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Decreased glial reactivity could be involved in the antipsychotic-like effect of cannabidiol

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ABSTRACT

NMDA receptor hypofunction could be involved, in addition to the positive, also to the negative symptoms and cognitive deficits found in schizophrenia patients. An increasing number of data has linked schizophrenia with neuroinflammatory conditions and glial cells, such as microglia and astrocytes, have been related to the pathogenesis of schizophrenia. Cannabidiol (CBD), a major non-psychotomimetic constituent of *Cannabis sativa* with anti-inflammatory and neuroprotective properties induces antipsychotic-like effects. The present study evaluated if repeated treatment with CBD (30 and 60 mg/kg) would attenuate the behavioral and glial changes observed in an animal model of schizophrenia based on the NMDA receptor hypofunction (chronic administration of MK-801, an NMDA receptor antagonist, for 28 days). The behavioral alterations were evaluated in the social interaction and novel object recognition (NOR) tests. These tests have been widely used to study changes related to negative symptoms and cognitive deficits of schizophrenia, respectively. We also evaluated changes in NeuN (a neuronal marker), Iba-1 (a microglia marker) and GFAP (an astrocyte marker) expression in the medial prefrontal cortex (mPFC), dorsal striatum, nucleus accumbens core and shell, and dorsal hippocampus by immunohistochemistry. CBD effects were compared to those induced by the atypical antipsychotic clozapine. Repeated MK-801 administration impaired performance in the social interaction and NOR tests. It also increased the number of GFAP-positive astrocytes in the mPFC and the percentage of Iba-1-positive microglia cells with a reactive phenotype in the mPFC and dorsal hippocampus without changing the number of Iba-1-positive cells. No change in the number of NeuN-positive cells was observed. Both the behavioral disruptions and the changes in expression of glial markers induced by MK-801 treatment were attenuated by repeated treatment with CBD or clozapine. These data reinforces the proposal that CBD may induce antipsychotic-like effects. Although the possible mechanism of action of these effects is still unknown, it may involve CBD anti-inflammatory and neuroprotective properties. Furthermore, our data support the view that inhibition of microglial activation may improve schizophrenia symptoms.

1. Introduction

Individuals suffering from schizophrenia manifest a range of behavioral changes, including positive (delusions and hallucinations) and negative symptoms (social withdrawal, anhedonia), as well as cognitive impairment. Cognitive deficits and negative symptoms are present even before the onset of psychosis and are frequently associated with poor long-term outcome (Elvevag and Goldberg, 2000; Lesh et al., 2011). While the existing medications have proven effective in treating positive symptoms, their efficacy on negative symptoms and cognitive deficits is limited (Elvevag and Goldberg, 2000; Hanson et al., 2010), indicating a great need for new psychopharmacologic agents.

Although the etiology of schizophrenia is still unknown, evidence suggests that an impaired function of the prefrontal cortex mediated by a glutamate NMDA receptor hypofunction could be involved in the negative and cognitive symptoms of schizophrenia (Gonzalez-Burgos and Lewis, 2012; Nakazawa et al., 2012). This proposal is based essentially on studies showing that acute and chronic administration of NMDA receptor antagonists, such as phencyclidine, ketamine, and MK-801, in animals and healthy volunteers induces schizophrenia-like

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signs (Krystal et al., 1994; Jentsch and Roth, 1999). Moreover, when administered to schizophrenia patients these drugs can worse the psychotic symptoms (Krystal et al., 2005). Thus, animal models based on administration of these drugs have been widely used. However, even if most studies have employed acute administration of NMDA receptor antagonists, the effects induced by chronic treatment with these drugs are proposed to better represent the behavioral, neurochemical and neuroanatomical changes observed in schizophrenia patients (Jentsch and Roth, 1999).

An increasing number of clinical, epidemiological, and experimental data have linked schizophrenia with inflammatory conditions. In this context, glial cells, such as microglia and astrocytes, have been related to the pathogenesis of schizophrenia (Schnieder and Dwork, 2011; Monji et al., 2013). Microglia and astrocytes are the major immune cells in the central nervous system (CNS), regulating the induction as well as the limitation of inflammatory processes (Sofroniew and Vinters, 2010; Graeber et al., 2011).

Cannabidiol (CBD), a major non-psychotomimetic compound from *Cannabis sativa*, presents potential therapeutic effects in schizophrenia with several pre-clinical studies indicating that this drug induces antipsychotic-like effects (for review see Campos et al., 2012). These effects have also been described in open-label clinical studies (Zuardi et al., 1995, 2006) and in a recent controlled, randomized, double-blind clinical trial (Leweke et al., 2012). The mechanism of these effects is still unknown (Campos et al., 2012). However, besides its antipsychotic properties, CBD also induces anti-inflammatory and neuroprotective effects, which could contribute for its beneficial effects in schizophrenia. Indeed, a considerable number of preclinical studies have indicated that CBD attenuated increased glial reactivity associated to pathological conditions (Mecha et al., 2013; Perez et al., 2013; Schiavon et al., 2014). Yet, the involvement of these mechanisms in CBD antipsychotic effects has not been evaluated in animal models of schizophrenia.

Based on these pieces of evidence, we investigated whether repeated CBD treatment would attenuate the impairment in social interaction and novel object recognition (NOR) tests induced by chronic administration of the NMDA receptor antagonist MK-801. These tests have been widely used to study the negative symptoms and cognitive deficits, respectively, in animal models of schizophrenia (Ellenbroek and Cools, 2000; Rajagopal et al., 2014). Additionally, given that neuroinflammatory processes in schizophrenia may involve abnormal astrocyte and microglia functions (Rothermundt et al., 2009; Schnieder and Dwork, 2011; Monji et al., 2013; Catts et al., 2014) and that NMDA receptor antagonists induce neuronal damage and alter the expression of astrocyte and microglial markers (Nakki et al., 1995, 1996), we also measured changes in the expression of neuronal (NeuN) and glial markers (GFAP, astrocytes; Iba-1, microglia) in brain structures related to the neurobiology of schizophrenia, such as the medial prefrontal cortex (mPFC), dorsal striatum (dSTR), nucleus accumbens (NAc) core and shell and dorsal hippocampus (dentate gyrus — DG, CA1 and CA3). CBD effects were compared to those induced by the atypical antipsychotic clozapine.

2. Material and methods

2.1. Animals

The experiments were performed using male C57BL/6J mice with 6 weeks of age at the beginning of treatment. Animals were housed in groups of four per cage (41 × 33 × 17 cm) in a temperature-controlled room (24 ± 1 °C) under standard laboratory conditions with free access to food and water and a 12 h light/dark cycle (lights on at 06:00 a.m.). Procedures were conducted 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 and politics. The Institution's Animal Ethics Committee approved housing conditions and experimental procedures (process number: 165/2010).

2.2. Drugs

The following drugs were used: cannabidiol (CBD; THC Pharm, Germany), clozapine (Tocris, USA) and MK-801 (Sigma-Aldrich, USA). CBD was diluted in 2% Tween 80 in saline, while clozapine was diluted in saline supplemented with 30 μL of 0.1 M hydrochloric acid (pH was adjusted to a value close to neutrality when necessary). MK-801 was diluted in saline. Drugs were injected intraperitoneally (ip) in a 10 mL/kg volume. Body weight was measured daily.

2.3. Experimental design

We investigated whether repeated treatment with CBD (30 and 60 mg/kg) or clozapine (1 mg/kg) would attenuate social withdrawal and deficits in the novel object recognition (NOR) test induced by chronic treatment with MK-801 1 mg/kg for 28 days (n = 7–9/group). CBD or clozapine treatment began on the 6th day after the start of MK-801 administration and continued until the end of the treatment. The doses and treatment schedule were based on a previous study from our group (Gomes et al., 2015) and were those able to prevent the prepulse inhibition impairment induced by repeated MK-801 treatment. CBD, clozapine or vehicle were administered 30 min before MK-801 or saline, resulting in the following groups: vehicle + saline, CBD 60 mg/kg + saline, clozapine + saline, vehicle + MK-801, CBD 30 mg/kg + MK-801, CBD 60 mg/kg + MK-801, and clozapine + MK-801. One day after the end of the treatment, animals were submitted to the social interaction test. Four hours later they were submitted to the habituation session of the NOR test. The acquisition and test trials of the NOR test were performed 24 h later. On the day following the NOR test, all animals were euthanized and their brains were processed to assess changes in the expression of neuronal (NeuN) and glial markers (astrocytes — GFAP, and microglia — Iba-1) by immunohistochemistry. For this, only the dose of 60 mg/kg of CBD was evaluated, since it was more effective in mitigating MK-801-induced behavioral changes. A diagrammatic representation of the experimental design is presented in Fig. 1.

To evaluate a possible interference of anxiety-related behaviors and locomotor activity changes, independent groups of mice were submitted to a similar treatment schedule and evaluated in the elevated plus-maze (EPM) and open field tests 24 and 48 h, respectively, after the end of the treatments.

2.4. Procedure

2.4.1. Social interaction test

The social interaction test was carried out in a rectangular arena (28 × 17 × 13 cm). The animals (an experimental mouse and an unfamiliar conspecific mouse) were placed on opposite sides of the arena to freely explore it for 10 min. The time of active social behavior of the experimental mouse such as sniffing, following, grooming and climbing on or under the other mouse was recorded. The experimental animals had not been previously exposed to the arena and to the unfamiliar animal.

2.4.2. Novel object recognition (NOR) test

The NOR test was carried out in a Plexiglas circular arena (40 cm diameter and 40 cm height). One day before the test session each animal was habituated in the arena for 15 min. On the test day, animals were submitted to two trials separated by a 1 h-intertrial interval. During the first trial (acquisition trial, T1), mice were placed in the arena containing two identical objects for 10 min. For the second trial (test trial, T2), one of the objects presented in T1 was replaced by an unknown object (novel object). Animals were then placed back in the arena for 5 min. The behavior was recorded on video for blind scoring of object exploration, which was defined as situations where the animal is directing its face to the object in a distance of approximately 2 cm

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