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Abstract

Cannabidiol (CBD), a *Cannabis sativa* constituent, may present a pharmacological profile similar to mood stabilizing drugs, in addition to anti-oxidative and neuroprotective properties. The present study aims to directly investigate the effects of CBD in an animal model of mania induced by D-amphetamine (D-AMPH). In the first model (reversal treatment), rats received saline or D-AMPH (2 mg/kg) once daily intraperitoneal (i.p.) for 14 days, and from the 8th to the 14th day, they were treated with saline or CBD (15, 30 or 60 mg/kg) i.p. twice a day. In the second model (prevention treatment), rats were pretreated with saline or CBD (15, 30, or 60 mg/kg) regime i.p. twice a day, and from the 8th to the 14th day, they also received saline or D-AMPH i.p. once daily. In the hippocampus CBD (15 mg/kg) reversed the D-AMPH-induced damage and increased (30 mg/kg) brain-derived neurotrophic factor (BDNF) expression. In the second experiment, CBD (30 or 60 mg/kg) prevented the D-AMPH-induced formation of carbonyl group in the prefrontal cortex. In the hippocampus and striatum the D-AMPH-induced damage was prevented by CBD (15, 30 or 60 mg/kg). At both treatments CBD did not present any effect against D-AMPH-induced hyperactivity. In conclusion, we could not observe effects on locomotion, but CBD protect against D-AMPH-induced oxidative protein damage and increased BDNF levels in the reversal model and these effects vary depending on the brain regions evaluated and doses of CBD administered.

Keywords

BDNF, bipolar disorder, cannabidiol, mania, oxidative stress

Introduction

Bipolar disorder (BD) is a relatively common condition afflicting approximately 1% of the general population, and is considered a chronic disease that may require lifetime treatment. According to several guidelines or consensus statements, lithium, anticonvulsivants such as valproic acid and carbamazepine, and the second generation antipsychotics are recommended for the pharmacological treatment of BD. Antidepressants such as selective serotonin reuptake inhibitors (SSRIs), can be added if mood stabilizers are not sufficient, particularly in the depressive phase. Although there have been substantial advances in the pharmacotherapeutics of this condition over the last 10–15 years, the benefits have been predominantly in terms of tolerability and safety (Mitchell and Malhi, 2006). All of such medications have important disadvantages such as careful dosage control, low adherence, recurrence of symptoms on withdrawn, important risks during pregnancy and breastfeeding and many unwanted side-effects (Goodwin, 2003). In addition, BD symptoms are often poorly controlled by the existing standard medications and frequently involve a combination of drugs. Thus, the investigation of newer pharmacological agents for use in the acute and maintenance phases of BD is clearly necessary.

It is well known that cannabis can cause adverse effects, including psychosis, anxiety and mania (Frankhauser, 2002; Zuardi et al., 2006a,b), although anecdotal reports suggest that some patients claim that the use of herbal cannabis

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preparations may alleviate depression and/or mania symptoms (Ashton et al., 2005; Ware et al., 2005). However, there is no substantial epidemiological evidence that cannabis abuse serves as a kind of self-medication for BD.

Cannabidiol (CBD), one of the main constituents from the cannabis plant, was previously proposed as a cannabinoid devoid of psychopharmacological activity. CBD can antagonize some behavioral effects of Δ9-THC, such as catalepsy and impairment of variable-interval schedule performance (Formukong et al., 1988; Zuardi and Karniol, 1983). Moreover, CBD blocks psychotomimetic and anxiogenic effects of Δ9-THC in humans (Karniol et al., 1974; Zuardi et al., 1982), an effect that probably involves pharmacodynamic rather than pharmacokinetic interactions (Hunt et al., 1981).

The antiepileptic effect of CBD was one of the first pharmacological actions described with such cannabinoid, both in experimental animals by a variety of procedures (Ashton and Young, 2003; Carlini et al., 1973; Izquierdo et al., 1973; Porter et al., 1999; Turkanis et al., 1974) and later in epilepsy patients who do not achieve complete control of (disabling) seizures (Cunha et al., 1980). Potential antidepressant (Musty et al., 2002), hypnotic (Monti, 1977) and anxiolytic (Crippa et al., 2004; Fusar-Poli et al., 2009; Guimarães et al., 1990; Moreira et al., 2006; Onaivi et al., 1990; Zuardi et al., 2006a,b) effects of CBD have also further been suggested based on preclinical and clinical data and it was suggested that CBD may exhibit a profile similar to atypical antipsychotic drugs (Bhattacharyya et al., 2009; Borgwardt et al., 2008; Zuardi et al., 2006a,b). More recently, CBD have also been reported to have anti-oxidative properties (Hampson et al., 1998), which may account to provide neuroprotection in acute and chronic neurodegeneration reported in different animal models (Garcia-Arencibia et al., 2007; Lastres-Becker et al., 2005). The anticonvulsivant and protective effects of CBD against glutamate toxicity may have a mood stabilizing action similar to some other antiepileptic drugs of proven value in BD (Ashton and Young, 2003; Porter et al., 1999).

Previous studies have suggested that oxidative stress may play a role in the pathophysiology of BD (Andreazza et al., 2008; Frey et al., 2006b; Machado-Vieira et al., 2007). It has been demonstrated that valproate and the prototype mood stabilizer lithium, both first line in the pharmacological treatment of BD, increase brain-derived neurotrophic factor (BDNF) content in rat hippocampus and frontal cortex. BDNF is a key regulator of synaptic plasticity and hence is thought to be uniquely important for neuroprotection. In addition, it was suggested that these mood stabilizers exert neuroprotective effects against oxidative stress, indicating that the regulation of neurotrophic factors might be associated with their pharmacological effects.

As the pharmacological profile of CBD has several characteristics in common with drugs known to benefit BD, it was hypothesized that CBD may have mood stabilizing properties (Ashton et al., 2005). Therefore, the aim of the present study was to directly investigate for the first time to the best of our knowledge, if the administration of CBD can reverse and/or prevent in rats the behavioral and oxidative stress effects of chronic use of the indirect dopaminergic agonist D-amphetamine, in an animal model of mania (Frey et al., 2006a, b).

Methods

In vivo studies were performed in accordance with National Institute of Health guidelines and with approval of Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil.

Animals

Male Wistar rats (age, 2–3 months; weight, 250–320 g) were used in this study. They were housed five to a cage with food and water available and libitum, and were maintained on a 12 h light/dark cycle (lights on at 7.00 a.m.) in a temperature controlled (22°C) colony room. These conditions were maintained constant throughout the experiments.

Drugs

CBD (THC-Pharm, Frankfurt, Germany) was suspended in polyoxyethylenesorbitan monooleate (Tween 80) 2% saline. D-AMPH (Sigma, St. Louis, MO, USA) was dissolved in saline (NaCl 0.9%). The solutions were prepared immediately before use and were protected from the light during the experimental session.

Reversal treatment

In this model, we reproduced the treatment of an acute manic episode according to an animal model of mania from Frey (Frey et al., 2006a). Rats received either a daily injection of D-amphetamine, 2 mg/kg, or saline for 14 days. Between the 8th and the 14th days, the animals were divided into four experimental groups (15 animals per group): CBD (15, 30 or 60 mg/kg) intraperitoneal (i.p.), twice a day, with an interval of 12 h or saline i.p., twice a day with an interval of 12 h. Locomotor activity was assessed 2 h after last injection.

Prevention treatment

The second model, we reproduced the maintenance treatment of BD according to an animal model of mania from Frey (Frey et al., 2006a). Rats received cannabidiol (15, 30 or 60 mg/kg) or saline i.p. twice a day, in an interval of 12 h for 14 days. The animals were then divided into two groups (15 animals per group). Between the 8th and the 14th days, each group received one daily i.p. injection of D-amphetamine, 2 mg/kg, or saline. Locomotor activity was assessed 2 h after the last injection.

Locomotor activity

We used the open-field task to assess locomotor activity. The task was performed in a 40 × 60 cm open field surrounded by 50 cm high walls. The floor of the open field was divided into 12 equal rectangles by black lines. The animals were gently placed on the left rear rectangle and were allowed to explore the arena. Crossings of the black lines and rearings were counted for 5 min (Frey et al., 2006a). The open field box was cleaned with alcohol 70% among between the sessions.

Biochemical measures

Measurement of protein carbonyls: The oxidative damage to proteins was assessed in prefrontal cortex and hippocampus by the determination of carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH) as previously described (Levine et al., 1990). Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid and redissolved in DNPH. The quantification of protein carbonyls in the samples was determined in the absorbance of 370 nm. The protein content was normalized by quantification according Lowry method (Lowry et al., 1951).

Measurement of BDNF levels: BDNF levels in hippocampus were measured by anti-BDNF sandwich ELISA, according to the manufacturer instructions (Chemicon, USA). Briefly, brain slices were homogenized in phosphate-buffered saline (PBS) with 1 mM phenylmethylsulfonyl fluoride (PMSF) and 1 mM ethylene glycol tetraacetic acid (EGTA). Microtitre plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1:2 in sample diluent and standard curve ranged from 7.8 to 500 pg/ml of BNDF. The plates were then washed four times with sample diluent and a monoclonal anti-BNDF rabbit antibody diluted 1:1000 in sample diluent was added to each well and incubated for 3 h at room temperature. After washing, a peroxidase conjugated anti-rabbit antibody (diluted 1:1000) was added to each well and incubated at room temperature for 1 h. After addition of streptavidin enzyme, substrate and stop solution, the amount of BDNF was determined by absorbance in 450 nm. The standard curve demonstrates a direct relationship between optical density (OD) and BDNF concentration. Total protein was measured by Lowry's method using bovine serum albumin as a standard.

Statistical analysis

All data are presented as mean ± SEM. Differences among experimental groups in experiment evaluating BDNF levels were determined by ANOVA. Multiple comparisons were determined by a Tukey test. In all experiments, p-values <0.05 were considered to indicate statistical significance.

Results

In the reversal experiment: D-AMPH increased locomotor and rearing behaviors (Figure 1A and B) in animals treated with this drug, $F=6.910$; $p<0.0001$ for crossings; $F=7.11$, $p<0.0001$ for rearings. CBD did not reverse D-AMPH-induced hyperactivity. The D-AMPH alone administration increased formation of protein oxidation products in this treatment in the brain regions analyzed (Figure 1C). In the prefrontal cortex the D-AMPH-induced damage was increased with the treatment of CBD 15, 30 or 60 mg/kg. In the hippocampus CBD 15 mg/kg reversed the D-AMPH-induced damage, but CBD 30 or 60 mg/kg increased this damage. In the striatum, no effects in the treatment with CBD 15 or 30 mg/kg was observed, but CBD 60 mg/kg increased D-AMPH-induced formation of carbonyl group. The D-AMPH alone had no effect on BDNF levels in rat hippocampus (Figure 1D), but CBD 30 mg/kg increased BDNF expression after AMPH administration. However CBD 15 or 60 mg/kg had no effect on BDNF levels in D-AMPH-treated animals. CBD 15, 30 or 60 mg/kg also had no effect on BDNF levels in saline-treated animals.

In the prevention experiment D-AMPH increased locomotor and rearing Figure 2A, B behavior in animals treated with this drug, $F=14.63$; $p<0.0001$ for crossings; $F=8.934$; $p<0.0001$ for rearings. CBD did not reverse D-AMPH-induced hyperactivity. The D-AMPH alone administration increased formation of protein oxidation products in this treatment in the brains regions analyzed, Figure 2C. The D-AMPH-induced formation of carbonyl group in the prefrontal cortex was prevented by CBD (30 or 60 mg/kg), and no effect was observed with CBD 15 mg/kg pretreatment. In the hippocampus and striatum the D-AMPH-induced damage was prevented by CBD 15, 30 or 60 mg/kg. The D-AMPH alone had no effect on BDNF levels in rat hippocampus (Figure 2D). CBD 15, 30 or 60 mg/kg had no effect on BDNF levels in AMPH- or saline-pretreated animals.

Discussion

In the present study, CBD neither reversed (reversal treatment model) nor prevented (prevention treatment model) amphetamine-induced hyperactivity in a valid animal model of mania (Frey et al., 2006a). These data are in line with the results we have observed in two BD female patients in manic episodes with psychotic features, who were treated with CBD for 25 days (initial oral dose of 600 mg reaching 1200 mg/day). Both patients showed no symptoms improvement during CBD monotherapy with any dose during the trial (Zuardi et al., 2008a). These preliminary data suggest that CBD may not be effective for the manic episode of BD.

Nevertheless, in this study we demonstrated that CBD (15 mg/kg) reversed amphetamine-induced damage and increased BDNF expression levels (30 mg/kg) after AMPH administration in rat hippocampus. Moreover, the D-AMPH-induced damage in the prefrontal cortex was prevented by 30 or 60 mg/kg of CBD and with all doses tested in the hippocampus and striatum. Interestingly, we have previously observed that CBD, like clozapine, induced c-Fos immunoreactivity in prefrontal cortex in rats, distinctively from haloperidol, that promotes it in dorsal striatum (Guimarães et al., 2004). Therefore, using the present model, we were able to reproduce previous findings of the neuroprotective and antioxidant effects of CBD.

Recently, it was observed that CBD reduces glutamate toxicity mediated by *N*-methyl-D-aspartate receptors (NMDAr), 2-amino-3-(4-butyl-3-hydroxyisoxazol-5-yl) propionic acid receptors (AMPA) or kainate receptors. This neuroprotection action of CBD seems to be independent of the CB1 receptor, the central known cannabinoid receptor, as it has not been affected by SR-141716A, a CB1 receptor antagonist (Hampson et al., 1998). Former studies had also demonstrated that the glutamate toxicity may be prevented by antioxidants (Cheng et al., 2008; Kuhlmann et al., 2008). Consistent with this observation, CBD has proven to reduce hydroperoxide-induced oxidative damage as well as or better than other antioxidants. CBD has shown to be more protective against

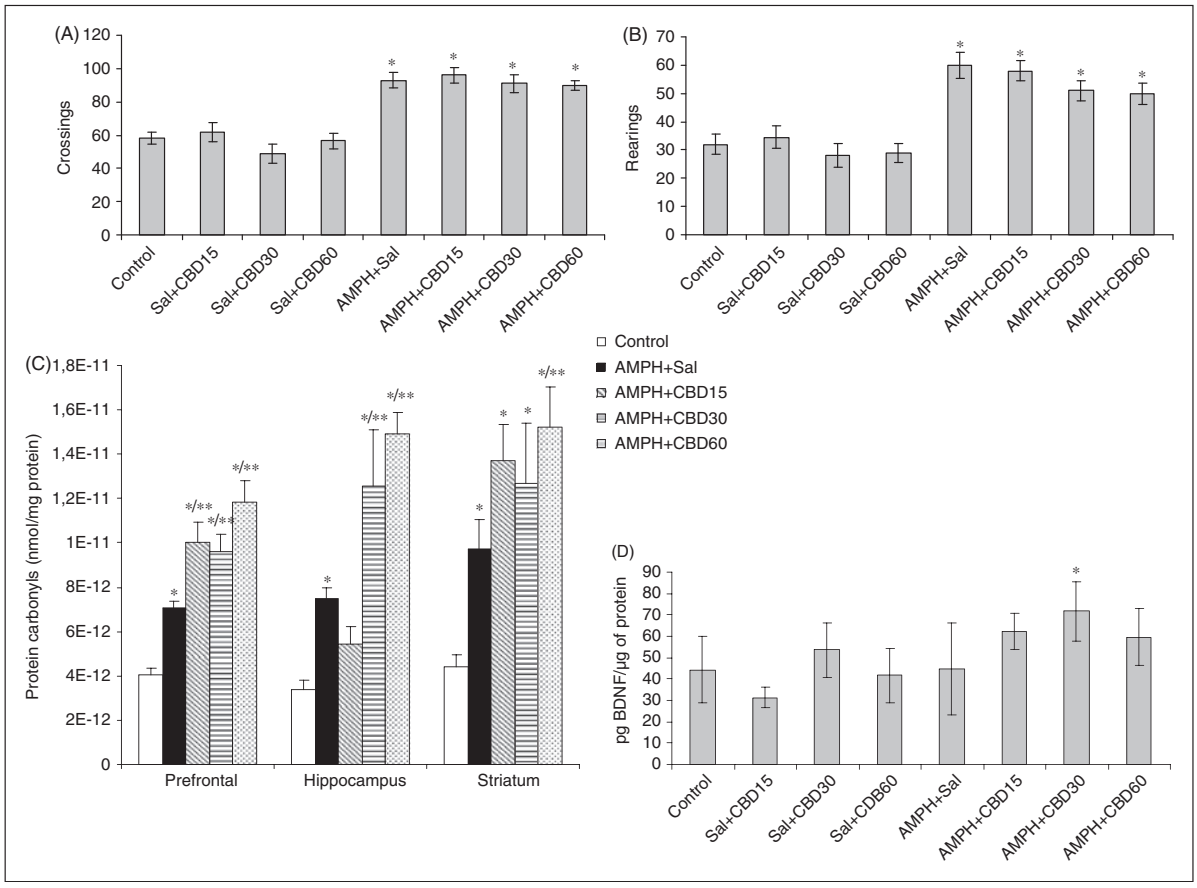

Figure 1. (A) Number of crossings. (B) Rearings ($n = 15$ for each group). (C) Protein carbonyl assessment ($n = 5$ for each group). (D) BDNF levels ($n = 5$ for each group) in the reversal model. Rats were pretreated with amphetamine (D-AMPH) for seven days and then treated with amphetamine plus cannabidiol (15, 30 or 60 mg/kg) between the 8th and 14th days. CBD = cannabidiol, control = vehicle + saline. Bars represent means; error bars represent standard error of the means (SEM). *Different to the saline group. **Different to the D-AMPH group.

glutamate neurotoxicity than the classical antioxidant compounds ascorbate and α-tocopherol (Hampson et al., 1998).

It was hypothesized that the anti-oxidative action of CBD could be responsible for the neuroprotection reported in animal models of Parkinson's disease (PD), as the sub-chronic administration of CBD reduces toxic effects caused by a unilateral injection of 6-hydroxydopamine into the medial forebrain bundle (Lastres-Becker et al., 2005). In this model of PD, CBD led to an up-regulation of mRNA levels of Cu/Zn-superoxide dismutase, a key enzyme in endogenous defense against oxidative stress. It was concluded that the antioxidant effects of CBD can provide neuroprotection against the progressive degeneration of nigrostriatal dopaminergic neurons that occur in this movement disorder (Garcia-Arencibia et al., 2007). This observation was corroborated by the fact that CBD reduced the striatal atrophy caused by 3-nitropropionic acid, in vivo, through mechanisms independent of the activation of vanilloid TRPV1, cannabinoid and adenosine A2A receptors (Sagredo et al., 2007). Using proton magnetic resonance spectroscopy in cannabis users, it was recently found a strong positive correlation of NAA/tCr and CBD in the putamen/globus pallidum and could reflect CBD's enhancement of neuronal and axonal integrity in these brain regions (Hermann et al., 2007). Thus, the prevention of D-AMPH-induced damage in the striatum with all CBD doses tested observed in the present study is in line with neuroprotective properties of CBD, which were also observed in in vitro model studies of Parkinson's disease.

Considering the relevance of these preclinical data and the observed antipsychotic effect of CBD in clinical and preclinical data, we have evaluated, for the first time, the efficacy, tolerability and safety of CBD in PD patients with psychotic symptoms. In an open-label pilot study, the PD patients have shown a significantly decrease both in the psychotic symptoms and in the motor function under CBD treatment. These preliminary data suggests that CBD may be effective for the treatment of PD (Zuardi et al., 2008b).

The evidences of possible neuroprotective properties of CBD in both in vitro (Esposito et al., 2006a, b; Iuvone et al., 2004) and in vivo (Esposito et al., 2007) led to the importance of studies on the therapeutic potential of this cannabinoid in Alzheimer's disease (AD), as this brain disorder is strongly related with oxidative stress.

Therefore, considering that the hippocampus neurodegeneration has a key role in AD, the ability of CBD in reversing and preventing amphetamine-induced damage, and in increasing BDNF expression levels in hippocampus further highlights that this compound is very promising to AD prevention.

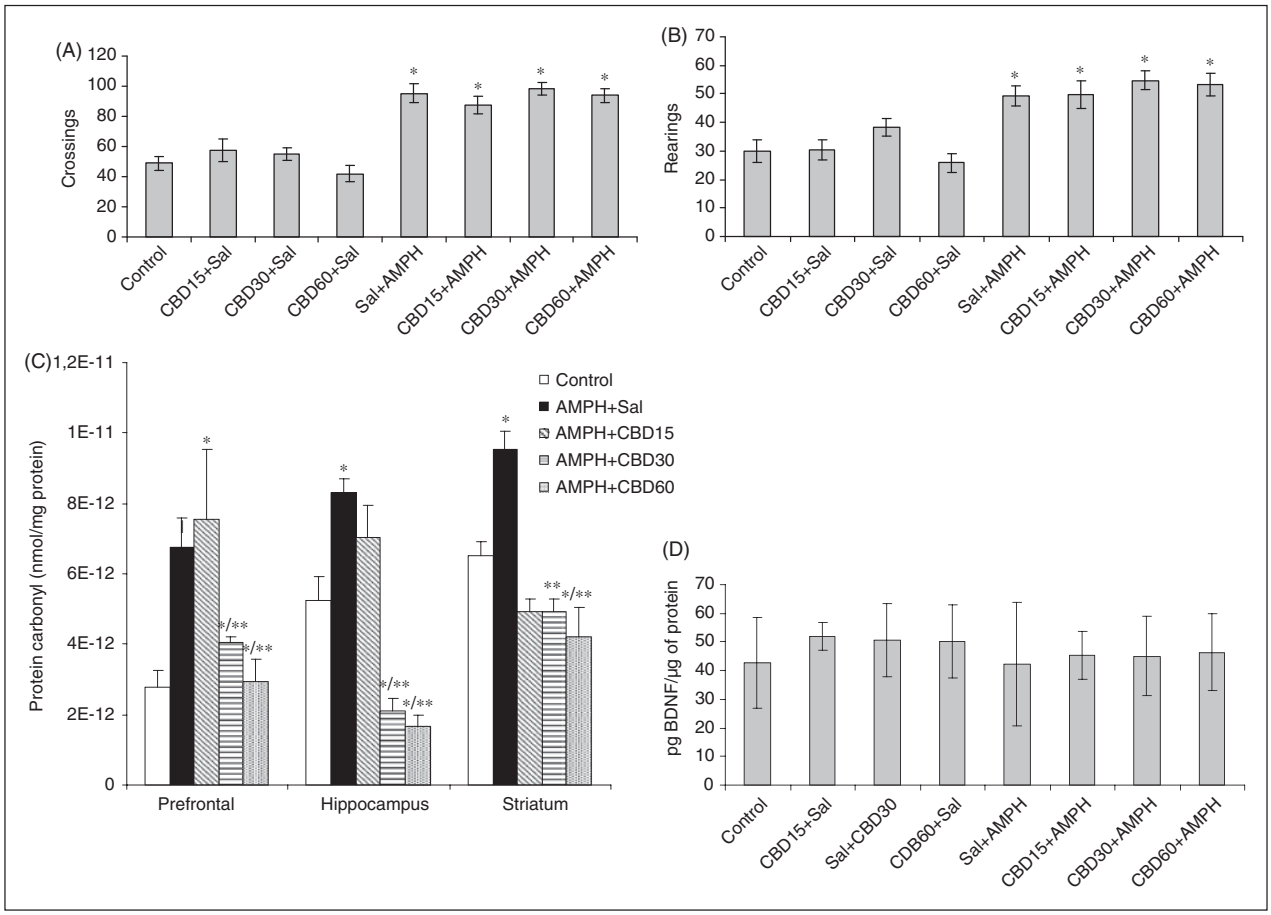

Figure 2. (A) Number of crossings. (B) Rearings ($n = 15$ for each group). (C) Protein carbonyl assessment ($n = 5$ for each group). (D) BDNF levels ($n = 5$ for each group) in the prevention model. Rats were pretreated with cannabidiol (15, 30 or 60 mg/kg) for seven days and then treated with amphetamine plus amphetamine (D-AMPH) the 8th and 14th days. CBD = cannabidiol, control = vehicle+saline. Bars represent means; error bars represent standard error of the means (SEM). *Different to the saline group. **Different to the D-AMPH group.

Interestingly, using functional neuroimaging we have previously observed that the anxiolytic-like effect induced by CBD is mediated by an action in the left para-hippocampal gyrus and left amygdala-hippocampus complex (Crippa et al., 2004).

Despite these observed anti-oxidant findings, in the present study we have also found that the co-administration of CBD with amphetamine can increase D-AMPH-induced formation of carbonyl group, suggesting that their effects on oxidative stress vary depending on the brain region, treatment and doses regimen. In fact, these contrasting neuroprotective and antioxidant CBD effects observed here can be explained by its multiple mechanisms of action and the fact that many of the effects of CBD draw a bell-shaped dose–response curve, suggesting that the dose is a key factor in CBD research (Zuardi et al., 2008b). Moreover, these contrasting results have also been previously verified with other compounds that exert neuroprotective effects such as valproate and lithium (Frey et al., 2006b).

In conclusion, we demonstrated that CBD did not modify D-AMPH-induced manic-like hyperactivity, but could protect D-AMPH-induced damage through oxidative stress. In addition, CBD increased levels of BDNF in the reversal experiment. However, these protective effects depend on the brain region analyzed, treatment and doses regimen. Our findings further support the notion that CBD may have neuroprotective effects, although more research is still needed to clarify its precise mechanisms that underlie this potentially beneficial effect of CBD.

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