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REVIEW

Control of excessive neural circuit excitability and prevention of epileptic seizures by endocannabinoid signaling

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Abstract

Progress in research on endocannabinoid signaling has greatly advanced our understanding of how it controls neural circuit excitability in health and disease. In general, endocannabinoid signaling at excitatory synapses suppresses seizures by inhibiting glutamate release. In contrast, endocannabinoid signaling promotes seizures by inhibiting GABA release at inhibitory synapses. The physiological distribution of endocannabinoid signaling molecules becomes disrupted with the development of epileptic focus in patients with mesial temporal lobe epilepsy and in animal models of experimentally induced epilepsy. Augmentation of endocannabinoid signaling can promote the development of epileptic focus at initial stages. However, at later stages, increased endocannabinoid signaling delays it and suppresses spontaneous seizures. Thus, the regulation of endocannabinoid signaling at specific synapses that cause hyperexcitability during particular stages of disease development may be effective for treating epilepsy and epileptogenesis.

Keywords Endocannabinoid · 2-AG · CB_1 · Epilepsy · Epileptogenesis · Seizure

Introduction

Epilepsy is a chronic neurological disease with various causes, characterized by paroxysmal recurrent episodes of symptoms, termed epileptic seizures. The estimated proportion of the general population with epilepsy at any given time is between 3 and 7 per 1000 people [1] and its incidence is 50.4 per 100,000 people per year [2]. Treatment of epilepsy usually starts with medications to prevent the occurrence of seizures. Most conventional antiepileptic drugs such as valproate, carbamazepine, phenytoin, and benzodiazepines act via blockade of Na^+ and/or Ca^{2+} channels, or activation of $GABA_A$ receptors [3]. However, approximately 30 percent of patients have uncontrollable episodes of seizures with current medications [4]. Hence, there is a critical unmet need for developing new anti-epileptic drugs with novel mechanisms of action.

Endocannabinoid signaling is a potential candidate for the development of a novel anti-epileptic treatment [5–7]. Endocannabinoids are a group of lipid substances that act as endogenous ligands for cannabinoid receptors to which active components of marijuana bind. Endocannabinoid signaling is known to regulate neuronal excitability via multiple mechanisms. In this review, we will first provide a brief overview of the physiological roles of endocannabinoid signaling in the control of synaptic transmission, synaptic plasticity, and neuronal excitability. We will then review and discuss how endocannabinoid signaling is involved in seizures and epileptogenesis.

Endocannabinoids

Marijuana is prepared from the plant *Cannabis sativa* and has been used for recreation as well as treatment of a variety of diseases for thousands of years. In 1964, Δ-9-tetrahydrocannabinol (Δ^9-THC) was identified as the main psychoactive chemical component in marijuana [8]. Almost 3 decades after the discovery of THC, cannabinoid type 1 (CB_1) and type 2 (CB_2) receptors were cloned in 1990 [9]

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and 1993 [10], respectively. In parallel with these events, the endogenous ligands for cannabinoid receptors were sought. *N*-arachidonoyl ethanolamine (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG) were eventually identified as the endogenous ligands for cannabinoid receptors in 1992 [11] and 1995 [12, 13], respectively.

Physiological roles of CB_1 receptor-mediated endocannabinoid signaling

The CB_1 receptor is a seven-transmembrane receptor coupled to $G_{i/o}$ protein [9] and is mainly expressed in the central nervous system. Activation of the CB_1 receptor is known to influence a wide range of effector molecules including voltage-gated Ca^{2+} channels, K^+ channels, and protein kinase A [14–16]. In 2001, endocannabinoids were discovered to function as retrograde messengers that mediate signals from depolarized postsynaptic neurons to presynaptic CB_1 receptors and causing a transient suppression of synaptic transmission [17–19]. Subsequently, endocannabinoid-mediated long-term suppression of synaptic transmission was reported [20–22]. Many studies thereafter have revealed detailed mechanisms of endocannabinoid-mediated retrograde suppression of inhibitory and excitatory synaptic transmission. In particular, the finding that endocannabinoid-mediated retrograde synaptic suppression is abolished in mice deficient in diacylglycerol lipase α (DGLα), a major enzyme that produces 2-AG from diacylglycerol [23], clarified that 2-AG is the retrograde messenger for synaptic suppression [24, 25]. Although the majority of short-term and long-term depression (STD and LTD, respectively) of synaptic transmission through the CB_1 receptor is mediated by 2-AG [26, 27], AEA is reported to be necessary for LTD at several types of synapse [28, 29]. As the mechanism of endocannabinoid-mediated regulation of synaptic transmission has been described in detail in several excellent reviews [27, 30, 31], we will briefly describe the 2-AG- and AEA-mediated STD and LTD of excitatory and inhibitory synaptic transmission.

Production of 2-AG is induced by increased activity of postsynaptic neurons. 2-AG-mediated STD occurs when the postsynaptic neuron is depolarized and intracellular Ca^{2+} levels are elevated following Ca^{2+} influx through voltage-gated Ca^{2+} channels (Fig. 1). This form of 2-AG-mediated STD of excitatory or inhibitory synaptic transmission is called depolarization-induced suppression of excitation (DSE) or inhibition (DSI), respectively (Fig. 1). DSE/DSI is completely abolished in mice deficient in DGLα [24, 25]. Calcium influx into the postsynaptic neuron through NMDA receptors can also induce 2-AG-mediated STD [32] (Fig. 1). The increase in intracellular Ca^{2+} levels to several μM alone can trigger the biosynthesis of 2-AG, termed Ca^{2+}-driven endocannabinoid release (Ca-ER) [33–35]. 2-AG then acts retrogradely upon CB_1 receptors on presynaptic terminals,

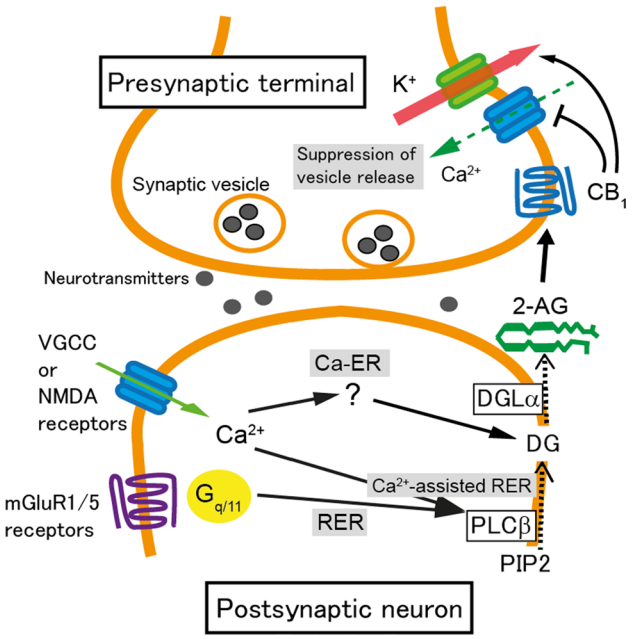

Fig. 1 Schematic illustration of the molecular mechanisms underpinning endocannabinoid-mediated short-term synaptic plasticity. When a large Ca^{2+} elevation is caused by the activation of voltage-gated Ca^{2+} channels (VGCC) or NMDA receptors, 2-arachidonoyl glycerol (2-AG) is generated in a diacylglycerol lipase (DGL) α-dependent manner. This process is termed Ca^{2+}-driven endocannabinoid release (Ca-ER). The target enzyme, which is activated by Ca^{2+} elevation to produce diacylglycerol (DG), has yet to be identified. When phospholipase C (PLC) β is stimulated by the activation of metabotropic glutamate receptors (mGluR) 1/5 or other $G_{q/11}$-coupled receptors, DG is generated from phosphatidylinositol 4,5-bisphosphate (PIP2) and converted to 2-AG by DGLα (receptor-driven endocannabinoid release; RER). When the activation of $G_{q/11}$-coupled receptors and intracellular Ca^{2+} elevation occur simultaneously, the production of 2-AG is accelerated through PLCβ-dependent pathways (Ca^{2+}-assisted receptor-driven endocannabinoid release; Ca^{2+}-assisted RER). 2-AG is released from postsynaptic neurons, activates presynaptic CB_1 receptors, and induces transient suppression of transmitter release through inhibition of voltage-gated Ca^{2+} channels and additionally through activation of K^+ channels

which suppresses neurotransmitter release mainly by inhibiting presynaptic voltage-gated Ca^{2+} channels (Fig. 1). Another pathway for 2-AG production is via $G_{q/11}$ protein-coupled receptors such as group I metabotropic glutamate receptors (mGluRs) and M_1/M_3 muscarinic acetylcholine receptors. This pathway is termed receptor-driven endocannabinoid release (RER) [34–37]. Activation of these receptors induces degradation of phosphatidylinositol into diacylglycerol by phospholipase Cβ, followed by production of 2-AG from diacylglycerol by DGLα (Fig. 1) [24, 25, 38]. The production of 2-AG is strongly elevated when the activation of $G_{q/11}$ protein-coupled receptors occurs simultaneously with the elevation of intracellular Ca^{2+} levels, termed Ca^{2+}-assisted receptor-driven endocannabinoid release (Ca-RER) (Fig. 1) [33–35, 39]. 2-AG is thought to pass through

the plasma membrane due to its lipophilic nature and diffuse towards presynaptic terminals. However, the precise details of these processes remain unknown. The amount of 2-AG may be regulated in postsynaptic neurons by 2-AG-hydrolyzing enzymes, namely α/β-hydrolase domain containing (ABHD) 6 and 12 [40], and by a 2-AG-oxygenizing enzyme, cyclooxygenase (COX)-2 [41].

At the presynaptic terminal, 2-AG binds to and activates CB_1 receptors as a full agonist [42] and suppresses neurotransmitter release mainly by inhibiting voltage-gated Ca^{2+} channels [14, 16]. Furthermore, 2-AG suppresses neurotransmitter release through CB_1 receptors by inhibiting the action of a presynaptic protein, Munc18-1, through phosphorylation by extracellular-regulated kinase (ERK) [43]. The actions of 2-AG are terminated upon degradation by monoacylglycerol lipase (MGL). MGL is densely expressed in the cytoplasm of subsets of presynaptic terminals and in astrocytes surrounding excitatory and inhibitory synapses [44–46]. Due to the highly heterogeneous expression pattern of MGL, 2-AG is thought to be degraded in a synapse non-specific manner [45].

In addition to STD, presynaptic forms of LTD are induced in several brain regions after activation of CB_1 receptors by 2-AG derived from postsynaptic neurons. Production and release of 2-AG in LTD occur in a similar manner to that in STD, but prolonged 2-AG production and resultant CB_1 receptor activation for several minutes are required for the induction of LTD. At inhibitory synapses in the hippocampus, LTD induction requires 5–10 min of continuous activation of CB_1 receptors and presynaptic activity [47]. The activation of CB_1 receptors mobilizes $G_{i/o}$ protein, decreases cAMP production and PKA signaling, and eventually results in persistent reduction of the efficacy of neurotransmitter release [48]. Once this machinery is switched on, the suppression is sustained without further activation of CB_1 receptors. Presynaptic proteins such as RIM1α [48], calcineurin [49], and potassium channels [50] are involved in the expression of 2-AG-mediated LTD. Furthermore, it has recently been shown that protein synthesis at presynaptic sites is necessary for the expression of CB_1-mediated LTD at inhibitory synapses in the hippocampus [51].

As described above, phasic suppression of synaptic transmission at excitatory and inhibitory synapses may occur when the activity of postsynaptic neurons is elevated. In addition, endocannabinoid signaling is reported to mediate tonic suppression of synaptic transmission at inhibitory synapses in the CA3 area of the hippocampus [52]. In this report, the authors tested the fidelity of synaptic transmission from cholecystokinin-positive inhibitory interneurons onto CA3 pyramidal cells, which usually show extremely low fidelity of synaptic transmission, in acute hippocampal slices treated with a CB_1 antagonist (AM251; 10 μM). They demonstrated that the CB_1 antagonist raised the fidelity of inhibitory synaptic transmission, suggesting that persistently active cannabinoid receptors mute the output of cholecystokinin-positive inhibitory interneurons in the hippocampal CA3 area.

In addition to 2-AG, the other major endocannabinoid AEA is crucial for the induction of CB_1-mediated LTD at inhibitory synapses in the striatum [28] and amygdala [53]. In addition, AEA-mediated LTD through the vanilloid receptor TRPV1 was reported at perforant path-granule cell synapses in the dentate gyrus [54] and in dopamine D2 receptor-positive medium spiny neurons in the nucleus accumbens [55]. Biochemical pathways for the synthesis of AEA are not fully understood, but a relevant signaling cascade involves the synthesis of *N*-acylphosphatidylethanolamine (NAPE) from phospholipid by *N*-acyltransferase [56] and the lysis of NAPE by NAPE-specific phospholipase D (NAPE-PLD) into AEA [57, 58]. AEA is cleaved by fatty acid amide hydrolase (FAAH) into arachidonic acid [59]. Similar to 2-AG, AEA is also degraded by COX-2 at postsynaptic sites [60].

CB_2 receptor-mediated signaling

The CB_2 receptor is $G_{i/o}$ coupled and is the other canonical cannabinoid receptor that is involved in 2-AG-mediated signaling. This receptor is mainly expressed in immune cells such as T cells, B cells, and monocytes, and is responsible for the anti-inflammatory effects of cannabis. However, recent reports describe CB_2 receptor mRNA expression in neurons using fluorescent in situ hybridization [61, 62]. Although the expression of CB_2 receptor protein in neurons remains to be confirmed, several studies using CB_2 knockout mice demonstrated that CB_2 receptors were involved in the regulation of neuronal excitability and synaptic transmission. Li and Kim [63] demonstrated that the amplitude of field excitatory post-synaptic potentials and the magnitude of LTP at Schaffer collateral-CA1 pyramidal cell synapses were reduced in CB_2 knockout mice. In contrast, there was no difference in paired-pulse facilitation at the same synapses between CB_2 knockout and wild-type littermates, suggesting that the decrease in excitatory synaptic transmission is of postsynaptic origin. The authors claim that CB_2 receptor signaling is required for maintaining excitatory synaptic transmission. In contrast, Stempel et al. compared CB_2 knockout mice to DGLα knockout mice and reported that CB_2 receptor signaling mediated activity-dependent long-lasting hyperpolarization and inhibition of CA3 pyramidal cells [62]. This study suggests that CB_2 receptor signaling has inhibitory actions on the excitability of hippocampal CA3 pyramidal neurons. In line with this study, excitability of dopamine neurons in the ventral tegmental area was reported to decrease through activation of CB_2 receptors

[64]. Thus, the effects of CB_2 receptor signaling on neuronal excitability may differ in different brain areas and cell types.

Because of the suppressive effect of CB_1 receptor signaling on synaptic transmission and the potential regulation of neuronal excitability by CB_2 receptors, many studies have been conducted regarding the roles of endocannabinoid signaling in the control of neuronal hyperexcitability, seizures, and epilepsy. Next, we provide an overview of studies on human epileptic patients with respect to endocannabinoid signaling.

Endocannabinoid signaling and epilepsy

Possible involvement of endocannabinoid signaling in epileptic patients

The brains of patients with mesial temporal lobe epilepsy (mTLE) with hippocampal sclerosis show reduced expression of DGLα mRNA compared to specimens from a non-epileptic control group [65] (Fig. 2). In contrast, no differences were found between control and epileptic patients in the expression levels of NAPE-PLD, MGL, or FAAH mRNA. These results suggest that there may be a selective reduction in 2-AG production, whereas 2-AG degradation, AEA production, and AEA degradation remain normal in the hippocampi of patients with mTLE. On the other

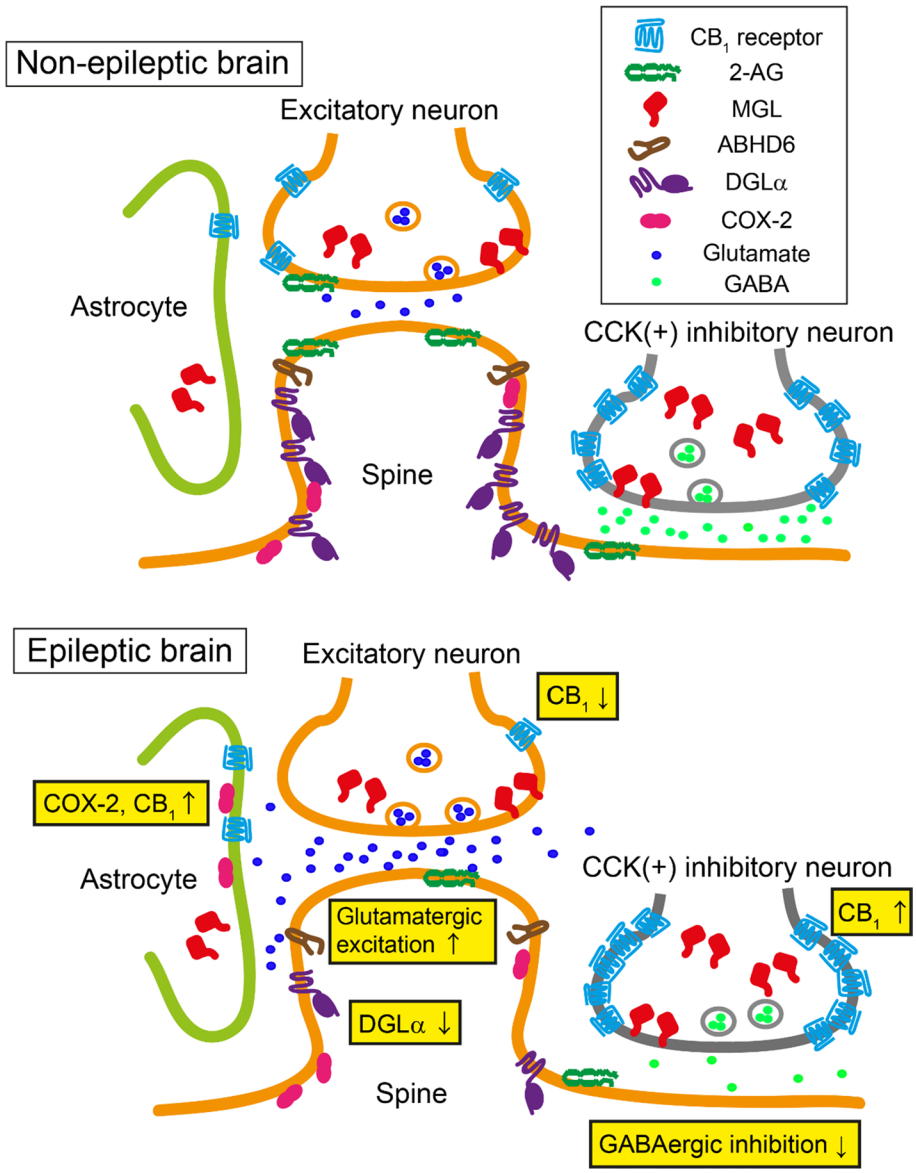

Fig. 2 Schematic illustration of changes in 2-AG and CB_1 receptor signaling observed in epileptic patients and animal models of epilepsy. Localization and expression of molecules involved in endocannabinoid production and degradation around synapses in a non-epileptic control brain (upper panel) and in an epileptic brain (lower panel). A significant decrease in the expression of DGLα may result in decreased production of 2-AG during the ictal phase. The expression of CB_1 receptors decreases at excitatory synapses, whereas it increases at axon terminals of cholecystokinin-positive (CCK+) interneurons. The expression of CB_1 receptors is detectable in hippocampal astrocytes in epileptic patients, whereas it is hardly detectable in non-epileptic control tissue. The expression of monoacylglycerol lipase (MGL) and α/β-hydrolase domain containing 6 (ABHD6) remains unchanged. These changes are thought to underlie the excessive glutamatergic transmission and scarce GABAergic inhibition in the epileptic brain. The expression of cyclooxygenase (COX)-2 increases in hippocampal astrocytes in severe epileptic patients, which may reduce CB_1 receptor signaling in the astrocytes of these patients

hand, the concentration of AEA in the cerebrospinal fluid is reduced in untreated patients with mTLE, whereas the level of 2-AG was similar between untreated patients and control subjects [66]. In this report, however, the concentrations of 2-AG and AEA were measured in the non-ictal state. It is, therefore, possible that the concentrations may differ during ictal activity when the production of endocannabinoids may be enhanced.

Ludányi et al. reported that the mRNA level of CB_1 receptors in the hippocampus was reduced in patients with mTLE compared to control subjects [65]. This reduction was even more significant in mTLE patients with hippocampal sclerosis, suggesting a negative correlation between mRNA levels and the severity of disease. Immunohistochemical analysis of CB_1 receptor expression in the inner molecular layer of the dentate gyrus demonstrated a significant reduction in mTLE patients, and the expression had almost completely disappeared in sclerotic hippocampal samples [65]. Further analysis with electron microscopy revealed that in addition to a decrease in the total number of excitatory synapses, the loss of CB_1 receptors in excitatory axon terminals was responsible for the reduced expression of CB_1 receptors in the inner molecular layer (Fig. 2) [65]. No changes were observed in the expression levels of CB_1 receptors at inhibitory axon terminals or in the number of inhibitory axon terminals in the dentate gyrus. In the hippocampus proper of mTLE patients with hippocampal sclerosis, an increase in the expression of CB_1 receptors was observed in the stratum oriens of the hippocampal CA2 and CA3 regions [65].

In contrast to the report described above, Maglóczky et al. found an increase in the expression of CB_1 receptors in the hippocampal dentate gyrus in patients with mTLE [67]. A previous study [68] using the same antibody against CB_1 receptors reported that the antibody exclusively labeled CB_1 receptors at inhibitory presynaptic terminals that expressed a high density of CB_1 receptors. Therefore, it is reasonable to assume that the expression of CB_1 receptors at inhibitory synapses may be increased in patients with mTLE (Fig. 2). However, it was unclear in the report by Maglóczky et al. [67] whether CB_1 receptors were expressed at excitatory synapses in the dentate gyrus of mTLE patients. As mentioned previously, increased CB_1 receptor expression at inhibitory synapses potentially causes disinhibition of postsynaptic neurons in a tonic and phasic manner. Consistent with the report by Maglóczky et al. [67], another study using positron emission tomography showed that in mTLE patients with hippocampal sclerosis, the availability of CB_1 receptors in the ipsilateral temporal lobe was increased compared to healthy controls [69]. Based on these results, the disruption or strengthening of endocannabinoid signaling at excitatory synapses or inhibitory synapses, respectively, may potentially underpin the increased excitability of neural circuits which underlies the chronic susceptibility to seizures observed in epileptic patients.

Recently, it was reported that over half of the hippocampal specimens taken from epileptic patients expressed CB_1 receptors in astrocytes. In contrast, there was minimal expression of CB_1 receptors in hippocampal astrocytes in non-epileptic control subjects (Fig. 2) [70]. In mice, activation of astrocytic CB_1 receptors caused glutamate release from astrocytes [71], suggesting that the elevated expression of CB_1 receptors in astrocytes observed in epileptic patients may increase the excitability of neural circuits. However, the influence of increased astrocytic CB_1 receptor expression may be small in patients with hippocampal sclerosis, since the expression of an endocannabinoid degradation enzyme, COX-2, is elevated in the hippocampal astrocytes of these patients [72].

Studies on genetic mutations in human epileptic patients may reveal causal relationships between the changes in endocannabinoid signaling and epilepsy. It is known that patients with mTLE often have histories of febrile seizures. When the frequency of single nucleotide polymorphisms (SNPs) in *CNR1* gene was compared between patients who experience febrile seizures and normal control subjects, no significant differences were observed [73], suggesting that SNPs in *CNR1* gene are not the cause of febrile seizures. Recently, a paper investigating the contribution of human genetic variations in *DAGLA* and *CNR1* to disease phenotypes was published [74]. In this study, a high incidence of seizures was observed in people with mutations in *DAGLA*, suggesting that the disruption of 2-AG-mediated endocannabinoid signaling in humans may cause epilepsy. The *DAGLA* mutation observed in this study may also contribute to the decrease in mRNA reported by Ludányi et al. [65].

In the next section, we review how alterations in endocannabinoid signaling are implicated in animal models of epilepsy.

Epileptogenesis

Epilepsy is caused by various factors, ranging from hereditary genetic mutations to traumatic brain injuries in adults. A group of patients with epilepsy have a specific episode such as febrile seizures, central nervous system infections, or traumatic brain injuries several years before the onset of paroxysmal, recurrent episode of seizures. The period between the initial insult and the onset of epileptic seizures is called the latent period. During the latent period, it is hypothesized that anatomical and physiological changes gradually occur, resulting in the development of epileptic foci from which seizures arise. This process is known as epileptogenesis.

To investigate epileptogenesis, two different strategies are typically employed in animal models. One strategy involves the application of severe initial insults such as sustained,

generalized tonic–clonic seizures called status epilepticus (SE), prolonged febrile seizures, and traumatic brain injury. These severe insults trigger epileptogenic changes in neural circuits and usually result in spontaneous seizures several weeks after the initial insults. Spontaneous seizures in these models are also useful for the evaluation of ictogenesis in the epileptic brain. The other strategy is kindling, in which sub-convulsive stimuli are initially applied in an intermittent fashion instead of strong initial insults. This makes it possible to observe the process of epileptogenesis in a stepwise manner [75]. In conventional kindling, electrical stimulation to the lateral amygdala or to the perforant path is used as a model of mTLE. Other protocols for kindling include transcorneal electrical stimulation and chemical kindling with pentylenetetrazole (PTZ) or picrotoxin. PTZ and picrotoxin are $GABA_A$ receptor antagonists and are considered to evoke seizures by strong disinhibition. All of the kindling protocols fulfill the condition of "seizure beget seizure", meaning that a seizure promotes epileptogenesis.

The disruption of long-term synaptic plasticity via blockade of NMDA receptor or mGluR1/5 receptor signaling affects epileptogenesis [76–78]. Moreover, electrical stimulation protocols used for the induction of LTD delay kindling epileptogenesis [79, 80]. As endocannabinoid signaling mediates long-term synaptic plasticity, it is possible that the initial insult strongly mobilizes 2-AG and AEA, which may induce long-term synaptic plasticity and affect the process of epileptogenesis. In addition, intense and prolonged activation of CB_1 receptors by 2-AG can cause downregulation of cannabinoid receptors [81–83], possibly resulting in the disruption of 2-AG signaling. Furthermore, endocannabinoids are known to exert protective effects on neurons [84]. Given that neuronal damage caused by brain injury, infection, seizures, or hypoxia can trigger epileptogenesis, neuroprotection by endocannabinoid signaling may prevent epileptogenesis.

In this section, we provide an overview of studies on the involvement of endocannabinoid signaling in epileptogenesis.

Involvement of 2-AG and AEA (Fig. 2, Table 1)

The activation of mGluR1/5 receptors and the increase in intracellular Ca^{2+} concentration induce the production of 2-AG. It is highly likely that this process occurs during SE or febrile seizures. Indeed, the production of 2-AG is enhanced by seizures [85, 86]. Intraperitoneal (i.p.) administration of pilocarpine, a muscarinic acetylcholine receptor agonist, causes SE. When the concentration of 2-AG was assessed in rat hippocampi 15 min after the initiation of pilocarpine-induced SE (375 mg/kg, i.p.) with high-performance liquid chromatography, the level of 2-AG exhibited an approximately 1.5-fold increase compared to that of control [85]. Elevation of 2-AG levels was also observed in the brains of animals with kainate-induced epilepsy [86]. Kainate is an agonist of the kainate-type ionotropic glutamate receptor and triggers SE when administered intraperitoneally in a manner similar to pilocarpine. In the aforementioned report, the authors measured the level of 2-AG in brain tissue 100 min after kainate (30 mg/kg, i.p.) or vehicle injection in 8-week-old rats and observed that the concentration was 1.5-fold higher in the kainate-treated group than in the control group

Table 1 Epileptogenesis and ictogenesis modulated by manipulations of endocannabinoid signaling

	Epileptogenesis		Ictogenesis			
	Kainate Pilocarpine Febrile seizure Trauma Spontaneous	Kindling	Kainate Pilocarpine	Pentylenetetrazole Bicuculine	Electrical stim.	Spontaneous seizure
2-AG↑	× [95]	○ [90, 92, 93]	○ [90]	○ [117], ≈ [93]	○ [90, 92, 93]	○ [90, 117]
2-AG↓	○ [95]	× [90]	× [90], ≈ [114]	?	× [90]	?
AEA↑	○ [100], × [101]	≈ [99]	○ [86, 122], × [101]	○ [123], × [101]	○ [121]	?
CB_1↑	○ [111], × [101]	○ [99]	○ [130, 131]	× [123], ○ [136]	○ [134, 135]	○ [85]
CB_1↓	○ [105, 108, 110] × [113]	≈ [90, 112]	× [87, 132] × Excitatory [129] ≈ Inhibitory [129]	≈ [117], × [123]	× Excitatory [112] ○ Inhibitory [112]	× [85, 90]
CB_2↑	?	?	?	× [139]	?	?
CB_2↓	× [113]	≈ [90]	× [90]	× Kindled rat[138] × [117]	≈ [137] × [90]	?

Cells in the leftmost column indicate the manipulations and those in the first and second rows denote the types of animal models. The observed effects are represented as follows: ○: suppresses ictogenesis or epileptogenesis, ≈: no change, ×: promotes ictogenesis or epileptogenesis, ?: unknown. Excitatory: results observed in mice with excitatory neuron-specific deletion of cannabinoid type 1 (CB_1) receptors; inhibitory: results observed in mice with inhibitory neuron-specific deletion of CB_1 receptors

[86]. They also measured the enzymatic activity of DGLα, MGL, NAPE-PLD, and FAAH under the same schedule and found that the enzymatic activity of DGLα increased, whereas that of NAPE-PLD decreased after kainate administration. In contrast, the activity of MGL and FAAH was the same between the kainate and vehicle-treated groups. Interestingly, the changes in endocannabinoid levels showed the opposite trend in 14-day-old rats, whereby 2-AG decreased whereas AEA increased significantly after kainate treatment [86], suggesting that the changes may depend on the age of animal.

In contrast to these reports, other studies have failed to report changes in 2-AG levels in kainate-induced epilepsy. Marsicano et al. used the kainate-induced epilepsy model and measured the concentration of 2-AG and AEA [87]. They reported that the concentration of 2-AG in the hippocampi of adult mice 20 min after kainate injection (30 mg/kg, i.p.) did not change, while that of AEA showed a significant rise up to 300% of pre-treatment levels [87]. The concentration of AEA returned to baseline within 1 h after kainate injection [87]. A recent study [88] assessed the levels of various lipid substances 1 h after kainate injection (30 mg/kg, i.p.) and reported that the levels of AEA and 2-AG remained unchanged in the cerebral cortex, striatum, hippocampus, thalamus, hypothalamus, and cerebellum compared to those of the vehicle-treated group [88]. The reasons for these variable results regarding 2-AG levels in the brain after kainate application remain unclear. In contrast, the increase in brain AEA levels after kainate application may reflect the production of AEA through activation of the postsynaptic kainate receptor, GluK2. Lourenço et al. applied kainate (3 μM) to hippocampal slices for 100 s and observed a significant increase in AEA levels with no change in 2-AG levels [89]. This increase was abolished in GluK2 knockout mice or by manipulating the chelation of Ca^{2+} in postsynaptic neurons [89], suggesting that AEA was mobilized by GluK2 activation and elevation of postsynaptic Ca^{2+} concentration.

The enzymes involved in 2-AG production and degradation undergo changes in expression during epileptogenesis. We recently reported a significant decrease in the expression of DGLα in the hippocampal CA1 and dentate gyrus of mice that received unilateral intrahippocampal kainate injections (0.2 μg) 4 weeks before immunohistochemical analysis [90]. The decrease in DGLα in the hippocampus is consistent with the findings observed in the hippocampi of mTLE patients, which may result in insufficient 2-AG production when it is required for retrograde suppression of excitatory synaptic transmission. On the other hand, there were no changes in 2-AG and AEA levels in the hippocampus after 4 weeks of PTZ kindling [91], suggesting that basal levels of 2-AG and AEA during the inter-ictal period were maintained during the course of epileptogenesis.

Decreased expression of DGLα in patients with mTLE and animal models of kainate-induced epilepsy provide potential explanatory mechanisms for the process of epileptogenesis. Recently, we demonstrated that kindling epileptogenesis with electrical stimulation of the perforant path was significantly promoted in DGLα knockout mice compared to wild-type littermates [90]. In line with these results, manipulations that increase 2-AG levels suppress kindling epileptogenesis (Table 1). For example, the MGL inhibitor JZL184 (4 mg/kg, i.p., twice/day) significantly delayed epileptogenesis induced by perforant path kindling in wild-type mice [90]. This result is consistent with the effect of JZL184 (8 mg/kg/day, i.p.) on the amygdala kindling model [92] and with that of the MGL inhibitor SAR127303 [30 mg/kg/day, per os (p.o.)] on the corneal kindling model [93]. Furthermore, in cultured hippocampal slices with intense activation of NMDA receptors (50 μM NMDA for 4 h), the increase in 2-AG levels by JZL184 (1 μM) prevented cell death in the CA1 region [94]. These results indicate that 2-AG prevents epileptogenesis by kindling procedures, and imply that elevation of 2-AG levels can delay epileptogenesis and prevent cell death.

However, contrasting results regarding the effect of 2-AG have been reported in epileptogenesis after SE. When DGLα was blocked by RHC80267 (1.3 μmol, intracerebroventricular; i.c.v.) for 7 days commencing immediately after the termination of pilocarpine-induced SE, the frequency of spontaneous seizures and the occurrence of cell death decreased [95], suggesting that 2-AG promotes SE-induced epileptogenesis (Table 1). The pro-epileptogenic effect of 2-AG during the initial 7 days after SE may be ascribed to downregulation of CB_1 receptor signaling. In rat organotypic cultured hippocampal slices, the concentration of 2-AG increased 24–72 h after NMDA treatment (50 μM) [94]. Moreover, 5 days of 2-AG upregulation by JZL treatment (16 mg/kg, i.p.) was sufficient to induce downregulation of CB_1 receptor signaling in non-epileptic wild-type mice [83]. It is, therefore, likely that downregulation of CB_1 receptor signaling occurs due to a sustained increase in 2-AG concentration in the initial stage of SE-induced epileptogenesis.

In an in vitro model of epilepsy [96–98], continuous epileptiform high-frequency bursts were induced in primary cultures of hippocampal neurons by the application of low Mg^{2+} artificial cerebrospinal fluid (aCSF) for 3 h. After restoration of normal Mg^{2+} concentration, spontaneous recurrent burst activity appeared within 5 min and persisted for more than 24 h. Application of a synthetic cannabinoid agonist, WIN55212-2, for 24 h after restoration of Mg^{2+} significantly increased neuronal burst activity and decreased the expression of CB_1 receptors [96]. These results are consistent with CB_1 receptor activation after initial insults downregulate CB_1 receptor expression.

In contrast to 2-AG, an increase in AEA by the FAAH inhibitor URB597 (3 mg/kg, i.p.) did not change kindling epileptogenesis [99]. However, when kainate-induced seizures were mild, elevation of AEA levels by i.cv. administration of URB597 (10 μg/μl for 10 days beginning 24 h after kainate administration) was able to reduce kainate-induced cell death in the hippocampal CA1 [100]. In contrast, when the concentration of AEA was further elevated by the deletion of FAAH combined with AEA administration (50 mg/kg, i.p.), severe neuronal cell death was observed in the hippocampus after administration of kainate (15 mg/kg, i.p.) or bicuculline (4 mg/kg, i.p.) [101]. Surprisingly, neuronal cell death after bicuculline administration was prevented by injection of a CB_1 antagonist, SR141716A (3 mg/kg, i.p.) [101]. These results suggest that the effects of AEA on epileptogenesis may be concentration-dependent.

Involvement of CB_1 and CB_2 receptors (Fig. 2, Table 1)

Similar to DGLα, the expression of CB_1 receptors is affected by epileptogenic processes. Pilocarpine-induced SE (375 mg/kg, i.p.) induced a time-dependent redistribution of hippocampal CB_1 receptors in rats [102, 103]. Within 1 week after SE, there was a pronounced loss of CB_1 receptor expression throughout the hippocampus. By 2 weeks after SE, the decreased CB_1 receptor expression was mostly restored in the CA1 but not in the dentate gyrus. By 1 month after SE, pilocarpine-treated rats began to show spontaneous seizures. Concurrently, a characteristic redistribution of CB_1 receptors was observed in chronic epileptic rats, whereby CB_1 receptor immunoreactivity in the dentate gyrus inner molecular layer and the CA1 pyramidal cell layer was specifically decreased; conversely, that in the strata oriens and radiatum of the CA1–3 was increased [103]. This unique redistribution of CB_1 receptors persisted for up to 6 months in chronic epileptic rats, which is consistent with the distribution of CB_1 receptors in the hippocampi of chronic mTLE patients [65, 67]. As described, we also investigated the distribution of CB_1 receptors 4 weeks after kainate-induced SE in mice [90]. The pattern of CB_1 receptor distribution was similar to that observed in rat 7 days after pilocarpine-induced SE: i.e., a pronounced loss in CB_1 receptor expression throughout the hippocampus. Since we were able to observe spontaneous seizures 4 weeks after kainate-induced SE, we propose that the decrease in CB_1 receptors in the inner molecular layer of the dentate gyrus and the pyramidal cell layer of the hippocampal CA1 are responsible for the occurrence of spontaneous seizures, rather than a successive increase of CB_1 receptors in the strata oriens and radiatum of the CA1–3.

Chen et al. [104] used a rat model of mTLE induced by febrile seizures and observed changes in CB_1 receptor expression. On postnatal day 10, the body temperature of rat pups was raised to 41–42 °C to evoke febrile seizures for approximately 20 min. Febrile seizures induced persistent enhancement of DSI in hippocampal CA1 pyramidal cells for up to 5 weeks [104], which resulted from an increase in CB_1 receptors associated with inhibitory inputs from cholecystokinin-positive interneurons. Following the period of febrile seizures, no significant effects were observed either on DSE in CA1 pyramidal neurons at 5 weeks or on 2-AG and anandamide levels at 1 week. These results suggest that febrile seizures induce changes in CB_1 receptor expression, which may disinhibit hippocampal neural circuits thus contributing to the development of epileptic foci. In this model, decreased CB_1 receptors in the inner molecular layer of the dentate gyrus and pyramidal cell layer of the hippocampal CA1 was not observed. Therefore, decreased suppression of excitatory synaptic transmission is unlikely to be the cause of increased seizure susceptibility in this model. Instead, disinhibition due to increased CB_1 receptor expression could be the major cause of heightened seizure susceptibility.

Chen et al. monitored DSI following a single injection of a CB_1 antagonist, SR141716A (1 mg/kg, i.p.) into rat pups 1 h before the start of febrile seizures [105]. Although the biological half-life of SR141716A measured in blood samples is 4.95 h in mice [106] and 6–9 days in humans [107], the acute injection of SR141716A blocked febrile seizure-induced enhancement of DSI, increase in CB_1 receptors, and worsening of acute kainate-induced seizures 6 weeks after the febrile seizure [105]. These results underscore the pertinence of the immediate anatomical and physiological changes in the endocannabinoid system after febrile seizures for elucidating key epileptogenic processes as well as the development of novel therapeutics.

As discussed previously, prolonged activation of CB_1 receptors causes their downregulation, which accounts for the decrease in CB_1 receptors at the initial stage of SE-induced epileptogenesis. However, at later stages of SE- or febrile seizure-induced epileptogenesis, CB_1 receptor expression is increased. Notably, this effect can be blocked by CB_1 receptor antagonists in the febrile seizure model. Therefore, heightened activation of CB_1 receptors at the initial stage of epileptogenesis may trigger the elevation of CB_1 receptor expression at later stages as well as the decrease in receptor expression at the initial stage. The detailed molecular mechanisms underlying these phenomena are unclear. It has been reported that an increase in interleukin β was observed up to 12 h after febrile seizures and administration of an interleukin 1 receptor antagonist (100 ng, i.c.v.) prevented the increase in CB_1 receptors after febrile seizures in rats [108].

Almost 10% of patients with traumatic brain injury later acquire epilepsy during their lifetime [109]. In animal models, brain injury also induces persistent hyperexcitability of neural circuits. In a rat model of traumatic brain injury,

Echegoyen et al. demonstrated that treatment with a CB_1 antagonist, SR141716A (10 mg/kg, i.p.), immediately after injury to the cerebral cortex prevented the long-term increase in seizure susceptibility induced by kainate (5 mg/kg, i.p.) [110]. This result is consistent with the findings in the febrile seizure model described above,and indicates that short-term blockade of CB_1 receptors may be broadly applicable for the prevention of epileptogenesis.

The evidence presented suggests that epileptogenesis may be promoted when CB_1 receptor signaling is activated for several hours immediately after the initial insult. In contrast, epileptogenesis may be suppressed when CB_1 receptor activation is commenced sub-acutely and continues for longer periods (Table 1). Di Maio et al. reported that treatment with a CB_1 agonist, WIN55212-2 (2 mg/kg/day, i.p.), for 2 weeks starting 1 day after pilocarpine-induced SE (360 mg/kg i.p.) significantly reduced the number of seizures observed 1–6 months after SE [111].

CB_1 receptor expression was increased by approximately three- to fourfold in the hippocampal CA1 and dentate gyrus after amygdala kindling [112]. The role of CB_1 receptors in kindling epileptogenesis has also been examined using knockout mice or pharmacological interventions (Table 1). Examination of amygdala kindling in conditional CB_1 receptor knockout mice in forebrain principal neurons revealed that CB_1 receptors expressed in these neurons decreased the duration of afterdischarges by amygdala stimulation [112]. However, there was no difference between conditional CB_1 knockout mice and their control littermates in the number of kindling stimuli necessary for the development of generalized tonic–clonic seizures [112]. Thus, the lack of CB_1 receptor signaling in principal neurons does not affect kindling epileptogenesis. The role of CB_1 receptors in inhibitory neurons has also been investigated by crossing Dlx5-Cre mice and CB_1-floxed mice. In these crossed transgenic mice, the specific deletion of CB_1 receptors in inhibitory terminals shortened the duration of afterdischarge without affecting the development of the kindling response. These findings are in contrast with the results obtained from DGLα knockout mice that present faster kindling development [90]. In addition, both WIN 55212-2 (4 mg/kg, i.p.) [99] and 2-AG augmentation [90, 92, 93] exert suppressive effects on kindling epileptogenesis. The reasons for the phenotypic differences between mouse models with genetic manipulation of CB_1 receptor expression and those of 2-AG levels remain unclear. However, our recent results suggest that CB_2 receptors may be involved in kindling development [90]. We reported that blockade of CB_1 or CB_2 receptors alone with AM251 (20 mg/kg, i.p., twice/day) or AM630 (2 mg/kg, i.p., twice/day), respectively, did not promote epileptogenesis in the perforant path kindling model [90]. In contrast, blockade of both CB_1 and CB_2 receptors significantly reduced the number of perforant path stimuli required to develop generalized tonic–clonic seizures, which is consistent with the results obtained in DGLα knockout mice [90]. A recent study demonstrated that spontaneous seizures were present in CB_1 and CB_2 double knockout mice [113]. Importantly, CB_1 or CB_2 single knockout mice did not show spontaneous seizures, demonstrating complementary roles played by CB_1 and CB_2 receptors on the prevention of epileptogenesis [113].

Collectively, these results suggest that CB_2 and CB_1 receptor signaling act in concert to block epileptogenesis. Further studies using CB_2 receptor knockout mice and/or CB_1 and CB_2 receptor double knockout mice will be required to elucidate the precise role of each receptor in epileptogenesis.

Ictogenesis

Ictogenesis is the process of transition from the non-ictal state to a seizure. This process is often investigated in animals showing spontaneous seizures or reduced seizure thresholds. Ictogenesis is also investigated by applying chemical convulsants or electrical stimulation to non-epileptic animals or tissues.

Involvement of 2-AG (Table 1)

To clarify how 2-AG signaling influences ictogenesis, we used mouse models of acute kainate administration or acute electrical stimulation [90]. After administration of kainate (30 mg/kg, i.p.), DGLα knockout mice exhibited faster development of tonic–clonic seizures and a higher mortality rate than wild-type mice. Furthermore, afterdischarges in the dentate gyrus evoked by perforant path stimulation were longer in DGLα knockout mice than in wild-type mice. Combined administration of the CB_1 blocker AM251 (20 mg/kg, i.p.) and the CB_2 blocker AM630 (2 mg/kg, i.p.) abolished the difference in the duration of afterdischarge between DGLα knockout and wild-type mice. These results indicate that 2-AG reduces the duration of afterdischarge through both CB_1 and CB_2 receptor-dependent mechanisms. Taken together, 2-AG seems to be crucial for the suppression of seizures [90].

Contrasting results have been reported in mice with virus-mediated overexpression of MGL in excitatory neurons of the dentate hilus, CA1, and CA3 [114]. In these mice, DSE was abolished in pyramidal cells, although DSI showed no difference compared to wild-type mice. Therefore, 2-AG signaling in these mice was functionally comparable to that in mice with conditional deletion of CB_1 receptors at hippocampal excitatory synapses. In mice with MGL overexpression, no differences were observed in the severity of kainate-induced seizures (35 mg/kg i.p.), which contrasts with the aforementioned results in DGLα knockout mice

[90]. It may be possible that the difference in seizure susceptibility between the mice with MGL overexpression and DGLα knockout mice is due to different concentrations of 2-AG in hippocampal tissue. In the hippocampi of MGL-overexpressed mice, the level of 2-AG was approximately half that of control mice, while that in DGLα knockout mice was reduced to approximately 1/10 that of wild-type mice [24]. In hippocampal homogenate samples, the efficiency of G protein activation by CB_1 receptors was higher at glutamatergic synapses than at inhibitory synapses [115]. However, it has been clearly shown that the cannabinoid sensitivity of excitatory presynaptic terminals is considerably lower than that of inhibitory synaptic terminals in hippocampal slices and cultures. Therefore, DSE requires a substantially higher transient elevation of endocannabinoid levels than DSI [116]. Thus, even if DSE is absent in the hippocampus of mice with MGL overexpression, the occurrence of kainate-induced seizures may be alleviated by the remaining levels of 2-AG, which are presumably sufficient to trigger normal DSI.

The augmentation of 2-AG concentration above physiological levels can ameliorate seizures. Administration of the MGL inhibitor JZL184 (40 mg/kg, i.p.) to wild-type mice prior to kainate injection significantly reduced the incidence of tonic–clonic seizures [90]. Moreover, administration of JZL184 (40 mg/kg, i.p.) shortened the duration of afterdischarge and reduced the minimal current intensity required to elicit afterdischarges in response to electrical stimulation of the amygdala or perforant path [90, 92]. A different MGL inhibitor, SAR127303 (30 mg/kg, p.o.), suppressed seizures caused by transcorneal electrical stimulation [93]. In mice with PTZ-induced seizures (50 mg/kg, i.p.), the severity of seizures was reduced by administration of WWL123 (10 mg/kg, i.p.) [117], an inhibitor of a postsynaptic 2-AG degrading enzyme, ABHD6. This indicates that augmented 2-AG effectively suppresses seizures evoked by disinhibition of neural circuit activity by the $GABA_A$ receptor blocker PTZ. Surprisingly, as this effect was observed in CB_1 and CB_2 knockout mice, the suppressive effect on seizures seems to be independent of CB_1 or CB_2 receptor-mediated mechanisms. It has been reported that 2-AG acted directly on $GABA_A$ receptors and enhanced the inhibitory effect of GABA [117]. The blockade of ABHD6 and the resulting increase in postsynaptic 2-AG levels seem to be crucial for 2-AG-mediated enhancement of $GABA_A$ receptor signaling. Conversely, increased 2-AG levels in presynaptic terminals by the application of the MGL inhibitor SAR127303 (30 mg/kg, p.o.) did not change the minimal dose of PTZ required to induce seizures [93].

The effects of 2-AG signaling on the occurrence of spontaneous seizures in the epileptic brain have been investigated. As discussed in the previous section, expression of DGLα and CB_1 receptors may have been altered from physiological levels during the onset of spontaneous seizures. Therefore, it is necessary to investigate whether 2-AG signaling exerts similar suppressive effects on seizures in the epileptic brain. We have recently reported that the MGL inhibitor JZL184 (4 mg/kg, i.p.) has a suppressive effect on the frequency of spontaneous seizures after kainate-induced SE (0.2 μg in 100 nl saline, intra-hippocampus) [90]. Naydenov et al. (2014) investigated the R6/2 mouse strain [117], which has spontaneous seizures without any preceding treatment. Although the etiology of these seizures in the R6/2 mouse strain is unknown, they demonstrated that pharmacological blockade of ABHD6 with WWL123 (10 mg/kg, i.p.) completely suppressed the occurrence of spontaneous seizures. These results suggest that augmentation of 2-AG signaling effectively suppresses ictogenesis in the epileptic brain.

The mechanisms underlying the suppressive effect of 2-AG signaling on ictogenesis can be classified into cannabinoid receptor-dependent and -independent mechanisms. Cannabinoid receptor-dependent mechanisms consist of CB_1 receptor-mediated suppression of synaptic transmission, as well as mechanisms involving CB_2 receptor signaling. The CB_1 receptor-dependent mechanism is considered to be dominant relative to CB_2 receptor-dependent mechanisms, since the deletion of CB_1 receptors can explain the majority of the epileptic phenotypes in DGLα knockout mice [90]. The contribution of each cannabinoid receptor to seizures will be discussed in the following section. On the other hand, cannabinoid receptor-independent mechanisms include enhancement of $GABA_A$ receptor-mediated currents by 2-AG [117, 118]. In Xenopus oocytes, Sigel et al. showed that 2-AG increased GABA (1 μM)-induced currents through interaction with β2 subunits [118], indicating that 2-AG exerts allosteric modulation of GABAergic inhibition. However, this effect was not corroborated at synaptic $GABA_A$ receptors [118]. A possible explanation for the cannabinoid receptor-independent suppressive effect of 2-AG on ictogenesis is the modulation of AMPA receptor currents via the postsynaptic degradation enzyme, ABHD6. ABHD6 is integrated into the AMPA receptor channel complex as an auxiliary protein [119]. Inactivation of ABHD6 by short hairpin RNAs increased the frequency of AMPA receptor-mediated miniature excitatory post-synaptic currents (EPSCs) in hippocampal neurons [120]. Therefore, dampening AMPA receptor signaling by the binding of 2-AG to ABHD6 protein during seizures may be beneficial in epilepsy. However, application of WWL70 (10 μM), a specific inhibitor of ABHD6-mediated degradation of 2-AG, onto hippocampal slices did not affect the suppressive effect of ABHD6 on AMPA receptor-mediated miniature EPSCs [120], suggesting that 2-AG binding to ABHD6 may not alter AMPA receptor currents.

Involvement of AEA (Table 1)

The threshold of acute seizures induced by transcorneal electrical stimulation increased when AEA (300 mg/kg, i.p.) was directly administered or its degradation was blocked by the FAAH inhibitor O-1812 (5 mg/kg, i.p.) before the induction of seizures [121]. Since these suppressive effects were no longer observed when the CB_1 antagonist SR141716A (10 mg/kg, i.p.) was co-administered, the suppressive effect of AEA on seizure seems to be mediated by CB_1 receptors. Moreover, subsequent studies in rats showed that an FAAH inhibitor, AM374 (8 mg/kg, i.p.), alleviated acute seizures induced by kainate (10 mg/kg, i.p.) [122], and a different FAAH inhibitor, URB-597 (0.3–3 mg/kg, i.p.), reduced the dose of PTZ required to induce seizures [123].

Disparate results regarding the effect of AEA on seizures in FAAH knockout mice have been reported. Degradation of AEA was slower and AEA levels in the cortex, hippocampus, and cerebellum were 10 times higher in FAAH knockout mice than in wild-type mice. However, seizures induced by intraperitoneal administration of 30 mg/kg of kainate were more severe in FAAH knockout mice than in wild-type mice [101]. Interestingly, seizures did not worsen when the $GABA_A$ receptor antagonist bicuculline (4 mg/kg, i.p.) was administered to naïve FAAH knockout mice. When AEA (25 mg/kg, i.p.) was administered to FAAH knockout mice to further increase the concentration of AEA, seizures induced by intraperitoneal administration of 15 mg/kg of kainate or 4 mg/kg of bicuculline worsened. Furthermore, kainate-induced cell death was enhanced by pre-treatment with AEA. These results indicate that AEA aggravates seizures, presumably through suppression of GABA release from inhibitory presynaptic terminals. In support of this, kainate suppresses evoked inhibitory postsynaptic currents in the hippocampal CA1 region through activation of CB_1 or $GABA_B$ receptors [89]. Suppression of inhibitory postsynaptic currents by kainate (3 μM) was not blocked by the DGLα inhibitor THL (5 μM) or the MGL inhibitor JZL184 (1 μM). However, the FAAH inhibitor URB597 (1 μM) significantly prolonged the effect of kainate, suggesting that AEA causes disinhibition of hippocampal CA1 neurons after administration of kainate.

AEA acts as a partial agonist of the CB_1 receptor [42] as well as a full agonist of the TRPV1 receptor [124], a cation channel that depolarizes cells when activated. TRPV1 activation suppressed 2-AG synthesis and increased tonic inhibition through the reduction of tonic endocannabinoid signaling in CA1 pyramidal cells [125]. Moreover, it increased AMPA receptor endocytosis at excitatory synapses and decreased excitatory synaptic transmission in dentate granule cells [54]. These results suggest that TRPV1 activation suppresses activity of CA1 and dentate granule cells. However, the overall effect of TRPV1 activation seems to be pro-convulsive, since i.c.v. application of the TRPV1 agonist, capsaicin (10 or 100 μg, i.c.v.), aggravated seizures [126].

Involvement of CB_1 receptors (Table 1)

Early studies reported suppressive effects of Δ^9-THC on ictogenesis [127, 128]. However, Marsicano et al. were the first to clearly demonstrate a protective effect of CB_1 receptor signaling against acute seizures [87]. Administration of kainate (30 mg/kg, i.p.) exacerbated acute seizures in CB_1 knockout mice compared to wild-type mice. Moreover, acute seizures induced by kainate (30 mg/kg i.p.) were more severe in mice carrying CaMKII-positive neuron-specific CB_1 receptor deletion, as well as in virus-mediated conditional knockout mice carrying CB_1 deletion specifically in hippocampal excitatory neurons compared to control mice [129]. Nevertheless, there was no observable difference compared to control mice when CB_1 receptors were knocked out specifically in inhibitory neurons [129]. This indicates that CB_1 receptors in hippocampal excitatory neurons are necessary to suppress kainate-induced seizures. Overexpression of CB_1 receptors in pyramidal cells of the hippocampal CA1, CA2, and CA3 regions; and in hilar mossy cells in the dentate gyrus significantly alleviated kainate-induced SE (30 mg/kg, i.p.) [130], suggesting that CB_1 receptors at these excitatory synaptic terminals are involved in the suppression of ictogenesis. Furthermore, expression of CB_1 receptors in excitatory neurons of the cerebral cortex, hippocampus, and amygdala of global CB_1 receptor knockout mice prevented the exacerbation of seizures induced by kainate (30 mg/kg, i.p.) [131]. Taken together, CB_1 receptors at excitatory synaptic terminals in the hippocampus are crucial for the suppression of kainate-induced seizures.

Seizure and cell death induced by administration of pilocarpine (250 mg/kg, i.p.) were more severe in CB_1 knockout mice than in wild-type littermates [132]. Moreover, pretreatment of wild-type mice with the CB_1 antagonist SR141716 (10 mg/kg) exacerbated pilocarpine-induced acute seizures [132]. In contrast, administration of the CB_1 agonist WIN55212-2 (10 mg/kg, i.p.) ameliorated pilocarpine-induced seizures (350 mg/kg, i.p.) [133]. These results indicate that endocannabinoid signaling mediated by CB_1 receptors effectively suppresses seizures induced by kainate receptor activation and those induced by activation of muscarinic acetylcholine receptors with pilocarpine.

Seizures induced by electrical stimulation of the amygdala were also more severe in excitatory neuron-specific CB_1 knockout mice than in wild-type mice [112]. Conversely, seizures induced by amygdala stimulation were milder in inhibitory neuron-specific CB_1 knockout mice than in wild-type mice [112]. Moreover, augmentation of CB_1 receptor signaling effectively suppressed seizures evoked

by electrical stimulation [134, 135]. These results indicate that the activation of CB_1 receptors at excitatory synapses prevent, whereas those at inhibitory synapses exacerbate, seizures induced by electrical stimulation.

In marked contrast to seizures induced by kainate, pilocarpine, or electrical stimulation, no significant difference was found between CB_1 receptor knockout mice and wild-type littermates in the severity of disinhibition-induced seizures by administration of PTZ (70 mg/kg, i.p.) [117]. This suggests that PTZ-induced seizures do not involve CB_1 receptor signaling. Other reports have shown conflicting effects of CB_1 receptor modulation on PTZ-induced seizures. For instance, administration of a CB_1 agonist, WIN55212-2 (1 mg/kg, i.p.) or a CB_1 antagonist, ACEA (4 mg/kg i.p.) [123] exacerbated PTZ-induced seizures, whereas WIN55212-2 (10 µg, i.c.v.) was also shown to ameliorate PTZ-induced seizures [136]. At present, it is difficult to conclude whether CB_1 receptor signaling has anti- or pro-convulsive effects on PTZ-induced seizures. Future studies should aim to clarify these issues.

As discussed in the previous section, 2-AG signaling suppresses the occurrence of spontaneous seizures in the epileptic brain. In rats with pilocarpine-induced SE, the number of spontaneous seizures was significantly decreased by the endocannabinoid agonist WIN55212-2 (5 mg/kg, i.p.) and was significantly increased by the CB_1 receptor antagonist SR141716A (10 mg/kg, i.p.) [85]. In SE induced by intrahippocampal injection of kainate (0.2 µg), we showed that CB_1 receptor signaling played an important role in the suppression of ictogenesis [90]. We administered a CB_1 receptor antagonist, AM251 (20 mg/kg, i.p.), and counted the number of spontaneous seizures that occurred 2–3 weeks after SE. We observed a significant increase in the number of spontaneous seizures in AM251-treated mice compared to vehicle-treated mice. These results suggest that CB_1 receptor signaling in the epileptic brain suppresses the occurrence of spontaneous seizures.

Involvement of CB_2 receptors (Table 1)

In situ hybridization studies have reported CB_2 mRNA expression in granule cells and inhibitory interneurons of the dentate gyrus, pyramidal cells in CA3, and pyramidal cells and inhibitory interneurons in the CA1 region [61]. Acute administration of AM630 (2 mg/kg, i.p.), a CB_2 receptor antagonist, had no impact on seizures in the hippocampal dentate gyrus after electrical stimulation of the perforant path in anesthetized rats [137]. We also observed that AM630 (2 mg/kg, i.p.) alone had no effect on kainate-induced acute seizures (30 mg/kg, i.p.) in wild-type mice [90]. However, when AM630 was administered to wild-type mice pretreated with the CB_1 receptor antagonist AM251 (20 mg/kg, i.p.), or to CB_1 receptor knockout mice, kainate-induced acute seizures became more severe. These results suggest that the suppressive effect of CB_2 receptor signaling on ictogenesis may become obvious in neural circuits with increased excitability caused by the disruption of CB_1 receptor signaling. This notion is consistent with the results of a study that investigated the effects of AM630 on evoked seizures in kindled rats. Repeated PTZ administration increases susceptibility to seizures, a process termed PTZ kindling. Intraventricular administration of the CB_2 receptor antagonist AM630 (5 µg/kg) to fully kindled rats resulted in longer PTZ-induced (37.5 mg/kg, i.p.) seizures [138]. Taken together, these results suggest that CB_2 receptor signaling may be more potent at alleviating seizures when the excitability of neural circuits is higher.

In contrast, the CB_2 receptor agonist AM1241 (1 and 10 µg) increased the duration of PTZ-induced acute seizures (70 mg/kg, i.p.). This effect was blocked by pretreatment with the CB_2 receptor antagonist AM630 (1 mg/kg, i.p.), suggesting a pro-convulsant effect of CB_2 receptor signaling [139]. In this report, the incidence of generalized seizures was lower and the latency to generalized tonic–clonic seizures was longer than those of PTZ-induced seizures in other reports [117, 138]. It is, therefore, possible that CB_2 receptor signaling may be pro-epileptic in the mildly epileptic brain. However, in the latter PTZ-induced seizure models, the number of generalized tonic–clonic seizures evoked by PTZ (70 mg/kg, i.p.) was significantly higher in CB_2 knockout mice than in their wild-type littermates [117]. It is, therefore, conceivable that CB_2 receptor signaling has suppressive effects on ictogenesis in the severely epileptic brain.

Medical marijuana

There has been interest in the medical use of cannabinoids as potential antiepileptic treatments. However, evidence for the use of marijuana in the treatment of epilepsy is still insufficient. There are several excellent reviews on the medical use of marijuana and cannabinoids for epilepsy, and we direct readers to them [140, 141]. Here, we briefly summarize the possible interaction of two major cannabinoids, Δ^9-THC and cannabidiol (CBD), with endocannabinoid signaling during epileptogenesis and ictogenesis. Acute treatment with Δ^9-THC (0.25 mg/kg in cats, 20–100 mg/kg in rats, i.p.) suppresses ictogenesis [127, 142, 143]. However, subchronic treatment with Δ^9-THC for several days is sufficient to induce tolerance and a loss of suppressive effects on seizures [142, 143]. Tolerance may be caused by decreased number [144] and/or desensitization of CB_1 receptors [145] in a dose-dependent manner [146], and may reduce the suppressive effect of endocannabinoids on epileptogenesis and ictogenesis.

On the other hand, CBD has been reported to have antiepileptic effects without generating tolerance [143]. Recently, a

series of randomized, double-blind, placebo-controlled trials of CBD have been published [147, 148]. These trials demonstrated that CBD was significantly more effective than placebo in the treatment of seizures in Dravet syndrome [147] and Lennox-Gastaut syndrome [148]. However, the mechanism by which CBD suppressed seizures in these patients remains unknown. CBD has very low affinity for CB_1 and CB_2 receptors [149] and acts as an inverse agonist of these receptors when it binds to them [150]. However, CBD blocks the activity of FAAH [151] and increases serum levels of AEA in humans (200–800 mg of CBD per day) [152]. Furthermore, injection of CBD (3 nmol) into the periaqueductal gray in rats increases the concentration of 2-AG at the injection site up to 2.6-fold higher than that of vehicle-injected rats [153]. Therefore, CBD may exert its suppressive effect on seizures by increasing endocannabinoids.

Concluding remarks

Endocannabinoid signaling exerts multifaceted effects on epileptogenesis and ictogenesis. Endocannabinoid signaling promotes epileptogenesis in the initial stage and suppresses ictogenesis in already established epileptic foci as well as non-epileptic brains. The roles of endocannabinoid signaling change from pro-epileptic to anti-epileptic during the course of epileptogenesis. Homeostatic downregulation of endocannabinoid signaling molecules such as DGLα and CB_1 receptors appears to be induced by increased production of AEA acutely, and 2-AG subacutely, after initial insults. Suppression of epileptogenesis by antagonists of DGLα or CB_1 receptors during the initial period of epileptogenesis may provide novel therapeutic targets. In kindling models, presumably due to the mild seizure phenotypes compared to SE models, augmentation of 2-AG or CB_1 receptor signaling can suppress epileptogenesis. However, it should be noted that the deletion of DGLα worsens kindling epileptogenesis, while deletion of CB_1 receptors does not, suggesting possible involvement of other signaling molecules such as CB_2 and/or $GABA_A$ receptors. AEA seems to have less contribution to epileptogenesis compared to 2-AG.

As for the regulation of ictogenesis, endocannabinoid signaling at excitatory synapses has important roles in suppressing seizures. In particular, 2-AG suppresses seizures through multiple downstream signaling pathways including CB_1, and possibly CB_2 receptors. In contrast, activation of CB_1 receptors at inhibitory synapses promotes ictogenesis. On the other hand, because of its partial agonist activity at CB_1 receptors and full agonist activity at TRPV1 receptors, AEA may have a smaller suppressive effect, or may potentially even promote ictogenesis. After animals become epileptic and exhibit spontaneous seizures, augmentation of 2-AG or CB_1 receptor signaling may prove to be an effective treatment, although excessive activation of these signaling pathways may cause downregulation of CB_1 receptors and worsen seizures.

Future perspectives

Three important issues remain unresolved. First, are CB_2 receptors involved in ictogenesis and epileptogenesis; and if so, how? CB_2 receptor knockout mice showed more severe PTZ-induced seizures than wild-type mice. However, it is necessary to investigate global or conditional CB_2 receptor knockout mice in several different models of ictogenesis and epileptogenesis to determine their precise roles. Moreover, there is a paucity of specific CB_2 antibodies that reliably reflect their cellular and subcellular localizations in immunohistochemical analyses.

Second, how does the expression of CB_1 receptors increase during the course of epileptogenesis? It is unlikely that the increased expression of CB_1 receptors is a compensatory response for increased excitability, as it seems to occur predominantly at inhibitory synapses, which leads to the reduction of tonic and phasic suppression of inhibitory synaptic transmission. Although involvement of CB_1 receptor signaling and interleukin β signaling in this process has been reported [108], possible interactions between these two molecular pathways are unclear.

Third, what molecules are involved in epileptogenesis and ictogenesis? Since CB_1 receptor-mediated STD and LTD use different molecular machinery, the molecules involved in epileptogenesis may differ from those in ictogenesis. Understanding the molecular processes of epileptogenesis and ictogenesis related to endocannabinoid signaling will contribute to developing novel anti-epileptic and anti-epileptogenic drugs.

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