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Review

Exploiting cannabinoid and vanilloid mechanisms for epilepsy treatment

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ABSTRACT

This review focuses on the possible roles of phytocannabinoids, synthetic cannabinoids, endocannabinoids, and "transient receptor potential cation channel, subfamily V, member 1" (TRPV1) channel blockers in epilepsy treatment. The phytocannabinoids are compounds produced by the herb *Cannabis sativa*, from which Δ^9-tetrahydrocannabinol (Δ^9-THC) is the main active compound. The therapeutic applications of Δ^9-THC are limited, whereas cannabidiol (CBD), another phytocannabinoid, induces antiepileptic effects in experimental animals and in patients with refractory epilepsies. Synthetic CB_1 agonists induce mixed effects, which hamper their therapeutic applications. A more promising strategy focuses on compounds that increase the brain levels of anandamide, an endocannabinoid produced on-demand to counteract hyperexcitability. Thus, anandamide hydrolysis inhibitors might represent a future class of antiepileptic drugs. Finally, compounds that block the TRPV1 ("vanilloid") channel, a possible anandamide target in the brain, have also been investigated. In conclusion, the therapeutic use of phytocannabinoids (CBD) is already in practice, although its mechanisms of action remain unclear. Endocannabinoid and TRPV1 mechanisms warrant further basic studies to support their potential clinical applications.
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1. Introduction

Cannabis sativa has a long history as a drug of abuse and a potential herbal medicine [1]. This plant produces more than a hundred compounds (phytocannabinoids), with different pharmacological applications, among which Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabidiol (CBD) are of particular interest. The Δ^9-THC accounts for most of the *Cannabis* effects (such as abuse potential, memory impairment, sedation, hyperphagia), whereas CBD lacks these typical "Δ^9-THC-like" properties [2].

The Δ^9-THC and its derivatives (synthetic cannabinoids) modify brain functions mainly through agonism (or partial agonism) at the CB_1 cannabinoid receptor [3,4]. Another cannabinoid receptor, termed CB_2 receptor, has also been characterized [5]. Both are metabotropic receptors activated in the brain by *N*-arachidonoyl ethanolamide (anandamide) and 2-arachidonoylglycerol (2-AG), which are termed endocannabinoids [6]. The actions of anandamide and 2-AG are terminated by neuronal internalization followed by the cleavage by the hydrolytic enzymes fatty acid amide hydrolase (FAAH) and monoacylglyceride lipase (MAGL), respectively [7–9]. Contrary to classical neurotransmitters, endocannabinoids function as retrograde messengers. After a calcium influx in the postsynaptic neuron, they are synthesized on-demand and released in the synaptic cleft to activate the CB_1 cannabinoid receptor in presynaptic neurons and modulate neuronal activity [10,11]. The cannabinoid receptors, the endocannabinoids, and their related enzymes are part of the so-called endocannabinoid system (Fig. 1), which has been extensively reviewed [12–14].

The endocannabinoid system may include additional ligands and receptors. Of particular interest is the "vanilloid channel" or "transient receptor potential cation channel, subfamily V, member 1" (TRPV1). The TRPV1 is a cation-permeable ion channel activated by heat, acid pH, and capsaicin (the pungent compound from the chili pepper *Capsicum frutescens*). Although it was initially identified as an "orphan" receptor, anandamide has been proposed as its main endogenous agonist [15–17]. Remarkably, whereas anandamide binding to the CB_1 receptor inhibits neuronal activity, TRPV1 activation depolarizes neurons and promotes neurotransmitter release [18].

This review article focuses on the potential of cannabinoids and related compounds for epilepsy treatment. Since the evidence comes mainly from experimental settings, we will present a brief overview on animal models useful for preclinical studies with antiepileptic

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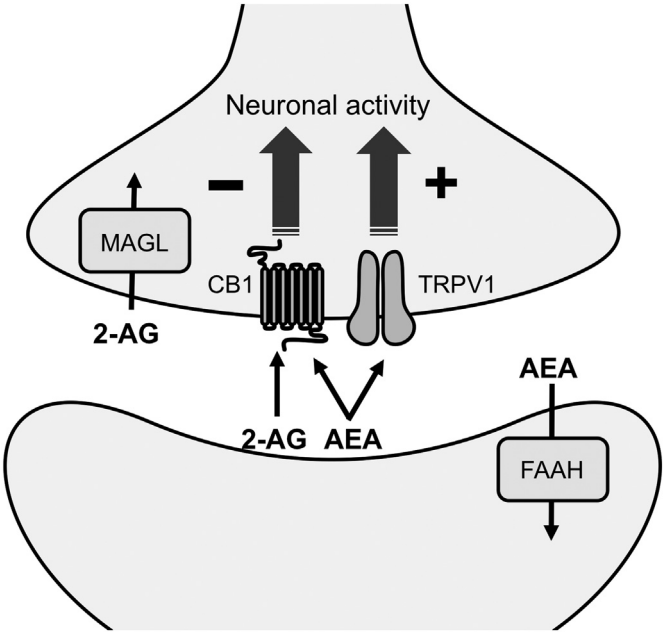

Fig. 1. A simplified view of the main components of the endocannabinoid system. The endocannabinoids *N*-arachidonoyl ethanolamide (AEA, anandamide) and 2-arachidoyl glycerol (2-AG) are synthesized on-demand from postsynaptic neuronal membranes and released in the synaptic cleft. 2-AG activates presynaptic CB_1 receptor, whereas anandamide activates both the CB_1 receptor and the TRPV1 channel, which inhibits and facilitates excitatory neuronal activity, respectively. 2-AG and anandamide effects are terminated by the enzymes monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH) respectively.

drugs. Next, we will review the evidence for the potential use of phytocannabinoids, synthetic cannabinoids, endocannabinoid hydrolysis inhibitors, and TRPV1 blockers in epilepsy treatment. Finally, we will briefly discuss the few clinical trials available and summarize the main conclusions.

2. Animal models for studying and developing antiepileptic drugs

Epilepsy is neurological disease characterized primarily by a predisposition to epileptic seizures. These, in turn, are transient signs and/or symptoms resulting from abnormal excessive or synchronous neuronal activity in the brain if an abnormally long seizures occur, it is characterized as a *status epilepticus*. Spontaneous epileptic seizures may result from a multifactorial process, termed epileptogenesis. Certain types of epilepsy include other features and can be characterized as epileptic syndromes [19].

The treatment of epilepsy and epileptic seizures consists mainly in pharmacological approaches. The antiepileptic drugs restrain neuronal activity through various mechanisms, including blockage of sodium channels, inhibition of excitatory neurotransmission (mainly glutamate), or facilitating inhibitory neurotransmission (mainly gamma-aminobutyric acid). Their clinical use, however, is limited by side effects and the fact that about one-third of patients remain untreated (refractory epilepsies) [20]. Thus, there is an urge for new antiepileptic drugs, whose development relies on preclinical research in experimental animals.

There are several types of animal models for studying and developing antiepileptic drugs [21–24]. Most of them consists in inducing epileptic seizures in laboratory animals by applying physical stimuli (acoustic, thermal or electrical) or injecting chemicals, such as, pentylenetetrazol (PTZ), pilocarpine, kainic acid, cocaine, methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate (DMCM), or 4-aminopyridine (4-AP). Genetic approaches in animals have also been instrumental to model specific types of seizures (such as, audiogeneic seizures) and epileptic syndromes (including Dravet and Lennox-Gastaut Sydromes) [25]. There are protocols applying either acute or repeated stimuli. Acute protocols quantify seizures induced either immediately at the delivery of the stimulus (such as the PTZ model) or "spontaneous" seizures occurring after the stimulus (pilocarpine model). The repeated protocols entail a gradual increase in seizure severity as a stimulus with constant intensity is applied repeatedly (kindling), although specific protocols vary across studies regarding brain region investigated, intensity of stimulus, and time course [22].

From a pharmacological standpoint, these models are useful for the screening of new antiepileptic drugs and the investigation of the underlying mechanisms. The expected readout for an antiepileptic drug can be an increase in the intensity of stimulus required to induce seizure or a reduction in seizure duration, frequency, severity, and lethality after a fixed stimulus is applied. Notwithstanding the application of these models, each of them has disadvantages and limitations regarding face, construct, and predictive validity [21,24]. A short summary of the animal models of epilepsy cited in this review is presented in Table 1.

3. Phytocannabinoids

The initial studies with cannabinoids in the treatment of epilepsy focused on CBD, a compound with low efficacy at the CB_1 receptor and a safer pharmacological profile as compared to Δ^9-THC [13]. An early publication from Carlini's group in Brazil reported the protective effect of CBD against convulsive agents in rodents [26]. After several years, the interest in preclinical studies with this compound has been renewed; CBD reduced seizures induced by cocaine intoxication [27,28] as well as pilocarpine-induced status epilepticus (SE) [29]. In addition, it prevented both seizures and electroencephalogram (EEG) activity induced by PTZ [30,31]. This phytocannabinoid was also effective in animal models of seizures induced by electrical stimulation, including the lamotrigine-resistant amygdala kindling [32]. Cannabidiol also decreased the duration, the severity, and the frequency recurrent seizures in a genetic mouse model of Dravet syndrome [33]. One recent study

Table 1
Summary of the main animal models of acute epileptic seizures, epilepsy and epileptic syndromes mentioned in this review.

Chemical stimuli (clinical implications)	Physical stimuli (clinical implications)	Genetic modification (clinical implications)
4-aminopyridine, bicuculin, methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate pentylenetetrazole, picrotoxin (acute seizures)	Electrical stimulation of the hippocampus or the amygdala (acute seizures/temporal lobe epilepsy)	Wistar audiogenic rat strain (reflex epilepsy and temporal lobe epilepsy)
Pilocarpine (temporal lobe epilepsy)	Electrical stimulation of the cornea (acute seizures)	*SCN1a* mutant mice (Dravet Syndrome)
Kainic acid (temporal lobe epilepsy)	Thermal stimulus (febrile seizures)	
Cocaine (cocaine intoxication)	Acoustic stimulus (audiogenic seizures)	

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investigated the effects of CBD in a range of animal models of chemically and electrically induced seizures, further demonstrating the antiepileptic effects of this compound in mice and rats [34].

The mechanisms underlying CBD antiepileptic activity remain unclear. It has low affinity and efficacy at the CB_1 receptor and may interfere with various other targets in the brain [35]. Accordingly, its anticonvulsant effects were not reversed by CB_1 receptor antagonists in electrically induced seizures [36]. Other authors suggest that anticonvulsant effect of CBD is related to its actions on voltage-gated sodium channels [37], a common antiepileptic drug target [38]. Similarly, the protective effect of CBD in a mouse genetic model of Dravet syndrome was not prevented by CB_1 antagonists [33]. However, in the PTZ model, its effects were reversed by CB_1, CB_2, and TRPV1 selective antagonists, suggesting that one potential mechanism might be the facilitation of the endocannabinoid system [31]. In this model, 5-HT_{1A} and 5-HT_{2A} antagonists failed to prevent CBD effects [30]. Finally, one possible intracellular mechanism comprises the facilitation of the mammalian target of rapamycin (mTOR) pathway with consequent reduction of glutamate release [27].

Other phytocannabinoids have also been investigated, although to a much less extent than CBD. Δ^9-tetrahydrocannabivarin reduced PTZ-induced seizures in rats [39]. Similarly, cannabidivarin-rich *Cannabis* extracts exerted anticonvulsant effects in the PTZ- and the pilocarpine-induced seizure models [40]. Finally, β-caryophyllene, a cannabinoid presented in several other plants, prevented PTZ-induced seizures [41] was well as the kainic acid- and the electroshock-induced seizures in mice [42].

4. Synthetic cannabinoids

Similar to phytocannabinoids, synthetic cannabinoids have been investigated in various animal modes of seizure and epilepsy. The WIN-55,212-2, a nonselective compound, showed efficacy in a CB_1-dependent manner in electrically-induced seizures [36] and in spontaneous recurrent epileptiform discharges in vitro [43,44]. However, the absence of anticonvulsant activity after prolonged treatment may indicate a tolerance to its anticonvulsant effects [43]. In pilocarpine-induced SE in mice, the WIN-55,212-2 reduced the frequency of excitatory postsynaptic currents, an effect blocked by CB_1 antagonists [45]. This compound also delayed seizure in the amygdala kindling model of temporal lobe epilepsy [46]. Finally, it was shown to improve survival and to reduce the incidence of early seizures in the lithium–pilocarpine SE model [47].

Other studies have focused on compounds that selectively activate the CB_1 receptor. Arachidonoyl-2-chloroethylamide (ACEA) enhanced the anticonvulsant activity of phenobarbital in electrically induced seizures in mice [48] and suppressed DMCM-induced seizure in rats [49]. Similarly, arachidonoylcyclopropylamide increased the threshold for PTZ-induced seizures [50]. However, some contrasting results indicate that CB_1 agonism may also facilitate seizures instead of decreasing them. The WIN-55,212-2 and ACEA reduced the threshold for myoclonic seizures induced by PTZ and enhanced epileptiform EEG activity in rats [51]. Moreover, AM2201 induced epileptiform behavior in mice, which was accompanied by abnormal spike–wave discharges and an increase in extracellular glutamate concentration in hippocampus. These effects were suppressed by CB_1 antagonism, but not by CB_2 or vanilloid receptor antagonists [52].

The reasons for these discrepancies remain unclear. One possible explanation is the presence of CB_1 receptors in both inhibitory gamma-aminobutyric acid ("GABAergic") [53] and excitatory glutamatergic terminals [54,55]. Thus, cannabinoids might act in certain dose ranges through the suppression of glutamate release in terminals located in the dentate gyrus [45]. Accordingly, the genetic deletion of CB_1 from principal neurons of the forebrain caused longer seizure duration in the kindling model of temporal lobe epilepsy in mice, while the deletion of CB_1 from GABAergic forebrain neurons resulted in the opposite effect [56].

5. Endocannabinoid hydrolysis inhibitors

Several lines of evidence point to the endocannabinoid system as an endogenous anticonvulsant mechanism, including the demonstration that anandamide is recruited on-demand in the brain to promote CB_1-mediated defense against excitotoxic stimuli [54]. Thus, the endocannabinoid system has been proposed as a brain circuit breaker, counteracting hyper-excitatory activity [57]. Accordingly, CB_1 antagonism facilitated electrically-induced seizures in mice [58]. Similarly, both anandamide and 2-AG reduced frequency of spontaneous and tetrodotoxin-resistant excitatory postsynaptic currents in mice with temporal lobe epilepsy in a CB_1-dependent manner [45]. In addition, the levels of anandamide are reduced in the cerebrospinal fluid of drug-naive patients affected by temporal lobe epilepsy [59]. Also, the injection of kainic acid is able to induce an increase in anandamide levels in the brain, which could be part of a brain protective response [60]. These results suggest a role of the endocannabinoid signaling in protecting the brain against seizure activity.

Thus, compounds that selectively inhibit the endocannabinoid-hydrolyzing enzymes have emerged as potential new pharmacological approaches to treat epilepsies. The majority of studies have focused on the inhibition of anandamide hydrolysis. In the kainic acid-induced seizure model, the FAAH inhibitor AM5206 and the dual FAAH/MAGL inhibitor AM6201 were able to reduce behavioral seizure scores and cytoskeletal damage [60,61]. Moreover, the FAAH inhibitor URB597 increased the threshold for PTZ-induced seizures and EEG epileptiform activity in rats [51]. This compound also inhibited seizure and cell death induced by cocaine intoxication in mice, both effects being prevented by CB_1 receptors antagonists [28]. In rats, URB597 prevented the seizure-induced impairment of synaptic plasticity in a CB_1-dependent manner [62]. As for the 2-AG hydrolysis inhibitors, the MAGL inhibitor JZL184 delayed the development of generalized epileptic seizures in the kindling model of temporal lobe epilepsy in mice. Its effects were abolished in the CB_1 receptor knockout mice [63].

These results indicate that the inhibition of endocannabinoid hydrolysis may confer protection against seizures and excitotoxicity. However, contrasting results showed that mice lacking FAAH exhibit enhanced seizure responses to kainic acid, which was increased by anandamide administration [64]. In addition, URB597 did not affect the development of seizures in the amygdala kindling model of temporal lobe epilepsy [46]. Thus, further studies are required to characterize the doses and the types of seizures in which endocannabinoid-hydrolysis inhibitor might be effective.

6. TRPV1 channel blockers

In addition to activating the CB_1 receptor, anandamide has been proposed as an endogenous agonist at the TRPV1 channel, although with lower affinity [15]. However, contrary to CB_1, TRPV1 activation tends to facilitate, rather than reduce, seizures. In experiments with in vitro preparations, anandamide induced an increase in spontaneous excitatory postsynaptic currents through TRPV1 channel activation [65]. Moreover, capsaicin, a TRPV1 agonist, enhanced spontaneous excitatory postsynaptic current frequency in mice with temporal lobe epilepsy, resulting in an increased glutamate release in dentate gyrus granule cells [65]. Accordingly, TRPV1 knockout mice are less susceptible to PTZ-induced seizures induced by early-life hyperthermia challenge [66]. In addition, pilocarpine-induced SE produced an upregulation of TRPV1 in hippocampus, while activation and inhibition of TRPV1 induced an increase and decrease, respectively, in the synaptic transmission in CA1 and CA3 of epileptic animals [67].

These results suggest that TRPV1 blockers might exert antiepileptic effects. The few results available so far seem to support this possibility.

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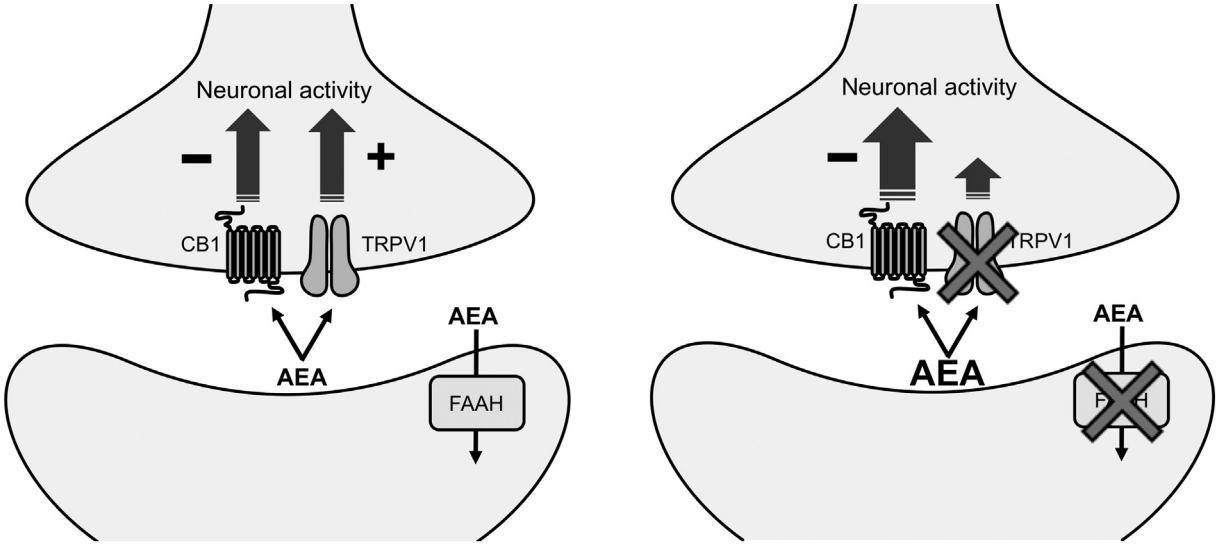

Fig. 2. A hypothesis on how dual FAAH/TRPV1 blockade might represent a potential treatment for epilepsy. Drugs acting through this mechanism increase *N*-arachidonoyl ethanolamide (AEA, anandamide) levels to selectively activate the CB_1 receptor, and inhibit neuronal activity. They simultaneously block the TRPV1 channel, which mediates the excitatory effects of anandamide.

The TRPV1 blockers induced anticonvulsant effects in 4-AP-induced epileptiform activity in vitro and in vivo [68]. They also reduced PTZ-induced seizures [69,70] as well amygdala-induced kindling in rats [71]. Finally, TRPV1 blockade also inhibited acoustically evoked seizures in the genetically epilepsy-prone rat [72]. One possible mechanism through which TRPV1 blockers reduce seizures is the reduction of Ca^{2+} influx. Accordingly, both PTZ and TRPV1 agonists increased Ca^{2+} influx in the hippocampus and the dorsal root ganglion of rats [73,74].

The possible role of anandamide as an endogenous agonist at both the CB_1 receptor and TRPV1 channel can be exploited for the development of drugs with a dual mechanism. In the PTZ-induced seizures model in mice, anandamide administration induced a biphasic effect, whereas a FAAH inhibitor combined with a TRPV1 blocker reduced seizure in mice [75]. Accordingly, the simultaneous blockade of FAAH and TRPV1 with the dual blocker arachidonoyl-serotonin (AA-5HT) alleviates seizures in the PTZ-model in mice, an effect reversed by CB_1 antagonism, but not completely mimicked by TRPV1 inhibition [76]. Finally, the anticonvulsant effects of WIN-55,212-2 were potentiated by TRPV1 blockade in a model of temporal lobe epilepsy [77]. Thus, dual FAAH/TRPV1 blockers warrants further investigation as putative new antiepileptic drugs.

7. Clinical studies

So far, the only cannabinoid-related compound to reach the clinics is CBD. Early clinical trials dated from the 70s to 80s reported improvement of refractory epilepsy after CBD treatment [78,79]. However, this compound remained under-investigated until some years ago, when clinical trials involving patients with different epileptic-related syndromes started to report its beneficial effects. Cannabidiol induced a significant reduction of seizures in patients with Lennox–Gastaut Syndrome [80,81] and in Dravet syndrome patients after a 14-week treatment [82]. In addition, CBD-enriched *Cannabis* extracts reduced the frequency of seizures in children diagnosed with different epilepsy syndromes and resistant to classical antiepileptic drugs [83]. Cannabidiol seems to induce adverse effects of moderate severity, such as, sedation, decreased appetite and fatigue [84]. More extensive studies are still required, including randomized, double-blind, placebo-controlled trials, comparing CBD to conventional antiepileptic drugs.

8. Conclusion

Antiepileptic drugs exert their effects by interacting with various targets, although there are some major common mechanisms, such as, blockade of sodium and calcium channels, glutamatergic inhibition, and GABAergic facilitation. Unfortunately, they induce a myriad of adverse effects and a subset of patients fails to respond to any of these treatments [20]. In this context, new pharmacological approaches must be pursued to advance the field and bring relief to patients.

Among the phytocannabinoids, CBD has been approved in some countries for the treatment of drug-resistant epileptic syndromes (Dravet and Lennox–Gastaut Syndromes). However, its mechanisms of action remain to be fully elucidated. Other phytocannabinoids are also under investigation. As for the synthetic cannabinoids, they are unlikely to represent promising strategies, due to their "*Cannabis*-like" side effects and even seizure-inducing activity. Alternatively, anandamide hydrolysis inhibitors (FAAH inhibitors), which exploit endocannabinoid on-demand defensive mechanisms, might represent interesting approaches. The TRPV1 blockers also warrant further investigation, particularly if combined with FAAH inhibitions (Fig. 2). This concept has been discussed elsewhere for the treatment of anxiety and mood disorders [18], and might be applied also in the search for new epilepsy treatments.

Declaration of competing interest

The authors have no conflict of interest to report.

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