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

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A B A C

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 con-

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

achieved tonic–clonic seizure (4 or 5), and 5) percentage mortality. Mortality was defined by the subject ceasing all respiratory and cardiac function at some point during the 30-min test. The group variables were based upon those described in Jones et al. [5].

2.3. Statistical analyses

Latencies were analyzed with a 2 × 2 analysis of variance (ANOVA). Median maximum seizure severities were analyzed with both a Kruskal–Wallis test and a median test, following by post-hoc Mann–Whitney tests. Group percentages were analyzed using multiple non-parametric binomial tests (as in Jones et al.), using the Holm–Bonferroni method to adjust alpha for multiple comparisons [5].

3. Results

3.1. Experiment 1

Table 1 summarizes the results of Experiment 1.

Fig. 1 displays the mean (± SEM) latency to first seizure event (for those subjects that experienced a seizure) for the four treatment groups. A 2 (CBD treatment) × 2 (WAY-100635 treatment) ANOVA on these data revealed no significant main effect of CBD ($F(1,36) = 3.27$, $p = 0.08$), no main effect of WAY-100635 ($F(1,36) = 0.58$, $p = 0.45$), and no interaction ($F(1,36) = 0.004$, $p = 0.95$).

Fig. 8

the behavior of these subjects compared to either the CBD-treated group or the CBD + WAY group. This was unexpected, given previous evidence that 5HT1A receptor agonists are often anticonvulsant [12]. However, the literature in this area has been inconsistent. For example, Lopez-Meraz et al. (2005) tested the effects of two 5HT1A agonists (8-OH-DPAT and Indorenate) and one antagonist (WAY-100635) on PTZ-induced (60 mg/kg) seizures in male Wistar rats [21]. 8-OH-DPAT treatment dose-dependently reduced the percentage of rats with tonic extension and the mortality rate. Conversely, 1 mg/kg 8-OH-DPAT significantly increased the number of clonic seizures (an effect which was blocked by WAY-100635). Indorenate dose-dependently increased seizure latencies and reduced the percentage of subjects presenting clonic seizure, tonic extension, and death. WAY-100635 (1 mg/kg, SC) by itself had no significant effect on seizure activity.

There are additional findings that other 5HT1A antagonists may possess anticonvulsant efficacy under certain experimental conditions. For example, Moreau at el. reported that (S)-UH-301, a selective 5HT1A antagonist, exhibited dose-dependent anticonvulsant activity in two different mouse models of acute seizure (audiogenic seizures and ICV administration of NMDA in DBA/2J mice) [31]. Graf et al. (2004) noted an anticonvulsant effect of WAY-100635 in WAG/Rij rats, which exhibit spontaneously occurring spike–wave discharges (SWDs) and behavioral symptoms similar to those seen in human absence epilepsy [32]. Subjects were given 0.2 mg/kg of WAY-100635 (IP) prior to 5 h of EEG and EMG recordings. Interestingly, WAY-100635 caused an initial increase in SWDs and paroxysms (30–60 min after administration), but then significantly reduced seizure activity in the following 4 h. These results suggest that there may be biphasic effects of 5HT1A antagonism on seizure activity.

Brain 5HT1A receptors can be either pre-synaptic, inhibitory autoreceptors (within the raphe nuclei, for instance), or post-synaptic (within forebrain/limbic terminal regions). Dose-dependent and biphasic effects of 5HT1A antagonists may, therefore, result from differential activation of pre- vs. post-synaptic receptors and potentially distinctive roles of these receptor categories in regulating seizure

exocytosis, as well as neuroprotection against damage occurring during seizure [8,41]. We must not be thwarted by the complexity of CBD, but continue exploring the various mechanisms by which it exerts its clinical effects, if CBD and other cannabinoids are to gain traction within the biomedical community as viable treatment options for neurological disorders.

5. Conclusion

Further validating previous preclinical studies [5,6], we found that 100 mg/kg of CBD significantly attenuated the most severe aspects of acute seizure induced by PTZ in male Wistar Kyoto rats. Many researchers have speculated that CBD's anticonvulsant properties may, in part, be linked to agonism at either the serotonergic 5HT1A or 5HT2A receptor [2,8]. Our study is the first to explore these specific hypotheses regarding CBD's mechanism of action. We found no evidence that CBD worked through 5HT1A or 5HT2A to reduce seizure activity in this model.

Conflicts of interest

None of the authors has any conflict of interest to disclose.

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