Expert Review of Neurotherapeutics

ISSN: 1473-7175 (Print) 1744-8360 (Online) Journal homepage: http://www.tandfonline.com/loi/iern20

The potential role of cannabinoids in epilepsy treatment

Carmen De Caro, Antonio Leo, Rita Citraro, Caterina De Sarro, Roberto Russo, Antonio Calignano & Emilio Russo

To cite this article: Carmen De Caro, Antonio Leo, Rita Citraro, Caterina De Sarro, Roberto Russo, Antonio Calignano & Emilio Russo (2017) The potential role of cannabinoids in epilepsy treatment, Expert Review of Neurotherapeutics, 17:11, 1069-1079, DOI: 10.1080/14737175.2017.1373019

To link to this article: http://dx.doi.org/10.1080/14737175.2017.1373019

Accepted author version posted online: 26 Aug 2017.
Published online: 04 Sep 2017.

EXPERT REVIEW OF NEUROTHERAPEUTICS, 2017
VOL. 17, NO. 11, 1069–1079
https://doi.org/10.1080/14737175.2017.1373019

Taylor & Francis
Taylor & Francis Group

REVIEW

The potential role of cannabinoids in epilepsy treatment

Carmen De Caro[a], Antonio Leo[a], Rita Citraro[a], Caterina De Sarro[a], Roberto Russo[b], Antonio Calignano[b] and Emilio Russo[a]

[a]Department of Science of Health, School of Medicine and Surgery, University "Magna Graecia" of Catanzaro, Catanzaro, Italy; [b]Department of Pharmacy, University of Naples Federico II, Naples, Italy

ABSTRACT

Introduction: Epilepsy is one of the world's oldest recognized and prevalent neurological diseases. It has a great negative impact on patients' quality of life (QOL) as a consequence of treatment resistant seizures in about 30% of patients together with drugs' side effects and comorbidities. Therefore, new drugs are needed and cannabinoids, above all cannabidiol, have recently gathered attention.

Areas covered: This review summarizes the scientific data from human and animal studies on the major cannabinoids which have been of interest in the treatment of epilepsy, including drugs acting on the endocannabinoid system.

Expert commentary: Despite the fact that cannabis has been used for many purposes over 4 millennia, the development of drugs based on cannabinoids has been very slow. Only recently, research has focused on their potential effects and CBD is the first treatment of this group with clinical evidence of efficacy in children with Dravet syndrome; moreover, other studies are currently ongoing to confirm its effectiveness in patients with epilepsy. On the other hand, it will be of interest to understand whether drugs acting on the endocannabinoid system will be able to reach the market and prove their known preclinical efficacy also in patients with epilepsy.

ARTICLE HISTORY
Received 22 June 2017
Accepted 25 August 2017

KEYWORDS
Cannabidiol; cannabidivarin; THC; endocannabinoids system; Dravet syndrome; childhood refractory epilepsy; FAAH

1. Introduction

The International League Against Epilepsy and the International Bureau for Epilepsy define epilepsy as 'Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures, and by the neurobiologic, cognitive, psychological, and social consequences of this condition. The definition of epilepsy requires the occurrence of at least one epileptic seizure' [1]. Often, patients with epilepsy are also affected by sensorimotor, cognitive, psychological, and social impairments contributing with other factors (e.g. drugs' side effects) to an impaired quality of life and being correlated to an increased risk of premature death [2].

About 1% of world population is estimated to be affected by epilepsy (prevalence), which makes this disease one of the most common neurological disorders [3–5]. Antiepileptic drugs (AEDs), better named antiseizure drugs (ASDs), target different biological substrates such as voltage-gated Na^+-channels (e.g. phenytoin, carbamazepine, etc.), GABAergic or glutamatergic neurotransmissions (e.g. phenobarbital, benzodiazepines, and perampanel), and voltage-gated Ca^{2+}-channels (e.g. ethosuximide and gabapentin). Polytherapy and high dosages are often used causing side effects above all in patients with treatment-resistant epilepsy, which still account for about 30% of all patients [3,6]. Furthermore, no drugs able to prevent the development of epilepsy or with disease-modifying properties have been so far discovered or entered the market. Therefore, understanding the underlying mechanisms is crucial for the development of new effective therapies.

In this review, we have summarized and reviewed the potential role of cannabinoids in epilepsy treatment. In fact, recently, mounting anecdotal reports and media coverage have sparked intense interest among parents, patients, and the scientific community regarding the potential of cannabis and its derivatives to treat seizures. The cannabis plant (*Cannabis sativa*) contains about 100 compounds known as phytocannabinoids, and a part of research on cannabis products able to treat epilepsy was focused on the main psychoactive component, tetrahydrocannabinol (THC). To discover new ASDs, recent research interest has been dedicated in investigating those compounds, which do not have psychoactive properties [7,8], such as cannabidiol (CBD) and cannabidivarin (CBDV). These phytocannabinoids are structurally similar but different for their pharmacological effects by which they have anticonvulsant properties. Finally, the endocannabinoid system (ECS) and drugs affecting its functioning have also been considered; however, only preclinical results are currently available.

2. Cannabinoids and the ECS: an overview

2.1. The ECS

The ECS is involved in modulating excitatory and inhibitory synaptic transmission in the brain [9,10]. ECS consists of two

CONTACT Emilio Russo erusso@unicz.it Chair of Pharmacology, Department of Science of Health, School of Medicine, University of Catanzaro, Italy Via T. Campanella, 115, 88100 Catanzaro, Italy

G-protein-coupled receptors, CB1 and CB2, with two known endogenous ligands, namely 2-arachidonoylglycerol (2-AG) and *N*-arachidonoylethanolamide (anandamide or AEA) [10,11]. AEA and 2-AG are synthesized from postsynaptic membrane phospholipid precursors and released when required (*on demand*) in an activity-dependent manner. Levels of intracellular calcium are increased by depolarization of the postsynaptic cell or by direct activation of metabotropic glutamate receptors, which trigger second messenger cascades and endocannabinoid synthesis [12–16].

AEA is produced via phospholipase D-mediated hydrolysis of *N*-arachidonoylphosphatidylethanolamine and degraded by the fatty acid amide hydrolase (FAAH) [17–19]. 2-AG is synthesized via diacylglycerol (DAG) lipase (DAGL) α-mediated hydrolysis of DAG and degraded by monacylglycerol lipase (MAGL) into arachidonic acid and glycerol (Figure 1) [18–20]. However, other serine hydrolases including ABHD6 and ABHD12 take part to the brain 2-AG degradation [21]. CB1 receptors are highly expressed in limbic structures (amygdala, hippocampus, cingulate), cerebral cortex, basal ganglia, and selected areas of the midbrain and medulla and are G-protein-coupled receptors, which can modulate both ion channels activity and neurotransmitter release [22].

CB1 receptors are mainly expressed presynaptically on both glutamatergic and GABAergic interneurons in the central nervous system (CNS) and the periphery. However, evidence also supports a postsynaptic localization for CB1 receptors [23,24]. They are responsible for THC's psychoactive effects and play an important physiological role in modulating stress responses, pain, lipogenesis, and energy regulation. Several studies suggest that CB1 receptors are involved in epilepsy-regulating neurotransmitters release above all in the hippocampus where they are abundantly expressed; furthermore, they are also expressed on microglia, astrocytes, and oligodendrocytes where they modulate inflammatory responses and this may also participate to their role in epilepsy [10,23,25]. In fact, numerous studies reported that AEA and 2-AG have been identified on GABAergic and glutamatergic

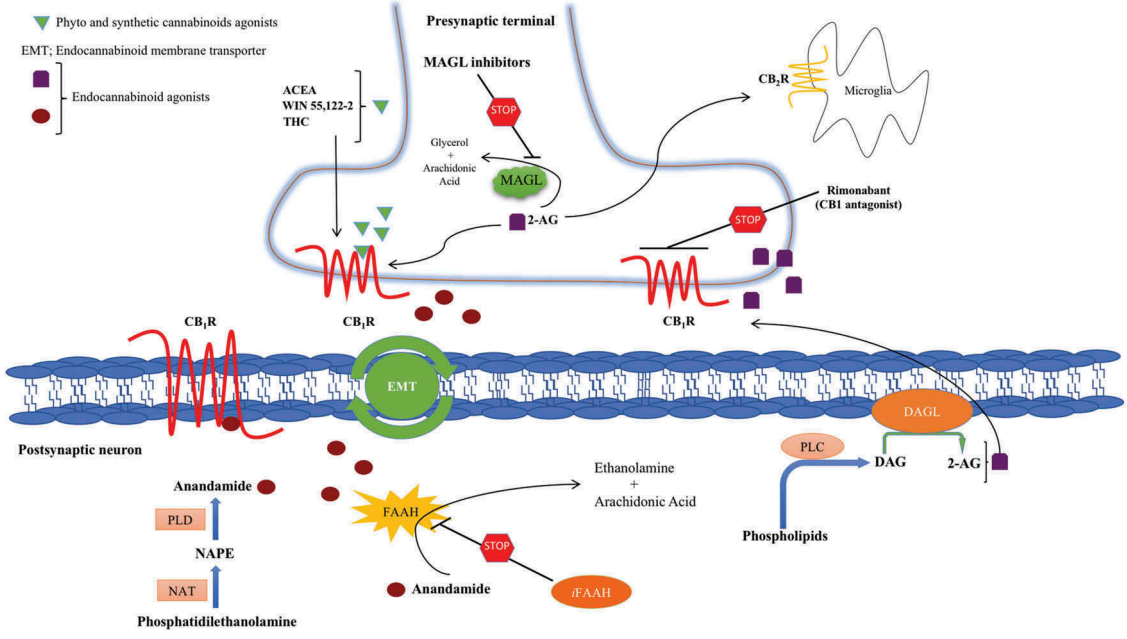

Figure 1. The endocannabinoids are small lipid messengers involved in several signaling processes. They are synthesized '*on demand*' in an activity-dependent manner through cleavage of membrane phospholipids. Afterwards, they are quickly released without being stored in vesicles. To date, two cannabinoid receptors (CB1 and CB2) have been identified. CB1 receptors are widely expressed in the CNS. They are mainly localized on presynaptic terminals, where they are able to modulate ion channels activity and neurotransmitters release. However, evidence also supports a postsynaptic localization for CB1 receptors. CB2 receptors have an expression level lower than CB1 receptors in the CNS. In fact, they are mainly located outside the CNS. Recently, evidence have demonstrated that CB2 receptors are expressed by microglia during inflammatory processes as well as in brainstem neurons. However, CB2 receptors activity in the CNS remains still unclear. Up to now, in spite of several endocannabinoids have been identified, only two were widely studied: Arachidonoylethanolamide (AEA; Anandamide) and 2-Arachidonoylglycerol (2-AG) are the main endocannabinoids and are synthesized and metabolized by separate pathways. AEA is mainly produced by the hydrolysis, catalyzed by Phospholipase D (PLD), of a membrane phospholipid precursor called N-arachidonoylphosphatidylethanolamine (NAPE), which is produced by the enzyme N-acyltransferase (NAT) in a Ca^{2+} dependent manner. In detail, NAT catalyzes the migration of an arachidonic acid group from the SN-1 position of Phosphatidylcholine to the Phosphatidylethanolamine. 2-AG can be synthesized through more than one route. The major route for the biosynthesis of 2-AG comprises the hydrolysis, by Phospholipase C (PLC), of Phosphatidylinositol (PI) to yield 1,2-Diacylglycerol (DAG). Subsequently, the enzyme Diacylglycerol lipase (DAGL) catalyzes the transformation of DAG to 2-AG. AEA and 2-AG can diffuse passively through phospholipid bilayer of neurons; this process can be enhanced by a selective endocannabinoid membrane transporter (EMT), which is located both in neurons and in glial cells. Afterwards, inside the cells, these endocannabinoids may be metabolized to inactive products by distinct hydrolases. AEA is hydrolyzed by Fatty acid amide hydrolase (FAAH) that is mainly located in postsynaptic structures. At odds, the Monoacylglycerol lipase (MAGL), which catalyzes the hydrolysis of 2-AG, seems to be largely associated with nerve endings. FAAH inhibitors (*i*FAAH) as well as MAGL inhibitors, by preventing the breakdown of endocannabinoids and therefore acting as indirect agonists, represent potential pharmacotherapeutic agents.

neurons that are involved in regulating excitability and seizures [26]. The effects of drugs modulating the ECS and its involvement in epilepsy are summarized in Section 3.1.

2.2. Tetrahydrocannabinol

THC, or tetrahydrocannabinol (THC), is the molecule responsible for most of cannabis' psychological effects; it has been studied extensively since its synthesis in 1964. Many studies demonstrated that THC, the main psychotropic constituent of cannabis, is a CB1 and CB2 receptors partial agonist and its related effects can be affected by endogenous cannabinoids release and the density and signaling efficiency of CB receptors [27,28].

In addition to its effects on CB receptors, THC also acts as a 5-HT3A-receptor antagonist as well as an allosteric modulator of μ and δ-opioid receptors. These latter properties are shared with CBD [29]. Moreover, THC is a peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist and this mechanism seems also to contribute to its vascular (relaxation) and antitumor effects. THC is also able to modulate GRP55 and GRP18 receptors; however, the effects of this interaction are not clear. Furthermore, THC also acts on glycine receptors contributing to its analgesic effects in behavioral mice models.

THC does not elicit a response at the vanilloid type 1 receptor (TRPV1 [transient receptor potential of vanilloid type 1], also known as the capsaicin receptor), but it has an agonistic effect on TRPV2, TRPV3, and TRPV4 channels, and it is an antagonist of the melastatin receptor (TRPM8) [30]. The consequences of activation of these targets by THC *in vivo* are not completely understood. There are *in vivo* and *in vitro* studies demonstrating that THC may have effects on experimental models of seizures through the modulation of systems in the CNS, including both GABAergic and glutamatergic neurotransmission (see Section 3.2) [31].

2.3. CBD and CBVD

The isolation and identification of the plant cannabinoids revived interest in studying efficacy for crude cannabis extracts in the treatment of convulsive disorders, prompting a particularly active area of preclinical research into the therapeutic potential of phytocannabinoids in epilepsy. Over 100 well-characterized compounds have been isolated from the cannabis plant. THC, CBD, and CBDV are the most prevalent natural cannabinoids and have received the most attention [32].

CBD is the main non-psychoactive cannabis constituent, it has been evaluated in both preclinical and clinical studies in several pathologies including epilepsy [33–35]. Different mechanisms of action for CBD with potentially anticonvulsant properties have been identified, though the exact mechanism by which CBD possesses anticonvulsant activity remains unknown. However, it is clear from some studies that while THC acts as an anticonvulsant (in some cases as a pro-convulsant) through CB1 receptors, CBD mechanism of action is different having a low affinity for these receptors [36–38], although it has been demonstrated that CBD is able to antagonize CB1 and CB2 receptor agonists *in vitro* with unexpectedly high potency [39]. However, CBD interacts with many other non-endocannabinoids signaling systems and should be considered a 'multi-target or multimodal' drug. There are many molecular mechanisms that are yet to be completely identified and may explain the effects of CBD on neuronal hyperexcitability. It was demonstrated that CBD could increase the activation of 5-HT1A receptors by endogenous release of serotonin [40]. At high micromolar concentrations, CBD activates the nuclear PPAR-γ and the TRPV1 andTRPV2 channels while also inhibits cellular uptake and FAAH-catalyzed degradation of AEA [27,41]. A complete review of the potential CBD's mechanisms of action in neurological disorders was published by Ibeas Bih et al. [38].

Similarly to CBD, CBDV is a non-psychoactive cannabinoid; it is a homolog of CBD with the side chain shortened by two methylene bridges. CBDV has proven to minimize the severity and duration of seizures in some animal models of epilepsy/seizures. As an example, CBDV (200 mg/kg; i.p.) administered with sodium valproate or ethosuximide was well tolerated and retained its own additive anticonvulsant actions in the pentylenetetrazole (PTZ) model (for a detailed description of its anticonvulsant properties see Section 3.2) [42–44].

CBDV exerts its effects via a CB1-receptor-independent mechanism [44,45]. Like THC, CBDV is also effective in reducing nausea and vomiting resulting from a variety of pharmaceutical drugs, treatments, and conditions. Like CBD, this molecule interacts with TRPV1, TRPV2, and TRPA1 channels, but its molecular pharmacology and mechanisms of action are less well understood than these of CBD [30,46].

Summarizing, mechanisms by which CBD and/or CBDV exert their antiseizure effects are not fully known though several potential targets have been suggested with the most relevant being: modulation of intracellular calcium through interaction with targets such as TRP channels [47], GPR55, or VDAC1 [48] among others [38]. Careful pharmacological studies are needed to further elucidate mechanisms and targets.

3. Cannabinoids and epilepsy: preclinical studies

3.1. ECS and epilepsy

Public interest in the use of cannabis for the treatment of epilepsy has burgeoned in the last few years [49]. Animal models provide powerful assays to demonstrate the efficacy of cannabinoids (as well as any other treatment) in preventing seizures and reducing mortality in epilepsy [35]. Indeed, animal models have their own limitations [50].

Endocannabinoids release prevents seizure-induced neurotoxicity. Kainic acid (KA; 30 mg/kg)-induced seizure increases AEA's levels in wild-type mice (20 min postinjection) and has a protective effect on wild-type hippocampal neurons [51]; similarly, high levels of 2-AG can be observed following pilocarpine (375 mg/kg)-induced seizures [36]. Accordingly, mice with CB1Rs' deletion on excitatory principal neurons only (but not interneurons) presented more severe KA-induced seizures (30 mg/kg) than wild-type controls and a higher death rate, which were accompanied by a reduced production of hippocampal AEA in comparison to control littermates [51]. The lack of CB1Rs increased gliosis and apoptosis following

KA-induced seizures and prevented activation of protective genes (e.g. c-Fos, Zif268, brain-derived neurotrophic factor). The relevance of ECS and its receptors was further confirmed by experiments demonstrating that blocking the endocannabinoid catabolic enzyme FAAH (AM374; 8 mg/kg) increases AEA levels and protects against KA (10 mg/kg)-induced hippocampal seizures [20]. Similarly, inhibition of FAAH (by AM374) or of the AEA reuptake transporter (by AM404) prevents α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid-induced excitotoxic damage in rat hippocampus [52]. Finally, AM6701 (5 mg/kg) inhibits FAAH and DAGLα raising AEA and 2-AG brain levels also protects against KA (9.8 mg/kg)-induced seizures [25].

Overall, animal models suggest that activation of CB1Rs and/or ECS reduces seizure severity and their expression on hippocampal glutamatergic (but not GABAergic) inputs is necessary and sufficient to protect against KA-induced seizures [53]. Furthermore, overexpression of CB1R on hippocampus reduced KA-induced seizure severity, pyramidal cell death, and mortality. This evidence demonstrates that CB1 could restrict seizure activity and protects neurons from cell death and gliosis and therefore they have now long been believed to be a suitable target for antiepileptic/antiepileptogenic drugs [54]. In fact, the ECS plays an important role against network hyperexcitability and excitotoxicity in acute brain insults [53].

Based on this background, several direct synthetic cannabinoids have been tested in animal and *in vitro* models. It was demonstrated that a single dose of WIN55,212-2 (5 mg/kg; a selective CB1R agonist) administered 4 h after the termination by pentobarbital of status epilepticus (SE) in the lithium–pilocarpine model reduced the total number of early seizures (but not latency and duration) and mortality in the first two post-SE days; however, despite the demonstration of some neuroprotective hippocampal effects, the number of spontaneous seizures 2 weeks after SE was not modified by the early acute treatment. These results suggest that a pharmacological stimulation of the ECS may have some beneficial effects; however, this protocol does not permit to understand whether this drug has antiseizure effects or even some potential antiepileptogenic properties considering the observed neuroprotection [55].

In the brain, ECS is responsible for retrograde synaptic signaling via CB1Rs [56]. Endocannabinoids are released from the postsynaptic neurons in an activity-dependent manner, and bind to presynaptic CB1, thereby suppressing transmitter release from presynaptic terminals [57]. Controversial data were published regarding the effects of cannabinoids in epilepsy. On one hand, in an animal model of temporal lobe epilepsy, CB1Rs agonists displayed antiepileptic effects [36], in addition, CB1 on glutamatergic axon terminals were shown to mediate anticonvulsant effect, by modulating glutamatergic transmission [53]. On the other hand, proconvulsive effects of CB1 agonists were also described [58]. Moreover, a CB1 antagonist was shown to prevent the long-term increase in seizure susceptibility when applied in a certain time-window [59].

Overall, initial experiments demonstrated the role of ECS and above all CB1Rs in controlling neuronal excitability and potential antiepileptic effects in animal models. However, more recent research articles and experiments highlighted potential controversial results indicating that ECS has a fine-tuning role in the brain and not necessarily its manipulation will lead to reduced hyperexcitability and seizures. Further research is warranted in this area before we can finally understand how to use this important system as a suitable pharmacological target for epilepsy treatment.

3.2. Phytocannabinoids and epilepsy in animal models: THC, CBD, and CBDV

Evidence demonstrates that THC, CBD, and its homologue CBDV offer protection from seizures in various preclinical animal models in mice and rats [34,44,60]; however, some controversial results for THC have been reported and its real efficacy has still to be proven. Furthermore, prolonged THC treatment causes desensitization and downregulation of CB1Rs [61–65] and this has been linked to the development of tolerance to not only THC but also other CB1Rs agonists [66,67]. Moreover, abrupt suspension of chronic THC treatment may trigger rebound seizures, anxiety, symptoms of aggressiveness, hyperirritability, and anorexia [68]. Unlike THC, CBD does not seem to produce significant intoxication [69], tolerance, or withdrawal effects [70].

Overall, these data suggest that using THC, or CB1Rs, may lead to tolerance and withdrawal symptoms [35]; indeed, more studies are needed. Furthermore, as abovementioned, some studies have previously evidenced that THC might also be proconvulsant in some cases [7,8,34]; this point also deserves further studies in order to determine whether this may be specific for some kind of seizures or epilepsies or due to pharmacological effects and therefore linked to the dose and treatment schedule. Overall, THC does not seem the best candidate between cannabinoids in the field of epilepsy even though it may prove to be efficacious in some clinical situations. Similarly, CB1Rs agonists may also be very problematic for clinical management considering their toxicity.

On the other hand, CBD reduces tonic but not clonic seizures in classical animal models of epilepsy, such as PTZ and maximal electroshock (MES) tests. This suggests that CBD might decrease and influence seizures onset [71,72]. Furthermore, it was demonstrated that CBD can increase the effects of phenytoin and reduce the anticonvulsant potencies of others ASDs including ethosuximide and clonazepam in the MES model [73,74].

In the PTZ model, CBD pretreatment (100 mg/kg; i.p.) is able to decrease the occurrence of PTZ-induced seizures [75]. Further studies in the same mouse model confirmed that CBD pretreatment (0.2–200 ng/mouse; i.c.v.) determines anticonvulsant effects, which are mitigated by coadministration of paxilline, an antagonist of voltage and Ca^{2+}-activated K^{+} (BK)-channels [76]. This latter interaction was not observed in the MES model, while CBD pretreatment (20, 100, and 200 ng/mouse; i.c.v.) possessed anticonvulsant effects. These results suggest that CBD's effects may be due to BK channels and this mechanism is only relevant for the PTZ model but not the MES model [77]. It is not surprising that a single mechanism of action in a drug with multiple mechanisms may be more relevant in some models and not others as previously

demonstrated for other drugs with multiple mechanisms of action [78].

CBD effects have also been studied in some models of chronic epilepsy. In the PTZ kindling model, rats pretreated with CBD (20 and 50 mg/kg) showed a reduction of PTZ-induced seizures, and a lower neuronal death in CA1 and CA3 hippocampal regions [79]. In the pilocarpine model, CBD pretreatment (1 and 100 mg/kg; i.p./rat) reduced tonic–clonic seizures, without influencing the percentage of mortality. In the penicillin model of seizures, CBD treatment (≥10 mg/kg; i.p.) prevented the occurrence of both tonic–clonic seizures and mortality with minimal side effects on motor performances [80]. CBD's antiseizure/antiepileptogenic effects in the pilocarpine rat model were further investigated in two different experimental protocols. In the first, a group received CBD for five consecutive days (100 ng; i.c.v.) started during the silent phase after pilocarpine-induced SE. In the second, a different group received acute administration of CBD (100 ng; i.c.v.) during the chronic phase of epilepsy after spontaneous seizures development. In both groups, CBD reduced seizures and surprisingly repeated administration of CBD delayed the onset of spontaneous recurrent seizures possibly through autophagy and antioxidant defense in hippocampal cells, evoking potential antiepileptogenic effects [81]. This latter point deserves further investigation considering the ability of cannabinoids also to module inflammatory responses in the brain and the potential involvement of mTOR pathway, which currently represents a promising target for antiepileptogenic drugs development [82,83].

CBDV, as well as CBD, is a non-psychoactive cannabinoid and quite similarly, it is also a powerful treatment able to minimize the severity and duration of seizures resulting from many conditions both *in vitro* and in animal models. CBDV (1–100 μM) has antiseizure effects in hippocampal slice models of epilepsy induced by 4-AP and Mg^{2+}-free solutions [84]. In another study, it has been suggested that CBDV antiepileptic effects may be due to activating and desensitizing TRPV1 channels [46,85]. In patch-clamp analysis using transfected HEK293 cells, it was demonstrated that CBDV (3–30 μM) dose dependently activated desensitized TRPV1–2 and TRPA1, and these effects were reverted by TRP antagonists. In hippocampal brain slices exposed to Mg^{2+}-free solution, CBDV reduced both epileptiform amplitude and duration. However, when it was used as an TRPV1 antagonist, CBDV's effects were not reverted, this suggests that its effects are not uniquely mediated by TRPV1 and other mechanisms also contribute to its antiepileptic efficacy as in the case of CBD [46,86].

CBDV pretreatment (1 h before seizure induction) possesses not dose-dependent antiseizure properties in PTZ (CBDV = 100 mg/kg; i.p.), audiogenic (CBDV = 50 mg/kg; i.p.), and MES (CBDV = 100 mg/kg; i.p.) models. However, CBDV (200 mg/kg; i.p.) alone is not effective against pilocarpine-induced acute seizures while it potentiates the effects of some other ASDs (e.g. phenobarbital or valproate) [44,45]; interestingly, in the post-SE pilocarpine model, it was reported that ACEA (a selective CB1R agonist) was reported to increase neurogenesis when administered together with valproic acid suggesting a potential impact of these drugs behavioral alterations dependent on hippocampus [87].

Concluding, CBD and CBDV possess antiepileptic efficacy in preclinical studies both *in vitro* and *in vivo* models; their mechanisms of action still remain to be completely elucidated and may have a different relevance in different animal models; finally, results in animal models of epileptogenesis are nearly completely lacking and this point may be extremely relevant considering epilepsy progression.

4. Clinical studies between cannabinoids and epilepsy

Cannabis was one of the primary compounds employed to treat several diseases including epilepsy; specifically, if some historical reports are considered believable, this relationship can span four millennia [88]. To date, the possible application of cannabis and cannabinoid derivatives in several diseases, such as pharmacologically refractory epilepsy, represents an exciting challenge. Up to now, various preclinical studies widely support the role of cannabinoids, above all CBD, and ECS in epilepsy. At odds, the current available clinical studies supporting the role of cannabinoids in the management of human epileptic patients are still limited. As previously described in several reviews, published up to 2016, this clinical evidence is mainly obtained by case reports, patients or caregivers surveys, anecdotal cases, and epidemiological studies. Overall, these studies report the positive effects of CBD and diverse cannabis preparations, containing high ratio of CBD: THC, in the management of resistant epilepsy without psychotropic effects and with a good tolerability [34,35,42,89]; however, several bias can be recognized in these articles and therefore they cannot be considered clinically relevant.

Kaplan et al. [90] reported that CBD could be an adjunctive therapy to treat refractory seizures in young patients with Sturge–Weber syndrome. In the five patients recruited, motor seizures frequency, quality of life, and side effects were recorded during the pretreatment period, after reaching CBD maintenance dose and up to the last visit. Visits were carried out at the enrollment (week −8), at week 0, weekly during weeks 1–6, and at weeks 10, 14, 20, 26, 38, and 48.

Motor seizures frequency has been significantly reduced in four subjects treated with CBD (dose range 5–25 mg/kg), who also reported an improvement in quality of life. The adverse effects related to CBD treatment were transient and not considered serious. However, the fifth patient was withdrawn from the study for lack of efficacy and the appearance of side effects [90]. Similarly, an improvement in the quality of life, distinct from CBD's ability to reduce seizure frequency, was reported in a prospective, open-label clinical study. This improvement was reported by caregivers of 48 young epileptic patients treated with CBD for 12 weeks [91]. Very recently, in Mexico, an online survey was performed within parents administering cannabis, together with other ASDs, to their children (between 9 months and 18 years of age), suffering of several types of refractory epilepsies. The results of this study indicate a reduction in convulsive seizures in 81.3% of patients treated with cannabis (extract): 7 (16%) seizure-free patients, 22 patients (51%) had a moderate to significant improvement, 7 patients (16%) had a low improvement, whereas in 5 patients (11.6%) there were no effects, and 2

patients (4.6%) had a worsening of convulsive seizures. The number of ASDs was decreased in 20.9% of young patients who received cannabis. Only mild side effects were reported in this survey. On average, cannabis (extract) used among these parents/patients was formed by CBD 76.6% and less than 0.1% of THC [92]. Likewise, a Facebook survey was also performed in Australia to evaluate the experience using cannabis products for the management of refractory epilepsy. The amount of responses collected was 976 and 60.1% concerned adult patients with epilepsy, whereas the remaining were children with epilepsy. Ninety percent of adults and 71% of children using cannabis reported a reduction of seizure frequency as well as a more favorable adverse-effect ratio in comparison to other ASDs [93]. Despite this kind of survey can gather useful information, the results reported are biased by the expectancy of patients/parents and subjective measures in short-term observations. Furthermore, extract standardization and therefore dosages used may vary also within the same patient.

Sulak et al. [94] have reported observational data on efficacy and side effects of cannabis in subjects with pharmacologically resistant epilepsy in the United States. The majority of patients were treated with CBD enriched with the addition of THC or THC acid. Eighty-six percent of 272 patients with medically refractory epilepsy reported benefit and good tolerability from the use of cannabis, whereas only 4% of patients experienced increased seizures. The effective doses of CBD ranged from 0.05 to 9 mg/kg/day, whereas the effective plasma levels of CBD ranged from 1.8 to 80 ng/ml.

To be noted out of these studies, cannabis extracts also apparently containing an high ratio of CBD:THC can aggravate seizures in some patients. This has not been reported in preclinical models for CBD but only of THC and other drugs acting on ECS. It is not clear therefore whether also CBD can aggravate some types of seizures and this potential effect should be better defined.

Conversely, adequate clinical studies on the use of THC in the management of epilepsy do not exist. Moreover, it should be bear in mind that several adverse effects such as cognitive impairment and psychiatric disorders were reported in humans after THC use. Likewise, controversial preclinical data exist on the potential role of THC in epilepsy [8,49,95]. Consistently, Crippa et al. [70] have reported the cases of two children with refractory epilepsy showing initial seizures remission using a CBD-enriched extract, followed by seizures exacerbation and typical toxicity by THC after a longer term exposure of the same extract. Subsequently, seizures remission and an improvement of toxicity were attained when the amount of THC (in the extract) was replaced by the same amount of CBD. In conclusion, the authors affirmed the need, for randomized clinical studies, to have high-quality preparations to establish the safety profile and the beneficial effects of cannabis.

Overall, these studies performed in patients with epilepsy by using several formulations containing CBD indicated the treatment as a good candidate for epilepsy treatment; in fact, after several months of CBD treatment at high doses (200–300 mg/kg/day), a reduction in seizures without psychotropic effects as well as small number of side effects has been described [34,35,42,96]. However, a drawback is represented by the pharmacokinetic profile of CBD, which has both a significant first-pass metabolism in the liver and instability in the gastric pH and/or low water solubility, which lead to an interpatients variability in gastrointestinal absorption [97,98]. In spite of this, in the majority of clinical studies, CBD has been administered orally in an oil-based formulation. Its oral bioavailability has been estimated around 10%, while after oral administration, the maximum plasma concentration is reached at 90–120 min (T_{max}) [99]. At odds, in people who smoked cannabis, the bioavailability of inhaled CBD was about 31%, whereas the T_{max} was about 10 min [100]. CBD shows a high lipophilicity that is responsible to the fast accumulation in fat tissues including the brain. As a consequence, its distribution volume is about 30 l/kg, whereas the half-life has been estimated in the range 18–32 h [101]. Moreover, CBD is highly bound to plasma proteins (99%) and it shows a plasma clearance, evaluated after i.v. administration, ranging between 960 and 1560 ml/min [102]. Phytocannabinoids are widely metabolized by several isoforms of cytochrome P450 enzymes (CYPs) in the liver. Among these enzymes, CYP2C9, CYP2C19, and CYP3A4 isoforms are the most widely implicated in the metabolism of CBD and its metabolites are mainly excreted in the feces. CBD is able to inhibit several isoforms of CYP450 leading to drug–drug interactions that deserve particular attention [34,43,103]. Accordingly, Geffrey et al. [104] have studied the interaction between clobazam and CBD in 13 subjects with refractory epilepsy. CBD's ability to inhibit CYP2C19 and CYP3A4 was considered responsible for an increased level of clobazam and norclobazam, which is about 20–100% as potent as clobazam, in children treated with CBD. According to the authors, this drug–drug interaction could be useful in the treatment of refractory epilepsy and contributes to its efficacy. However, the ability of CBD to interact with other ASDs could also be linked to pharmacodynamics mechanisms [34]. At the moment, a phase 2 clinical study is ongoing to investigate the levels of clobazam and its major metabolite (norclobazam) as a consequence of using CBD (ClinicalTrials.gov: NCT02565108). In any case, CBD will probably interact also with many other drugs.

Finally, a multicentric open-label interventional study was performed in 214 patients (aged 1–30 years) with pharmacoresistant epilepsy including those with a diagnosis of Dravet or Lennox–Gastaut syndrome. These patients received oral CBD at 2–5 mg/kg/day titrated up to a dose of 25–50 mg/kg/day. One hundred and thirty seven of 214 patients were analyzed to study the efficacy of this drug, whereas 162 of 214 patients were analyzed to evaluate the safety profile of CBD. Following the administration of CBD (mean dose 22.7 mg/kg/day), motor seizures reduction was 36.5% with 4% of seizure-free patients over the 12 weeks of follow-up. Moreover, a good side effects profile was reported. The most common side effects were fatigue, drowsiness, convulsion, decreased appetite, and gastrointestinal disturbances [105]. Unfortunately, this was an uncontrolled study, and thus not suitable to evaluate the efficacy and safety profile of CBD; however, it was the best at that time. Accordingly, a few years before (2012), a Cochrane review of all available trials on CBD (cannabinoids in epilepsy) concluded that no reliable conclusions on CBD efficacy could be obtained; however, it seemed to be a well-

tolerated treatment. Thereafter, with the aim to better evaluate the efficacy and the safety of CBD, a series of well-designed double-blind randomized clinical trials for refractory epilepsy have been performed and completed [34,89,106], while some others are ongoing [43,107,108].

GW pharmaceuticals together with clinical investigators are performing powered placebo-controlled randomized clinical trials, some of which are showing encouraging effects of a formulation named Epidiolex® (100 mg/ml of purified CBD), in treating several catastrophic forms of resistant epilepsy including Dravet syndrome, Lennox–Gastaut syndrome, tuberous sclerosis complex, and infantile spasms with adequate safety profiles for patients [34,43,106]. Recently, US FDA assigned Epidiolex as orphan drug for the treatment of these types of refractory epilepsies [108]. Very recently, Devinsky et al. [106] reported the data regarding the first well-controlled phase 3 clinical trial of Epidiolex in Dravet syndrome, a rare and 'catastrophic' form of epilepsy. This was a multinational, powered placebo-controlled randomized clinical trial performed in 120 subjects (mean age 9.8) affected by inadequately controlled Dravet syndrome. These 120 patients were randomly assigned to receive either Epidiolex at a dose of 20 mg/kg/daily or placebo as add-on therapy for 14 weeks of treatment. The median of ASDs taken by patients was 3 (range 1–5). Among these ASDs, the most commonly used was clobazam (65% of patients). Forty-three percent of patients taking CBD experienced at least a 50% of seizure frequency reduction, whereas in the placebo group, the seizure frequency reduction was 27%. Furthermore, during the treatment period, 5% of patients became seizures free, whereas in the placebo group, no seizure-free patients were reported. Additionally, in these patients, CBD was able to reduce all type of seizures except nonconvulsive seizures. The most common side effects reported in the CBD group were fatigue, somnolence, elevation of liver-enzyme levels, and gastrointestinal disorders [106]. Therefore, these results, confirming the efficacy and good tolerability of CBD observed in a previous open-label study (see above), could represent a milestone in the treatment of Dravet syndrome. However, additional data regarding the long-term efficacy and safety of CBD for this syndrome are needed.

Overall, CBD is slowly reaching a good level of clinical evidence for efficacy and very shortly, when results from other trials will be available, we will finally be able to ascertain whether this drug has maintained the promise.

CBDV is another active phytocannabinoid that has demonstrated antiepileptic effects in several models of seizures. To date, CBDV was studied in a double-blind, randomized, placebo-controlled, phase 2 clinical trial, registered in ClinicalTrials.gov, for focal seizures in adult patients. This trial was divided in two parts: in the part A (ClinicalTrials.gov: NCT02369471), the possible induction or inhibition of CBDV metabolism by other concomitant ASDs in adult patients (18–65 years) affected by inadequately controlled focal seizures was studied. These patients were randomized in three groups: group 1, concomitant inducers; group 2, concomitant inhibitors; and group 3, neither inducers nor inhibitors. Ongoing part B was planned to examine the possible antiseizure effects of CBDV as add-on therapy in adult patient (18–65 years) with uncontrolled focal seizures (ClinicalTrials.gov: NCT02365610) [108]; results are unfortunately not yet available.

5. Conclusions

Cannabis and its derivatives together with the ECS and its chemical modulators have gathered attention and have been widely studied in several diseases including neurological disorders. As reviewed in this article, research in the epilepsy field has been spread over time with some peaks of interest and a new current enthusiasm. We should distinguish results from the modulation of the ECS to the one obtained with phytocannabinoids (excluding THC which mainly acts through the EC system) considering the difference in their mechanisms of action.

Drugs acting on CB1 receptors have generally demonstrated some efficacy against seizures in animal models of epilepsy and similarly, also drugs potentiating the ECS are also effective increasing endogenous endocannabinoid levels. However, several controversial results have been reported and these molecules have never been used in clinical studies with the exception of THC, which has some spontaneous reporting of not randomized blinded clinical trials. These latter are far from being convincing and THC is burdened by too many side effects including its psychotropic action. Furthermore, some ECS modulators have reached clinical development and nearly all of them possessed serious side effects which lead to their failure in further clinical studies.

On the other hand, we have reviewed the evidence about the potential clinical efficacy of some phytocannabinoids, namely CBD and CBDV, in the field of epilepsy. These drugs were found to be potent anticonvulsant drugs in several animal models of epilepsy with a mechanism not involving the ECS. They do not possess psychotropic effects and CBD in some anecdotal, observational, and one randomized clinical trial has also demonstrated clinical efficacy against seizures in children with refractory epilepsy and in some cases also in adults.

In conclusion, despite the several limitations that can be found in some clinical studies and the unexplainable holes in the study of these drugs in epilepsy field, it should be concluded that a big step through has been accomplished with CBD approval as an orphan drug and all the research performed so far are encouraging further future studies.

6. Expert commentary

Many years of prohibition have set away cannabis-derived therapies from being evaluated and developed for clinical use in several pathologies including epilepsy. In the last few years, there have been many disagreements on cannabis and its derivatives considering whether an illegal drug would provide patients with a level of therapeutic relief comparable to other pharmaceutical treatments. Indeed, regulatory and bureaucratic paths represent a current limitation for the study of such compounds and derivatives in many countries. In any case, the actual major problem resides in the still unsatisfactory clinical data analyzed and the current hopes and beliefs of patients and their relatives. This discrepancy has been driven by media and public engagement with patients and relatives

gathering information from not reliable sources and requesting support to their clinicians. It is not surprising considering that nowadays, patients retrieve information trying to improve their therapies overriding clinicians; this, however, implies a continuous request and consultancy eliciting in some cases issues between patients and clinicians. Therefore, there is now an urgent need for reliable clinical data to help clinicians to both correctly use such treatments and solve their everyday relationship with patients. In fact, there are a number of patients self-administering cannabis and derivatives without any proper medical advice and control.

In this light, the very recent demonstration of CBD efficacy in patients with Dravet syndrome represents the first step which will however require further studies to elucidate the potential of this treatment also in other epileptic conditions such as adult patients with pharmacoresistant epilepsy. Considering this step forward, scientific community knows that many more studies are undoubtedly needed to understand the real potential of this drug and its applicability to clinical practice. Furthermore, long-term efficacy and short and long-term tolerability also need to be thoroughly studied and considered before clinicians can obtain the best out of CBD and possibly other cannabis derivatives. On the other hand, patients and their relatives will feel their opinion further corroborated and therefore their request reinforced. Indeed, the main limitation is currently due to the technical time to perform reliable clinical studies.

Preclinical development will be based on the identification and study of other cannabinoids with potential clinical utility both in epilepsy and other diseases. This area of research is also linked to the study of not only pure molecules but also to standardized extracts containing mixtures of cannabinoids which are practically easier to obtain considering the problem of isolating a single molecule but that are more difficult to manage considering the necessity to standardize such formulations in the long term and in different laboratories. Accordingly, most of the studies reported in literature regarding the potential effects of cannabis extract are not easy to replicate or even have an internal bias due to the poor quality of such formulations.

Finally, while the data coming from the study of THC and synthetic derivatives acting on the ECS appear very interesting and worth of further investigation, too many controversies have been evidenced and clinical development has been not surprisingly slowed down due to neurological and psychiatric side effects. Overall, we probably still need to better understand the functioning of the ECS in relation to its potential as a target in epilepsy and try to define whether it would be possible to avoid psychotropic effects even if this could be acceptable in view of a high beneficial effect.

7. Five-year view

It is clear that we have two different lines of research; the first is the one regarding the study of molecules acting on the ECS including receptors and enzymes, the second and currently most advanced is the study of phytocannabinoids and cannabis extracts without psychotropic effects.

Research in the area of the ECS has accumulated very interesting results both from the efficacy point of view and the knowledge of the mechanisms involved. Indeed, we do not have clinical trials and all the evidence is based on preclinical models of epilepsy and therefore, as abovementioned, transferability to clinical settings is not so direct and easy. Further research is warranted and the aim will be that of better defining the mechanisms involved in the anticonvulsant effects of compounds acting on the ECS. Of interest is also the study of their potential effects on neuroinflammation and their neuroprotective effects which have been observed in various models not only of epilepsy. Accordingly, such drugs might have potential antiepileptogenic and/or disease modifying properties.

The second area of research, which is the one that most recently has gained attention and investments by companies, is represented by the study of phytocannabinoids with not psychotropic effects in which the most studied molecules are so far CBD and CBDV. CBD has already available results on randomized clinical trials and it appears to be efficacious in patients with Dravet syndrome but has also several reports in other forms of pharmacoresistance epilepsy. CBDV is currently in phase 2 clinical trials but its development is currently supported by very positive results in preclinical models. It is very interesting that the two drugs do not seem to share a common mechanism of action or at least not between the studied so far. The next future will probably see the further validation and study of both drugs in clinical trials of pharmacoresistant epilepsy both in children and adults clarifying the real potential of these treatments. On the other hand, other active compounds have been isolated from cannabis and will be studied for a variety of pathologies based on some of the proposed therapeutically effects of cannabis. Finally, very relevant will be the study of their mechanisms of action which, as it has happened in the past, may be useful for the determination of new pharmacological targets for epilepsy treatment. Indeed, these treatments all appear to act on more than one single target and this may contribute to their efficacy in various models as in the case of valproic acid.

Ultimately, considering their current study in children epilepsies, their impact as disease-modifying drugs and on other neurological/psychiatric comorbidities such cognitive deficit will be studied and will impact on their use.

Key issues

- Recently, cannabis and its derivatives together with the endocannabinoid system and its chemical modulators have gathered attention in the epilepsy field.
- The endocannabinoid system and its modulators seem to play a key role in epilepsy treatment and pathophysiology.
- Cannabidiol and Cannabidivarin have demonstrated efficacy to reduce seizures in several animal models of epilepsy.
- Several Clinical trials are ongoing to better evaluate the possible role of cannabidiol and cannabidivarin in refractory epilepsy.
- Cannabidiol is effective in treating seizures in patients with Dravet Syndrome.

Funding

This paper was not funded.

Declaration of interest

E Russo has received speaker fees from Eisai, Lundbeck, and Almirall. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

References

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Fisher RS. Commentary: operational definition of epilepsy survey. Epilepsia. 2014;55:1688.
2. Fisher RS, van Emde Boas W, Blume W, et al. Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia. 2005;46:470–472.

• **Latest approved (ILAE) definition of seizures and epilepsy.**

3. Perucca E, French J, Bialer M. Development of new antiepileptic drugs: challenges, incentives, and recent advances. Lancet Neurol. 2007;6:793–804.
4. Behr C, Goltzene MA, Kosmalski G, et al. Epidemiology of epilepsy. Rev Neurol (Paris). 2016;172:27–36.
5. Singh A, Trevick S. The epidemiology of global epilepsy. Neurol Clin. 2016;34:837–847.
6. Pitkanen A, Loscher W, Vezzani A, et al. Advances in the development of biomarkers for epilepsy. Lancet Neurol. 2016;15:843–856.

• **Defining the role of biomarkers in epilepsy and reviewing the most relevant advances.**

7. Rosenberg EC, Tsien RW, Whalley BJ, et al. Cannabinoids and epilepsy. Neurotherapeutics. 2015;12:747–768.
8. Rosenberg EC, Patra PH, Whalley BJ. Therapeutic effects of cannabinoids in animal models of seizures, epilepsy, epileptogenesis, and epilepsy-related neuroprotection. Epilepsy Behav. 2017;70:319–327.
9. De Chiara V, Motta C, Rossi S, et al. Interleukin-1beta alters the sensitivity of cannabinoid CB1 receptors controlling glutamate transmission in the striatum. Neuroscience. 2013;250:232–239.
10. Di Marzo V, Stella N, Zimmer A. Endocannabinoid signalling and the deteriorating brain. Nat Rev Neurosci. 2015;16:30–42.

•• **Review of the role of the endocannabinoid system in the diseased brain**

11. Pertwee RG. The pharmacology of cannabinoid receptors and their ligands: an overview. Int J Obes (Lond). 2006;30(Suppl 1):S13–S18.
12. Alger BE. Retrograde signaling in the regulation of synaptic transmission: focus on endocannabinoids. Prog Neurobiol. 2002;68:247–286.
13. Brown SP, Brenowitz SD, Regehr WG. Brief presynaptic bursts evoke synapse-specific retrograde inhibition mediated by endogenous cannabinoids. Nat Neurosci. 2003;6:1048–1057.
14. Maejima T, Ohno-Shosaku T, Kano M. Endogenous cannabinoid as a retrograde messenger from depolarized postsynaptic neurons to presynaptic terminals. Neurosci Res. 2001;40:205–210.
15. Melis M, Perra S, Muntoni AL, et al. Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. J Neurosci. 2004;24:10707–10715.
16. Katona I, Freund TF. Endocannabinoid signaling as a synaptic circuit breaker in neurological disease. Nat Med. 2008;14:923–930.
17. Di Marzo V, Fontana A, Cadas H, et al. Formation and inactivation of endogenous cannabinoid anandamide in central neurons. Nature. 1994;372:686–691.
18. Di Marzo V, Deutsch DG. Biochemistry of the endogenous ligands of cannabinoid receptors. Neurobiol Dis. 1998;5:386–404.
19. Stella N, Schweitzer P, Piomelli D. A second endogenous cannabinoid that modulates long-term potentiation. Nature. 1997;388:773–778.
20. Karanian DA, Karim SL, Wood JT, et al. Endocannabinoid enhancement protects against kainic acid-induced seizures and associated brain damage. J Pharmacol Exp Ther. 2007;322:1059–1066.
21. Savinainen JR, Saario SM, Laitinen JT. The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signalling through cannabinoid receptors. Acta Physiol (Oxf). 2012;204:267–276.
22. Tsou K, Brown S, Sanudo-Pena MC, et al. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience. 1998;83:393–411.
23. Maa E, Figi P. The case for medical marijuana in epilepsy. Epilepsia. 2014;55:783–786.
24. Piomelli D. The molecular logic of endocannabinoid signalling. Nat Rev Neurosci. 2003;4:873–884.
25. Naidoo V, Karanian DA, Vadivel SK, et al. Equipotent inhibition of fatty acid amide hydrolase and monoacylglycerol lipase - dual targets of the endocannabinoid system to protect against seizure pathology. Neurotherapeutics. 2012;9:801–813.
26. Castillo PE, Younts TJ, Chavez AE, et al. Endocannabinoid signaling and synaptic function. Neuron. 2012;76:70–81.

•• **Role of the endocannabioid system in brain function.**

27. Pertwee RG. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. Br J Pharmacol. 2008;153:199–215.

•• **The different effects of phytocannabinoids on CB receptors.**

28. Colizzi M, Fazio L, Ferranti L, et al. Functional genetic variation of the cannabinoid receptor 1 and cannabis use interact on prefrontal connectivity and related working memory behavior. Neuropsychopharmacology. 2015;40:640–649.
29. Kathmann M, Flau K, Redmer A, et al. Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. Naunyn Schmiedebergs Arch Pharmacol. 2006;372:354–361.
30. Morales P, Hurst DP, Reggio PH. Molecular targets of the phytocannabinoids: a complex picture. Prog Chem Org Nat Prod. 2017;103:103–131.
31. Alger BE. Seizing an opportunity for the endocannabinoid system. Epilepsy Curr. 2014;14:272–276.
32. Turner SE, Williams CM, Iversen L, et al. Molecular pharmacology of phytocannabinoids. Prog Chem Org Nat Prod. 2017;103:61–101.
33. Iseger TA, Bossong MG. A systematic review of the antipsychotic properties of cannabidiol in humans. Schizophr Res. 2015;162:153–161.
34. Leo A, Russo E, Elia M. Cannabidiol and epilepsy: rationale and therapeutic potential. Pharmacol Res. 2016;107:85–92.
35. Devinsky O, Cilio MR, Cross H, et al. Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. Epilepsia. 2014;55:791–802.
36. Wallace MJ, Blair RE, Falenski KW, et al. The endogenous cannabinoid system regulates seizure frequency and duration in a model of temporal lobe epilepsy. J Pharmacol Exp Ther. 2003;307:129–137.
37. Wallace MJ, Martin BR, DeLorenzo RJ. Evidence for a physiological role of endocannabinoids in the modulation of seizure threshold and severity. Eur J Pharmacol. 2002;452:295–301.
38. Ibeas Bih C, Chen T, Nunn AV, et al. Molecular targets of cannabidiol in neurological disorders. Neurotherapeutics. 2015;12:699–730.

•• **Analysis of the potential mechanisms of action of CBD in epilepsy and the brain.**

39. Thomas A, Baillie GL, Phillips AM, et al. Cannabidiol displays unexpectedly high potency as an antagonist of CB1 and CB2 receptor agonists *in vitro*. Br J Pharmacol. 2007;150:613–623.
40. Mishima K, Hayakawa K, Abe K, et al. Cannabidiol prevents cerebral infarction via a serotonergic 5-hydroxytryptamine1A receptor-dependent mechanism. Stroke. 2005;36:1077–1082.
41. Leweke FM, Piomelli D, Pahlisch F, et al. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl Psychiatry. 2012;2:e94.

42. dos Santos RG, Hallak JE, Leite JP, et al. Phytocannabinoids and epilepsy. J Clin Pharm Ther. 2015;40:135–143.
43. Lippiello P, Balestrini S, Leo A, et al. From cannabis to cannabidiol to treat epilepsy, where are we? Curr Pharm Des. 2016;22:6426–6433.
44. Hill AJ, Mercier MS, Hill TD, et al. Cannabidivarin is anticonvulsant in mouse and rat. Br J Pharmacol. 2012;167:1629–1642.

•• **Effects of CBDV in animal models of epilepsy and seizures.**

45. Hill TD, Cascio MG, Romano B, et al. Cannabidivarin-rich cannabis extracts are anticonvulsant in mouse and rat via a CB1 receptor-independent mechanism. Br J Pharmacol. 2013;170:679–692.

•• **Effects of CBDV in animal models of epilepsy and seizures.**

46. Iannotti FA, Hill CL, Leo A, et al. Nonpsychotropic Plant Cannabinoids, Cannabidivarin (CBDV) and Cannabidiol (CBD), activate and desensitize Transient Receptor Potential Vanilloid 1 (TRPV1) channels *in vitro*: potential for the treatment of neuronal hyperexcitability. ACS Chem Neurosci. 2014;19:1131–1141.

•• **Effects of CBD and CBDV on TRPV channels.**

47. Gonzalez-Reyes LE, Ladas TP, Chiang CC, et al. TRPV1 antagonist capsazepine suppresses 4-AP-induced epileptiform activity in vitro and electrographic seizures *in vivo*. Exp Neurol. 2013;250:321–332.
48. Rimmerman N, Ben-Hail D, Porat Z, et al. Direct modulation of the outer mitochondrial membrane channel, voltage-dependent anion channel 1 (VDAC1) by cannabidiol: a novel mechanism for cannabinoid-induced cell death. Cell Death Dis. 2013;4:e949.
49. O'Connell BK, Gloss D, Devinsky O. Cannabinoids in treatment-resistant epilepsy: a review. Epilepsy Behav. 2017;70:341–348.
50. White HS, Loscher W. Searching for the ideal antiepileptogenic agent in experimental models: single treatment versus combinatorial treatment strategies. Neurotherapeutics. 2014;11:373–384.
51. Marsicano G, Goodenough S, Monory K, et al. CB1 cannabinoid receptors and on-demand defense against excitotoxicity. Science. 2003;302:84–88.
52. Karanian DA, Brown QB, Makriyannis A, et al. Dual modulation of endocannabinoid transport and fatty acid amide hydrolase protects against excitotoxicity. J Neurosci. 2005;25:7813–7820.
53. Monory K, Massa F, Egertova M, et al. The endocannabinoid system controls key epileptogenic circuits in the hippocampus. Neuron. 2006;51:455–466.
54. Guggenhuber S, Monory K, Lutz B, et al. AAV vector-mediated overexpression of CB1 cannabinoid receptor in pyramidal neurons of the hippocampus protects against seizure-induced excitoxicity. PLoS One. 2010;5:e15707.
55. Suleymanova EM, Shangaraeva VA, van Rijn CM, et al. The cannabinoid receptor agonist WIN55.212 reduces consequences of status epilepticus in rats. Neuroscience. 2016;334:191–200.
56. Mackie K, Stella N. Cannabinoid receptors and endocannabinoids: evidence for new players. AAPS J. 2006;8:E298–E306.
57. Mackie K. Distribution of cannabinoid receptors in the central and peripheral nervous system. Handb Exp Pharmacol. 2005;168:299–325.
58. Gordon E, Devinsky O. Alcohol and marijuana: effects on epilepsy and use by patients with epilepsy. Epilepsia. 2001;42:1266–1272.
59. Echegoyen J, Armstrong C, Morgan RJ, et al. Single application of a CB1 receptor antagonist rapidly following head injury prevents long-term hyperexcitability in a rat model. Epilepsy Res. 2009;85:123–127.
60. dos Santos NF, Arida RM, Filho EM, et al. Epileptogenesis in immature rats following recurrent status epilepticus. Brain Res Brain Res Rev. 2000;32:269–276.
61. Breivogel CS, Scates SM, Beletskaya IO, et al. The effects of delta9-tetrahydrocannabinol physical dependence on brain cannabinoid receptors. Eur J Pharmacol. 2003;459:139–150.
62. McKinney DL, Cassidy MP, Collier LM, et al. Dose-related differences in the regional pattern of cannabinoid receptor adaptation and *in vivo* tolerance development to delta9-tetrahydrocannabinol. J Pharmacol Exp Ther. 2008;324:664–673.
63. Sim-Selley LJ. Regulation of cannabinoid CB1 receptors in the central nervous system by chronic cannabinoids. Crit Rev Neurobiol. 2003;15:91–119.
64. Sim-Selley LJ, Schechter NS, Rorrer WK, et al. Prolonged recovery rate of CB1 receptor adaptation after cessation of long-term cannabinoid administration. Mol Pharmacol. 2006;70:986–996.
65. Villares J. Chronic use of marijuana decreases cannabinoid receptor binding and mRNA expression in the human brain. Neuroscience. 2007;145:323334.
66. Ten Ham M, Loskota WJ, Lomax P. Acute and chronic effects of beta9-tetrahydrocannabinol on seizures in the gerbil. Eur J Pharmacol. 1975;31:148–152.
67. Blair RE, Deshpande LS, Sombati S, et al. Prolonged exposure to WIN55,212-2 causes downregulation of the CB1 receptor and the development of tolerance to its anticonvulsant effects in the hippocampal neuronal culture model of acquired epilepsy. Neuropharmacology. 2009;57:208–218.
68. Aceto MD, Scates SM, Lowe JA, et al. Cannabinoid precipitated withdrawal by the selective cannabinoid receptor antagonist, SR 141716A. Eur J Pharmacol. 1995;282:R1–R2.
69. Robson P. Abuse potential and psychoactive effects of delta-9-tetrahydrocannabinol and cannabidiol oromucosal spray (Sativex), a new cannabinoid medicine. Expert Opin Drug Saf. 2011;10:675–685.
70. Crippa JA, Crippa AC, Hallak JE, et al. Delta9-THC intoxication by cannabidiol-enriched cannabis extract in two children with refractory epilepsy: full remission after switching to purified cannabidiol. Front Pharmacol. 2016;7:359.
71. Carlini EA, Leite JR, Tannhauser M, et al. Letter: cannabidiol and Cannabis sativa extract protect mice and rats against convulsive agents. J Pharm Pharmacol. 1973;25:664–665.
72. Consroe P, Benedito MA, Leite JR, et al. Effects of cannabidiol on behavioral seizures caused by convulsant drugs or current in mice. Eur J Pharmacol. 1982;83:293–298.
73. Consroe P, Wolkin A. Cannabidiol–antiepileptic drug comparisons and interactions in experimentally induced seizures in rats. J Pharmacol Exp Ther. 1977;201:26–32.
74. Scuderi C, Filippis DD, Iuvone T, et al. Cannabidiol in medicine: a review of its therapeutic potential in CNS disorders. Phytother Res. 2009;23:597–602.
75. Jones NA, Hill AJ, Smith I, et al. Cannabidiol displays antiepileptiform and antiseizure properties *in vitro* and *in vivo*. J Pharmacol Exp Ther. 2010;332:569–577.

•• **CBD effects on animal models of epilepsy and seizures**

76. Ahrens J, Demir R, Leuwer M, et al. The nonpsychotropic cannabinoid cannabidiol modulates and directly activates alpha-1 and alpha-1-Beta glycine receptor function. Pharmacology. 2009;83:217–222.
77. Leo A, Citraro R, Constanti A, et al. Are big potassium-type Ca(2+)-activated potassium channels a viable target for the treatment of epilepsy? Expert Opin Ther Targets. 2015;19:911–926.
78. Russo E, Constanti A, Ferreri G, et al. Nifedipine affects the anticonvulsant activity of topiramate in various animal models of epilepsy. Neuropharmacology. 2004;46:865–878.
79. Mao K, You C, Lei D, et al. High dosage of cannabidiol (CBD) alleviates pentylenetetrazole-induced epilepsy in rats by exerting an anticonvulsive effect. Int J Clin Exp Med. 2015;8:8820–8827.
80. Jones NA, Glyn SE, Akiyama S, et al. Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures. Seizure. 2012;21:344–352.

•• **CBD effects on animal models of epilepsy and seizures.**

81. Hosseinzadeh M, Nikseresht S, Khodagholi F, et al. Cannabidiol post-treatment alleviates rat epileptic-related behaviors and activates hippocampal cell autophagy pathway along with antioxidant defense in chronic phase of pilocarpine-induced seizure. J Mol Neurosci. 2016.
82. Citraro R, Leo A, Constanti A, et al. mTOR pathway inhibition as a new therapeutic strategy in epilepsy and epileptogenesis. Pharmacol Res. 2016;107:333–343.

83. Leo A, Constanti A, Coppola A, et al. mTOR signaling in epilepsy and epileptogenesis: preclinical and clinical studies. In: Maiese K, editors. Molecules to medicine with mTOR: translating critical pathways into novel therapeutic strategies. London, Uk: Elsevier Inc.; 2016. p. 123–142.
84. Yamaguchi S, Rogawski MA. Effects of anticonvulsant drugs on 4-aminopyridine-induced seizures in mice. Epilepsy Res. 1992;11:9–16.
85. Shu HF, Yu SX, Zhang CQ, et al. Expression of TRPV1 in cortical lesions from patients with tuberous sclerosis complex and focal cortical dysplasia type IIb. Brain Dev. 2013;35:252–260.
86. De Petrocellis L, Ligresti A, Moriello AS, et al. Effects of cannabinoids and cannabinoid-enriched Cannabis extracts on TRP channels and endocannabinoid metabolic enzymes. Br J Pharmacol. 2011;163:1479–1494.
87. Andres-Mach M, Zagaja M, Haratym-Maj A, et al. A long-term treatment with arachidonyl-2′-chloroethylamide combined with valproate increases neurogenesis in a mouse pilocarpine model of epilepsy. Int J Mol Sci. 2017;18.
88. Russo EB. Cannabis and epilepsy: an ancient treatment returns to the fore. Epilepsy Behav. 2017;70:292–297.
89. Friedman D, Devinsky O. Cannabinoids in the treatment of epilepsy. N Engl J Med. 2016;374:94–95.
90. Kaplan EH, Offermann EA, Sievers JW, et al. Cannabidiol treatment for refractory seizures in sturge-weber syndrome. Pediatr Neurol. 2017;71:18–23.e2.
•• **CBD effects in patients with epilepsy.**
91. Rosenberg EC, Louik J, Conway E, et al. Quality of life in childhood epilepsy in pediatric patients enrolled in a prospective, open-label clinical study with cannabidiol. Epilepsia. 2017.
92. Aguirre-Velazquez CG. Report from a survey of parents regarding the use of cannabidiol (Medicinal cannabis) in Mexican children with refractory epilepsy. Neurol Res Int. 2017;2017:2985729.
93. Suraev AS, Todd L, Bowen MT, et al. An Australian nationwide survey on medicinal cannabis use for epilepsy: history of antiepileptic drug treatment predicts medicinal cannabis use. Epilepsy Behav. 2017;70:334–340.
94. Sulak D, Saneto R, Goldstein B. The current status of artisanal cannabis for the treatment of epilepsy in the United States. Epilepsy Behav. 2017;70:328–333.
95. Volkow ND, Baler RD, Compton WM, et al. Adverse health effects of marijuana use. N Engl J Med. 2014;370:2219–2227.
96. Cunha JM, Carlini EA, Pereira AE, et al. Chronic administration of cannabidiol to healthy volunteers and epileptic patients. Pharmacology. 1980;21:175–185.
97. Lodzki M, Godin B, Rakou L, et al. Cannabidiol-transdermal delivery and anti-inflammatory effect in a murine model. J Control Release. 2003;93:377–387.
98. Consroe P, Kennedy K, Schram K. Assay of plasma cannabidiol by capillary gas chromatography/ion trap mass spectroscopy following high-dose repeated daily oral administration in humans. Pharmacol Biochem Behav. 1991;40:517–522.
99. Bialer M, Johannessen SI, Levy RH, et al. Progress report on new antiepileptic drugs: a summary of the Twelfth Eilat Conference (EILAT XII). Epilepsy Res. 2015;111:85–141.
100. Ohlsson A, Lindgren JE, Andersson S, et al. Single-dose kinetics of deuterium-labelled cannabidiol in man after smoking and intravenous administration. Biomed Environ Mass Spectrom. 1986;13:77–83.
101. Castaneto MS, Wohlfarth A, Desrosiers NA, et al. Synthetic cannabinoids pharmacokinetics and detection methods in biological matrices. Drug Metab Rev. 2015;47:124–174.
102. Huestis MA. Pharmacokinetics and metabolism of the plant cannabinoids, delta9-tetrahydrocannabinol, cannabidiol and cannabinol. Handb Exp Pharmacol. 2005;168:657–690.
103. Zendulka O, Dovrtelova G, Noskova K, et al. Cannabinoids and cytochrome P450 interactions. Curr Drug Metab. 2016;17:206–226.
104. Geffrey AL, Pollack SF, Bruno PL, et al. Drug-drug interaction between clobazam and cannabidiol in children with refractory epilepsy. Epilepsia. 2015;56:1246–1251.
•• **Interaction between CBD and clobazam.**
105. Devinsky O, Marsh E, Friedman D, et al. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. Lancet Neurol. 2016;15:270–278.
•• **CBD effects in patients with epilepsy.**
106. Devinsky O, Cross JH, Laux L, et al. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. N Engl J Med. 2017;376:2011–2020.
•• **CBD effects in patients with epilepsy.**
107. Gloss D, Vickrey B. Cannabinoids for epilepsy. Cochrane Database Syst Rev. 2014;3:CD009270.
108. Bialer M, Johannessen SI, Levy RH, et al. Progress report on new antiepileptic drugs: a summary of the Thirteenth Eilat Conference on New Antiepileptic Drugs and Devices (EILAT XIII). Epilepsia. 2017;58:181–221.