Review Article

Phytocannabinoids and epilepsy

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SUMMARY

What is known and objective: **Antiepileptic drugs often produce serious adverse effects, and many patients do not respond to them properly. Phytocannabinoids produce anticonvulsant effects in preclinical and preliminary human studies, and appear to produce fewer adverse effects than available antiepileptic drugs. The present review summarizes studies on the anticonvulsant properties of phytocannabinoids.**
Methods: **Literature search using the PubMed database to identify studies on phytocannabinoids and epilepsy.**
Results and discussion: **Preclinical studies suggest that phytocannabinoids, especially cannabidiol and cannabidivarin, have potent anticonvulsant effects which are mediated by the endocannabinoid system. Human studies are limited in number and quality, but suggest that cannabidiol has anticonvulsant effects in adult and infantile epilepsy and is well tolerated after prolonged administration.**
What is new and conclusion: **Phytocannabinoids produce anticonvulsant effects through the endocannabinoid system, with few adverse effects. Cannabidiol and cannabidivarin should be tested in randomized, controlled clinical trials, especially in infantile epileptic syndromes.**

WHAT IS KNOWN AND OBJECTIVE

The medicinal properties of cannabis have been known in China and India for thousands of years, and by the XIX century, the therapeutic use of cannabis derivatives reached Europe and the United States.[1–9] Nowadays, despite the fact that cannabis is the most consumed illegal recreational drug worldwide,[10–12] there is increasing interest in its medicinal potentials.[1,5,8,13–17]

Cannabis contains over 100 compounds called phytocannabinoids, which are unique to the plant.[1,16,18] The main cannabinoids are delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD). THC is responsible for producing most subjective effects of cannabis, whereas CBD lacks the psychoactivity of THC.[19–22]

Epilepsy is a neurological condition characterized by recurrent seizures.[23,24] There is evidence that THC, delta-9-tetrahydrocan-

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nabivarin (THCV), cannabidivarin (CBDV), delta-8-tetrahydrocannabinol (delta-8-THC), cannabinol (CBN) and especially CBD have anticonvulsant effects.[13–17,23–29] Considering that currently available anti-epileptic drugs are not efficient for many patients and that these drugs often produce several side effects, there is a clear need to explore other compounds with higher efficacy and lower toxicity.[23,24] Therefore, the present text presents a review of the studies on the anticonvulsant effects of phytocannabinoids.

METHODS

A literature search was performed in the PubMed database using the words 'cannabis', 'marijuana', 'cannabinoids', 'tetrahydrocannabinol', 'THC', 'cannabidiol', 'CBD', 'cannabidivarin', 'CBDV', 'seizures' and 'epilepsy', until October 2014. No date or language limitation was used in the search. Only full peer-reviewed articles were included in the review, abstracts and letters being excluded. Among selected articles, a selection was made based on the quality and relevance of the study.

RESULTS AND DISCUSSION

Anticonvulsant effects of cannabis

Reports of the anti-epileptic potential of cannabis have thousands of years of age.[1,4,5,8,16,26,28,30,31] Anecdotal reports suggest that individuals using cannabis to treat their epilepsy may precipitate the re-emergence of convulsive seizures when they stop cannabis use, while resuming cannabis consumption controls epilepsy.[13,16,23,24,26,28–30,32–36]

As there is only anecdotal evidence supporting the anti-epileptic effects of cannabis, and considering that there are only a small number of case reports describing this therapeutic potential, there is insufficient information to make any conclusions regarding the anticonvulsant effects of cannabis. Moreover, cannabis has several other substances, including other phytocannabinoids and non-cannabinoid compounds, with unknown effects on epilepsy. Anecdotal evidence and case reports also suggest that, at least in some patients, cannabis may not affect epilepsy at all or may even produce seizure exacerbation.[16,23,24,26,28–30,34,37]

Even considering the absence of controlled studies, cannabis is currently licensed in several states in the United States and in Canada for the treatment of epilepsy.[23,24,33,35] This indicates that some physicians approve the use of cannabis on epileptic syndromes and also suggests the potential efficacy of cannabis in at least some patients. Indeed, many epileptic patients use cannabis as a complementary or alternative anticonvulsant

medicine.[26,29,35] However, given the quality of the available data, no reliable conclusions can be drawn from the available studies.

Anticonvulsant effects of THC

THC is the main active compound in cannabis. THC acts as a partial agonist at cannabinoid CB_1 receptors, found primarily in the central nervous system (CNS), and CB_2 receptors, found primarily on cells of the immune system.[5,19,21,22,26]

The anticonvulsant activity of THC was originally investigated in the 1970s. In these studies, THC produced primarily anticonvulsant effects, but in other studies, there was no effect or even proconvulsant effects.[26,28,29,38–43]

THC (2·5–10 mg/kg) decreased the susceptibility of rat dorsal hippocampus to seizures discharges caused by afferent stimulation.[38] In a study in mice, the anticonvulsant potential of THC was assessed utilizing the maximal electroshock seizure test (MES) and the pentylenetetrazole (PTZ) seizure test.[39] THC (1–80 mg/kg) afforded no protection against PTZ-induced seizures, but was effective against electroshock-induced seizures (160–200 mg/kg). THC significantly potentiated the anticonvulsant effectiveness of phenytoin against electroshock seizures, but no potentiation of phenobarbital effectiveness could be demonstrated in the PTZ-induced seizure test. THC (20–75 mg/kg) also significantly lengthened rather than shortened the hindlimb extensor phase of the electroshock seizures.

The anticonvulsant effects of THC were assessed after acute and chronic (6 days) administration utilizing a seizure sensitive strain of gerbils.[40] Although 20 mg/kg THC did not reduce seizure patterns, 2 h after the first injection of the higher THC dose (50 mg/kg), no seizures were seen in any of the animals. Nevertheless, complete tolerance developed to the anticonvulsant effect of THC by the sixth day, which could limit the clinical use of this compound, as anti-epileptic drugs are used in a daily basis for prolonged periods of time.

In mice, several doses of THC (0·3125–107 mg/kg) were compared with diphenylhydantoin, phenobarbital and chlordiazepoxide using the MES test and in seizures induced by PTZ, strychnine and nicotine.[41] In the MES test, THC blocked convulsions, increased their latency and prevented mortality. Seizures and mortality induced by PTZ or by strychnine were enhanced by THC, and none of the drugs prevented seizures in the nicotine test.

In another study,[42] rats were exposed to the MES test and to the audiogenic seizure test (AS), and the median effective potency (ED_{50}) for THC anticonvulsant effect was calculated. In the MES test, THC produced anti-epileptic effects with an ED_{50} of 35 mg/kg. In the AS test, THC produced anti-epileptic effects with an ED_{50} of 21 mg/kg.

In a study utilizing rats rendered chronically epileptic by bilateral implantation of cobalt into frontal cortices,[43] 10 mg/kg THC was administered twice daily from day 7 through 10 after cobalt implantation, at which time generalized seizure activity was maximal. THC markedly reduced the incidence of seizures on the first and second days of administration. According to the authors, the effects of the first few injections of THC were dramatic: within 15–30 min after administration, generalized seizure activity was completely abolished. Activity of the cobalt focus, on the other hand, was actually enhanced by THC (20 mg/kg). Moreover, on the third and fourth days, tolerance developed to the effects of THC, with a return of seizure frequency to values not significantly different from those of controls. As previously commented, the development of tolerance could limit the clinical use of THC.

Although early studies reported anticonvulsant effects of THC, there was also evidence of proconvulsant effects.[26,28,29,38–43] The neurochemical basis for these opposite effects could depend on the preferential action of THC on CB_1 receptors located in glutamatergic or GABAergic neurons. Thus, THC could produce anticonvulsant effects by inhibiting the glutamatergic excitatory transmission and proconvulsant effects by inhibiting the release of GABA.[26,30,44] These findings suggest that activation of CB_1 receptors may not be sufficient to yield therapeutic benefits for epilepsy patients. Moreover, the development of tolerance and the subjective effects of THC limited the investigation of this compound in clinical trials.

Anticonvulsant effects of CBD

Together with THC, CBD is one of the most important phytocannabinoids. CBD pharmacology is not completely understood, as it has multiple mechanisms of action and produces several pharmacological effects. CBD effects in the endocannabinoid system do not seem to depend directly on $CB_{1/2}$ receptors. CBD possesses very low (micromolar range) affinity for $CB_{1/2}$ receptors, but antagonizes $CB_{1/2}$ agonists in the nanomolar range.[14,25–28] CBD also inhibits the uptake of anandamide at micromolar concentrations and inhibits its enzymatic hydrolysis. CBD also antagonizes the putative novel cannabinoid receptor GPR55 at nanomolar concentrations.[14,25–28]

At micromolar concentrations, CBD activates $5-HT_{1A}$ receptors, inhibits the uptake of serotonin, activates $TRPV_{1/2}$ and $TRPA_1$ channels, inhibits the uptake of adenosine, noradrenaline, dopamine and GABA, stimulates the activity of the inhibitory glycine-receptor and antagonizes α_1-adrenergic and μ-opioid receptors.[14,25–28,45]

Moreover, CBD reduces hydroperoxide-induced oxidative damage, tissue cyclooxygenase (COX) activity, the production of nitric oxide (NO), T-cell responses, the release of bioactive tumour necrosis factor (TNF), the production of prostaglandin E2 (PGE2), cytokine interferon γ (IFN-γ) and tumour necrosis factor (TNF) and also blocks voltage-gated Na^+ channels.[14,25–28,46]

The multipharmacological profile of CBD corresponds well with the wide range of therapeutic potentials reported for this compound, including its anti-epileptic activity. However, as many of these effects are produced at the micromolar range, they are of uncertain relevance for the pharmacological effects of CBD.

Although further research is needed to clarify the precise mechanisms that underlie CBD therapeutic effects, including its anti-epileptic potentials, in the last decades, a growing number of studies have reported that CBD may act as an sedative, anxiolytic, antipsychotic, anti-inflammatory, antioxidative, neuroprotector, anti-emetic, anticancer, antidepressant and mood stabilizer, and with therapeutic action on movement disorders, ischaemia and diabetes, and on cannabis withdrawal syndrome.[1,8,13–15,17,18,25] Moreover, there are 18 clinical trials involving the administration of CBD, including studies on with multiple sclerosis (six studies), schizophrenia and bipolar mania (four studies), social anxiety (two studies), neuropathic and cancer pain (two studies), cancer anorexia (one study), Huntington's disease (one study), insomnia (one study) and epilepsy (one study).[25]

CBD anticonvulsant effects: preclinical studies

The anti-epileptic effects of CBD were one of the first pharmacological actions described for this compound, still in the

Table 1. Anti-epileptic effects of CBD in animal models

Model	Animals	Results[a]		Dose (mg/kg)	References
Convulsant hippocampal discharges	Rats	Reduced susceptibility	(+)	5	38
Leptazol-induced seizures	Mice	Seizure and mortality reduction	(+)	200	47
Electroshock-induced seizure	Mice	Seizure reduction	(+)	ED_{50}[b] = 120	48
Electroshock-induced seizure	Rats	Seizure reduction	(+)	?	42
Electroshock-induced seizure transcorneal	Mice	Reduction of tonic seizures	(+)	ED_{50} = 267	49
Seizures induced by strychnine sulphate	Mice	No protection against seizures or death	(−)	–	49
Picrotoxin-induced seizures	Mice	Reduction of tonic seizures	(+)	ED_{50} = 194·4	49
Seizures induced by 3-mercaptopropionic acid	Mice	Reduction of tonic seizures	(+)	ED_{50} = 122·5	49
Pentylenetetrazol-induced seizures	Mice	Reduction of tonic seizures	(+)	ED_{50} = 304·8	49
Seizures induced by isonicotinic acid hydrazide	Mice	Reduction of tonic seizures	(+)	ED_{50} = 266·1	49
Bicuculline-induced seizures	Mice	Reduction of tonic seizures	(+)	ED_{50} = 379·9	49
Epileptiform activity in hippocampal tissue	*In vitro* (Rats)	Protection	(+)	1–100[c]	50
Pentylenetetrazol-induced seizures	Rats	Seizure and mortality reduction	(+)	100	50
Pilocarpine-induced seizures	Rats	Seizure reduction	(+)	1–100	51
Partial seizures induced by intraventricular penicillin	Rats	Seizure reduction	(+)	10–100	51
Electroshock-induced seizure	Mice	Increased threshold	(+)	20–200[d]	52
Pentylenetetrazol-induced seizures	Mice	Increased threshold	(+)	200[d]	52

[a](+) anticonvulsant action, (−) no anticonvulsant action.
[b]median effective doses.
[c]μM.
[d]ng.
?, not known.

1970s.[1,28,29,38,42,47,48] According to previous studies in rodents, CBD is an effective and relatively potent anticonvulsant.[29,38,42,47,48]

Table 1 shows that preclinical evidence clearly attests the protective effect of CBD in respect to seizures induced by a number of agents in laboratory animals.

CBD decreased the susceptibility of rat dorsal hippocampus to seizures discharges caused by afferent stimulation and significantly protected mice from the proconvulsant effects of leptazol.[38,47]

In a study in rats using the MES and the AS tests, CBD enhanced the anticonvulsant effects of drugs clinically effective in major seizures (phenytoin) and reduced the effects of drugs effective in minor seizures (chlordiazepoxide, clonazepam, trimethadione and ethosuximide).[42] In the MES test, CBD produced anti-epileptic effects with a median effective dose (ED_{50}) of 12 mg/kg. In the AS test, CBD produced anti-epileptic effects with an ED_{50} of 17 mg/kg. CBD interactions with other anti-epileptic drugs may result from pharmacokinetic or pharmacodynamic mechanisms. Nevertheless, CBD is a potent inhibitor of multiple cytochrome P450 enzymes including CYP1A2, CYP2B6, CYP2C9, CYP2D6 and CYP3A4,[25] which suggest that pharmacokinetic mechanisms are involved.

In a study in mice using a transcorneal electroshock current or convulsant drug administration to induce seizures,[49] 50–600 mg/kg CBD pretreatment prevented tonic convulsions caused by the electroshock current and by GABA-inhibitors, 3-mercaptopropionic acid, picrotoxin, isonicotinic acid hydrazine, pentylenetetrazol and bicuculline.[49] Nevertheless, in a study utilizing rats rendered chronically epileptic by bilateral implantation of cobalt into frontal cortices, 60 mg/kg CBD did not alter the frequency of appearance of seizures.[43]

In an *in vitro* study, the electrophysiological effects of CBD on epileptiform activity were assessed by means of extracellular multi-electrode array recordings using the Mg^{2+}-free and 4-aminopyridine (4-AP) models of epilepsy in the mammalian hippocampus, a key epileptogenic brain region.[50] CBD (0·01–100 μM) produced concentration-related and region-dependent attenuation of epileptiform activity in both seizure models. This study also examined the effects of CBD *in vivo* using the PTZ test, reporting that 100 mg/kg CBD reduced the incidence of severe seizures and mortality in rodents. Moreover, this study assessed CBD affinity for cannabinoid CB_1 receptors, reporting that CBD acted with only low affinity at cannabinoid CB_1 receptors. This last result suggests that CBD anticonvulsant effects are produced by CB_1 receptor-independent mechanisms.

In another study in rodents, the anticonvulsant potential of CBD was evaluated using the acute pilocarpine model of temporal lobe seizures and the penicillin model of partial seizures.[51] In the pilocarpine model, CBD (1–100 mg/kg) reduced the incidence of the most severe seizures, but did not reduce mortality. In the penicillin model, CBD produced anticonvulsant effects, reduced mortality and reduced the proportions of animals developing the most severe seizure types. CBD had very little effect on motor function tests, suggesting a better safety profile when compared to currently available anti-epileptic drugs, which may cause significant motor side effects.

A recent rodent study using the MES and the PTZ tests reported anticonvulsant effects of CBD (0·2–200 ng/mouse) in both seizure models.[52] This study also evaluated the possible interactions between CBD and the potassium BK channel blocker paxilline, reporting that co-administration of CBD and paxilline attenuated the anticonvulsant effects of CBD in PTZ test. In the MES test, there was no interaction between both substances. These results suggest a BK channel-mediated anticonvulsant action of CBD in the PTZ test, where CBD could act by decreasing intracellular calcium levels.

The effects of CBD and the structurally similar cannabinoid cannabigerol (CBG) on voltage-gated Na^+ (Na_V) channels were

investigated in rat hippocampal neurons, mouse cortical neurons, human neuroblastoma cells and recombinant Na$_V$ channels.[46] The effect of CBG on PTZ-induced seizures was assessed in the rat. CBD (10 μM) blocked Na$_V$ currents in mouse neurons, human cells and recombinant cell lines, affected spike parameters in rat neurons and decreased membrane resistance. CBD effects were retained in the presence of a CB$_1$ receptor antagonist, with the exception of the decreased membrane resistance. CBG blocked Na$_V$ to a similar degree to CBD in both human and mouse recordings, but had no effect (50–200 mg/kg) on PTZ-induced seizures. These results indicate that the anticonvulsant effects of CBD are independent of Na$_V$ blockade and CB$_1$ receptor activation.

In resume, CBD produced anticonvulsant effects in several preclinical studies, suggesting that this compound may have therapeutic effects in different epileptic syndromes.

CBD anticonvulsant effects: human studies

Although there is a growing number of preclinical studies and several case reports reporting the anti-epileptic action of CBD, only a small number of placebo-controlled clinical trials were published.[1,13–15,17,23–25,28,29,53,54] Overall, trials reported reduction in seizures and few side effects after 4–12 months of 200–300 mg/day CBD.[1,13–15,17,23–25,28,29,53,54]

Human studies on the effectiveness of CBD in epilepsy are shown in Table 2.

The study by Cunha et al.[53] seems to be the only double-blind, placebo-controlled clinical trial on the anti-epileptic effects of CBD that was fully published in a peer-reviewed journal.[1,13–15,17,23–25,28,29,53,54] In other studies, few methodological details are given, and the overall quality of the reports is low.[14,17,23–25,29,53]

Cunha et al.[53] evaluated fifteen patients (11 women; aged 14–49 years; average 24 years) suffering from secondary generalized epilepsy with temporal lobe focus that was unresponsive to prescribed anti-epileptic drugs. Patients continued to take their regular anti-epileptic drugs throughout the study period. Patients participated in a double-blind, placebo-controlled study, where

eight patients received 200–300 mg/day oral CBD for 8–18 weeks and the other seven individuals received placebo. In this study, four of eight CBD-treated patients evidenced significant improvement in their condition, remaining virtually convulsion-free for the duration of the study. The other three CBD-treated subjects exhibited partial improvement in their clinical condition. Moreover, three CDB-treated patients showed improvements in electroencephalographic (EEG) measures. In the placebo group, only one patient improved. CBD was well tolerated by all participants.

A recent survey investigated the use of CBD-enriched cannabis in children with treatment-resistant epilepsy.[33] The researchers presented a survey to parents who used CBD-enriched cannabis to treat their child's seizures. Nineteen cases were reported in the study: thirteen children had Dravet syndrome, four had Doose syndrome, one had Lennox–Gastaut syndrome, and one had idiopathic epilepsy. The average number of anti-epileptic drugs tried was 12 (range 4–17), and seizure frequency ranged from 2 per week to 250 per day. The children experienced a variety of seizure types including focal, tonic–clonic, myoclonic, atonic and infantile spasms. In most cases, the children experienced treatment-resistant epilepsy for more than 3 years. The treatment period with CBD-enriched cannabis ranged from 2 weeks to over 1 year. Sixteen (84%) of the 19 parents reported a reduction in their child's seizure frequency. Of these, two (11%) reported complete seizure freedom, eight (42%) reported a greater than 80% reduction in seizure frequency, and six (32%) reported a 25–60% seizure reduction. Other beneficial effects included increased alertness, better mood and improved sleep. Side effects were mild and included drowsiness and fatigue.

The case of a girl with SCN1A-confirmed Dravet syndrome was recently reported.[16,27,28] Adjunctive therapy with a high CBD concentration strain of cannabis reduced the girl's seizure frequency from nearly 50 convulsive seizures per day to 2–3 nocturnal convulsions per month. According to the report, this effect has persisted for 20 months.[16]

Clinical studies with CBD focusing on children with intractable epileptic syndromes such as Dravet and Lennox–Gastaut syndromes are currently underway (ClinicalTrials.gov Identifier:

Table 2. Human studies on the effectiveness of CBD in epilepsy

Study design	Sample	Results	Dose	Reference
Retrospective assessment with questionnaires completed by parents of children treated with Cannabis extract rich in CBD	19 children with treatment-resistant epilepsy[a]	84% reported seizure reduction	?	33
Double-blind, placebo-controlled trial[b]	15 adults with treatment-resistant epilepsy	7 of 8 patients improved with CBD 1 of 7 improved with placebo	200–300 mg/day (add-on) 8–18 weeks	53
Open label trial	27 children or young adults (up to 18 years) with treatment-resistant epilepsy[c]	Compared with baseline: 15% – no seizures 22–90% reduction 41–70% reduction 48–50% reduction	5–20 mg/kg/day 12 weeks	GW Pharmaceuticals, 2014

[a]12 children with Dravet syndrome, four with Doose syndrome, one with Lennox–Gastaut syndrome, one with mental retardation, and one with early-onset idiopathic epilepsy.
[b]single clinical trial fully published in a peer-reviewed journal.
[c]predominantly with Dravet syndrome (n = 9).
?, not known.

NCT02091206, NCT02091375, NCT02224560, NCT02224573, NCT02224690 and NCT02224703).

Anticonvulsant effects of CBDV

A major advance in the last few years is the potential of CBDV, a CBD analogue derived from cannabigerovarin (CBGV), as an anti-epileptic agent.[45,55–57] Several preclinical studies reported that CBDV has anticonvulsant properties that are apparently independent of CB_1 receptors, as CBDV binds to these receptors with only very weak affinity.[45,55–57] Moreover, CBDV inhibits the cellular uptake of anandamide at micromolar concentrations, activates $TRPV_{1/2}$ and $TRPA_1$ channels and inhibits the synthetic enzyme of the endocannabinoid 2-arachidonoylglycerol (2-AG) at nanomolar concentrations and may also act via CB_2 receptors.[26,45,55–57] However, the pharmacological and clinical relevance of these effects is still uncertain.

The anticonvulsant profile of CBDV was investigated in vitro using multi-electrode array recordings of epileptiform local field potentials induced in rat hippocampal brain slices by 4-aminopyridine application or Mg^{2+}-free conditions.[55] CBDV (1–100 μM) significantly decreased the amplitude and duration of local field potentials.

CBDV effects were investigated in four rodent seizure models: MES and AS tests in mice and PTZ- and pilocarpine-induced seizures in rats.[55] CBDV effects on rat seizures were also assessed in combination with commonly used anti-epileptic drugs (valproate, ethosuximide and phenobarbital). CBDV had significant anticonvulsant effects on the MES ($\geq$100 mg/kg), AS ($\geq$50 mg/kg) and PTZ tests ($\geq$100 mg/kg). On the PTZ test, CBDV significantly reduced mortality (100–200 mg/kg). CBDV (200 mg/kg) alone had no effect against pilocarpine-induced seizures, but produced significant anticonvulsant effects when co-administered with ethosuximide in the PTZ model and even greater effects when co-administered with valproate in the pilocarpine model. CBDV did not affect the effects of phenobarbital in the pilocarpine model and had only very limited effects on the onset of seizures when co-administered with valproate before PTZ treatment. No negative interactions between CBDV and the anti-epileptic drugs were observed.

The motor side effect profile of CBDV (50–200 mg/kg) was investigated using static beam test to assess motor coordination and a grip strength test to assess drug-induced muscle relaxation and functional neurotoxicity.[55] CBDV had no effect on motor function, suggesting a better side effect profile compared to current available anti-epileptic drugs, which often produce motor side effects. CBDV was administered orally to suppress PTZ seizures, as a prerequisite for human epilepsy treatment is that a drug is effective after oral administration. CBDV (400 mg/kg) significantly reduced the severity of PTZ-induced seizures.[55]

A study in rats evaluated CBDV effects on the PTZ test and quantified expression levels of several epilepsy-related genes in tissue from hippocampus, neocortex and prefrontal cortex.[56] CBDV (400 mg/kg) significantly decreased seizure severity and increased latency to the first seizure sign. PTZ treatment upregulated mRNA expression coding for Fos, Egr1, Arc, Ccl4 and Bdnf in all brain regions tested. Clear correlations between seizure severity and mRNA expression were observed for these genes in the majority of brain regions, and mRNA expression of these genes was suppressed in the majority of brain regions after CBDV treatment.

Another study in rodents investigated the anticonvulsant profiles of cannabis extracts rich in CBDV in three animal models of acute seizure and also assessed the binding of CBDV-rich cannabis extracts and their components at CB_1 receptors.[57] CBDV-rich cannabis extracts' effects on motor function were investigated using static beam and grip strength assays, and purified CBDV and CBD were evaluated for potential pharmacological interactions. On the rat PTZ test, 200–275 mg/kg CBDV-rich cannabis extracts had a significant anticonvulsant effect on seizure severity and significantly reduced seizure associated mortality. Both $\geq$50 mg/kg purified CBDV and 100 mg/kg CBDV-rich cannabis extracts significantly suppressed seizure severity, and mortality was significantly reduced by both purified CBDV and CBDV-rich cannabis extracts ($\geq$100 mg/kg). CBDV-rich cannabis extracts ($\geq$87 mg/kg) exerted significant anticonvulsant effects in the mice AS test, and $\geq$100 mg/kg purified CBDV significantly reduced seizure incidence. CBDV-rich cannabis extracts suppressed pilocarpine-induced convulsions in the rat ($\geq$100 mg/kg). The anticonvulsant effects of $\geq$116 mg/kg purified CBDV and $\geq$27 mg/kg CBD were linearly additive when co-administered. CBDV-rich cannabis extracts produced some motor effects on static beam performance, but no effects on grip strength. This study also reported that CBDV binds to CB_1 receptors with only very weak affinity, suggesting that the anticonvulsant mechanisms of action of CBDV are not mediated by CB_1 receptors.

A recent study evaluated whether the epileptiform activity of CBDV was related to activation of transient receptor potential (TRP) channels.[45] Patch-clamp analysis in transfected HEK293 cells demonstrated that CBDV (3–30 μM) dose-dependently activated and rapidly desensitized $TRPV_{1–2}$ and $TRPA_1$, and these effects were blocked by TRP antagonists. When tested on epileptiform neuronal spike activity in hippocampal brain slices exposed to a Mg^{2+}-free solution using multi-electrode arrays, CBDV reduced both epileptiform burst amplitude and duration. CBDV effects on burst amplitude were not reversed by a selective $TRPV_1$ antagonist, suggesting that they are not uniquely mediated by $TRPV_1$.

In resume, CBDV showed anticonvulsant properties in several preclinical models and produced few motor effects. Thus, CBDV could be effective in a variety of epileptic syndromes and may be less toxic than currently available anti-epileptic drugs.

Anticonvulsant effects of CBN

Few studies investigated the anticonvulsant properties of CBN. Similar to CBD and CBDV, micromolar concentrations of CBN inhibit cellular uptake of anandamide.[26] In one study,[42] rats were exposed to the MES and AS tests and the ED_{50} for CBN anticonvulsant effect was calculated. In the MES test, CBN produced anti-epileptic effects with an ED_{50} of 18 mg/kg. CBN showed only minimal effectiveness in the AS test.

Anticonvulsant effects of delta-8-THC

Delta-8-THC results from the isomerization of THC and has a similar pharmacology, although it appears to be less active.[5] In a study utilizing rats rendered chronically epileptic by bilateral implantation of cobalt into frontal cortices, 10 mg/kg delta-8-THC markedly reduced the incidence of seizures.[43] Within 15–30 min after delta-8-THC administration, generalized seizure activity was completely abolished. Nevertheless, tolerance developed after chronic (6 days) delta-8-THC administration.

Anticonvulsant effects of delta-9-THCV

Similar to CBDV, delta-9-THCV is derived from cannabigerovarin (CBGV).[26] Binding assays suggested a relatively high-affinity interaction of delta-9-THCV with CB_1 receptors but a lack of agonist action, leading to its description as a CB_1 antagonist (although with evidence of agonist properties at higher doses).[26,58] Delta-9-THCV is also a potent CB_2 receptor partial agonist.[26]

A study assessed the anticonvulsant potential of delta-9-THCV in an *in vitro* model of epileptiform activity induced by Mg^{2+}-free extracellular media.[58] This study also investigated the effects of delta-9-THCV in the PTZ test. Delta-9-THCV (20–50 μM) significantly reduced burst complex incidence and the amplitude and frequency of paroxysmal depolarizing shifts (PDSs), and slices pretreated with 10 μM delta-9-THCV exhibited significantly reduced burst complex incidence and PDS peak amplitude. Delta-9-THCV (0·25 mg/kg) significantly reduced seizure incidence in the PTZ test.

Anticonvulsant effects of CBG

CBG is the precursor of THC and CBD. Micromolar concentrations of CBG inhibit the uptake of anandamide, and this phytocannabinoid also activates $TRPV_{1/2}$ channels and is an agonist at α_2-adrenoceptors and an antagonist at $5-HT_{1A}$ receptors.[26]

The effects of CBG on Na_V channels were investigated in mouse cortical neurons and human neuroblastoma cells.[46] The effect of CBG on PTZ-induced seizures was assessed in the rat. CBG blocked Na_V in both human and mouse recordings, but had no effect (50–200 mg/kg) on PTZ-induced seizures. These results indicate that Na_V blockade *per se* does not correlate with anticonvulsant effects.

The endocannabinoid system modulates cortical excitability

The endocannabinoid system represents a compelling target for development of future anti-epileptic therapies, as it is intimately involved in the regulation of cortical excitability, is altered in epilepsy or by epileptic seizures, and its modulation can alter seizure activity or change the development of epileptogenesis in various *in vitro* and *in vivo* models.[30,44]

In a study in mice, the anticonvulsant effects of the endocannabinoid anandamide and of its metabolically stable analogue O-1812 were assessed on seizure threshold and severity in the maximal electroshock model.[59] Anandamide (50–300 mg/kg) and O-1812 (5 mg/kg) produced potent anticonvulsant effects, which were mediated by cannabinoid CB_1 receptor activation, as a CB_1 receptor-specific antagonist blocked the anticonvulsant activity of these compounds. Furthermore, administration of the CB_1 receptor antagonist alone produced a reduction of maximal seizure threshold, providing evidence for an endogenous cannabinoid tone modulating the brain's excitability. Interestingly, high concentrations of anandamide are detected in the hippocampus, an area with high cannabinoid CB_1 receptor expression and which is known to be a major brain region involved in epileptogenesis and seizure disorders.[30,50,59–63]

A study in rodents investigated the role of the endocannabinoid system in modulating the brain's excitability using the kainic acid-induced seizures model in mutant mice lacking CB_1 receptor expression in the majority of cortical glutamatergic neurons, including hippocampus, neocortex and amygdala.[60] Mutant mice showed stronger seizures following kainic acid treatment as compared to wild-type mice, suggesting that glutamatergic cortical neurons are the main target of CB_1-dependent protection against acute excitotoxic seizures. Moreover, this study reported that functional CB_1 protein is abundantly present on glutamatergic hippocampal terminals in the inner molecular layer of the dentate gyrus.

An *in vitro* study evaluated the effects of the endocannabinoids methanandamide (a stable analogue of anandamide) and 2-AG, and of the anti-epileptic drugs phenobarbital and phenytoin, on refractory status epilepticus using the low-Mg^{2+} hippocampal neuronal culture model.[61] Phenobarbital and phenytoin were ineffective in completely blocking status epilepticus at the high micromolar range. On the other hand, methanandamide (300 nM– 1 μM) and 2-AG (1–10 μM) inhibited status epilepticus in a very potent, dose-dependent manner, at nanomolar concentrations. Moreover, the effects of methanandamide and 2-AG were mediated by agonism at the cannabinoid CB_1 receptor, as they were blocked by a CB_1 receptor antagonist.

Another rodent study evaluated the long-term effects of status epilepticus on CB_1 receptor expression, binding and G protein activation in the rat pilocarpine model of acquired epilepsy, a model of partial complex or limbic epilepsy in humans.[62] Status epilepticus produced long-term redistribution of hippocampal CB_1 receptors and regionally selective functional changes in CB_1 receptor binding and G protein activation. According to the authors, these results suggest that CB_1 receptor redistribution may play an important role in the permanent plasticity changes associated with brain injury from status epilepticus and epileptogenesis.

In a study in mice, the pilocarpine-induced status epilepticus mouse model of temporal lobe epilepsy was used to study the effect of endogenous cannabinoid agonists on recurrent excitatory circuits of the dentate gyrus using electrophysiological recordings in hippocampal slices.[63] Anandamide (1–10 μM) and 2-AG (10 μM) reduced the frequency of excitatory post-synaptic currents, an effect that was blocked by a CB_1 receptor antagonist. 1 μM WIN55, 212-2, a CB_1 receptor agonist, also reduced the frequency of excitatory post-synaptic currents, an effect that was also blocked by a CB_1 receptor antagonist. Moreover, there was an upregulation of CB_1 receptors in the dentate gyrus of animals with temporal lobe epilepsy. These findings suggest that activation of CB_1 receptors present on nerve terminals can suppress recurrent excitation in the dentate gyrus.

A recent study investigated the role of cannabinoids in specific areas of the cortico-thalamic network involved in oscillations that underlie seizures in a genetic animal model of absence epilepsy, the WAG/Rij rat.[64] The study assessed the effects of focal injection of the endogenous cannabinoid anandamide, WIN55, 212-2, and of a selective CB_1 receptor antagonist/inverse agonist (rimonabant) into thalamic nuclei and primary somatosensory cortex of the cortico-thalamic network. Anandamide (1–5 μg/0·5 μL) and WIN55, 212-2 (0·1–1 μg/0·5 μL) reduced absence seizures independently from the brain focal site of infusion, whereas rimonabant increased absence seizures only when focally administered to the ventroposteromedial thalamic nucleus. These results support therapeutic potential for endocannabinoid system modulators in absence epilepsy and highlight that an attenuated endocannabinergic tone might be present in the cortico-thalamic circuit underlying absence epilepsy in WAG/Rij rats.

Finally, a recent study in rats used behavioural and video-electroencephalographic (EEG) analysis to investigate the modulatory potential of synthetic cannabinoids and anandamide hydrolysis inhibitors [fatty acid amide hydrolase (FAAH) inhibitors] on

seizures induced by PTZ.[65] WIN55, 212-2 (1 mg/kg) reduced myoclonic seizure ('minimal seizure') threshold, whereas other doses (0·3 and 3 mg/kg) did not alter seizure threshold. WIN55, 212-2 (1 mg/kg) also significantly increased EEG seizure duration. The administration of arachidonyl-2-chloroethylamide (ACEA; 1–4 mg/kg), a selective CB_1 receptor agonist, significantly decreased seizure threshold, whereas 2 mg/kg ACEA interfered with EEG seizure duration, significantly increasing epileptiform discharge duration. Rimonabant (0·3–3 mg/kg), a selective CB_1 antagonist/inverse agonist, did not alter myoclonic seizure threshold or epileptiform discharge duration and threshold. Contrary to the other cannabinoids, 3 mg/kg URB-597, a selective FAAH-inhibitor, significantly increased seizure threshold, and 0·3–3 mg/kg URB-597 reduced EEG epileptiform activity. WIN55, 212-2 and ACEA produced characteristic proconvulsant effects, whereas none of the cannabinoids changed the threshold or epileptiform EEG latency for tonic–clonic generalized ('maximal') seizures.

Integrating the data on cannabinoids and epilepsy

There is contradictory information that support both anti- and proconvulsant effects of smoked cannabis, and results from preclinical studies using isolated cannabinoid agonists are equally divergent.[13,16,23,24,27–30,32–37] Conflicting results have also been observed with cannabinoid antagonists, with some studies showing anticonvulsant effects,[58] others failing to report such effects,[65] others reporting a reduction of maximal seizure threshold after pharmacological blockade of CB_1 receptors[59] and others reporting increased seizure severity after genetic deletion of CB_1 receptors.[60]

Nevertheless, regarding phytocannabinoids specifically, the reviewed literature suggests that these compounds, especially CBD and CBDV, are in general potent anticonvulsants that produce few side effects. The contradictory reports regarding cannabis could be explained by the different phytocannabinoids, with potentially conflicting pharmacology. Furthermore, the preferential action of THC on CB_1 receptors located in glutamatergic or GABAergic neurons could produce anti- or proconvulsant effects, respectively.[26,30,44]

Regarding THC and synthetic cannabinoids, although these compounds seem to produce their anticonvulsant effects by activating CB_1 receptors, several preclinical studies reported that CBD, CBDV, CBN, delta-9-THCV and CBG showed anticonvulsant properties that are apparently independent of CB_1 receptors.[26,45,55–58] These compounds bind to CB_1 receptors with only very weak affinity, and their anticonvulsant effects seem to be mediated by activation of the endocannabinoids system through inhibition of the cellular uptake of anandamide and of its enzymatic hydrolysis.[26,45,55–58]

Moreover, the conflicting results could in part be mediated by several methodological aspects. Many of the reviewed studies used different animal models of seizures, which could interfere with results. Studies often used different rodent species, which could potentially represent differences in the availability of cannabinoid, GABAergic and glutamatergic receptors and endogenous neurotransmitters in different brain regions, potentially modifying results. Different rodent species may also present different metabolism regarding cannabinoids and several anti-epileptic drugs used in comparison studies, which could produce not only pharmacokinetic interactions, but also pharmacodynamic modifications. Differences in administered doses, routes of administration, mechanisms of action (i.e. agonist, antagonist, partial

agonist, inverse agonist) and binding affinities of phyto-, endo- and synthetic cannabinoids to cannabinoid receptors could also potentially cause differences in results. The potential action of cannabinoids in other neurotransmitter systems should also be considered, especially with high doses.

Evidence from preclinical studies suggests a physiological role for the endocannabinoid system in the modulation of seizure threshold and severity.[30,44] Cannabinoid antagonists modify seizure threshold and severity.[59,64] Anandamide has a protective action against electroshock seizures and reduces excitability in a hippocampal neuronal culture model.[59,61] Moreover, anandamide and 2-AG reduce the frequency of excitatory post-synaptic currents,[63] and anandamide hydrolysis inhibitors (FAAH-inhibitors) have anticonvulsant effects.[59] Genetic deletion of CB_1 receptors increases seizure severity,[60] and CB_1 receptors are upregulated in the dentate gyrus of animals with temporal lobe epilepsy.[63] Furthermore, status epilepticus produces long-term redistribution of hippocampal CB_1 receptors and regionally selective functional changes in CB_1 receptor binding and G protein activation.[62]

These results corroborate the hypothesis that on-demand synthesized endocannabinoids promote defence against acute excitotoxicity, and the signalling pathways through which these protective effects occur might involve opening K^+ channels and inhibition of inward Ca^{2+} currents, which are mediated by CB_1 receptors.[30,44,59–63,65] The endocannabinoid system could also produce neuroprotective effects by neurochemical mechanisms that are independent of the CB_1 receptor, such as inhibition of the cellular uptake of anandamide and of its enzymatic hydrolysis.[26,45,55–59,61,63]

Although several phytocannabinoids discussed present anticonvulsant potentials that deserve to be further investigated, CBD and CBDV appear to be the most promising phytocannabinoids regarding epilepsy treatment, as the anticonvulsant properties of these compounds were reported in several in vitro and animal studies, and also in a few small clinical trials.[14,17,26–29,53] CBD and CBDV have a multipharmacological profile, resulting in distinct mechanisms of action possibly responsible for their anticonvulsant effects.[13–17,23–29,45,55–58] CBD and CBDV anticonvulsant effects may result from numerous cannabinoid receptor-independent mechanisms, as these phytocannabinoids have little affinity for $CB_{1/2}$ receptors.[13–17,23–29,45,55–58] Thus, CBD may potentially modulate neuronal hyperexcitability via a number of different mechanisms, which include regulation of Ca^{2+} homeostasis, agonistic properties at $5-HT_{1A}$ receptors and reduction in adenosine reuptake.[14,25–29,50–52] Moreover, CBD and CBDV inhibit the uptake of anandamide and of its enzymatic hydrolysis.[26,45,55–59,61,63] Investigating the diverse mechanisms of action responsible for CBD and CBDV anticonvulsant effects may stimulate research with new anti-epileptic agents with a better therapeutic response and which produce few side effects than current available anti-epileptic drugs.

Considering that many patients report limited therapeutic efficacy with several currently available anti-epileptic drugs and that these drugs produce several side effects, it is necessary to investigate drugs with better therapeutic efficacy and improved safety. Phytocannabinoids are potential candidates for these future investigations.

WHAT IS NEW AND CONCLUSION

Phytocannabinoids, specially CBD and CBDV, have demonstrated anticonvulsant effects in vitro and in vivo, and at least one double-

blind study suggest that CBD reduces seizure frequency and is well tolerated in adult epileptic patients. Moreover, CBD and CBDV have demonstrated anticonvulsant properties in several preclinical models of seizures and produced few motor effects. Thus, CBD and CBDV could be effective in a variety of epileptic syndromes and may be less toxic than currently available anti-epileptic drugs.

On the other hand, given the small number of clinical trials with patients and the quality of the available data, no reliable conclusions can be drawn from the available studies regarding the possible therapeutic uses of phytocannabinoids in specific epileptic syndromes as standalone drugs or as adjunct treatments. However, anecdotal evidence strongly suggest that CBD may be effective in infantile intractable epileptic syndromes such as Dravet and Lennox–Gastaut syndromes, and clinical studies with CBD focusing on the syndromes are currently underway.

Given the good safety profile of CBD and CBDV, there is a clear need to perform new randomized, controlled clinical trials with these molecules.

CONFLICT OF INTEREST

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