Chapter 5

Do Cannabinoids Represent a Good Therapeutic Strategy for Epilepsy?

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Abstract

The medical use of cannabinoids has been proposed for the control of epilepsy. At present, several studies have focused on investigating how cannabinoids can regulate the expression of epileptic seizures as well as the epileptogenesis process. Some of them suggest that cannabinoids may represent a therapeutic approach for different types of epilepsy. However, experimental evidence indicates that the effects of cannabinoids depend on several experimental and pathological conditions. In this chapter, we provide an overview of these preclinical and clinical research.

Key words Endocannabinoid system, Anandamide (AEA), 2-Arachidonoyl glycerol (2-AG), Δ9-Tetrahydrocannabinol (Δ9-THC), Cannabidiol (CBD), CB1/CB2 receptors, Seizures, Epilepsy

1 Introduction

During centuries cannabis plants have been used for both medicinal and recreational uses as described in Chinese, Indian, and Arab pharmacopeias [1]. Presently, the medical use of *Cannabis* extracts has been approved in some European countries [2].

Cannabis plants present a mixture of chemical constituents, the C_{21} terpenophenolic compounds also called phytocannabinoids. Detailed chemical analysis has allowed the identification of about 70 molecular species of these phytocannabinoids [3] whose amounts depend on each plant and environmental conditions [4, 5]. The most important phytocannabinoids are the psychoactive Δ9-tetrahydrocannabinol (Δ9-THC) and the non-psychoactive cannabidiol (CBD). CBD was isolated in 1940, and its structure was elucidated in 1963 [6, 7]. The Δ9-THC was isolated by Yechiel Gaoni and Raphael Mechoulam [8] and was shown to account for the psychotropic effects of cannabis preparations in rhesus monkeys [9]. During the late 1980s, it was found that Δ9-THC exerts its effects through the activation of two G-protein-coupled receptors: cannabinoid type 1 (CB1) and cannabinoid type 2 (CB2)

Alan Talevi and Luisa Rocha (eds.), *Antiepileptic Drug Discovery: Novel Approaches*, Methods in Pharmacology and Toxicology, DOI 10.1007/978-1-4939-6355-3_5,

receptors [10, 11]. Thereafter, the endocannabinoids (eCBs) anandamide and 2-arachidonoylglycerol were identified as the endogenous ligands of CB1 and CB2 receptors [12–14].

A growing body of evidence supports that the eCB system is involved in several functions of the brain and that *Cannabis* and phytocannabinoids represent pharmacological strategies to induce neuroprotection and control of disorders such as epilepsy, migraine, and pain [15]. In the case of epilepsy, experimental evidence indicates a key role for eCB system in the modulation of neuronal excitability. The present review focuses on providing a better understanding of how and when pharmacological interventions with cannabinoids or phytocannabinoids may control epilepsy.

2 The Endocannabinoid System

The eCB system has a crucial role in different brain functions including cerebral development, cognition, learning, memory, motor behavior, appetite regulation, temperature regulation, and pain [16]. Experimental evidence indicates that the role of eCB in the regulation of physiological responses depends on the gender [17]. The eCB system consists of cannabinoid receptors, their endogenous lipid ligands (eCBs), and the enzymatic machinery for their biosynthesis, cellular uptake, release, and degradation [18].

2.1 Endocannabinoids

The first eCBs identified in the central nervous system (CNS) [19, 20] were the hydrophobic ligands N-arachidonoyl ethanolamide (anandamide, AEA) [12] and 2-arachidonoyl glycerol (2-AG) [13, 14]. The synthesis of these eCBs depends on specific enzymes using membrane phospholipids as precursors. The *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) is the enzyme responsible for the synthesis of AEA and other *N*-acylethanolamines [21], whereas different diacylglycerol lipases (DAGLs) are involved in the synthesis of 2-AG [22]. The effects mediated by eCBs are limited by their fast catabolism. The enzyme fatty acid amide hydrolase (FAAH) catabolizes AEA [23]. Monoacylglycerol lipase (MAGL) and serine hydrolase α/β--hydrolase domain 6 (ABHD) induce degradation of 2-AG in the brain [24–26]. Carrier-mediated transport systems are involved in clearing eCBs from the extracellular space [27–29], and their subsequent enzymatic degradation can proceed through either hydrolysis or oxidation [24, 30, 31].

Unlike other neuromodulators and traditional vesicular neurotransmitters, eCBs are believed to be synthesized "on demand" by changes in neural activity [32]. The synthesis of eCBs in postsynaptic neurons can be triggered by the increase in intracellular Ca^{2+} concentration subsequent to depolarization and activation of voltage-gated Ca^{2+} channels [33–37] and the activation of certain

Gαq/11 protein-coupled receptors [38–40]. Other studies suggest that intracellular storage organelles might accumulate pre-synthesized eCBs [41, 42].

2.2 Endocannabinoid Receptors

CB1 and CB2 receptors belong to the large superfamily of heptahelical G-protein-coupled receptors (GPCR) and couple to Gi/Go proteins. The CB2 receptor is predominately expressed in the immune system [43] and has very limited expression in the CNS. By contrast, the CB1 receptors are highly expressed at presynaptic levels in the brain, and its activation is implicated in inhibition of the synaptic neurotransmission [44–47]. Concerning this notion, it is known that the activation of presynaptic CB1 receptors reduces the release of neurotransmitters like glutamate and γ-aminobutyric acid (GABA) [48] as a consequence of the inhibition of Ca^{2+} channels and activation of K^+ channels [49–54], a situation that may modify the neuronal excitability [55].

Activation of CB1 receptors promotes its interaction with Go proteins, resulting in guanosine diphosphate/guanosine triphosphate exchange and subsequent dissociation of α and βγ subunits with a consequent reduction of adenylate cyclase and cyclic adenosine monophosphate production [56]; inhibition N-, P/Q-, and L-type voltage-gated Ca^{2+} channels [20, 46, 57, 58]; stimulation of A type K^+ channels [44, 59, 60], activation of G-protein-coupled inwardly rectifying K^+ channels [61, 62]; and inhibition of the vesicular release machinery [63].

While the CB1 receptor is responsible for the vast majority of the currently known effects of cannabinoids and eCBs in the CNS, it is worth noting that additional cannabinoid receptors may exist. The cannabinoid-sensitive receptor G-protein-coupled receptor 55 (GPR55), identified as a novel cannabinoid receptor that couples to Gα13 protein [64], is activated by some phytocannabinoids such as Δ9-THC. In the brain, GPR55 is present in the caudate, putamen, hippocampus, thalamus, pons, cerebellum, frontal cortex, and thalamus [64]. In human embryonic kidney cells, the activation of GPR55 triggers the release of intracellular Ca^{2+} from endoplasmic reticulum stores via a pathway dependent on Ras homolog gene family member A (RhoA), phospholipase C, and inositol 1,4.5-trisphosphate receptor [65]. The increases of intracellular Ca^{2+} levels that result from the activation of GPR55 by L-α-lysophosphatidylinositol (LPI, an endogenous agonist) augment the probability of vesicular release of glutamate at excitatory hippocampal synapses [66, 67]. These results support a relevant role of GPR55 in cerebral excitability.

3 Phytocannabinoids

CBD and Δ9-THC represent the most important phytocannabinoids contained in the *Cannabis* plants [3]. Δ9-THC is a partial agonist of CB1 receptors that induces most of the behavioral,

cognitive, and psychotropic effects of *Cannabis*. The mechanisms by which Δ9-THC induces these effects also involve the activation and desensitization of the transient receptor potential (TRP) channels of ankyrin type 1 (TRPA1) and vanilloids type 1 (TRPV1) and type 2 (TRPV2) [68–70].

CBD is considered a "multitarget" drug because of its interaction with many other non-eCB signaling systems. It acts as an agonist of TRPV1, TRPV2, and TRPA1 [68, 70–72], 5-hydroxytryptamine1α receptors [73], and glycine receptors [74]. CBD acts as an antagonist of TRP melastatin type-8 channels [69], T-type voltage-gated Ca^{2+} channels [75], and GPR55 receptors [76]. Also, it exerts dynamic control over intracellular Ca^{2+} stores [77, 78] and inhibits the uptake and enzymatic degradation of AEA via FAAH [79].

CBD may potentiate some effects induced by Δ9-THC such as analgesia, antiemesis, and anti-inflammation, but it also reduces Δ9-THC-induced psychoactive effects (impaired working memory, sedation, tachycardia, and paranoia) [80–82]. Cannabis products with a high content of CBD induce greater tolerability and lower incidence of psychosis when compared with those with high content of Δ9-THC [83].

4 Effects of Cannabinoids on Seizure Activity and Epilepsy

Several studies indicate that eCBs and cannabinoids play an important role in epilepsy. Here, we summarize evidence from preclinical and clinical studies focused on clarifying this situation.

Concerning experimental models of acute seizure activity, it is described that the i.c.v. administration of arachidonyl-2-chloroethylamide (ACEA, a CB1 receptor agonist) decreases the frequency of penicillin-induced epileptiform activity in rats, an effect blocked by AM-251 (a CB1 receptor antagonist) [84]. Compounds like Δ9-THC, WIN55,212-2, CBD, and AEA and their analog O-1812 induce anticonvulsant effects in the maximal electroshock seizure model [85, 86]. In in vitro models, the activation of CB1 receptors with agonists (methanandamide, 2-AG, AEA, or WIN 55,212-2) reduces the epileptiform activity induced by low or omission of Mg^{2+} and high K^{+} [87–89]. The cannabinoid agonist HU210 reduces the epileptiform synchronization in hippocampus induced by kainic acid administration, an effect avoided with the pretreatment with rimonabant, a CB1 receptor antagonist [90]. This group of evidence reveals that cannabinoids may modify both focal and generalized seizures blocking neuronal hypersynchronization associated with epileptic activity.

Studies reveal the participation of cannabinoids in the expression of seizure activity and the epileptogenesis process. Δ9-THC and the cannabinoid agonist WIN55,212 abolish spontaneous

epileptic seizures subsequent to pilocarpine-induced SE. Conversely, the administration of the CB1 receptor antagonist SR141716A increases both seizure duration and frequency [91]. The administration of WIN 55,212-2 during 15 days after pilocarpine-induced SE reduces the severity, duration, and frequency of spontaneous recurrent seizures, an effect associated with the preservation of GABAergic neurons, as well as absence of changes in the oxidative stress and expression of NMDA receptor subunits [92]. In the kindling model, the activation of CB1 receptors has been proposed to delay the acquisition of generalized seizures, whereas the inhibition of the enzymatic degradation of AEA did not affect the epileptogenesis process but reduces the neurogenesis associated to it [93]. All these findings support the idea that activation of CB1 receptors can suppress recurrent excitation during epileptogenesis.

Studies indicate that the activation of CB1 receptors can augment or reduce the seizure termination and duration, a situation that depends on the neuronal subpopulation [94]. CB1 receptors are also expressed in astrocytes [95, 96], and their activation is involved in the maintenance of epileptiform discharge [97].

eCBs may interact with other neurotransmitters and neuromodulators. Using the pentylenetetrazol-induced clonic seizure model, it was found that opioids are able to modulate the anticonvulsant effects of cannabinoids [98, 99]. In glutamatergic neurons, activation of CB1 receptors reduces the excitatory neurotransmission and the susceptibility to seizure activity [94, 100]. In experimental models of temporal lobe epilepsy (TLE), the activation of CB1 receptors with agonists (WIN 55,212-2, AEA, or 2-AG) decreases the epileptiform activity, the EPSCs evoked by glutamate, and the excitatory events evoked after antidromic electrical stimulation of mossy fibers in hilus [101].

Several experiments have focused on determining the role of eCB system on seizure activity by enhancing the availability of eCBs. Inhibition of AEA hydrolysis with URB-597, a FAAH inhibitor, results in anticonvulsive effects in the PTZ-induced seizures [102]. The inhibition of the 2-AG hydrolysis using WWL123 (an antagonist of ABHD6) reduces spontaneous seizures in R6/2 mice (a genetic model of juvenile Huntington's disease seizures) and PTZ-induced tonic-clonic convulsions [103]. Also, the increased levels of 2-AG that result of inhibition of degrading enzyme MAGL have been associated with a delay in the development of the kindling process [104]. The reduced metabolism of eCBs induced by the combination of AM404 (inhibitor of endocannabinoid reuptake) and URB597 (inhibitor of FAAH) results in decreased kainic acid-induced SE in guinea pigs [105]. These studies indicate that the blockage of specific enzymes can represent a new strategy to augment the anticonvulsant effects of eCBs.

In WAG/Rij rats, a genetic animal model of absence seizures, the administration of AEA or WIN55,212-2 (CB1 receptor agonists) reduces the seizure activity, while rimonabant (a CB1 receptor antagonist) increases it [106]. These results suggest that attenuated eCB function may contribute to the generation and maintenance of absence seizures.

5 eCBs and CB1 Receptors in Experimental Models of Seizure Activity and Epilepsy

Several studies indicate that seizure activity and epilepsy modify the eCB system and CB1 receptors. Concerning this issue, it is known that pilocarpine-induced SE increases 2-AG and CB1 receptor expression in hippocampus [91]. Acute seizures induced by kainic acid produce a rapid augmentation of AEA synthesis in the hippocampus and activation of CB1 receptors [107]. Studies indicate that seizure-induced changes in eCBs are age specific. Kainic acid-induced seizures in young rats augment the tissue content of AEA and their biosynthetic enzyme (NAPE-PLD) in the hippocampus, while adult rats present elevated tissue content of 2-AG and its biosynthetic enzyme DAGL [108]. Kindling-induced seizures augment CB1 receptor density in the pyramidal cell layer of the hippocampus [94]. Similar findings have been reported for different mouse models of epilepsy [109, 110]. In contrast, other studies indicate a low expression of CB1 receptors in certain neuronal subpopulation [111, 112]. These contradictory results can be explained by the different epilepsy models used and the period of evaluation after induction of seizures.

Upregulation of CB1 receptors in hippocampus is detected in mice with TLE subsequent to pilocarpine-induced SE [101]. Using the same experimental model of TLE in rats, it was found that spontaneous recurrent seizures are associated with a redistribution of CB1 receptors and changes in expression, binding, and G-protein activation in hippocampus [113]. This situation might depend on the time course of the SE-induced epileptogenesis process [111]. This group of evidence leads to suggest that the redistribution of CB1 receptors is associated with the cerebral plasticity involved in the epileptogenesis process.

6 eCBs and CB1 Receptors in Patients with Epilepsy

In dogs with idiopathic epilepsy, high concentrations of AEA were found in the cerebrospinal fluid, a situation that correlates with the severity of seizures and duration of the disease [114]. This study suggests an important activation of the cannabinoid systems as result of seizure activity. However, the low levels of AEA detected

in the cerebrospinal fluid of drug-naïve patients with TLE do not support this hypothesis [115].

Positron emission tomography (PET) imaging revealed increased availability of CB1 receptors in the ipsilateral temporal lobe of patients with TLE, a situation that was more evident in those subjects evaluated within short term after the last seizure and presenting higher number of seizures. These patients also show a decreased availability of CB1 receptors in the ipsilateral superior insular cortex, a condition that may restrict the seizure propagation [116]. However, it is important to consider that in vivo studies using PET imaging cannot avoid the presence of endogenous ligands and the enhanced availability of CB1 receptors can be associated with an increase in their number or affinity, or it is a consequence of low extracellular levels of eCBs.

The evaluation of hippocampal tissue obtained from patients with refractory TLE indicates a reduced expression of cannabinoid receptor-interacting protein-1a (CRIP1a) mRNA and the metabolic enzymes DGAL-α (enzyme involved in the synthesis of 2-AG). There is also a decrease in the mRNA and protein expression of CB1 receptors, mainly at glutamatergic axons, but not in GABAergic boutons, in the dentate gyrus [117]. Considering that CB1 receptors reduce the excitatory neurotransmission in glutamatergic neurons [94, 100], their lower expression at glutamatergic axons can facilitate the excitatory neurotransmission in the epileptic hippocampus. In contrast, CB1 receptors are preserved in dentate gyrus and CA1 region of patients with TLE, suggesting increased expression of these receptors in the GABAergic sprouting axons [118]. These results indicate that the disruption of the inhibitory effects of eCB system on GABAergic transmission in hippocampus of patients with TLE may facilitate the seizure activity.

Concerning TRPV1, studies revealed no significant changes in their expression in hippocampus of animals submitted to repetitive seizures [94]. However, patients with pharmacoresistant temporal lobe epilepsy show increased TRPV1 expression in the hippocampus [119]. Considering that cannabinoids may act as agonists of TRPV1, TRPV2, and TRPA1 [68, 70–72], the activation of these receptors by eCBs may contribute to the modulation of synaptic plasticity in human epileptic hippocampus.

7 The Phytocannabinoids and Epilepsy

There are new well-documented cases reporting remarkably strong beneficial effects of cannabinoids on seizure activity. This situation has triggered an upsurge in exploiting medical marijuana in patients with refractory epilepsy.

CBD is the major constituent of marijuana; it lacks psychoactive side effects and does not act as a CB1 receptor agonist. CBD induces anticonvulsant effects in the seizure activity induced by maximal electroshock test, pentylenetetrazol, pilocarpine-induced temporal lobe seizures, and penicillin [120–123]. However, CBD does not modify the seizure activity induced by cortical administration of cobalt [124]. In kindled rats, CBD reduces the seizure susceptibility and reduces the afterdischarge amplitude, duration, and propagation [125]. Clinical studies also support the anticonvulsant effect of CBD [126]. On the other hand, results obtained from in vitro and in vivo models indicate that cannabidivarin and Δ9-tetrahydrocannabivarin represent the two most important phytocannabinoids with therapeutic potential as anticonvulsant agents [127–131]. At present it is evident that CBD and other phytocannabinoids exert their antiseizure effects at CB1 receptors and other pharmacological targets [128].

In patients with Dravet syndrome, in which epilepsy is usually refractory to standard antiepileptic drugs, medical marijuana with a high CBD/Δ9-THC ratio has been successful to reduce the seizure activity [132]. CBD reduces the seizure frequency in patients with Lennox–Gastaut syndrome, who experience multiple refractory seizures everyday in spite of antiepileptic drugs [133]. Epidiolex (GW Pharmaceuticals), a new phytocannabinoid obtained from *Cannabis* extracts that contains about 98% of CBD and 2% of other cannabinoids, is now approved as a drug to be evaluated in pediatric patients with Dravet and Lennox–Gastaut syndromes [134]. However, proper controlled clinical trials are necessary to establish efficacy and safety of these phytocannabinoids in patients with epilepsy. In addition, future studies have to explore the cellular mechanisms and the signaling pathways involved in the anticonvulsant effects of CBD and other phytocannabinoids.

8 Is the Administration of Cannabinoids a Good Option to Control Epilepsy in Humans?

It is clear that epilepsy modifies the eCB system (e.g., CB1 receptors). However, as many other neuromodulatory systems, the activation of CB1 receptors can augment or reduce the seizure termination and duration, a situation that depends on the neuronal subpopulation and the experimental model used. Concerning this issue, cannabinoids may induce excitatory effects if CB1 receptors are overexpressed in GABAergic neurons. In contrast, the overexpression of these receptors in glutamatergic neurons can produce inhibitory effects. Therefore, the findings obtained from the evaluation of CB1 receptors in patients with epilepsy using PET or in in vitro conditions have to include a clear identification of the cells

in which those changes are produced. In addition, it is relevant to demonstrate that CB1 receptors are functionally active. This situation will help in the clarification of the mechanisms that underlie the anticonvulsant effects of cannabis and cannabinoids in different types of human epilepsy. It will also facilitate the establishment of compounds with therapeutic efficacy to reduce the seizure activity.

Although the results obtained from experimental models are relevant to understand the role of eCBs in epilepsy, they do not reproduce totally the pathological conditions of the human epilepsy. Therefore, the analysis of cerebral tissue obtained from patients with pharmacoresistant epilepsy and submitted to epilepsy surgery is essential to clarify if cannabinoids represent a good therapeutic strategy for epilepsy.

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