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Influence of single and repeated cannabidiol administration on emotional behavior and markers of cell proliferation and neurogenesis in non-stressed mice

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ABSTRACT

Therapeutic effects of antidepressants and atypical antipsychotics may arise partially from their ability to stimulate neurogenesis. Cannabidiol (CBD), a phytocannabinoid present in *Cannabis sativa*, presents anxiolytic- and antipsychotic-like effects in preclinical and clinical settings. Anxiolytic-like effects of repeated CBD were shown in chronically stressed animals and these effects were parallel with increased hippocampal neurogenesis. However, antidepressant-like effects of repeated CBD administration in non-stressed animals have been scarcely reported. Here we investigated the behavioral consequences of single or repeated CBD administration in non-stressed animals. We also determined the effects of CBD on cell proliferation and neurogenesis in the dentate gyrus (DG) and subventricular zone (SVZ). Single CBD 3 mg/kg administration resulted in anxiolytic-like effect in mice submitted to the elevated plus maze (EPM). In the tail suspension test (TST), single or repeated CBD administration reduced immobility time, an effect that was comparable to those of imipramine (20 mg/kg). Moreover, repeated CBD administration at a lower dose (3 mg/kg) increased cell proliferation and neurogenesis, as seen by an increased number of Ki-67-, BrdU- and doublecortin (DCX)-positive cells in both in DG and SVZ. Despite its antidepressant-like effects in the TST, repeated CBD administration at a higher dose (30 mg/kg) decreased cell proliferation and neurogenesis in the hippocampal DG and SVZ. Our findings show a dissociation between behavioral and proliferative effects of repeated CBD and suggest that the antidepressant-like effects of CBD may occur independently of adult neurogenesis in non-stressed Swiss mice.

1. Introduction

Adult neurogenesis is a remarkable example of activity-dependent neural plasticity that is influenced by factors such as stress, aging, voluntary exercise, an enriched environment, and brain injury (van Praag et al., 1999; Parent, 2003; Jun et al., 2012). In the adult mammalian brain, neurogenesis has been shown to occur in at least two different regions: the subventricular zone (SVZ) of the lateral ventricles and subgranular zone (SGZ) of the hippocampal dentate gyrus (DG) (Patrício et al., 2013). Neuroblasts that are born in the SVZ migrate along the rostral migratory stream (RMS), becoming mostly mature γ-aminobutyric acid (GABA)-ergic granule and periglomerular interneurons in the olfactory bulb (Lois and Alvarez-Buylla, 1993; Doetsch et al., 1999). The cells that are born in the adult SGZ migrate to the granular cell layer (GCL) of the DG and differentiate into excitatory granule cells (Balu and Lucki, 2009). In other brain regions, such as the cerebral cortex, amygdala, hypothalamus, and substantia nigra, neurogenesis has been detected at very low levels or under non-physiological conditions (Patrício et al., 2013).

Hippocampal neurogenesis has attracted particular interest. Newborn neurons contribute to enhanced neural plasticity, which can sustain specific brain functions, such as spatial learning and mood regulation (Santarelli et al., 2003; Llorens-Martin et al., 2006). Antidepressant drugs and lithium are used to treat mood disorders and have been shown to increase cell proliferation and perhaps promote the subsequent survival of hippocampal neurons, indicating that increased hippocampal neurogenesis may be a common action of antidepressant treatment (Malberg et al., 2000; Chen et al., 2000; Manev et al., 2001). Non-antidepressant psychotropics, such as haloperidol or opioids,

Abbreviations: % OAE, percentage of open arm entries; % OAT, percentage of time spent on the open arms; 5-HT_{1A}, 5-hydroxytryptamine 1A; BrdU, bromodeoxyuridine; CBD, cannabidiol; CNS, central nervous system; CUS, chronic unpredictable stress; DAB, 3-3'-diamino-benzidine; DCX, doublecortin; DG, dentate gyrus; DNA, deoxyribonucleic acid; EPM, elevated plus maze; FTS, forced swim test; GAMA, ϒ-aminobuyric acid; GCL, granular cell layer; H_2O_2, hydrogen peroxide; HCl, hydrochloric acid; PBS, phosphate buffered saline; RMS, rostral migratory stream; SEM, standard error of the mean; SGZ, subgranular zone; SVZ, subventricular zone; TRPV1, transient potential vanilloid type 1; TST, tail suspension test; Δ^9-THC, Δ^9-tetrahydrocannabinol.

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have no effect on or even decrease hippocampal neurogenesis (Eisch et al., 2000; Malberg et al., 2000; Benninghoff et al., 2013). Interestingly, atypical antipsychotics, such as olanzapine, risperidone (Wakade et al., 2002), clozapine (Halim et al., 2004), and ziprasidone (Benninghoff et al., 2013; Peng et al., 2013) increased cell proliferation in both neurogenic regions (i.e., the hippocampal SGZ and SVZ).

Cannabidiol (CBD) is a phytocannabinoid that is present in the plant *Cannabis sativa*. It produces several effects in the central nervous system (CNS). Cannabidiol blocked the anxiogenic effects of high doses of Δ^9-tetrahydrocannabinol (Δ^9-THC) (Zuardi et al., 1982) and reduced anxiety in healthy volunteers in a neuroimaging study and in a simulated public speaking procedure (Zuardi et al., 1993). In animals, CBD has anxiolytic-like effects in the Vogel conflict test (Moreira et al., 2006), contextual fear conditioning paradigm (Resstel et al., 2006), and predator exposure test (Campos and Guimarães, 2009). Preclinical studies have shown that CBD also exhibits a profile that is similar to atypical antipsychotic drugs. Cannabidiol reduced stereotyped behavior (Zuardi et al., 1991), reversed the disruption of prepulse inhibition (Long et al., 2006; Gururajan et al., 2011), and restored MK-801-induced behavioral deficits in the social interaction and novel object recognition tests (Gomes et al., 2015). In the clinical setting, CBD attenuated some aspects of schizophrenia without producing extrapyramidal side effects (Zuardi et al., 1995; Zuardi et al., 2006; Leweke et al., 2012).

To our knowledge, no clinical study has been conducted that has evaluated whether CBD decreases depressive symptoms. In the preclinical field, the results are also scarce. Systemic CBD administration decreased immobility time in mice that were subjected to the forced swim test (FST) (El-Alfy et al., 2010; Zanelati et al., 2010), a characteristic effect of antidepressant compounds. Cannabidiol also prevented the autonomic and behavioral consequences of inescapable stress (Resstel et al., 2009). The common drawback of these studies, however, was that the animals received single intraperitoneal CBD injections. Only two studies of which we are aware have reported the antidepressant-like effects of chronic CBD administration (Réus et al., 2011; Campos et al., 2013). Campos et al. (2013) tested the effects of repeated CBD administration (30 mg/kg for 14 days) on the behavioral consequences of chronic unpredictable stress (CUS) in C57/B6 mice, which included anhedonia and anxiety-like behavior. Repeated CBD administration exerted anxiolytic-like effects in chronically stressed rats but not control animals. These effects were shown to depend on the facilitation of hippocampal neurogenesis.

The behavioral consequences of repeated CBD administration in non-stressed animals have been scarcely studied. In the present study, we investigated the effects of CBD treatment after single (1 h) and repeated (15 days) dosing in the elevated plus maze (EPM) and tail suspension test (TST). The TST, which involves a stressful situation of the animals being hung in an uncontrollable fashion by their tail, has good predictive validity for antidepressant-like effects (Cryan et al., 2005). Because neurogenesis has been suggested to underlie the therapeutic efficacy of antidepressants and CBD (Campos et al., 2013), we also investigated the effects of repeated CBD administration on cell proliferation and survival in the DG of the hippocampus and SVZ in non-stressed Swiss mice.

2. Material and methods

2.1. Animals

Male Swiss albino mice (30–40 g, 35–45 days old) were obtained from the central vivarium of the State University of Maringá, Maringá, Brazil. Prior to and throughout the experiments, the animals were maintained under conditions of controlled temperature (22 ± 1 °C) with a 12 h/12 h light/dark cycle (lights on at 7:00 AM). The animals were housed in groups of 3–5 per cage and received standard commercial chow and tap water ad libitum. A total of 103 mice was included in the experiments. The experimental procedures were approved by the Ethics Committee on Animal Experimentation of the State University of Maringá (CEEA 042/2012) and were in accordance with the guidelines of the U.S. National Institutes of Health and Brazilian College for Animal Experimentation.

2.2. Drugs

Vehicle (0.9% NaCl with 1% Tween 80), imipramine (20 mg/kg, Sigma-Aldrich, St. Louis, MO, USA), and CBD (3, 10, and 30 mg/kg, THC Pharma, Frankfurt, Germany) were prepared immediately before use and injected intraperitoneally (i.p.) in a volume of 1 ml/kg. The treatment regimen and CBD doses were based on Campos et al. (2013). Imipramine doses were based upon those that reduced immobility in the FST and TST and increased hippocampal neurogenesis after repeated administration in mice (Santarelli et al., 2003; Surget et al., 2008; Han et al., 2011; Pechnick et al., 2011).

2.3. Experimental design

We investigated the effects of single and repeated CBD administration in Swiss mice in the EPM and TST (Scheme 1). The experiments were performed between 8:00 AM and 12:00 PM. Before the start of the behavioral tests, the animals were allowed to acclimate to the testing rooms for 1 h. Exploratory behavior in the EPM was measured using a contrast-sensitive video tracking system (ANYmaze, Stoelting, Wood Dale, USA).

The animals were randomly assigned to the treatment conditions and tested in a counterbalanced order. For single administration, vehicle (n = 9), CBD 3 mg/kg (n = 8), CBD 10 mg/kg (n = 10), CBD 30 mg/kg (n = 9) or imipramine (20 mg/kg; n = 8) were administered 1 h before the behavioral testing. For repeated treatment, we investigated the behavioral and neurogenic effects of vehicle (n = 14), CBD at lower and higher doses, i.e., CBD 3 mg/kg (n = 17) and 30 mg/kg (n = 16), respectively, or imipramine (n = 12) given i.p. during 15 days. The behavioral testing was performed 1 h after later the last drug administration. This 1 h interval between CBD i.p. injection was shown sufficient to ensure effective CBD blood and brain concentration in mice (Deiana et al., 2012).

After 15 days of drug administration and behavioral testing, 5–7 animals from each experimental group were randomly chosen for the immunohistochemical analysis. Their brains were processed to evaluate cell proliferation (Ki-67 and bromodeoxyuridine [BrdU] staining) and neurogenesis (doublecortin [DCX] staining) (Scheme 1B).

2.4. Procedure

2.4.1. Elevated plus maze

The EPM consisted of two open arms (57 cm × 8 cm) and two closed arms (57 cm × 8 cm × 21 cm) that extended from a common central platform (8 cm × 8 cm). The entire maze was elevated 90 cm above the floor. The number of closed arm entries, number of open arm entries, and time spent on the open arms were recorded for 5 min for each animal (Pellow and File, 1986; Lister, 1987). The percentage of open arm entries ($\%OAE = 100 \times \textit{open arm entries} / \textit{total entries}$) and percentage of time spent on the open arms ($\%OAT = 100 \times \textit{time spent on open arms} / [\textit{time spent on open arms} + \textit{time spent on closed arms}]$) were calculated.

2.4.2. Tail suspension test

Each mouse was suspended 40 cm above the floor for 6 min using adhesive tape that was placed approximately 1 cm from the tip of the tail. The latency to the first episode of immobility and duration of immobility in the last 4 min of the test were recorded. Immobility was operationally defined as the mouse hanging motionless (Steru et al., 1985).