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Anticonvulsant activity of β-caryophyllene against pentylenetetrazol-induced seizures

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ABSTRACT

Increasing evidence suggests that plant-derived extracts and their isolated components are useful for treatment of seizures and, hence, constitute a valuable source of new antiepileptic drugs with improved efficacy and better adverse effect profile. β-Caryophyllene is a natural bicyclic sesquiterpene that occurs in a wide range of plant species and displays a number of biological actions, including neuroprotective activity. In the present study, we tested the hypothesis that β-caryophyllene displays anticonvulsant effects. In addition, we investigated the effect of β-caryophyllene on behavioral parameters and on seizure-induced oxidative stress. Adult C57BL/6 mice received increasing doses of β-caryophyllene (0, 10, 30, or 100 mg/kg). After 60 min, we measured the latencies to myoclonic and generalized seizures induced by pentylenetetrazole (PTZ, 60 mg/kg). We found that β-caryophyllene increased the latency to myoclonic jerks induced by PTZ. This result was confirmed by electroencephalographic analysis. In a separate set of experiments, we found that mice treated with an anticonvulsant dose of β-caryophyllene (100 mg/kg) displayed an improved recognition index in the object recognition test. This effect was not accompanied by behavioral changes in the open-field, rotarod, or forced swim tests. Administration of an anticonvulsant dose of β-caryophyllene (100 mg/kg) did not prevent PTZ-induced oxidative stress (i.e., increase in the levels of thiobarbituric acid-reactive substances or the decrease in nonprotein thiols content). Altogether, the present data suggest that β-caryophyllene displays anticonvulsant activity against seizures induced by PTZ in mice. Since no adverse effects were observed in the same dose range of the anticonvulsant effect, β-caryophyllene should be further evaluated in future development of new anticonvulsant drugs.

1. Introduction

Epilepsy is a common neurological disease, which affects [1] and has been considered a major worldwide public health problem [2]. Recurrent epileptic seizures and behavioral comorbidities such as depression, anxiety, psychosis, and cognitive deficits largely affect the quality of life of the patients with epilepsy and their families [3]. There is the further complication that seizures in a significant percentage of patients remain inadequately controlled by currently available pharmacological treatments [4]. In addition, most anticonvulsant drugs display adverse effects such as ataxia, sedation, and cognitive dysfunction at serum concentrations within the therapeutic range for epileptic seizures [5]. Accordingly, discovery of a new anticonvulsant with better efficacy and improved safety profile is of fundamental importance [4]. In this context, several plant extracts and products may be useful for the treatment of convulsions or seizures, and therefore, natural products constitute a promising source of new antiepileptic drugs [6].

β-Caryophyllene is a natural bicyclic sesquiterpene that is a constituent of many plants [7]. Several biological activities have been reported for β-caryophyllene, including anti-inflammatory [7], anti-alcoholism [8], antinociceptive [9], anxiolytic, and antidepressant [10] properties. Interestingly, recent accumulating evidence indicates that β-caryophyllene is neuroprotective in several experimental paradigms [11–14]. For instance, administration of β-caryophyllene protects against cerebral ischemic injury in rats [11,14] and reduces astrogliosis and microglial activation in a transgenic mouse model of Alzheimer's disease [12]. Since neuroprotective compounds may display anticonvulsant activity

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and vice versa [15], and in order to further evaluate the potential therapeutic applications of β-caryophyllene, our present study aimed to test the hypothesis that this natural compound displays anticonvulsant effects. In addition, we investigated the effect of β-caryophyllene on selected behavioral parameters and on seizure-induced oxidative stress.

2. Materials and methods

2.1. Animals and reagents

Adult C57BL/6 mice (25–35 g, 60–90 day-old) of both genders were used. Animals were maintained under controlled light and environment (12:12 h light–dark cycle, 24 ± 1 °C, 55% relative humidity) with free access to water and food (Purotrato, Santa Maria, RS, Brazil). All experimental protocols aimed to keep the number of animals used to a minimum, as well as their suffering. These were conducted in accordance with national and international legislation (guidelines of Brazilian Council of Animal Experimentation — CONCEA — and of U.S. Public Health Service's Policy on Humane Care and Use of Laboratory Animals — PHS Policy), and with the approval of the Ethics Committee for Animal Research of the Federal University of Santa Maria (process 016/2014). Pentylenetetrazol (PTZ) and β-caryophyllene were purchased from Sigma (Sigma-Aldrich, St. Louis, MO, USA). All other chemicals were reagent grade and purchased from local suppliers.

2.2. Behavioral seizure evaluation

Animals were individually placed in glass boxes and injected with increasing doses of β-caryophyllene (10, 30, or 100 mg/kg; i.p.) or its vehicle (0.9% NaCl containing 0.05% Tween 80). Sixty minutes thereafter, PTZ (60 mg/kg, i.p.) was injected, and the animals were observed for 15 min. During this time, we recorded the latency to myoclonic jerks, latency to generalized seizure, and the duration of the first generalized seizure. All solutions were administered at 10 mL solution per kg of body weight. Doses and schedules for drug injections were selected based on the literature [11,14] and on pilot experiments.

2.3. Electroencephalographic (EEG) recordings

Seizure activity and the effect of β-caryophyllene were evaluated in a subset of animals (n = 3–4) by EEG recordings. For recording electrode implantation, animals were anesthetized with intraperitoneal ketamine (80 mg/kg) and xylazine (10 mg/kg) and placed in a rodent stereotaxic apparatus. Under stereotaxic guidance, two stainless steel screw electrodes were placed over the parietal cortex, along with a ground lead positioned over the nasal sinus. The electrodes were connected to the multipin socket and were fixed to the skull. Meloxicam (200 mg/kg, s.c.) and metamizole (100 mg/kg, s.c.) were administrated immediately before and for three days after the surgical procedure.

Six days after the surgery, each animal was transferred to a Plexiglas cage (25 × 25 × 40 cm) and habituated for 20 min before EEG recordings. The mouse was then connected to a 100× headstage preamplifier (model #8202-DSE3) in a low-torque swivel (Pinnacle Technology Inc., Lawrence, KS, USA), and the EEG was recorded using a PowerLab 16/30 data acquisition system running LabChart 7.2 software (AD Instruments, Castle Hill, Australia). Routinely, a 30-min baseline recording was obtained to establish an adequate control period. After the baseline recording, mice were injected with β-caryophyllene (100 mg/kg) or vehicle, 60 min before the injection of PTZ. Following convulsant injection, the EEG signals were recorded for 15 min. Electroencephalographic signals were amplified, filtered (0.1 to 50.0 Hz, bandpass), digitalized (sampling rate 1024 Hz), and stored in a PC for off-line analysis.

2.4. Behavioral tests

In order to investigate the effects of an anticonvulsant dose of β-caryophyllene (100 mg/kg) on exploratory behavior and motor skills of the mice, we evaluated performance in the open-field, object recognition, rotarod, and forced swim tests. Independent groups of mice were used in each test, and each animal was used only once.

2.4.1. Open-field test

Animals were placed in the central area of a round open field (56 cm in diameter), which had its floor divided into 10 equal areas. Five areas of the apparatus had their borders limited by the walls of the arena and were considered as peripheral areas. The remaining five areas that had no contact with the walls of the apparatus were considered as central areas. β-Caryophyllene (100 mg/kg) or its vehicle were injected 60 min before the beginning of the test, and the number of crossed areas (crossings) as well as the number of rearing responses (animal stands on its hind legs) were recorded for 5 min.

2.4.2. Object recognition test

The object recognition test consisted of three sessions, namely, habituation #1 (first training session), habituation #2 (second training session, 4 h after training), and memory evaluation (test session, 24 h after habituation #1). During the first training session, two identical objects (transparent cylindrical plastic bottles) were equidistantly placed in the center of the same open-field arena described above, and the time spent in exploration of each object was recorded for 10 min. Four hours thereafter (habituation #2), one of the bottles was replaced for a new object (plastic red apple), and the time spent in exploration of each object was measured for 10 min. Finally, 24 h after training, the plastic red apple was replaced with another new object (triangular plastic cup), and the time spent in exploration of each object was recorded for 10 min (test session). Any subjects that failed to complete a minimum of 10-second exploration time in the test trial (three vehicle-treated and three β-caryophyllene-treated animals) were excluded from the analysis. The object recognition index was calculated with the following formula: recognition index = (time spent in new object) / (time spent in the new object + time spent in the familiar object). β-Caryophyllene (100 mg/kg) or its vehicle were injected 60 min before the beginning of the test session.

2.4.3. Rotarod test

Fine motor coordination was assessed by using the rotarod test. The task consisted of one training session and one testing session, carried out 24 h apart. Trial starts with the mouse being placed in the apparatus (3.7 cm rod diameter, 8 rpm constant speed) and ends when the mouse falls off the rod or after reaching the cutoff time of 60 s two consecutive times. During the training session, the maximum number of attempts was 10. A resting time of 60 s was allowed between each trial. In the test session, mice were observed for 4 min, and the latency to the first fall was recorded. β-Caryophyllene (100 mg/kg) or its vehicle were injected 60 min before the beginning of the test session.

2.4.4. Forced swim test

Mice were placed in individual, clear polyvinyl chloride (PVC) cylinders (30 cm tall × 10 cm diameter) containing 23–25 °C water (20 cm-deep to prevent the mouse's tail from touching the cylinder bottom). Water was changed between subjects. The immobility time during the 5 min of test was recorded. Immobility was assigned when no additional activity was observed other than that required to keep the mouse's head above water. β-Caryophyllene (100 mg/kg) or its vehicle were injected 60 min before the beginning of the test session.