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Therapeutic effects of cannabinoids in animal models of seizures, epilepsy, epileptogenesis, and epilepsy-related neuroprotection

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Abstract

The isolation and identification of the discrete plant cannabinoids in marijuana revived interest in analyzing historical therapeutic claims made for cannabis in clinical case studies and anecdotes. In particular, sources as old as the 11th and 15th centuries claimed efficacy for crude marijuana extracts in the treatment of convulsive disorders, prompting a particularly active area of preclinical research into the therapeutic potential of plant cannabinoids in epilepsy. Since that time, a large body of literature has accumulated describing the effects of several of the >100 individual plant cannabinoids in preclinical models of seizures, epilepsy, epileptogenesis, and epilepsy-related neuroprotection.

We surveyed the literature for relevant reports of such plant cannabinoid effects and critically reviewed their findings. We found that acute CB_1R agonism in simple models of acute seizures in rodents typically produces anti-convulsant effects whereas CB_1R antagonists exert converse effects in the same models. However, when the effects of such ligands are examined in more complex models of epilepsy, epileptogenesis and neuroprotection, a less simplistic narrative emerges. Here, the complex interactions between (i) brain regions involved in a given model, (ii) relative contributions of endocannabinoid signaling to modulation of synaptic transmission in such areas, (iii) multi-target effects, (iv) cannabinoid type 1 and type 2 receptor signaling interactions and, (v) timing, (vi) duration and (vii) localization of ligand administration suggest that there is both anti-epileptic therapeutic potential and a pro-epileptic risk in up- and down-regulation of endocannabinoid signaling in the central nervous system. Factors such receptor desensitization and specific pharmacology of ligands used (e.g. full vs partial agonists and neutral antagonists vs inverse agonists) also appear to play an important role in the effects reported. Furthermore, the effects of several plant cannabinoids, most notably cannabidiol (CBD) and cannabidavarin (CBDV), in models of seizures, epilepsy, epileptogenesis, and neuroprotection are less ambiguous, and consistent with reports of therapeutically beneficial effects of these compounds in clinical

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Conflict of interest

The authors do not have any conflict of interests to declare.

studies. However, continued paucity of firm information regarding the therapeutic molecular mechanism of CBD/CBDV highlights the continued need for research in this area in order to identify as yet under-exploited targets for drug development and raise our understanding of treatment-resistant epilepsies.

The recent reporting of positive results for cannabidiol treatment in two Phase III clinical trials in treatment-resistant epilepsies provides pivotal evidence of clinical efficacy for one plant cannabinoid in epilepsy. Moreover, risks and/or benefits associated with the use of unlicensed Δ^9-THC containing marijuana extracts in pediatric epilepsies remain poorly understood. Therefore, in light of these paradigm-changing clinical events, the present review's findings aim to drive future drug development for newly-identified targets and indications, identify important limitations of animal models in the investigation of plant cannabinoid effects in the epilepsies, and focuses future research in this area on specific, unanswered questions regarding the complexities of endocannabinoid signaling in epilepsy.

Keywords

Cannabinoid; Epilepsy; Seizure; Epileptogenesis; Neuroprotection; Animal models

1. Introduction

In order to understand the justification for modern, preclinical investigations of the effects of cannabinoids in animal models of epilepsy and its associated symptoms and features, some appreciation of the historical, anecdotal use of marijuana (cannabis) in convulsive disorders is required. There is general consensus that the origins of marijuana use in the treatment of convulsions lie in reports from the Middle East that were ascribed to the scholar al-Mayusi [1] in 1100 and the historian Ibn al-Badri in 1464 [2]. It was not until 1649 that Nicholas Culpeper translated the *Pharmacopoeia Londonensis* from Latin into English, and suggested marijuana as a treatment of “inflammation of the head” [3]. Thereafter, there appears to be no further mention of this therapeutic use of marijuana until its introduction to Western medicine in the 19th century by William O'Shaughnessy. Here, alongside other reports from the same period describing the control seizures with marijuana extracts [4–6], O'Shaughnessy described successful treatment of infantile seizures with a cannabis tincture [7]. Similarly, J. R. Reynolds described marijuana as ‘*the most useful agent with which I am acquainted*’ in the treatment of ‘*attacks or violent convulsions …* (and) *…may be stopped with a full dose of hemp*’ [6] while William Gowers commented that ‘*Cannabis indica…is sometimes, although not very frequently, useful. It is of small value as an adjunct to the bromide*, *but is sometimes of considerable service given separately*’ [8].

Despite these admittedly anecdotal reports of efficacy in convulsive episodes, only very limited investigation of the anti-convulsant effects of marijuana were undertaken in animal models prior to the 1980s [9,10]. Arguably, it was the isolation, identification, and subsequent synthesis of the two most abundant cannabinoids derived from marijuana, Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD), in the 1960s [11,12] which has driven modern studies of their pharmacological effects in a variety of models of central nervous system disease, including those of epilepsy, seizures, epileptogenesis, and epilepsy-

related neuroprotection reviewed here. Recent reports of positive effects in properly controlled human clinical trials of CBD in the treatment resistant epilepsies have provided the first definite evidence of clinical efficacy for one plant cannabinoid in epilepsy. Given the pivotal nature of these clinical findings, critical review of the preclinical literature associated with this topic is warranted and addressed herein.

2. Methods

To identify effects of cannabinoids in pre-clinical animal models of seizures, epilepsy, epileptogenesis, and neuroprotection, we searched for peer-reviewed, primary literature using a PubMed search. Results were obtained using the keywords “CB1R,” “CB2R,” “cannabinoid”, “cannabidiol”, “THC”/“Tetrahydrocannabinol”, “anandamide”, “2-AG”, “FAAH”/“Fatty acid amide hydrolase”, and “MAG lipase” plus the terms “seizures,” “epilepsy,” “epileptogenesis,” and “neuroprotection.” We excluded primarily in vitro studies and clinical studies in humans (clinical trials, case reports, observational studies). Studies were evaluated based on their scientific rigor and use of physiologically relevant drug concentrations to in vivo studies [13]. Summary details of all studies examined in the present review are presented in Supplemental Table 1.

3. Results

3.1. Pre-clinical models of seizures and epileptogenesis

Early studies from the 1970s–1980s demonstrated that plant cannabinoids (‘phytocannabinoids’) derived from cannabis exerted anticonvulsant effects in both acute animal models of seizures [14–18] and chronic models of epileptogenesis [19–23]. These studies initiated clinical and scientific inquiry into mechanisms mediating potential anti-seizure effects of cannabinoids, albeit using unstandardized animal models and variable routes of cannabinoid administration and doses. The isolation of the target receptors of the major phytocannabinoid, Δ^9-THC, (CB_1R [24]and CB_2R [25]) and the discovery of an endogenous “endocannabinoid” signaling network [26–28] inspired the use of synthetically derived compounds to specifically target cannabinoid receptors and modulate endocannabinoid function. Thus, within the past few decades, there has been a renewed interest in investigating which particular components of marijuana, target receptors, and endocannabinoids mediate potential pro- and anti-convulsant effects of cannabinoids in pre-clinical models. Adding to the complexity, differences in CB_1R expression patterns in different areas of the brain, as well as differential expression on excitatory vs inhibitory synaptic terminals [29] may mediate the variable responses in different seizure model studies. Additionally, a diversity of animal models of both acute and chronic epilepsy, as well as time, route, and frequency of drug administration produce complex and often contradictory results.

To address these concerns, we reviewed the current literature describing the use of modulators of endocannabinoid function, synthetic agonists and antagonists of CB_1R/CB_2Rs, and phytocannabinoids in both acute models of seizure and epilepsy (Section 3.3), chronic models of epileptogenesis (Section 3.4), and epilepsy-related neuroprotection (Section 3.5). We considered studies involving “epileptogenesis” as those in which drugs are

administered during the “latent” phase following a trigger that mediates long-term, spontaneous recurrent seizure. In some cases (e.g. genetic animal models of seizure), clear divisions between acute seizures and epileptogenesis are somewhat ambiguous. Each pre-clinical model was evaluated for completeness and scientific rigor, and the resulting responses (pro-convulsive, anti-convulsive, mixed effect, or no significant effect) were tabulated (Suppl. Tables 1, 2) and summarized (Fig. 1), within “acute seizure” and “epileptogenesis” conditions.

3.2. The endocannabinoid system

The endocannabinoid system plays an important physiological role in modifying excitatory and inhibitory synaptic transmission in the brain. The canonical endocannabinoid system consists of two G protein coupled receptors, CB_1R and CB_2R, with endogenous ligands 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamide (anandamide or AEA), each with unique degradation machinery [30,31]. Of the two cannabinoid receptor subtypes, CB_1R is most widely expressed in the central nervous system, particularly in the mossy cell-granule cell synapses of the hippocampus. However, of relevance to epileptogenesis, CB_1Rs are also present, to a lesser extent, on microglia, astrocytes and oligodendrocytes [31]. Once thought to be exclusively expressed outside the central nervous system, current research suggests that CB_2Rs are also expressed in the brain [32], mediating neuronal excitability [33] and inflammation in microglia [34]. Importantly, in addition to acting via the canonical cannabinoid receptors, the endocannabinoids can also act via interactions with other receptor types such as the orphan G-protein coupled receptor, GPR55, and the transient receptor potential vanilloid receptor (type 1), TRPV1 [35].

Of importance in activity-dependent pathophysiological processes such as epileptogenesis and epilepsy, the synthesis of endocannabinoids typically occurs “on demand” from postsynaptic membrane phospholipids although pre-synthesized endocannabinoid reserves are also contained within intracellular storage organelles [36,37]. However, most commonly, postsynaptic neuronal depolarization triggers membrane phospholipid breakdown by the enzymes diacylglycerol lipase (DAGL) and N-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) to form 2-AG and AEA respectively [38,39]. Following their synthesis, the endocannabinoids passively diffuse across the presynapse in a retrograde fashion to orthosterically bind to and activate presynaptically located CB_1R to inhibit the release of glutamate or GABA from principal or GABAergic neurons respectively [35,40]. The endocannabinoid-mediated inhibitions of excitatory glutamate or inhibitory GABA release are respectively known as depolarized-induced suppression of excitation (DSI) or inhibition (DSI) [35].

Endocannabinoid signaling is thought to play an important role in epileptogenesis and the subsequently developed epilepsy. Supplementing depolarization-induced postsynaptic synthesis, endocannabinoids are also synthesized following activation of metabotropic glutamate and muscarinic acetylcholine receptors [41,42]. Therefore, in addition to the activity-dependent phasic control of neurotransmitter release described previously, this tonic control of endocannabinoid release mediated by G-protein couple receptors may play an important role in epileptogenesis [35]. Furthermore, the complexities of endocannabinoid

signaling also extend to the pharmacological properties of the two principal endocannabinoids. Here, 2-AG (K_i = 472 nM), while acting as a full agonist at CB_1R, exhibits lower affinity for CB_1R than AEA (K_i = 32 nM) which acts as a partial agonist, although 2-AG levels are, on average, ~170-fold higher in brain than AEA [43]. Therefore, overall effects on the development of seizure may be driven by relative levels of the endocannabinoids in particular brain areas [44,45]. Finally, following dissociation from the CB_1R, endocannabinoids are rapidly degraded [46]; 2-AG and AEA are catabolized by the enzymes monoacylglycerol lipase (MAGL) and alpha/beta-hydrolase domain containing 6 (ABHD6) [47] or fatty acid amide hydrolase (FAAH), respectively [48,49].

Furthermore, activation of TRPV1 by AEA can trigger enhanced glutamate release following an increase in intracellular Ca^{+2} concentrations [50], although the concentration of AEA required to activate TRPV1 is greater than that required for CB_1R activation [51,52]. Moreover, TRPV1 activation rapidly leads to desensitization which, in the presence of persistently raised AEA levels, could lead to reduced neuronal activity [53].

3.3. Seizures and epilepsy

By regulating the biochemical synthesis and degradation of endocannabinoids (2-AG and AEA), as well as AEA reuptake, recent studies have demonstrated anti-seizure effects in several pre-clinical acute models of seizure (Suppl. Table 1, Fig. 1). Inhibition of FAAH, the degradative enzyme of AEA, via the synthetic blocker URB597 reduced seizures in a mouse maximal electroshock (MES) model (at 0.05–1 mg/kg, i.p., 30 min before testing) [54], elevated the threshold and reduced duration of pentylenetetrazole (PTZ)-induced seizure (at 0.3–3 mg/kg, i.p. URB597 in rats, 30 min before testing) [55], and reduced seizure severity, duration, and amplitude associated with kainic acid (KA)-induced seizures (2.4 mM/guinea pig cannula, 5 min before, 2 h after testing) [56]. Other studies demonstrated either no effect of URB597 (10–100 μg/rat, i.c.v.) on PTZ seizures [54], or a dose-dependent mixed effect with lower concentrations (1–40 μg/rat, i.c.v.) lowering seizure incidence, and higher concentrations (80–160 μg/rat, i.c.v.) elevating seizure incidence. The authors proposed that this contradiction may be attributable to different dose-dependent mechanisms: low range effects were blocked by the CB_1R antagonist AM251 (2 μg/rat, i.c.v), and higher range effects were blocked by the TRPV1 antagonist capsazepine (1 μg/rat, i.c.v) [52]. In one study, inhibition of MAGL, the catabolic enzyme for 2-AG, via URB602 (10–500 μg/rat, ic.v, 5 min before testing) elevated seizure latency in rats [54]. However, another study with the MAGL inhibitor, SAR127303 (10, 30 mg/kg, p.o., 1 h prior to testing), demonstrated no significant effect on MES or PTZ-induced seizures in mice [57]. Blockade of another 2-AG degradative enzyme, ABHD6, via WWL123 (10 mg/kg i.p., 4 h before injection) also reduced PTZ-induced seizure incidence. This effect was blocked by the $GABA_AR$ antagonist, picrotoxin (1 mg/kg, i.p), but persistent in CB_1R KO mice, suggesting that elevated 2-AG may exert anti-convulsive effects via a CB_1R–independent mechanism mediated through actions on $GABA_ARs$ [58].

Inhibition of AEA reuptake also demonstrated anti-convulsive or minimal effects in several models. Administration of the AEA reuptake inhibitor AM404 (40 mM/guinea pig, cannula, 5 min, 2 h before testing) reduced KA-induced seizure severity, duration, and amplitude

[56]. Other studies with AM404 demonstrated a mixed effect on PTZ–induced seizures (as before with URB597), with a CB_1R-dependent anti-convulsant effect at lower doses (1–40 μg i.c.v., 2 min before injection) and a TRPV1-dependent pro-convulsant effect at higher doses (80–160 μg, i.c.v., 2 min before injection) [52]. Furthermore, another AEA reuptake inhibitor (VDM11, 1, 10 mg/kg, i.p., mice, 30 min before testing) exerted no significant effect on seizures [59]. Collectively, these results suggest that elevating levels of the endocannabinoids 2-AG and AEA may have an anti-convulsive (or negligible) effect that may be only partially mediated via a CB_1R-dependent mechanism, although further characterization of which target receptors mediate anti-seizure properties is indicated.

Direct agonism of CB_1R via the synthetic compound WIN55,212 produced an anti-convulsant effect in several animal models of acute seizure. In mice with induced MES, WIN55,212 reduced seizure incidence when administered 2 h before testing (1–100 mg/kg, i.p.) [60] or 3 min before testing (0.5–4 mg/kg, i.p.) [61]. Additionally, WIN55,212 (5–10 mg/kg, i.p., 2 min before MES seizure in mice) increased the anti-convulsant effects of co-administered anti-seizure medications: valproate (125–275 mg/kg, i.p.), carbamazepine (4–16 mg/kg, i.p.), phenobarbital (8–30 mg/kg, i.p.), phenytoin (4–12 mg/kg, i.p.), lamotrigine (2–7 mg/kg, i.p.), pregabalin (25–150 mg/kg, i.p.), and topiramate (10–60 mg/kg, i.p.) [62,63]. WIN55,212 increased the threshold (5–15 mg/kg, i.p., 60 min before testing) [64] and latency (1–100 μg/rat i.c.v., 20 min before testing) [54] of PTZ-induced seizure in rats, and increased the anti-convulsant effects of ethosuxamide (75–175 mg/kg, i.p.), phenobarbital (6–30 mg/kg, i.p.), and valproate (50–175 mg/kg, i.p.), when administered 15 mg/kg, i.p., 20 min before testing [65]. However, other studies noted a pro-convulsant effect of WIN55,212 (1 mg/kg, i.p., 30 min before testing) in PTZ seizure induced in rats: reducing the seizure threshold and increasing seizure duration [55]. In KA-induced seizure in juvenile (P20) rats, WIN55, 212 reduced seizure severity, burst frequency, and amplitude at the lower dose (0.5 mg/kg, i.p.), but increased seizure and behavioral impairments at the higher dose (5 mg/kg, i.p.), when given 90 min before testing [66].

Another direct CB_1R agonist, arachidonyl-2'-chloroethylamide (ACEA, 2.5–10 mg/kg, i.p.), co-administered with the serine protease/FAAH inhibitor phenylmethane sulfonyl fluoride (PMSF, 30 mg/kg i.p.) 10 min before testing, increased the threshold for MES induction in mice, and increased the anti-convulsant effects of valproate (195 mg/kg, i.p.) [67], phenobarbital (10–24 mg/kg, i.p.) [68], ethosuxamide (75–175 mg/kg, i.p.), phenobarbital (6–30 mg/kg, i.p.), and valproate (50–175 mg/kg, i.p.) [69]. Furthermore, ACEA (2–8 mg/kg, i.p.) given 60 min before testing [70] or (10 mg/kg, i.p.) 30 min before testing [59] increased seizure threshold and latency to PTZ-induced seizure. As expected, the effects of CB_1R agonism were reversed by treatment with the CB_1R antagonist AM251 (1 mg/kg, i.p.) [71]. However, the anti-convulsant effects of ACEA were also enhanced by the nitric oxide precursor L-arginine (50, 100 mg/kg, i.p.), and blocked by the non-specific nitric oxide synthase (NOS) inhibitor L-NAME (15 and 30 mg/kg, i.p.) and the specific neuronal NOS inhibitor 7-NI (40 and 80 mg/kg, i.p.) [71]. Additionally, anti-convulsant effects of CB_1R agonists were potentiated by ultra-low doses of the opioid antagonist naltrexone (at ultra-low dose 1 pg/kg, i.p.) [70], and blocked at higher doses of naltrexone (1–10 mg/kg, i.p.) [72]. Taken together, these studies suggest that CB_1R agonists may interact with several other

systems to enhance anti-seizure properties. Notably, other reports demonstrate potential pro-convulsive effects of ACEA on PTZ seizure (1–4 mg/kg, i.p. 30 min before testing) [55].

In addition to the role of CB_1Rs in reducing seizure, recent studies suggest that activation of mixed CB_1R/ CB_2Rs, or CB_2Rs alone, may regulate excitability in the hippocampus, increase excitatory transmission [33], and trigger cell-type specific hyperpolarization following sustained stimulation [73]. Administration of a CB_2R agonist, AM1241 (1, 10 μg/rat i.c.v.), elevated PTZ seizure frequency and severity, and reduced seizure latency when administered 90 min before testing [74], suggesting a pro-convulsive effect. Studies with mixed CB_1R/CB_2R agonists (e.g. anandamide, or closely related palmitoylethanolamide) demonstrate primarily anti-convulsive effects in MES- [75,76] and PTZ-induced seizure [77]. Administration of AEA (6.25–50 mg/kg, i.p.) increased seizure severity and neurotoxicity in bicuculline- ($GABA_A$R antagonist) and KA-induced seizure models, but only in FAAH-deficient mice, suggesting that precise regulation of AEA levels is required to regulate excitability after ictal events. More studies are suggested to uncover potential roles for CB_2Rs in regulating excitability, as well as interactions between endocannabinoids and CB_1R/CB_2Rs in the brain.

Direct CB_1R antagonists (SR141716A, AM251) demonstrate primarily pro-convulsive effects in pre-clinical seizure models. 0.75–3 mg/kg, i.p. AM251, administered 1 h before testing, increased PTZ-induced seizure incidence in mice [72]. AM251 (20 mg/kg, i.p.) also reduced seizure latency, and increased burst duration, after-discharge duration, and mortality in mice with PTZ-induced seizure (15–30 min prior to testing); effects potentiated by addition of the CB_2R antagonist AM630 (2 mg/kg, i.p.) [78]. However, AM630 alone (1 mg/kg, i.p., 3 min before testing) had no significant effect on PTZ-induced seizure in rats [74]. In a KA seizures model in guinea pigs, 10 mM focal injection of AM251 (2 μl hippocampus, entorhinal cortex, basal nucleus of amygdala, medial septum) at 5 min before testing and 2 h after testing increased seizure incidence, without altering duration or frequency, producing no net effect on status epilepticus [56]. Other studies suggest that AM251 has no significant effect on MES seizure when given 15–30 min before testing [59,71], and a pro-convulsive effect in PTZ-induced seizure models at higher doses (5–10 μg, i.c.v.) [77].

Early studies suggest that, like the synthetic CB_1R agonists, Δ^9-THC exerts mixed effects on seizure susceptibility in multiple species. Δ^9-THC acts as a partial CB_1R/CB_2R agonist, and activates TRP channels and so may produce more complex effects on excitability than synthetic full agonists specific for a given CBR. Δ^9-THC (1–100 mg/kg, i.p.) reduced seizure incidence when administered 2 h before MES seizure induction in mice [60]. Other studies utilizing MES mouse seizure models indicated dose-dependent, mixed effects of Δ^9-THC on hindlimb extension [79] and increased seizure threshold in MES models and 6-Hz stimulation paradigms, but reduced threshold in 60-Hz stimulation (100 mg/kg i.p., daily for 3–4 days before testing) [15]. Δ^9-THC (25–50 mg/k, p.o.) increased the anti-convulsant effects of co-administered phenobarbital (9.3–40 mg/kg, i.p.) in the MES seizure model [14]. In PTZ-induced seizure, Δ^9-THC exerted no significant effect when administered 30 min before testing in mice (up to 80 mg/kg, i.p., Δ^9-THC) [80] and 0.5 or 2 h before 35 or 80 mg/kg PTZ in chickens (0.25–1 mg/kg, i.v., Δ^9-THC) [81]. Notably, Δ^9-THC (3 mg/kg,

i.p.) administered to a pregnant female mouse from 5 consecutive days at embryonic days 12.5–16.5 reduced seizure latency and reduced the required PTZ dose to induce seizure in offspring [82], raising the possibility that Δ^9-THC can cause pro-convulsive effects in future generations. Tetrahydrocannabivarin (Δ^9-THCV), a structurally related compound to Δ^9-THCV that acts as a CB_1R *antagonist* and CB_2R partial agonist, reduced seizure incidence when given 0.25 mg/kg i.p., 30 min prior to PTZ-induced seizure in rats. Collectively, Δ^9-THC and Δ^9-THC-related compounds produce variable effects in several models of seizure, potentially due to the promiscuous nature of receptor binding, and differences in activity at excitatory vs. inhibitory terminals (DSE vs DSI).

Unlike Δ^9-THC, cannabidiol (CBD) demonstrates primarily anticonvulsive effects in reported seizure models. Of note, CBD has minimal affinity at both CB_1Rs and CB_2Rs [83–86], and instead acts through various targets such as GPR55, VDAC1, and ENT1 (modulating adenosine transport) [13]. Cannabidiol reduced seizure incidence and increased seizure threshold in the MES model in mice and rats, when administered 0.5–6 h before testing [14,15,17,60]. In another study, CBD (5–400 mg/kg, i.p.) exerted anti-convulsive effects in six of eight acute mouse seizure models (MES, picrotoxin, isonicotinic acid, bicuculline, hydrazine, and PTZ), when given 1 h before testing [18]. In PTZ seizure models, Cannabidivarin reduced seizure severity and mortality (100 mg/kg, i.p.) [83] and reduced neuronal loss and astro-cyte hyperplasia (50 mg/kg, i.p.) [87], when provided 1 h before testing. A structurally related phytocannabinoid, cannabidivarin (CBDV), also demonstrated prominent anti-seizure properties in both mice and rats. Cannabidivarin reduced seizure severity when administered at 5–200 mg/kg i.p. 1 h before either MES seizure in mice or PTZ seizure in rats, as well as 400 mg/kg p.o. 3.5 h before PTZ seizure. At 200 mg/kg i.p., CBDV also potentiated the effects of valproate (50–200 mg/kg, i.p.) and ethosuximide (60–175 mg/kg, i.p.) [88]. Taken together, CBD and CBDV appear to be two potentially well-tolerated and highly anti-convulsive compounds in pre-clinical *acute* seizure models, with no documented cases of pro-convulsant effects.

3.4. Epileptogenesis

Epileptogenesis is an intricate phenomenon through which, following a suitable trigger, neuronal networks shift from a healthy, physiological state to an excitatory, epileptic state that culminates in the development and expression of spontaneous, recurrent seizures [89]. Numerous alterations take place in the brain during the latent period following the trigger such as neuronal loss and degeneration, gliosis, mossy fiber sprouting, scattering of dentate granule cells, and synaptic modifications in the dentate gyrus; all of which subserve the epilepto-genic process [90,91]. Moreover, due to increased neuronal activity, neurons experience oxidative stress and generated free radicals cause mitochondrial damage and apoptosis [92,93].

Both direct (i.e. CB_1R agonism) and indirect approaches (inhibition of endocannabinoid catabolism) can hinder epileptogenesis in animal models (Suppl. Table 2, Fig. 1). Chronic administration of the full CB_1R agonist WIN55,212-2 (4 mg/kg/day, i.p., for 11 days, 30 min before stimulation) exhibited anti-epileptogenic properties in the amygdala kindling model of temporal lobe epilepsy in mice by delaying the progression of seizures severity

[94]. A single dose of WIN55,212-2 (4 mg/kg, s.c.) administered at the start of the epileptogenic kindling process has also been shown to prolong seizures development by a period of two weeks in Krushinsky-Molodkina (KM) rats with genetic audiogenic epilepsy [95]. WIN 55,212-2 (10 μM) attenuated the frequency of excitatory postsynaptic currents in whole cell patch clamp recordings from granule cells in vitro and impeded recurrent excitation in the dentate gyrus of mice with pilocarpine-induced temporal lobe epilepsy (TLE), assessed after electric stimulation of mossy fibers in the hilus of hippocampal slices [96]. Again, repeated doses of WIN55,212-2 (2 mg/kg/day, s.c., for 15 days from 24 h after epileptogenic insult) suppressed the severity, duration, and frequency of spontaneous recurrent seizures that manifest at the end of the latent period in the pilocarpine-induced model of TLE in rats [97]. Furthermore, the same study showed that WIN55-212,2 reduced over-expression of NR2A and NR2B subunits of the NMDA receptor, characteristic of neurotoxicity in this model, suggesting a potential antiepileptogenic mechanism [97,98]. The study further demonstrated that WIN55-212,2 reduced the immature GABAergic cell population in the hippocampus observed 6 months after the *status epilepticus* (SE) event used to induce epilepsy in rats [97]. Importantly, immature GABAergic cells are excitatory in nature rather than inhibitory due to depolarizing GABA-evoked currents arising from a developmentally reversed chloride gradient [99], suggesting that CB_1R agonism may inhibit epileptogenesis via modifications to GABAergic signaling. However, while a single WIN55,212-2 injection (5 mg/kg, i.p.) administered four hours after the cessation of status epilepticus in the lithiumpilocarpine model of TLE reduced the onset of early seizures frequency, mortality, and cell death in dentate hilus in rats, it failed to reduce the frequency of the spontaneous recurrent seizures 1–4 months after SE, indicating only a partial effect on the epileptogenic process [100].

A selective and irreversible MAGL inhibitor of MAGL, JZL184 (8 mg/kg/day, i.p., administered 60 min before electric stimulation), delayed amygdala electric kindling acquisition (700 μA/day, 5 days a week) and impeded the development of generalized epileptic seizures, while reducing the duration of behavioral and electrographic seizures activity in mice [44]. In addition, JZL184 treatment (40 mg/kg/day, i.p.) decreased the first after-discharge duration and increased the stimulus intensity threshold in a perforant path kindling model in mice [78]. Moreover, a daily intraperitoneal injection of JZL184 (4 mg/kg for 10 days), two weeks after kainic acid (KA) injection (0.2 μg administered to the right dentate gyrus in 8 week old mice), reduced the frequency of spontaneous seizures in a KA-induced chronic mouse seizures model [78]. Another potent MAGL inhibitor, SAR127303 (30 mg/kg/day, p.o.), after repeated administration, also delayed kindling progression and decreased kindled seizures (8 mA/0.8 ms at 60-Hz sub-convulsive corneal stimulations for 2 s twice daily at least 90 min apart) in mice [57]. These two MAGL inhibitors are highly selective [57,101] and act specifically at those synapses where there is more ‘on demand’ production of 2-AG from the postsynaptic membrane following activation of an excitatory network [44]. Therefore, MAGL inhibition leads to enhanced 2-AG levels that produces sustained CB_1R activation to, most likely, inhibit glutamate release and produce the observed anti-epileptogenic effect. However, contrary to these findings, a longer-term use of a higher dose of JZL184 increased the frequency and duration of spontaneous seizures in a mouse model of pilocarpine induced temporal lobe epilepsy [102]. Here, it has been

speculated that the higher dose of JZL184 can lead to sufficient accumulation of 2-AG to induce downregulation and/or desensitization of CB_1R, leading to a loss of CB1R-mediated inhibition of excitatory neurotransmission and the resulting pro-convulsant effect reported in the latter study [44,103]. Alternatively, this exacerbation could also be explained by preferential 2-AG-mediated inhibition of synaptic transmission at GABAergic synapses, triggering hyperexcitability through disinhibition. It is plausible that the same rationale applies to the apparently pro-convulsant effects of Δ^9-THC in both healthy rats and mice after chronic administration [104].

The somewhat contradictory effects seen following potentiation of endocannabinergic transmission upon epileptogenesis also extend to CB_1R antagonism. Several studies indicate that CB_1R antagonists exert a pro-convulsive effect, as predicted by the primarily anti-epileptogenic effect of CB_1R agonists. Five days oral administration of CB_1R specific antagonist, SR141716A (rimonabant, 30 mg/kg), increased the number of rats susceptible to audiogenic seizures, as well as seizures duration, suggesting a pro-epileptogenic effect [105]. Additionally, SR141716A (10 mg/kg, i.p.) administered 2 h prior following pilocarpine treatment increased the frequency and duration of seizures [106]. Pharmacological blockade of CB_1 and CB_2 receptors by AM251 (20 mg/kg, i.p.) and AM630 (2 mg/kg, i.p.) respectively or genetic deletion of DAGLα increased seizures duration and reduced seizures latency in the kindling epileptogenesis model [78]. However, other results demonstrate an anti-epileptogenic effect of CB_1R antagonists, as these compounds may also restrain cannabinoid-mediated disinhibition. Febrile seizures in immature (P10) rats enhanced CB_1R expression at inhibitory presynapses more than excitatory presynapses, rendering inhibitory terminals more sensitive to depolarization-induced suppression of inhibition (DSI) [107], producing net hyperexcitability. Administration of the CB1R antagonist, SR141716A (1 mg/kg, i.p., 1 h before seizures induction), immediately prior to the hyperthermic insult blocked this increase in CB_1R expression and the resulting change in DSI in hippocampal slices [108]. A similar effect was observed in a model of traumatic brain injury induced by lateral fluid percussion in rats where a single rapid administration of SR141716A (1, 2 or 10 mg/kg, i.p.) attenuated long-term seizures susceptibility and suppressed the epileptogenic process [109,110]. It is notable that, in some cases, SR141716A treatment is only effective when administered prior to or during the insult used to trigger epileptogenesis; e.g. anti-epileptogenic effects were lost when administration was delayed to 20 min after head trauma [109]. However, a single dose of SR141716A (1 and 10 mg/kg, i.p) administered 7 days after the induction of febrile seizures in immature (P8) rats attenuated the maximal electrical shock-induced seizures stage [111]. SR141716A appears to limit hyperexcitability and epileptogenesis by inhibiting the long-term postsynaptic mGluR5 receptor overexpression and activating the dynorphin-KOR system [110]. Since seizures-induced neuronal depolarization stimulates sustained 2-AG synthesis at the postsynaptic membrane as a protective mechanism, this intense and sustained activation of endocannabinoid signaling could lead to subsequent downregulation of presynaptic CB_1R expression in principal cells [112]. Therefore, the positive effect of SR141716A in the inhibition of epileptogenesis might be attributed to the early prevention of the downregulation of the endocannabinoid system and potentiation of the dynorphin-KOR system to preserve the neuronal function [78, 110]. In contrast to the above studies,

SR141716A (10 mg/kg, i.p.) had no effect on epileptogenesis in a kainate model of temporal lobe epilepsy in rats even when administered immediately after the development of *status epilepticus* [113]. A possible explanation of this result might be that the brain insult employed was not sufficient to produce CB_1 receptor downregulation and so endocannabinoid signaling is not implicated in this model. Overall, while anti-convulsant effects of acute CB_1R agonism in acute seizures is evident, a more nuanced view is clearly required when considering the less clear relationship between acute CB_1R antagonism and acute seizures. In these cases, it is essential to consider the model, species, timing, dose, and brain area in which CB_1R antagonists are administered.

In addition to the potential for endocannabinoids to modulate the epileptogenic process, as described previously, several plant cannabinoids exert significant anti-convulsant effects, conceivably influencing epileptogenesis. As a partial CB_1R/ CB_2R agonist, Δ^9-THC reduces seizures severity and duration in spontaneously epileptic gerbils (*Meriones unguiculatus*) when given 50 mg/kg p.o. at six days before testing, although significant tolerance develops over time [19]. Furthermore, Δ^9-THC (10 mg/kg) reduced audiogenic seizures incidence when administered 15–45 min before the testing stimulus (audiogenic priming at postnatal day 19, stimulus at day 28 in mice) [20]. A single dose of Δ^9-THC (10 mg/kg, i.p.) immediately prior to EEG and behavioral monitoring prevented pilocarpine-induced spontaneously recurrent seizures in rats [106]. Δ^9-THC also exerted anti-epileptogenic activity when administered in an amygdala kindling model in adult rats (1–5 mg/kg i.p. Δ^9-THC) [21], cats (0.25 mg/kg, i.p. Δ^9-THC) ([114], and baboons (0.25–1 mg/kg, i.p. Δ^9-THC) [115], as well as limbic kindling (0.3–4 mg/kg, i.p. Δ^9-THC) [22] and cortical cobalt implantation (10 mg/kg, i.p. Δ^9-THC) [23] in rats. However, other studies demonstrate a pro-convulsive effect of Δ^9-THC; repeated administration of Δ^9-THC reliably induces convulsive seizures in healthy rats and mice [104].

Cannabidiol (CBD) and cannabidivarin (CBDV) exhibit anti-epileptogenic effects in several animal models through a CB_1R/CB_2R-independent mechanism. CBD (1, 10, 100 mg/kg, i.p.) reduced seizures incidence and mortality when administered 1 h before pilocarpine or penicillin-induced epileptogenesis in rats [116]. Additionally, 0.3–3 mg/kg i.p. CBD, given at 30-min intervals following limbic kindling (60–300 min), decreased seizures duration and amplitude, and increased seizures threshold in rats [22]. In other reports of pilocarpine-induced seizures, CBDV had no significant effect (200 mg/kg, i.p., 1 h before testing) [89], although cannabis-based botanically-derived substances enriched with CBDV reduced pilocarpine-induced seizures severity (200 mg/kg, i.p.). Furthermore, administration of the same enriched CBDV cannabis extract (50–200 mg/kg, i.p.) limited audiogenic seizures and mortality in DBA/2 mice, when given 1 h before testing [88,117].

Collectively, understanding the role of the endocannabinoid system in epileptogenesis requires careful consideration of the dosing schedule, time window of drug administration, age of the animal, and underlying seizures network [95]. CB_1R agonists demonstrate primarily anticonvulsive effects in chronic models, while CB_1R antagonists exert mixed effects, based on drug timing and dosage. CB_1R antagonism immediately before or after an epileptogenic insult or repeated administration of a low to medium dose of a CB_1R agonist could be of benefit in epileptogenesis, although the complexities associated with such a

dynamic signaling system make it difficult to extrapolate findings in these animal models to specific recommendations for clinical development. Phytocannabinoids such as Δ^9-THC, CBD, and CBDV may also provide a therapeutic option to limit epileptogenesis, although further study is required.

3.5. Neuroprotection

The sustained hyperexcitable state in which neurons are maintained during a seizures often leads to subsequent loss of function and/or excitotoxic cell death [118,119]. Furthermore, during prolonged seizures states (e.g. *status epilepticus*) sustained neuronal depolarization leads to energy deprivation and a consequentially hypoxic ischemic brain injury [120]. The endocannabinoid system plays a substantial role in neuroprotection in a variety of neurodegenerative diseases [40] and traumatic brain injury [121]. However, the extent of the literature describing endocannabinoid signaling and the therapeutic potential presented by its modulation in such neurodegenerative diseases goes beyond the scope of the present review. Therefore, the following evidence focuses specifically upon reports describing cannabinoid-mediated effects upon neuroprotection in epilepsy.

In the kainic acid (KA)-induced model of seizures in mice, endocannabinoids exert a neuroprotective action by activating presynaptic hippocampal CB_1R to inhibit excessive glutamate release and so hinder subsequent excitotoxicity [122]. Excitotoxic cell death is also limited after conditional CB_1R overexpression in an acute KA-induced model of seizures in the mouse [123]. In such cases, elevated AEA protected hippocampal principal cells from KA-induced seizures-mediated excitotoxicity in mice [124]; however, AEA levels decreased and 2-AG levels increased with increasing of age in KA-induced rats [125]. In support of these find-ings, inhibition of AEA hydrolysis by a single dose of the FAAH inhibitor AM5206 (8 mg/kg, i.p.) reduced cytoskeletal damage and retarded synaptic protein decline in KA-induced seizures in rats [126]. A single injection of a synthetic CB_1R agonist WIN55,212-2 (5 mg/kg, i.p.), 4 h after termination of SE, protected cells from death as observed in dentate hilar region of hippocampal slices five months after SE [100]. Further, WIN55,212-2 (10 mg/kg, i.p.) conferred significant protection against hyperexcitability in the maximal dentate activation (MDA) model of temporal lobe epilepsy in rats [53]. In addition, the same study demonstrated that co-administration of the TRPV1 antagonist, capsazepine (2 mg/kg, i.p.) enhanced the neuroprotective effect of WIN55,212-2 (10 mg/kg, i.p.) [53], suggesting a possible endocannabinoid-endovanilloid system interplay in neuroprotection. A previous study on soman- (pinacolylymethylphosphonofluoridate) induced seizures in rats demonstrated that the synthetic non-psychoactive analogue of the plant cannabinoid, Δ^9-THC, HU-211 (dexanabinol, 25 mg/kg, i.p., single dose) which acts as a CB_1R agonist, reduced piriform cortical neuronal damage although treatment failed to attenuate the seizures parameters [127].

A single dose of CBD (0.1 mg/kg, i.v., single dose), a non-psychoactive plant cannabinoid, significantly reduced neuronal loss due to hypoxic ischemic brain injury in newborn pigs [128]. Furthermore, WIN55,212-2 (0.1 mg/kg, s.c., single dose) also prevented neuronal damage due to hypoxic ischemia in rat pups [129]. As stated before, seizures yield hypoxic ischemic neuronal injury. Therefore, cannabinoids can be considered as neuroprotective

candidates even for seizures-induced brain injury. However, the role of cannabinoids in neuroprotection is not limited to the inhibition of excitotoxic insults but also supported by inhibition of free radical-induced oxidative damage, regulation of PI3K/Akt/GSK-3 signaling pathway, augmentation of microcirculation in brain, and protection of the microglial function [40,130–132].

4. Conclusion

In conclusion, we find that the evidence describing the effects of major plant cannabinoids that do not act as CBR ligands, most notably cannabidiol and cannabidavarin, exerts consistently beneficial therapeutic effects in preclinical models of seizures, epilepsy, epileptogenesis, and neuroprotection, consistent with emerging human clinical trial results. This recent clinical validation of the predictive nature of the preclinical models used to study these phytocannabinoids and the continued lack of information confirming their underlying anti-epileptic molecular mechanism fully justifies renewed efforts in preclinical research to identify these targets and so better understand treatment resistant epilepsy and the mechanisms through which it may be tackled.

Unlike the evidence base for CBD and CBDV, the evidence describing endocannabinoid system modulators is much less clear although holds considerable promise. Here, most typically, acute enhancement of endocannabinoid signaling (e.g. CB_1R agonism) is anti-convulsant while inhibition of this system (e.g. CB1R antagonism) is pro-convulsant in simple models of acute seizures in rodents. However, when such modulations are examined in more complex models of epilepsy, epileptogenesis, and neuroprotection, a less simplistic narrative emerges, most likely due to interplay between a number of pharmacological, pharmacodynamic, and pathophysiological factors. Interactions between the brain regions involved in a given disease model and the relative contributions made by endocannabinoid signaling (e.g. CB_1R:CB_2R expression and DSE vs DSI) in these regions appear to influence outcome; a complexity most often illustrated by species-specific differences in response. Furthermore, the pleiotropic pharmacology of many CBR ligands and the relative expression of such ‘off target’ signaling systems also play a role. The timing, duration, and any localization of treatment administration also produce variable results where factors such as desensitization of a variety of ligand targets interact to determine the therapeutic or adverse outcome in a given model. Taken together, the results suggest that there remains significant anti-epileptic therapeutic potential and a pro-epileptic risk in up- and down-regulation of endocannabinoid signaling in the central nervous system which require more detailed examination, particularly in models and systems that accurately reflect human endocannabinoid signaling (e.g. human tissue from surgical resections in epilepsy or stem-cell derived neuronal networks from people with epilepsy) or animal models validated thereupon.

Overall, our findings provide summary insight into a complex and sometimes conflicted preclinical literature that has not yet been fully exploited to drive future drug development for newly identified indications, identify important limitations of animal models in the investigation of plant cannabinoid effects in the epilepsies, and focus future research in this

area on specific, unanswered questions regarding the complexities of endocannabinoid signaling in epilepsy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

ABHD6	α-β-hydrolase domain 6
ACEA	arachidonyl-2′-chloroethylamide
AEA	anandamide
CBD	cannabidiol
CBDV	cannabidivarin
CB_1R	cannabinoid type 1 receptor
CB_2R	cannabinoid type 2 receptor
DAGL	diacylglycerol lipase
Δ^9-THC	Δ^9-tetrahydrocannabinol
DSE	depolarization-induced suppression of excitation
DSI	depolarization-induced suppression of inhibition
FAAH	fatty acid amide hydrolase
GABA	γ-Aminobutyric acid
GPR	G protein-coupled receptor
KA	kainic acid
KO	knock-out
MAGL	monoacylglycerol lipase
MDA	maximal dentate activation
MES	maximal electroshock
NAPE-PLD	N-acylphosphatidylethanolamine-hydrolyzing phospholipase D
PMSF	phenylmethane sulfonyl fluoride
PTZ	pentylenetetrazole
TLE	temporal lobe epilepsy
TRPV1	transient receptor potential vanilloid receptor (type 1)

VDAC voltage-dependent anion channel

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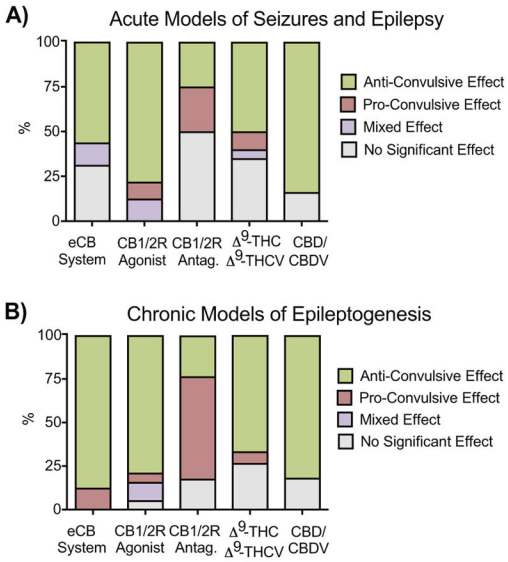
Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.yebeh.2016.11.006.



	Modulators of the eCB System	CB_1R/CB_2R Synthetic Agonists	CB_1R/CB_2R Synthetic Antagonists	Δ^9-THC/ Δ^9-THCV	CBD/ CBDV
# of Species	3	2	3	4	2
# of Discrete Conditions/Models	16	32	12	20	31
Anti-convulsant	9 (56.3%)	25 (78.1%)	3 (25.0%)	10 (50.0%)	26 (83.9%)
Pro-convulsant	0 (0.0%)	3 (9.4%)	3 (25.0%)	2 (10.0%)	0 (0.0%)
Mixed Effect	2 (12.5%)	4 (12.5%)	0 (0.0%)	1 (5.0%)	0 (0.0%)
No Significant Effect	5 (31.3%)	0 (0.0%)	6 (50.0%)	7 (35.0%)	5 (16.1%)

	Modulators of the eCB System	CB_1R/CB_2R Synthetic Agonists	CB_1R/CB_2R Synthetic Antagonists	Δ^9-THC/ Δ^9-THCV	CBD/ CBDV
# of Species	1	2	2	5	2
# of Discrete Conditions/Models	8	19	17	15	11
Anti-convulsant	7 (87.5%)	15 (78.9%)	4 (23.5%)	10 (66.7%)	9 (81.8%)
Pro-convulsant	1 (12.5%)	1 (5.3%)	10 (58.8%)	1 (6.7%)	0 (0.0%)
Mixed Effect	0 (0.0%)	2 (10.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
No Significant Effect	0 (0.0%)	1 (5.3%)	3 (17.6%)	4 (26.7%)	2 (18.2%)

Fig. 1.

Pre-clinical animal models of seizures, epilepsy, and epileptogenesis. Compiled data from synthetic and phytocannabinoids in 181 total animal models of A. Acute models of seizures and epilepsy. B. Chronic models of epileptogenesis. Pre-clinical interventions were subdivided into (1) Modulators of the Endocannabinoid System (“eCB System”), (2) CB_1/CB_2R Agonists, (3) CB_1/CB_2R Antagonists, (3) $D^9THC/THCV$, and (4) CBD/CBDV. See Suppl. Tables 1, 2 for further description of analyzed studies.