subjects factor and drug as the between-subjects factor. Box's epsilon function was employed to correct the degree of freedom of the repeated factors. Experiment two was analysed by two-way ANOVA using the first (WAY100635 or saline) and the second (CBD or vehicle) injections as main factors. In case of significant interaction between factors the treatment groups were compared using a one-way ANOVA followed by Duncan's *post hoc* test. For experiment three, BDNF levels were normalized to the total protein content and expressed as mean ± SEM. The results were compared using one-way ANOVA. The significance level was set at $P < 0.05$.

Materials

CBD (kindly supplied by THC-Pharma, Frankfurt, Germany): 3, 10, 30, 100 mg·kg^{-1}; imipramine hydrochloride (Sigma, St. Louis, MO, USA): 30 mg·kg^{-1} (dose based on Poleszak *et al.*, 2005); WAY100635 (WAY, Sigma, St. Louis, MO, USA): 0.1 mg·kg^{-1} (dose based on Kaster *et al.*, 2005). Imipramine and WAY100635 were dissolved in sterile isotonic saline solution and CBD was suspended in polyoxyethylenesorbitan monooleate (Tween 80) 2%-saline. The solutions were prepared immediately before use and injected i.p. in a volume of 10 mL·kg^{-1}.

Results

Effects of CBD or imipramine treatment in the forced swimming test and in the open field arena

There was a significant treatment effect in the forced swimming test ($F_{6,59} = 3.89$, $P < 0.01$), with imipramine and CBD (30 mg·kg^{-1}) significantly reducing the immobility time compared with the vehicle group (n = 8–12, Duncan $P < 0.05$; Figure 1).

In the open field arena the distance travelled decreased over time ($F_{1,42} = 49.12$, $P < 0.005$), but there was no difference between drug treatment and vehicle at any dose tested ($n = 8$ per group; $F_{5,42} = 0.59$, $P > 0.05$). Also, no interaction was observed between time and treatment effects ($F_{5,42} = 0.54$, $P > 0.05$; Figure 2).

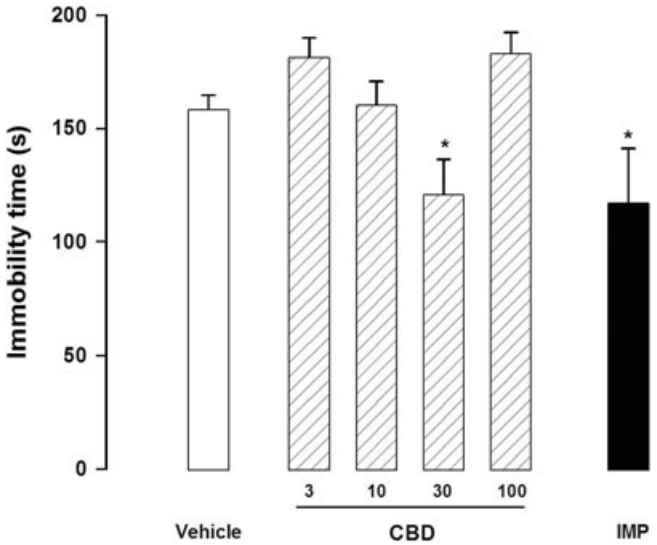

Figure 1 Cannabidiol (CBD, 30 mg·kg^{-1}) and imipramine (IMP, 15 mg·kg^{-1}) reduced immobility time in the forced swimming test. Mice (8–12/group) received i.p. injections of CBD (3–100 mg·kg^{-1}) or imipramine (IMP) and 30 min later were submitted to the forced swim. Data represent the mean ± SEM. * indicates $P < 0.05$ compared with vehicle group (ANOVA followed by Duncan).

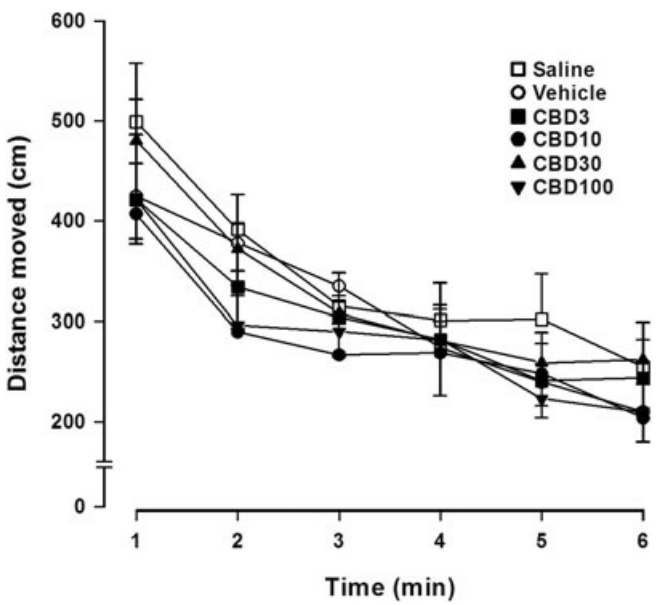

Figure 2 Cannabidiol (CBD, 3–100 mg·kg^{-1}) did not induce any significant change in the exploratory activity. Mice (8 per group) received i.p. injections of CBD and 30 min later the distance moved in an open arena was analysed over 6 min. Data represent the mean ± SEM.

Effects of CBD (30 mg·kg^{-1}), alone or in combination with WAY100635, in the forced swimming test

There was a significant interaction between the first and second injections ($F_{1,48} = 5.67$, $P = 0.02$). Confirming results

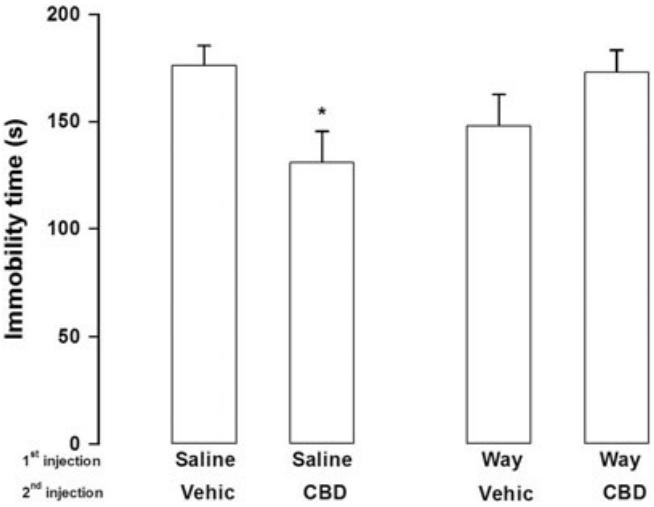

Figure 3 Pretreatment with WAY100635 (Way, 0.1 mg·kg^{-1}) prevented cannabidiol (CBD, 30 mg·kg^{-1}) effects in the forced swimming test. Mice (8–17 per group) received a first i.p. injection of WAY100635 or saline followed, 30 min later, by a second injection of CBD or vehicle (Vehic). The animals were submitted to the forced swim 30 min after the second injection. Data represents mean ± SEM. * indicates $P < 0.05$ compared with saline + vehicle group (ANOVA followed by Duncan).

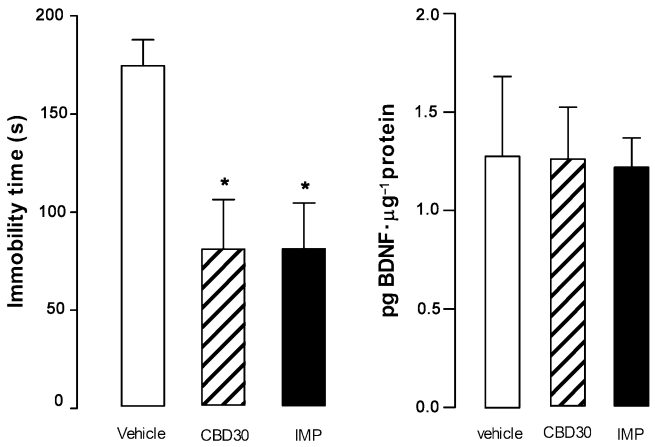

Figure 4 Cannabidiol-induced antidepressant-like effects were not associated with alterations in hippocampal BDNF levels. Mice (7–8 per group) received i.p. injection of cannabidiol (CBD, 30 mg·kg^{-1}) or imipramine (IMP, 30 mg·kg^{-1}) and 30 min later were submitted to the forced swim test (left graph). Immediately after, the animals were killed, their hippocampi removed and processed for BDNF measurements (right graph). Data represent the mean ± SEM. * indicates $P < 0.05$ compared with vehicle group (ANOVA followed by Duncan).

from the first experiment, saline + CBD (30 mg·kg^{-1}, $n = 11$) treatment reduced immobility time in the forced swimming test when compared with the saline + vehicle group ($n = 16$; Duncan $P < 0.05$). Effects of CBD were prevented by pretreatment with WAY100635 ($n = 8$, WAY100635 + CBD vs. saline + CBD: Duncan $P < 0.05$). WAY100635 + vehicle ($n = 17$) treatment did not induce significant changes in immobility time when compared with saline + vehicle group (Duncan, $P > 0.05$). See Figure 3.

Effects of CBD (30 mg·kg^{-1}) or imipramine on hippocampal BDNF levels

As observed in the previous experiments, CBD (30 mg·kg^{-1}) and imipramine reduced the immobility time in the forced swimming test ($F_{2,19} = 5.91$, $P < 0.05$; Figure 4A). However, these treatments failed to change hippocampal BDNF levels ($F_{2,19} = 0.013$, $P > 0.05$; Figure 4B). Moreover, there was no correlation between immobility time and hippocampal BDNF levels (Pearson correlation, $r = 0.33$, $n = 22$, $P > 0.05$, data not shown).

Discussion

The present results show that CBD reduces immobility time in the forced swimming test to a similar extent as a prototype antidepressant, imipramine. Thus, this work is the first to suggest that CBD has a favourable profile in a model predictive of antidepressant-like activity (Porsolt *et al.*, 1977). CBD, however, was effective only at the 30 mg·kg^{-1} dose, with smaller or higher doses producing no effect.

Considering that the forced swimming test is a paradigm based on evaluation of motor activity, drugs that induce changes in this parameter could confound data interpretation. In the present study, none of the CBD doses tested modified the distance moved in the open field arena. This datum is in agreement with previous reports showing that, at the doses tested, CBD does not induce significant motor changes (Guimarães *et al.*, 1990; Moreira and Guimarães, 2005). This indicates that the antidepressant-like effect of CBD is not secondary to changes in motor behaviour. The present results, therefore, suggest that, in addition to anxiolytic, hypnotic and antipsychotic effects (Zuardi *et al.*, 1982; Guimarães *et al.*, 1990; Resstel *et al.*, 2006), CBD could also have antidepressant properties.

The precise mechanisms underlying the effects of CBD are not well understood. CBD has a low affinity for cannabinoid CB_1 receptors (Petitet *et al.*, 1998; Thomas *et al.*, 1998), but may indirectly affect the endocannabinoid system by blocking anandamide reuptake (Bisogno *et al.*, 2001) and inhibiting its enzymatic hydrolysis (Watanabe *et al.*, 1998). It has recently been proposed, in addition, that CBD could act as an agonist at 5-HT$_{1A}$ receptors (Russo *et al.*, 2005). These receptors have been consistently related to the neurobiology of depression and to the mechanism of action of antidepressant drugs (Graeff *et al.*, 1996; Joca *et al.*, 2003; Savitz *et al.*, 2009).

In accordance with the proposed activity of CBD on 5-HT$_{1A}$ receptors, the present study showed that the antidepressant-like effect of CBD is inhibited by pre-treatment with WAY100635, a selective 5-HT$_{1A}$ receptor antagonist (Kaster *et al.*, 2005), at a dose that did not produce any effect by itself. This suggests that CBD effects in the forced swimming test depend on activation of 5-HT$_{1A}$ receptors. This is consistent with previous observation that neuroprotective (Hayakawa *et al.*, 2007) and anxiolytic (Campos and Guimarães, 2008; Resstel *et al.*, 2009) effects induced by CBD are sensitive to 5-HT$_{1A}$ receptor antagonists.

The mechanisms by which 5-HT$_{1A}$ receptors mediate adaptation to stress and induce antidepressant-like effects are not completely understood. 5-HT$_{1A}$ receptors are located

presynaptically (somatodendritic autoreceptors) in 5-hydroxytryptaminergic cell bodies in the raphe nuclei of the brain stem and post-synaptically, predominantly in limbic structures such as the hippocampus, hypothalamus, prefrontal cortex and amygdala (Chalmers and Watson, 1991). It has been suggested that stress and/or genetic factors might impair post-synaptic 5-HT_{1A}-mediated effects in limbic regions, such as the hippocampus, and predispose individuals to stress-induced behavioural consequences (Graeff *et al.*, 1996). In fact, several studies have found reduced number and/or affinity of post-synaptic 5-HT_{1A} receptors in the brains of depressed individuals (Sargent *et al.*, 2000; Szewczyk *et al.*, 2009) and of animals submitted to stress (Flugge, 1995; van Riedel *et al.*, 2003). On the other hand, the number of 5-HT inhibitory autoreceptors is increased (Stockmeier *et al.*, 1998). In agreement with the proposed hypothesis, chronic antidepressant treatment is shown to facilitate post-synaptic 5-HT_{1A} receptor-mediated function (Haddjeri *et al.*, 1998) and administration of 5-HT_{1A} receptor agonists into the hippocampus induces antidepressant-like effects in animal models, most likely by attenuating the emotional impact of aversive stimuli (Graeff *et al.*, 1996; Joca *et al.*, 2003; 2007). However, the mechanisms involved in 5-HT_{1A} receptor-mediated antidepressant effect are still a matter of debate. One possibility is that activation of 5-HT_{1A} receptors attenuates limbic hyperactivity, an effect that can be observed in stressed rats (Shumake *et al.*, 2002) and depressed humans (Mayberg *et al.*, 2000; Goldapple *et al.*, 2004). This effect could involve attenuation of local glutamate release (Strosznajder *et al.*, 1996).

In addition, antidepressant treatment may increase hippocampal neurogenesis and BDNF expression (see Duman and Monteggia, 2006), effects that are necessary for some of their behavioural effects (Santarelli *et al.*, 2003; Saaralainen *et al.*, 2003). However, it remains controversial whether their effects on stress-coping in the forced swimming test occur in parallel with increased BDNF expression. 5-HT_{1A} receptors are thought to mediate some of the trophic actions attributed to 5-HT, such as increased neurogenesis (Brezun and Daszuta, 1999; Radley and Jacobs, 2002) and BDNF release (Ivy *et al.*, 2003). Thus, we tested whether imipramine and CBD would increase hippocampal BDNF. However, the present study failed to detect any effect of CBD or imipramine on this variable. Despite these results, BDNF involvement in imipramine- and CBD-induced effects may not be ruled out as, even if acute effects of antidepressants on BDNF-mediated transmission have already been described (Saaralainen *et al.*, 2003; Rantamäki *et al.*, 2007; Dzitoyeva *et al.*, 2008; Shieh *et al.*, 2008), several studies have shown increased hippocampal BDNF expression only after subchronic or chronic antidepressant treatments (Castrén *et al.*, 2007). Experimental differences between studies, other than the treatment duration, might also have contributed to the observed results. For example, the use of extracts of the whole hippocampus could have masked treatment effects on BDNF levels, as molecular and functional differences among hippocampal subregions have been described (Bannerman *et al.*, 2004; Datson *et al.*, 2009). Moreover, the use of tissue homogenates impairs the distinction between intracellular and released BDNF pools and it is possible that only a small and local release of BDNF is required to cause behavioural effects (Saaralainen *et al.*, 2003).

The antidepressant-like effects of CBD were only evident at the dose of 30 $mg \cdot kg^{-1}$, with smaller or higher doses producing no effect. This inverted U-shape profile has been observed in several previous studies with CBD (Guimarães *et al.*, 1990; Moreira *et al.*, 2006) and is often observed with drugs that modulate the endocannabinoid system (Viveros *et al.*, 2005). The mechanisms for this effect are not yet understood, but probably reflect the complex pharmacology of this compound. Among the possible mechanisms there is an interaction with TRPV1 vanilloid receptors. CBD can activate these receptors in μM concentrations (Bisogno *et al.*, 2001) and they are expressed in several brain areas related to emotional responses such as the amygdala, hippocampus, prefrontal cortex and periaqueductal gray (Cristino *et al.*, 2006). TRPV1 receptors can facilitate glutamate release (Palazzo *et al.* 2002) and glutamate receptor antagonists have been shown to induce antidepressant-like effects (Joca *et al.*, 2007). Corroborating this possibility, activation of TRPV1 receptors has recently been implicated in the bell-shaped dose–response curve observed with the endocannabinoid anandamide, microinjected into the prefrontal cortex (Rubino *et al.*, 2008).

In conclusion, the results of the present study showed that CBD induces antidepressant-like effects in the forced swimming test, suggesting for the first time that this compound may possess antidepressant properties. Moreover, this work also suggests that CBD-induced effects are probably mediated by facilitation of 5-HT_{1A} receptor-mediated neurotransmission.

Acknowledgements

The authors wish to thank J.C. Aguiar and E.L.T. Gomes for technical support and FAPESP and CNPq for financial support. We also thank THC-Pharma for the kind supply of CBD.

Conflicts of interest

The authors declare no conflicts of interest.

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