Anticonvulsant effect of cannabidiol in the pentylenetetrazole model: Pharmacological mechanisms, electroencephalographic profile, and brain cytokine levels

Luciano R. Vilela a, Isabel V. Lima b, Érica B. Kunsch b, Hyorrania Priscila P. Pinto c, Aline S. de Miranda d, Érica Leandro M. Vieira e, Antônio Carlos P. de Oliveira b, Marcio Flávio D. Moraes e, Antônio L. Teixeira e, Isabel V. Lima b, Érica B. Kunsch b, Hyorrania Priscila P. Pinto c, Aline S. de Miranda d, Érica Leandro M. Vieira e, Antônio Carlos P. de Oliveira b, Marcio Flávio D. Moraes e

a Graduate School in Neuroscience, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Brazil
b Department of Pharmacology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Brazil
c Department of Physiology and Biophysics, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Brazil
d Department of Morphology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Brazil
e Department of Internal Medicine, School of Medicine, Universidade Federal de Minas Gerais, Brazil

A R T I C L E  I N F O
Article history:
Received 20 April 2017
Revised 1 July 2017
Accepted 7 July 2017
Available online xxxx

Keywords:
Epilepsy
Seizure
Anticonvulsants
Cannabis sativa
Cannabinoids

A B S T R A C T
Cannabidiol (CBD), the main nonpsychotomimetic compound from Cannabis sativa, inhibits experimental seizures in animal models and alleviates certain types of intractable epilepsies in patients. Its pharmacological profile, however, is still uncertain. Here we tested the hypothesis that CBD anticonvulsant mechanisms are prevented by cannabinoid (CB1 and CB2) and vanilloid (TRPV1) receptor blockers. We also investigated its effects on electroencephalographic (EEG) activity and hippocampal cytokines in the pentylenetetrazole (PTZ) model. Pretreatment with CBD (60 mg/kg) attenuated seizures induced by intraperitoneal, subcutaneous, and intravenous PTZ administration in mice. The effects were reversed by CB1, CB2, and TRPV1 selective antagonists (AM251, AM630, and SB366791, respectively). Additionally, CBD delayed seizure sensitization resulting from repeated PTZ administration (kindling). This cannabidiol also prevented PTZ-induced EEG activity and interleukin-6 increase in prefrontal cortex. In conclusion, the robust anticonvulsant effects of CBD may result from multiple pharmacological mechanisms, including facilitation of endocannabinoid signaling and TRPV1 mechanisms. These findings advance our understanding on CBD inhibition of seizures, EEG activity, and cytokine actions, with potential implications for the development of new treatments for certain epileptic syndromes.

© 2017 Elsevier Inc. All rights reserved.

1. Introduction
Cannabis sativa (marijuana) has raised interest due to its potential use for the treatment of certain types of epilepsies [1]. One of its constituents, Δ9-tetrahydrocannabinol (Δ9-THC), accounts for most cannabis effects by activating cannabinoid CB1 and CB2 receptors. These receptors are activated in the body by arachidonoyl ethanolamide (anandamide) and 2-arachidonyl glycerol (2-AG), collectively termed endocannabinoids. Anandamide and 2-AG actions are terminated by the enzymes fatty acid amidase hydrolase (FAAH) and monoacylglycerol lipase (MAGL). The cannabinoid receptors, the endocannabinoids, and the enzymes responsible for their metabolism constitute the endocannabinoid system [2]. Δ9-THC and its synthetic derivatives, which directly bind to cannabinoid receptors, may inhibit or facilitate experimental seizures, in addition to inducing potential side effect, which limit their potential use as anticonvulsant medicines [3,4]. The clinical applications of these compounds are further limited by psychotomimetic activity and other potential undesirable effects [2]. Cannabis, however, produces dozens of other compounds, termed phytocannabinoids, among which cannabidiol (CBD) is one of the most abundant. CBD does not act as a cannabinoid receptor agonist and thus lacks the typical deleterious effects of Δ9-THC [5]. Considering its safety profile, CBD has been investigated for the treatment of some neurological and psychiatric disorders [1,5,6].

Studies employing various in vivo and in vitro models report consistent anticonvulsant and antiepileptiform effects of CBD [6–12]. Its mechanisms of action, however, remain elusive. CBD has very low affinity for CB1 and CB2 receptors, although it may indirectly facilitate cannabinoid signaling by blocking anandamide uptake and hydrolysis [13]. CBD may
activate the transient receptor potential of vanilloid type 1 (TRPV1), which is also targeted by anandamide [13].

One approach for investigating the anticonvulsant mechanisms of pharmacological agents is the pentylenetetrazole (PTZ) model. PTZ induces convulsive seizures by blocking gamma-aminobutyric acid type A (GABA_A) channels, leading to occurrence of generalized tonic–clonic seizures [14]. This compound is useful for studying seizures in single- or repeated-injection protocols, as well as for detecting anticonvulsant or proconvulsant drug effects [15,16]. In addition, the PTZ model allows the studies of electroencephalographic (EEG) and neurochemical events associated with epileptic seizures. In this context, recent studies have highlighted the role of inflammation in the molecular and structural changes that contribute to seizures. Increased inflammatory mediators are produced secondarily to the epileptogenic insults, which are important in the development and maintenance of seizure responses. This notion is supported by studies demonstrating elevated brain levels of proinflammatory cytokines, adhesion proteins, and related molecules in convulsive seizures [17–22].

Considering evidence from in vitro experiments and the paucity of in vivo approaches, this study was designed to investigate the effects of CBD in PTZ-induced seizures in mice. First, we characterized the anticonvulsant effect of this phytocannabinoid against single and repeated PTZ treatment (kindling). Second, to investigate the underlying pharmacological mechanisms, we tested the hypothesis that CBD effect is reversed by specific antagonists of CB1, CB2, and TRPV1 receptors. Finally, we hypothesized that CBD prevents EEG activity and cytokine expression induced by PTZ.

2. Materials and methods

2.1. Animals and surgical procedures

All experiments were done in accordance with the Ethical Committee for Animal Experimentation (CETEA) of the Federal University of Minas Gerais (Universidade Federal de Minas Gerais – UFMG), which is in accordance with the ARRIVE and other international guidelines. All the procedures for animal care were previously approved by this organization under protocol number 242/2013.

Male Swiss mice weighing 20–30 g (from Centro de Bioterismo, CEBIO-UFMG) were kept on a 12 h:12 h dark/light cycle (lights on between 7 am and 7 pm) at 22 ± 1 °C with free access to food and water throughout the experiment. Electrophysiological studies were performed in accordance with the Brazilian Society for Neuroscience and Behavior Guidelines for Animal Experimentation. The animals were anesthetized with ketamine:xylazine (80 mg/kg:8 mg/kg, i.p., Syntec®, Cotia, Brazil) and positioned in a stereotaxic frame (David Kopf, model 960). Stainless steel bone-screw electrodes (Fine Sciences Tools mod. 19,010–00) were placed over parietal cortices and fixed to the skull with zinc cement and soldered to pin bars. Coordinates (AP = −2.0 mm, ML = ±2.0 mm referenced from the bregma suture) were derived from a stereotaxic atlas for mice [23]. A reference and a ground electrode were inserted into the nasal bone. The animals received prophylactic intramuscular injections of polyantibiotic (0.27 g/kg benzylpenicillin, streptomycin, and dihydrostreptomycin; Pentabiotic®, Fort Dodge, Brazil) and the nonsteroidal anti-inflammatory drug flunixinmeglumine (0.025 g/kg; Banamine®, Schering Plow, Brazil). They were allowed to recover for 4–5 days before the experiments.

2.2. Drugs

Pentylenetetrazole (PTZ), Sigma-Aldrich (St. Louis, MO, USA), was diluted in physiological saline [24]. Cannabidiol (CBD) was kindly supplied by THC-Pharma (Frankfurt Germany) and was diluted in physiological saline containing tween–80 at 2%. The CB1 antagonist, 1-[(2,4-dichlorophenyl)-5-[(4-isodophenyl)-4-methyl-N-(1-piperidyl)]pyrazole-3-carboxamide (AM251); the CB2 antagonist, 6-lodo-2-methyl-1-[2-(4-morpholino)[ethyl]-1H-indol-3-yl][4 methoxyphenyl] methane (AM 630), and the TRPV1 antagonist, 40-chloro-3-methoxyccinnamamide (SB-366791), were obtained from Cayman Chemical (Ann Arbor, MI, USA) and dissolved in cremophor–ethanol–saline at the proportion of 1:1:18 [25]. The solutions were prepared immediately before use, and all injections were administered intraperitoneally (i.p.) in a volume of 1 ml/kg body weight 30 min before PTZ injections.

2.3. Seizure evaluation

2.3.1. Intraperitoneal PTZ test

The experiments were conducted between 8 am and 1 pm. The animals were individually placed in glass boxes, injected with PTZ at dose of 60 mg/kg via i.p. route and observed for 15 min. Although there are several possible behavioral responses to PTZ [26], we have focused on recording the latency to the first generalized seizures and their total duration during the 15-min observation time [24]. Doses and schedules for drug injections were selected based on pilot experiments and literature [25]. Thirty minutes after PTZ administration, mice were decapitated without anesthesia and the hippocampus and prefrontal cortex (PFC) were dissected from freshly perfused brains, immediately frozen, and stored at −80 °C until the assay.

2.3.2. Subcutaneous PTZ test

The animals received a 60 mg/kg dose of PTZ subcutaneously into a loose fold of skin of the neck, between two shoulder blades, and were observed over the course of 15 min. The latency to the first generalized seizure and the total time in seizures were recorded. Animals not displaying seizures during this period were assigned a cutoff time latency of 30 min for the calculations of mean onset latency to generalized seizure.

2.3.3. Intravenous PTZ test

PTZ (10 mg/ml; infusion rate 0.2 ml/min) was infused to the tail vein of freely moving, unrestrained mice. The needle was secured in the tail vein with a tape. Infusion was blocked as soon as forelimb clonus occurred, and it was immediately followed by full clonus of the body. Seizure thresholds were calculated using the following formula: threshold dose of PTZ [mg/kg] = (PTZ concentration [mg/ml] × infusion rate [ml/s] × infusion duration [s] × 1000)/body weight [g] and were expressed as the dose of PTZ (in mg/kg) needed to produce a given endpoint as noted above [24]. The animals were euthanized immediately after the end of the infusion.

2.3.4. PTZ-induced kindling

To induce kindling, we injected PTZ in a subconvulsant dose (35 mg/kg, i.p.) on alternate days for 30 days. After each injection, the mice were placed in transparent plexiglass cages and were observed for 30 min, according to the Racine scale [24,27]. The severity of seizure response was evaluated using a five-point scoring system: Stage 0, no response; Stage 1, ear and facial jerks; Stage 2, myoclonic body jerks without upright position; Stage 3, myoclonic jerks, upright position with bilateral forelimb clonus; Stage 4, tonic–clonic seizures; and Stage 5, generalized tonic–clonic seizures, loss of postural control. The animals were considered kindled if they exhibited Stage 4 or (and) 5 of seizures on two consecutive trials. The animals received injections of vehicle or CBD at dose of 60 mg/kg 30 min before PTZ during all 15 injections.

2.4. Electroencephalographic recording and analysis

Video-EEG recordings were performed starting immediately after PTZ administration for a period of 10 min. The EEG signal was amplified (1000 ×), filtered (1 Hz high pass and 2000 Hz low pass), and digitized using an A/D converter set at a sampling rate of 1 kHz (Kananda® Ltda. Belo Horizonte, MG, Brazil). Data were stored in a personal computer,
and records were analyzed offline according to the latency to the onset and the duration of the epileptiform discharge by a person blind to animal status.

2.5. Cytometric bead array (CBA)

Cytokine levels were determined using a mouse Th1/Th2/Th17 cytometric bead array (CBA) kit (BD Biosciences, San Diego, CA, USA) and analyzed on a FACSCanto II flow cytometer. Standard curves for each cytokine were determined over a range of 20–5000 pg/ml. The lower limit of detection for the CBA was 0.1–2.0 pg/ml depending on the analyte. The levels of the cytokines interleukin-2 (IL-2), IL-6, IL-10, IL-17A, interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α) in the hippocampus and PFC were measured and analyzed with the FCAP Array software (BD Biosciences) [28].

2.6. Statistical analysis

The effects of CBD on PTZ-induced seizure, EEG activity, and cytokine levels were analyzed with Student t-test or ANOVA followed by the Newman–Keuls test, as appropriate. PTZ-induced kindling was analyzed with repeated-measures analysis of variance (ANOVA), considering treatment and time as experimental factors. All data are presented as mean ± SEM. The significance level was considered at p < 0.05. The statistical software used was the GraphPad Prism 6.0 Version for Windows (San Diego, CA, USA).

3. Results

CBD administration (i.p.) 30 min before PTZ (i.p.) induced anticonvulsant effects at the dose of 60 mg/kg. There was an increase in latency

Fig. 1. Effects of CBD (30–90 mg/kg, i.p.) on seizures induced by intraperitoneal administration of PTZ (60 mg/kg) in mice. CBD (60 mg/kg) increased seizure latency (upper panel) and reduced seizure duration (lower panel). *p < 0.05 and **p < 0.01 compared with Vehicle (Veh) group, one-way ANOVA, followed by Newman–Keuls test (n = 8, 7, 8, 8). Data are expressed as mean ± SEM.

Fig. 2. Effects of CBD (60 mg/kg, i.p.) on seizures induced by subcutaneous administration of PTZ (60 mg/kg) in mice. CBD increased seizure latency (upper panel) and reduced seizure duration (lower panel). *p < 0.05, Student t-test (n = 8/group). Data are expressed as mean ± SEM.

Fig. 3. Effects of CBD (60 mg/kg, i.p.) on seizures induced by intraperitoneal administration of PTZ (10 mg/mL) in mice. CBD increased the dose of intravenous PTZ solution (10 mg/mL) required to induce seizure. **p < 0.01, Student t-test (n = 5, 6). Data are expressed as mean ± SEM.

Fig. 4. Effect of CBD (60 mg/kg, i.p.) on intraperitoneal PTZ (35 mg/kg)-induced kindling in mice. The animals received PTZ injections every 2 days. Vehicle (Veh) or CBD were administered before each PTZ injection. CBD delayed the seizure progression induced by PTZ. Two-way ANOVA followed by Bonferroni test (n = 5, 6). Data are expressed as mean ± SEM.
to the first seizure ($F_{1,27} = 3.516, p = 0.02$; Fig. 1, upper panel) and a reduction in seizure duration ($F_{3,27} = 5.229, p = 0.005$; Fig. 1, lower panel).

In order to test if CBD promotes consistent anticonvulsant effects, we tested the effective dose (60 mg/kg, i.p.) against PTZ administered via other routes. CBD was effective in increasing seizure latency ($t_{14} = 2.343, p = 0.03$; Fig. 2, upper panel) and reducing seizure duration ($t_{14} = 2.828, p = 0.013$; Fig. 2, lower panel) induced by subcutaneous PTZ administration. Moreover, previous administration of this cannabinoid increased the dose of intravenous PTZ necessary to trigger convulsive seizures ($t_6 = 4.898, p = 0.0009$; Fig. 3).

Finally, we tested the effects of CBD in PTZ-induced kindling (Fig. 4). Repeated sub-threshold PTZ (35 mg/kg, i.p.) injection resulted in a gradual increase in convulsive activity (seizure score) culminating in generalized clonic–tonic seizures in all animals (time factor: $F_{14,165} = 39.28, p < 0.0001$). The first incidence of two consecutive seizures with score 4 or more was observed at the 12th injection, indicating kindled animals. There was a significant drug effect (drug factor: $F_{1,165} = 14.18, p = 0.0002$), implying that treatment with CBD (60 mg/kg, 30 min before each PTZ injection) delayed the progression of kindling. However, the maximal effect was not reduced. There was no interaction between time and drug factors ($F_{14,165} = 1.456, n.s.$).

Regarding the potential mechanisms of action of CBD in the i.p. PTZ-induced seizure model, pretreatment with the CB1 antagonist, AM251, at the dose of 1.0 mg/kg reversed the effects of CBD on seizure latency ($F_{3,38} = 3.462, p = 0.02$; Fig. 5A) and duration ($F_{3,38} = 3.903, p = 0.01$; Fig. 5B). Likewise, the CB2 antagonist, AM630 at the dose of 2.0 mg/kg also reversed CBD effects on seizure latency ($F_{2,28} = 6.696, p = 0.001$; Fig. 5C) and duration ($F_{2,28} = 8.979, p = 0.0003$; Fig. 5D).

The same was observed with the TRPV1 antagonist, SB366771, at the dose of 1.0 mg/kg both for latency ($F_{2,34} = 5.350, p = 0.004$; Fig. 5E) and duration ($F_{2,34} = 3.189, p = 0.03$; Fig. 5F).

In the EEG recordings, CBD increased the latency to the first seizure ($t_{11} = 4.686, p = 0.0007$; Fig. 6, upper panel) and reduced the effects of PTZ upon total seizure duration ($t_{11} = 2.413, p = 0.0344$; Fig. 6, lower panel). Representative recordings of EEG activity after vehicle or CBD injection are presented in Fig. 7.

As for the effect on cytokine levels, pretreatment with CBD (60 mg/kg, i.p.) prevented PTZ (60 mg/kg, i.p.)-induced increase in IL-6 in the PFC ($F_{2,25} = 1795, p < 0.0001$). PTZ also reduced IFN-γ levels, although this change was not prevented by CBD ($F_{2,25} = 4818, p < 0.0001$). There were no changes in the levels of other cytokines.

4. Discussion

In the present study we showed that pretreatment with CBD attenuated seizures and EEG activity changes induced by PTZ in mice. This effect is prevented by CB1 and CB2 receptor antagonists as well as by a TRPV1 channel blocker. Moreover, repeated administration of this...
cannabinoid delayed the development of PTZ-induced kindling, although the maximum response was not reduced. CBD also prevented PTZ-induced IL-6 increase in the PFC.

The antiepileptic potential of *C. sativa* preparations has been historically recognized, in spite of legal restrictions preventing its use [4,29]. Moreover, phyto cannabinoids, particularly CBD, are effective in preclinical animal models of seizures and epilepsy, including audiogenic seizures, maximal electroshock, transcorneal electroshock, and the chemical stimulants such as PTZ, penicillin, pilocarpine, and cocaine [11,12,30–32]. Studies in humans also show its efficacy and favorable safety profile [6,10,33]. The therapeutic potential of the CBD attracted renewed interest in the last years for the pediatric treatment of certain epileptic syndromes such as Dravet and Lennox–Gastaut syndromes [34]. Our study provides further support for the literature showing CBD protective effect against seizure induced by PTZ administered through different routes in mice. However, we found that CBD induced a U-shaped dose–response effect, in which only the intermediate dose (60 mg/kg) inhibited PTZ effect. This is in accordance with CBD effects in other pharmacological responses, particularly in models of anxiolytic activity [35]. This profile is expected considering the complex pharmacology of this cannabinoid, which binds to several targets depending on the concentration, including endocannabinoid-related enzymes, serotonin-1A receptor, TRP channels, and GPR55 receptor, among others [5]. Apart from these receptors, alternative mechanisms should also be considered. For instance, recent studies found that histamine-mediated process may contribute to the development of convulsive seizures [36]. Since cannabinoids interact with the histaminergic system [37,38], this possibility warrants further investigation.

Whereas most studies have focused only on single CBD injections, we have also tested if repeated treatment would interfere with seizure progression. Statistical analysis revealed a drug effect, in which CBD shifted PTZ curve to the right, delaying seizure progression. The maximal PTZ effect, however, was not reduced, indicating that CBD delayed, but failed to prevent, kindling. This is in contrast with a recent study showing a favorable CBD effect in the chronic phase of pilocarpine-induced epilepsy [39]. Thus, CBD effects should be further investigated in chronic models of seizure and epilepsy. This is particularly relevant considering the limits of single-injection models for the development of new treatments. Indeed, epilepsies are chronic neurological syndromes characterized by long-term molecular and cellular changes that precipitate spontaneous neural excitability. Thus, models employing long-term interventions have better predictive validity [15].

Previous studies have detected the effects of CBD in various experimental models of seizure and epilepsy. However, its mechanisms of action have remained scarcely investigated. Here, we found that its anticonvulsant effects were prevented by CB1 and CB2 receptor
antagonists, and by a TRPV1 blocker. These results should be discussed considering the molecular pharmacology of CBD. This cannabinoid does not act as an agonist at CB1 or CB2 receptor [6,13]. Thus, other mechanisms should be considered to explain its anticonvulsant effect. For instance, CBD inhibits anandamide uptake and hydrolysis [13] and may increase anandamide levels in the brain, indirectly facilitating both CB1 and CB2 receptor signaling to reduce PTZ-induced seizures. This notion is supported by previous studies showing that FAAH inhibitors (the main enzyme responsible for anandamide hydrolysis) exhibit anticonvulsant activity [24,25]. Increasing anandamide actions upon CB1 receptor may reduce excitatory neurotransmission and promote on-demand protection against seizure and neurotoxicity [40]. As for CB2, this receptor has also been implicated in the modulation of experimental seizures [41], although the precise neural mechanisms remain to be further investigated.

Regarding the role of TRPV1, this ion channel has been implicated in the modulation of experimental seizures and epilepsy [42]. Here we found that the selective blocker, SB366791, prevented the anticonvulsant effect of CBD, in line with molecular studies showing that this cannabinoid acts as a TRPV1 agonist [13]. Since this is an ion channel whose activation leads to calcium influx and increase in neuronal activity and glutamate release [43], it seems contradictory to speculate that its activation reduces seizures. Moreover, TRPV1 blockade exerts anticonvulsant activity in experimental models [42]. One possible explanation is that this ion channel can be rapidly desensitized after agonist binding, leading to responses that may actually result from channel inactivation [42]. This hypothesis is supported by in vitro studies showing that CBD reduced epileptiform activity by phosphorylating and thereby promoting desensitization of TRPV1 channels [44]. Therefore, CBD protective effects may occur by increasing anandamide levels, which in turn facilitate CB1 and CB2 receptor signaling, along with desensitization of TRPV1 channel.

As an additional measure of CBD anticonvulsant effect, we have recorded EEG activity in parallel to scoring convulsive responses. As expected, PTZ injection resulted in the occurrence of repeated high-amplitude spikes. Remarkably, CBD delayed the latency and reduced the duration of PTZ-induced EEG activity changes, paralleling the effects observed in behavioral seizures. This is in accordance with another study in which this compound reduced the in vivo electrophysiological effects of convulsant stimuli, including seizure spread in brain [32]. In vitro experiments also showed that CBD reduces epileptiform activity in hippocampal brain slices [11] and normalizes intracellular calcium increase in cells exposed to excitotoxic stimuli [45]. Based on the current results, CBD may reduce neural excitability by facilitating anandamide action upon cannabinoid receptor signaling and by direct desensitizing TRPV1 channels.

Apart from the behavioral and EEG recordings, we also demonstrated that CBD prevented PTZ-induced increase in the proinflammatory cytokine IL-6 in the PFC (Table 1). This might implicate that anti-inflammatory mechanisms contribute to the antiseizure effects of CBD. Supporting this possibility, clinical and experimental studies have strongly suggested that neuroinflammation participates in the pathophysiology of epilepsy [19–21,46]. There has been evidence for activation of both innate and adaptive immune systems, recruitment of microglia and astrocytes and production of inflammatory molecules [47]. Accordingly, epileptic seizures can induce the production of IL-6, which in turn may participate in the pathogenesis and course of epilepsy [20]. In addition, IL-6 treatment increases PTZ-induced seizure in rats [48]. As for CBD, this cannabinoid attenuates inflammatory cell migration/ infiltration (e.g., neutrophils) in most in vivo models of inflammation, in addition to reducing T cell responses, cyclooxygenase activity, and nitric oxide production [1]. The suppressive effects of CBD on cellular immune responses and on the production of pro-inflammatory mediators may indicate its usefulness in several inflammatory diseases [1].

In conclusion, CBD consistently reduced the effects of PTZ administration in various protocols. This effect possibly depends on indirect CB1 and CB2 receptor facilitation and TRPV1 channel desensitization.

CBD also prevented PTZ effects on EEG activity and IL-6 expression. These results reinforce previous studies suggesting the potential use of CBD for the treatment of certain types of epilepsies and unveil possible mechanisms underlying its anticonvulsant activity. This is particularly relevant considering the recent case reports and clinical trials showing the effectiveness of this cannabinoid against refractory epileptic syndromes [34,49,50].

### Authors’ contributions

LRV conducted behavioral experiments and analyzed data; IVL and EBK conducted behavioral experiments; HPPP conducted EEG experiments; ASdM and ELMV conducted neurochemical experiments; ACPO, MFDM, ALT, and FAM designed the experiments, supervised research, and analyzed data. All the authors participated and agreed to the final version of the paper.

### Funding

The authors thank FAPEMIG (APQ-01728-13), CNPq (477541/2012-7), INCT-TM for (2009) the financial support. Apart from providing funding, they have no involvement in any part of the study.

### Acknowledgments

The authors would like to acknowledge Dr. Alexandre Crippa for providing CBD.

### Conflict of interest

None.

### References


### Table 1

<table>
<thead>
<tr>
<th>Cytokine levels (pg/mL)</th>
<th>Veh-Veh</th>
<th>Veh-PTZ</th>
<th>CBD-PTZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2 Hipp</td>
<td>10.14 ± 0.74</td>
<td>9.440 ± 0.544</td>
<td>8.665 ± 0.345</td>
</tr>
<tr>
<td>PFC PP</td>
<td>7.83 ± 0.64</td>
<td>9.790 ± 0.398</td>
<td>10.07 ± 0.255</td>
</tr>
<tr>
<td>IL-4 Hipp</td>
<td>5.02 ± 0.16</td>
<td>4.685 ± 0.320</td>
<td>4.508 ± 0.166</td>
</tr>
<tr>
<td>PFC PP</td>
<td>5.31 ± 0.20</td>
<td>5.011 ± 0.397</td>
<td>5.027 ± 0.118</td>
</tr>
<tr>
<td>IL-6 Hipp</td>
<td>2.27 ± 0.24</td>
<td>2.264 ± 0.170</td>
<td>2.015 ± 0.049</td>
</tr>
<tr>
<td>PFC PP</td>
<td>2.68 ± 0.18</td>
<td>104.6 ± 2.270</td>
<td>2.376 ± 0.122</td>
</tr>
<tr>
<td>IL-10 Hipp</td>
<td>42.59 ± 11.04</td>
<td>33.45 ± 6.643</td>
<td>38.64 ± 7.193</td>
</tr>
<tr>
<td>PFC PP</td>
<td>14.87 ± 3.91</td>
<td>28.31 ± 3.103</td>
<td>29.98 ± 4.882</td>
</tr>
<tr>
<td>IL-17 Hipp</td>
<td>2.98 ± 0.17</td>
<td>3.217 ± 0.347</td>
<td>3.151 ± 0.2500</td>
</tr>
<tr>
<td>PFC PP</td>
<td>2.53 ± 0.16</td>
<td>3.049 ± 0.241</td>
<td>2.931 ± 0.182</td>
</tr>
<tr>
<td>TNF-α Hipp</td>
<td>4.46 ± 0.56</td>
<td>4.816 ± 0.540</td>
<td>3.383 ± 0.203</td>
</tr>
<tr>
<td>PFC PP</td>
<td>6.06 ± 0.38</td>
<td>5.058 ± 0.401</td>
<td>3.663 ± 0.279</td>
</tr>
<tr>
<td>IFN-γ Hipp</td>
<td>90.62 ± 1.87</td>
<td>1.454 ± 0.104</td>
<td>1.378 ± 0.054</td>
</tr>
<tr>
<td>PFC PP</td>
<td>1.49 ± 0.06</td>
<td>1.593 ± 0.086</td>
<td>1.544 ± 0.044</td>
</tr>
</tbody>
</table>

* p < 0.05 compared with Vehicle (Veh)-Veh group.
* # p < 0.05 compared with Veh-PTZ group.


