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Assessing the role of serotonergic receptors in cannabidiol's anticonvulsant efficacy

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ABSTRACT

Cannabidiol (CBD) is a phytocannabinoid that has demonstrated anticonvulsant efficacy in several animal models of seizure. The current experiment validated CBD's anticonvulsant effect using the acute pentylenetetrazol (PTZ) model. Furthermore, it tested whether CBD reduces seizure activity by interacting with either the serotonergic 5HT1A or 5HT2A receptor. 120 male adolescent Wistar-Kyoto rats were randomly assigned to 8 treatment groups in two consecutive experiments. In both experiments, subjects received either CBD (100 mg/kg) or vehicle 60 min prior to seizure testing. In Experiment 1, subjects received either WAY-100635 (1 mg/kg), a 5HT1A antagonist, or saline vehicle injection 80 min prior to seizure testing. In Experiment 2, subjects received either MDL-100907 (0.3 mg/kg), a specific 5HT2A antagonist, or 40% DMSO vehicle 80 min prior to seizure testing. 85 mg/kg of PTZ was administered to induce seizure, and behavior was recorded for 30 min. Seizure behaviors were subsequently coded using a 5-point scale of severity. Across both experiments, subjects in the vehicle control groups exhibited high levels of seizure activity and mortality. In both experiments, CBD treatment significantly attenuated seizure activity. Pre-treatment with either WAY-100635 or MDL-100907 did not block CBD's anticonvulsant effect. WAY-100635 administration, by itself, also led to a significant attenuation of seizure activity. These results do not support the hypothesis that CBD attenuates seizure activity through activation of the 5HT1A or 5HT2A receptor. While this work further confirms the anticonvulsant efficacy of CBD and supports its application in the treatment of human seizure disorders, additional research on CBD's mechanism of action must be conducted.

1. Introduction

One-third of the 50 million people with epilepsy experience seizures that cannot be controlled with available treatments [1]. Current antiepileptic drugs (AED's) also cause many negative side effects, including fatigue, nausea, decreased appetite, dizziness, difficulty concentrating, memory problems, aggression, and hyperactivity. There is a substantial need for novel treatments for seizure disorders, and cannabinoids represent one highly promising option.

Cannabidiol (CBD) is the second-most abundant phytocannabinoid derived from the *Cannabis sativa* plant. CBD possesses antipsychotic, anxiolytic, and neuroprotective effects, and has also demonstrated anticonvulsant efficacy in several experimental studies [2,3]. Unlike Δ9-tetrahydrocannabinol (THC), the primary psychoactive cannabinoid, CBD does not induce psychoactive effects and is not reinforcing. As such, it is an especially attractive drug candidate for the treatment of seizures in children and adolescents. Recently, an open-label trial reported that CBD reduced seizure frequency in patients with severe treatment-resistant epilepsy [4]. CBD's anticonvulsant properties have also been tested in several different animal models of seizure and epilepsy [5,6]. The proconvulsant compound, pentylenetetrazol (PTZ), forms the basis of one of the most common animal models of generalized seizure and is often used to screen putative anticonvulsants [7]. Jones et al. (2010) reported that a 100 mg/kg dose of CBD was highly effective at reducing seizure severity in PTZ-treated rats [5]. One goal of the current experiments was to validate this anticonvulsant effect of CBD using the PTZ-model.

A second goal was to explore two possible mechanisms by which CBD may reduce seizure activity. CBD is a pharmacologically complex compound that targets many different molecules in the central nervous system and may affect seizure activity through multiple mechanisms [8]. Intriguingly, CBD has minimal affinity and shows low agonist activity at central cannabinoid receptors (CB1 and CB2). Among other targets, CBD binds to several serotonin receptors in the brain, including the 5HT1A and 5HT2A [9]. CBD's ability to influence serotonergic activity is relevant to seizure researchers for several reasons. First, serotonin and its receptors participate in the pathophysiology of epilepsy [10]. For

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example, PET imaging studies have noted reduced expression of 5HT1A receptors in the hippocampus, entorhinal cortex, and parahippocampal gyrus of patients with temporal lobe epilepsy [11]. Numerous studies have documented that 5HT1A agonism attenuates seizure, although this literature is not entirely consistent [12]. Second, recent work has suggested that fenfluramine, a potent indirect 5HT agonist, can be an effective add-on treatment for Dravet's syndrome [13]. Fenfluramine works through multiple mechanisms to increase synaptic serotonin, but it is not known what post-synaptic effects are most relevant to its anticonvulsant efficacy. Third, there is growing awareness that 5HT2A receptor dysfunction may underlie comorbidity between major depressive disorder and epilepsy in human patients [14].

CBD's affinity for central 5HT1A receptors has been demonstrated through its ability to displace 8-OH-DPAT, and its efficacy has been observed through measurement of in vitro signal transduction [9]. Furthermore, antagonism of 5HT1A receptors blocks several of the physiological and behavioral effects of CBD administration. For instance, CBD's anti-depressant effects in mice were reversed following administration of the 5HT1A antagonist, WAY-100635 [15]. Similarly, CBD induced an anxiolytic effect in Wistar rats when injected directly into the bed nucleus of the stria terminalis (BNST) – an effect blocked by pretreatment with WAY-100635 [16]. Perhaps most pertinent to its anticonvulsant potential, Ledgerwood et al. (2011) noted that CBD inhibits basal synaptic transmission in acute hippocampal slices, and this effect was terminated by administration of WAY-100135, a non-selective 5HT1A antagonist [17]. Russo et al. (2005) also demonstrated that CBD is active at the 5HT2A receptor, albeit less so than at the 5HT1A [9]. It is unknown whether any of CBD's physiological or behavioral effects are caused by activity at the 5HT2A receptor.

The current studies were designed to further assess CBD's anticonvulsant efficacy and to specifically determine whether CBD is working through the serotonergic 5HT1A or 5HT2A receptor. Our study is the first attempt to explore these hypotheses and serves as a critical step in isolating CBD's mechanism of action. In two separate experiments, adolescent Wistar Kyoto rats were given PTZ to induce severe seizure. Half were given 100 mg/kg CBD, which has previously been shown to significantly reduce seizure severity [5]. In Experiment 1, we tested whether administration of the specific 5HT1A antagonist, WAY-100635, attenuates CBD's anticonvulsant effect. In Experiment 2, we conducted a similar experiment using MDL-100907, a specific 5HT2A antagonist. If CBD reduces seizure activity by serving as an agonist at either of these receptors, then pre-treatment with a suitable antagonist should block its anticonvulsant properties.

2. Material & methods

2.1. Subjects

120 male, Wistar Kyoto rats (Charles River Laboratories, Wilmington, MA) were used in two successive experiments. They were tested in two separate cohorts: 60 subjects in Experiment 1 and 60 in Experiment 2. Subjects arrived at 21 days of age and were pair-housed in plastic cages with corncob bedding within a temperature-controlled (23 ± 2 °C) vivarium. The vivarium was programmed with a 12:12 light–dark schedule (lights on 6:00–18:00 h). Food and water were available ad libitum, and all subjects' cages were environmentally enriched with a Nylabone® and a short length of PVC pipe. All experimental protocols were approved by the campus Institutional Animal Care and Use Committee in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.2. Procedure

Subjects were handled for several days prior to any experimental procedures. For four days leading up to the seizure tests, subjects were habituated to the seizure testing room and apparatus; each subject was placed in a seizure observation chamber for 15 min per day.

In Experiment 1, we tested whether the 5HT1A receptor is implicated in CBD's anticonvulsant activity. 60 subjects were randomly assigned to one of the four treatment conditions (n = 15 each), using a 2 (CBD vs. vehicle) × 2 (WAY-100635 vs. vehicle) design. The four groups were: 1) vehicle + vehicle, 2) CBD + vehicle, 3) CBD + WAY-100635, and 4) vehicle + WAY-100635. CBD was administered via intraperitoneal (IP) injection at a dose of 100 mg/kg, 60 min prior to seizure induction [18]. CBD was dissolved in a vehicle of 2:1:17 ethanol: Kolliphor: physiological saline. CBD was generously donated from GW Pharmaceuticals (Cambridge, UK). WAY-100635 (Sigma-Aldrich, St. Louis, MO) was administered via subcutaneous (SC) injection at a dose of 1 mg/kg, 20 min prior to CBD administration [19]. Vehicle was physiological saline. WAY-100635 was chosen because it is one of the most commonly used, selective 5HT1A antagonists used in behavioral pharmacology [20] and has been used previously to test the mechanism of action for other behavioral effects of CBD [15,16]. Dosage was based on previous research, including Lopez-Meraz et al. (2005), who used 1 mg/kg in male Wistar rats to assess the effects of various 5HT1A agonists on epileptic seizures in several different models [21]. Notably, 1 mg/kg of WAY-100635 did not significantly affect seizure incidence or intensity on its own.

In Experiment 2, we tested whether the 5HT2A receptor is implicated in CBD's anticonvulsant effects. 60 subjects were randomly assigned to one of four treatment conditions (n = 15 each), using a 2 (CBD vs. vehicle) × 2 (MDL-100907 vs. vehicle) design. These four groups were: 1) vehicle + vehicle, 2) vehicle + CBD, 3) MDL-100907 + vehicle, and 4) MDL-100907 + CBD. MDL-100907 (Sigma-Aldrich, St. Louis, MO) was administered via subcutaneous (SC) injection at a dose of 0.3 mg/kg, 80 min prior to seizure induction [22]. MDL-100907 was chosen because it is considered to be one of the most selective 5HT2A receptor antagonists available for preclinical studies [23–25]. With regard to dose, 0.3 mg/kg falls within the range used by previous researchers to successfully examine the functionality of the 5HT2A receptor in a variety of behavioral domains [25–27]. MDL-100907 was dissolved in a vehicle of 40% DMSO in physiological saline. CBD, as before, was administered via intraperitoneal (IP) injection at a dose of 100 mg/kg, 60 min prior to seizure induction.

The following procedures were identical for both Experiment 1 and Experiment 2. Between post-natal day (PND) 29–33, subjects were tested for seizure susceptibility. Seizure testing took place in a separate room under normal light conditions, beginning around 10:00 h. To induce seizure, subjects were administered a single intraperitoneal (IP) injection of 85 mg/kg of pentylenetetrazol (PTZ). Immediately after PTZ administration, each subject was transferred to a transparent plastic cage (27 × 16.5 × 12 cm) for observation. Four subjects — one from each treatment group — were tested simultaneously. A wooden divider was placed between cages so that subjects being tested simultaneously were not visible to each other. The seizure test lasted for 30 min. Tests were video-recorded using a tripod-mounted digital camera (NTSE SONY XC-EI50). Video was digitized at 60 Hz in 640 × 840 resolution onto a Macintosh computer. Video was processed using unpublished software (RatCam) using a frame-by-frame accurate timecode.

Seizure videos were subsequently coded by experimenters blind to the treatment condition of each subject. Individual seizures were scored on a 5-point severity scale appropriate for generalized seizures with forebrain origin [28,29]: 1, isolated myoclonic jerks; 2, atypical clonic seizure; 3, fully developed bilateral forelimb clonus; 3.5, forelimb clonus with tonic component and body twist; 4, tonic–clonic seizure with suppressed tonic phase, loss of righting; 5, fully developed tonic–clonic seizure with loss of righting. For each subject, latency to first sign of seizure and maximum seizure severity achieved were coded. The following group variables were then calculated: 1) mean latency to first seizure event, 2) median maximum seizure severity, 3) percentage of subjects that displayed any seizure activity (1 or greater), 4) percentage that