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

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ABSTRACT

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 (CB_1 and CB_2) 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 CB_1, CB_2, and TRPV1 selective antagonists (AM251, AM630, and SB366791, respectively). Additionally, CBD delayed seizure sensitization resulting from repeated PTZ administration (kindling). This cannabinoid 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.

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, Δ^9-tetrahydrocannabinol (Δ^9-THC), accounts for most cannabis effects by activating cannabinoid CB_1 and CB_2 receptors. These receptors are activated in the body by arachidonoyl ethanolamide (anandamide) and 2-arachidonoyl glycerol (2-AG), collectively termed endocannabinoids. Anandamide and 2-AG actions are terminated by the enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). The cannabinoid receptors, the endocannabinoids, and the enzymes responsible for their metabolism constitute the endocannabinoid system [2].

Δ^9-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 Δ^9-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 CB_1 and CB_2 receptors, although it may indirectly facilitate cannabinoid signaling by blocking anandamide uptake and hydrolysis [13]. CBD may

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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 CB_1, CB_2, 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; Pentabiotico®, 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 CB_1 antagonist, 1-(2.4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-(1-piperidyl)pyrazole-3-carboxamide (AM251); the CB_2 antagonist, 6-Iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1*H*–indol-3-yl(4 methoxyphenyl) methanone (AM 630), and the TRPV1 antagonist, 40-chloro-3-methoxycinnamanilide (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,