The CB2 receptor as a novel therapeutic target for epilepsy treatment

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Abstract: Epilepsy is characterized by repeated spontaneous reactions caused by hyper-excitability and neurons firing in high synchronization in the central nervous system. It seriously affects the quality of life of epileptic patients and nearly 30% of individuals are refractory to treatment of antiepileptic drugs. Therefore, there is an urgent need to develop new medicines to manage and control the refractory epilepsy. Cannabinoid ligands including selective cannabinoid receptor subtype (CB₁ or CB₂ receptor) ligands and non-selective cannabinoid (synthetic and endogenous) ligands may serve as the novel candidates for this need. Cannabinoid systems appear to regulating seizure activity in the brain through the activation of CB₁ and CB₂ cannabinoid receptors (CB₁R and CB₂R). An abundant series of cannabinoid analogues have been tested in various animal models, including a rat pilocarpine model of acquired epilepsy, in vitro hippocampal neuronal culture models of acquired epilepsy and status epilepticus, a pentylenetetrazole model of myoclonic seizures in mice and a penicillin-induced model of epileptiform activity in the rats. The accumulating lines of evidence show that cannabinoid ligands exhibit significant benefits to control seizure activity in different epileptic models. For this reason, we summarize the relationship between brain CB₂ receptors and seizures, and emphasize the potential mechanisms of their therapeutic effects involving affecting neurons, astrocytes, and microglia cells. The unique features of CB₂Rs, such as lower expression levels under physiological conditions and high inducibility under epileptic conditions, make it an important target for future research on drug-resistant epilepsy.

Keywords: cannabinoid receptor 2; epilepsy; cAMP, M-current; anti-inflammatory
1. Introduction

Epilepsy is the third most common chronic neurological disorder that affects over 70 million people worldwide [1]. Occurrence of epileptic seizure in brain different location may lead to loss of consciousness or intuition, motor or sensory disorders, emotional or cognitive dysfunction [2]. Despite much progress in medical treatment using antiepileptic drugs to control epileptic seizures, it still remains 30% of patients that fail to control by or respond to anti-epileptic drugs [3]. Thus, there is an urgent need to develop new medicines to manage and control the refractory epilepsy.

More understanding of underlying mechanisms in epileptogenesis has identified cellular and molecular targets for new therapies, for example, anti-inflammatory drugs that can overcome the limitations of current drugs and provide symptomatic control of epileptic seizures[4]. Accumulating data have demonstrated that cannabinoid systems, including endocannabinoids, anandamide, and 2-arachidonoyl glycerol, and their targets, the cannabinoid receptor subtype 1 (CB1R) and subtype 2 (CB2R) appear to regulate seizure activity.[5-13] The rationale for the antiepileptic effects of the cannabinoid system is that the CB1Rs (possibly also CB2Rs) are linked to an inhibitory G-protein (G\textsubscript{i/o}) signaling, which reduces neuronal excitability and/or neural synchronization. For example, the activation of brain CB1R modulates A-type K\textsuperscript{+} channels and N- and P/Q-type voltage-gated Ca\textsuperscript{2+} currents and stabilizes the membrane potentials[14, 15], and it modulates presynaptic neurotransmitter release.[16-18] Furthermore, cannabidiol has been shown to not only reduce the frequency of seizures in animal models of epilepsy but also greatly decrease the frequency of drop seizures among children and adults with Lennox–Gastaut syndrome [19]. Based on these concepts, numerous cannabinoid analogues have been examined in a variety of animal models.[5, 8, 20][21, 22][11, 23] However, although cannabinoid ligands and CB1R
agonists possess some antiepileptic effects, non-specific modulations of cannabinoid systems will limit their therapeutic use for treatment of human epilepsy because of their severe adverse effects. Therefore, significant attention is currently being directed toward the possibility of developing medicines from compounds that can selectively activate CB$_2$Rs and have important potential therapeutic applications at doses that induce little or no CB$_1$Rs.

In this review, we summarize the current state of knowledge on CB$_2$R expression and function, which could serve as an important means for modulating neuronal excitability and neuroinflammation.

2. CB$_2$R expression and inducible feature

Cannabinoid receptor type 2 (CB$_2$R) is a plasma membrane G-protein-coupled receptor that was characterized from spleen by Munro [24]. The expression and function of CB$_2$ in the brain have been debated due to early studies implying that CB$_2$Rs were deficiency in the central nerves system, since CB$_2$R mRNA contains were not measured in rat brain by using in situ hybridization [24]. In accordance with this result, northern blot analysis also failed to detect CB$_2$R mRNA in rat, mouse and human brain [25-27]. RT-PCR experiments demonstrated abundant CB$_2$R expression among peripheral immune tissues like on spleen T cells and on macrophages, but barely measurable levels in rodent brain [25, 26, 28, 29]. Little is revealed about CB$_2$Rs receptor expression in microglia, astrocytes, and astrocytomas, and the activation of these receptors affecting cellular function and activity[30]. Based on the above research, CB$_2$Rs have been classically considered as a ‘peripheral cannabinoid receptor [24, 31, 32]. Recently, this concept of CB2 deficiency within brain has been challenged along with the identification of CB$_2$Rs widespread the central nervous system (CNS), though they are expressed at lower densities than CB$_1$. Emerging evidence shows that
significant CB$_2$R mRNA can be detected by ISH in cultured granule cells among the
granule layer and Purkinje cell layer of the mouse cerebellum[33], in mouse retina[34],
and in the globus pallidus of non-human primates[35]. RT-PCR analysis has also been
applied to distinguish CB$_2$ mRNA expression in multiple brain regions, including the
retina[34], cortex[35-38], striatum[26, 38], hippocampus[35], amygdala[37, 38],
brainstem [39] and cerebellum[40]. Furthermore, two CB$_2$ isoforms CB$_{2A}$ and CB$_{2B}$
have been characterized in the rodent and human brain [36] along with a new CB$_2$
transcript that has been found in mouse and monkey B lymphocytes [41]. This suggests
the possibility that CB$_2$R expression not only exists in peripheral tissues, but also in the
brain. It has been reported that CB$_2$Rs manage a variety of important processes in
dopamine (DA)-related behaviors[42], including food intake [43-46], anxiety[37, 47],
depression[48], and schizophrenia-like behavior[38, 49]. Recent evidences emerging
from several laboratories, including ours, have indicated that brain CB$_2$Rs play a pivotal
role in the elimination of cocaine, alcohol and nicotine addiction [50-52]. Collectively,
these lines of evidence strongly suggest an important role of CB$_2$R in the
mesocorticolimbic system, as well as in various brain functions involving psychiatric,
cognitive and neurobiological activity. Comparing to CB$_1$Rs, central CB$_2$Rs display
the following unique features: (1) low expression grades, insinuating that they may not
modulate neural functions under physiological conditions; (2) high pathological
expression, meaning that under some pathological conditions (for example, addiction,
stroke, stress, schizophrenia, inflammation, anxiety), CB$_2$R expression increased in the
brain [53], suggesting the change of CB$_2$R expression/function is closely related to
various mental and neurological diseases; (3) Post-synaptic localization, CB$_2$R is
mainly expressed in neuronal somatodendritic area [54], whereas CB$_1$Rs are chiefly
expressed on neuronal terminals, especially on GABAergic terminals (presynaptic),
which leads to some opposing effects after activation by these two receptor subtypes [55]. In consideration of these features, CB2R brings out to be an important target for neuroprotection [56], and targeting CB2Rs likely provide a novel therapeutic strategy for treating neuropsychiatric and neurological diseases without typical CB1-mediated side-effects. However, to fulfill this possibility, an understanding of the functional effects of CB2Rs in brain is required. Unfortunately, the function of CB2Rs in the CNS has not been well established and studies of the functional effects of CB2Rs in neurons have ignited debate and controversy. A consensus has yet to emerge regarding the expression and function of CB2Rs in midbrain ventral tegmental area (VTA) neurons, which is the source of mesocorticolimbic dopamine (DA) signaling. In our recent study, we found that functional CB2Rs are expressed in VTA DA neurons, and the activation of these CB2Rs reduced the excitability of DA neurons through both intrinsic and synaptic mechanisms [57].

3. Drug resistance in epilepsy

The occurrence of epilepsy is usually related to abnormal release neurotransmitter, such as up-regulation of glutamate, acetylcholine, and down-regulation of GABA, serotonin. According to this concept, about 40 anti-seizure drugs are used for symptomatic treatment of epilepsy [58]. However, over one third of individuals resistant to multiple antiepileptic drug therapies. Drug-resistant epilepsy can be defined as a failure of multiple efficient treatments of tolerated, chosen, and appropriately used anti-epileptic drug guidelines [59]. Patients with drug-resistant epilepsy have increased risk of cognitive, spiritual, psychological, social functions, even death. Based on the inducement and hypothetical mechanisms of drug-resistant epilepsy, recent speculations of mechanisms of drug resistance include: 1) drug target variation: Drug
targets evoke the alteration of neurotransmitter receptors, voltage-dependent ion channels, and transporters participating in metabolic pathway involved in the metabolism of neurotransmitters [60]; 2) genetic mechanisms: The epigenome is a dynamic process, and endogenous mutations in receptor genes may be considered to cause the occurrence of drug-resistant epilepsy [61]. The roles in microbiome are also of great interest in epileptic disorders [62]. However, the underlying relationship between gut microbiota and human neuronal disorder is an intriguing topic, earlier research demonstrating the effect of linking through microbial ketogenic diets with seizure control[63]. 3) Drug target missed because most of the anti-epileptic drugs focus on the neuronal inhibition and excitation but not pay attention on the real pathogenesis caused by encephalitis or cancer. In view of patients suffer the serious harm caused by drug-resistant epilepsy, the alternative treatment methods mainly include (1) surgical treatment via removal of epileptic foci [64], with the development of epileptic area localization and imaging technology, as well as the in-depth research on the resistance mechanism of epilepsy, comprehensive treatment based on surgery will continue to be improved and promoted; (2) transcranial magnetic stimulation, the combination of transcranial magnetic stimulation (TMS) and motor cortical EEG enables biomarkers to provide cortical stimulation and suppression measures that are particularly relevant to epilepsy[65]; (3) embed stimulating electrode is applied on interference the synchronization process of abnormal nerve cells discharge [66] or provocation electrical current to the vague nerve [67]; and (4) Ketogenic diets imitate this fasting state by take-in fat as the chief fuel source therapy[68].

4. Cannabinoid system as a potential therapeutic target for treating epilepsy

4.1. Endocannabinoid system
The endocannabinoid system (ECS) is involved in regulation of excitatory and inhibitory synaptic transmission in the brain [69], and consists of two G protein-coupled receptors, CB1R and CB1R, with two known endogenous cannabinoid ligands, namely 2-arachidonoylglycerol (2-AG) and N-arachidonoylethanolamide (NAN), respectively [10,11]. In the past few years, scientists have drawn attention to using a treatment focus on ECS [70, 71]. There is already a comprehensive review indicating that the roles of ECS dysfunction-induced neuroinflammation in the epilepsy [72]. Anticonvulsant-like effects of cannabinoid receptor agonists are depended on CB1R. CB1 agonists could increase ATP-sensitive K+ channel (KATP) activation by decreasing mitochondrial ATP levels. CB1R-mediated regulation in neuronal excitability can exert antiepileptic effects [73]. Microglia are evoked by pathogens, products of damaged/inflammatory neurons, and destruction of the blood-brain barrier, as well as a diverse of chemical menace signals. However, there is also controversy about suppressing epilepsy by adjusting the activity of CB1 receptors to affect excitability. Owing to the extensive distribution and high level of CB1 in the CNS under physiological conditions develop the risk of side-effects when CB1Rs are activated and targeted.

4.2. Cannabinoid’s effects on epilepsy

Tetrahydrocannabinol (THC) and cannabidiol (CBD) have been the most researched at present, especially in the psychoactive pharmacological. THC and CBD have many similarities in structure, and their structure-activity relationship has an impact on mental activity. It has been reported that both have certain anticonvulsant effects. THC mainly activates the GPR55 receptor, in the meantime partially activating the CB1Rs and CB2Rs. However, due to the psychological effects of anxiety, such as coordination problems, slower reaction times, memory loss, anxiety and addiction. CBD can
completely alleviate these serious side effects caused by THC. Studies have reported that CBD treats the frequency of spontaneous seizures in DS mice mainly by improving the excitability of hippocampal interneurons, and the excitability of vertebral neurons in the dentate gyrus to strong depolarization stimulation is also reduced [70]. The pharmacological activity of CBD is mainly blocking the effect of GPR55 receptors to inhibit the effective effect of neurotransmission and significantly reduces the $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) mediated amplitude and frequency of induced excitatory postsynaptic currents (eEPSCs) and micro EPSCs (mEPSCs) [74]. CBD is also used clinically to treat epileptic seizures caused by Lennox-Gastaut syndrome[19]. However, the therapeutic effect of CBD on other types of epilepsy and the complete mechanism of action remain unclear. CBD also acts partial on CB$_1$R that causing drowsiness, changes in appetite, and dizziness. The peripheral side effects are mainly diarrhea. There are only indirect proofs implies that CBD could modulated endocannabinoid signaling but not promising data indicating a direct binding or interaction between CBD and CB receptors. CBD also has a partial CB$_2$R activation [75]. Negative allosteric modulator activity of CBD might interpret its action for antiepileptic and other neural disorders that provides us novel insights to develop its medical application [76]. Regarding CB$_2$R, CBD-DMH, a modification on different pharmacophoric sties, was considered to promote a conformational change in CB$_2$R which favors G-protein-dependent signaling rather than $\beta$-arrestin-dependent signaling [97]. But there are very few modulators about CB1R and CB2R reported in the literature.

4.3. CB$_2$R effects on preclinical epilepsy

Because CB$_2$Rs exhibit low expression levels in the brain under normal conditions, but are highly inducible during various disease states (including epilepsy), they appear
to be an important substrate for neuroprotection [77]. Targeting CB₂Rs will likely offer a novel therapeutic strategy for treating epileptic seizures without the typical CB₁R-mediated side effects [78, 79]. Emerging evidence has indicated that CB₂Rs are involved in epileptic activity in animal models. In an acute pentylenetetrazole (PTZ) rat seizure model, pretreatment with palmitoyl ethanolamide (PEA) increased the latency of seizure initiation and reduced the duration of seizures, and this antiepileptic effect was attenuated by CB₂R antagonist (AM630), suggesting that CB₂Rs mediate PEA’s effect [80]. In developing rats, Huizenga et al. examined the antiepileptic effects of a variety of cannabinoid ligands, and found that either combined CB₁R/CB₂R or selective CB₁R agonists exhibited antiepileptic effects in either chemo-convulsing methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate or PTZ seizure models of postnatal 10-day rats [81]. Although the CB₂R selective agonist HU-308 did not show an antiepileptic effect, the CB₂R selective antagonist AM630 did increase seizure severity [81]. In addition, a recent report showed that CB₁R knockout (KO) mice did not have an epilepsy phenotype, but co-KO of CB₁R and CB₂R caused animal epilepsy [82], suggesting that CB₂R plays a role in stabilizing the neuronal system. Recent studies have also explored the effects of modulating CB₂R activity on seizure susceptibility (Table 1). The activation of CB₂Rs decreases excitatory synaptic transmission in the CNS. The new roles for CB₂R have been identified in inducing hippocampal pyramidal cell hyperpolarization and inhibiting epileptic seizures [12]. The CB₂Rs expressed on hippocampal CA3 neurons also play a critical role in reduced neuronal excitation and oscillations [83]. WIN 55212-2, a non-selective CB receptor agonist, shows striking antiepileptic effects in a rat epileptic model [84] and CB₁R and CB₂R double-knockout mice show spontaneous or manual-evoked seizures [82]. CB₂R knockout mice including both heterozygous and homozygous exhibit enhanced
epileptic susceptibility, and a reduction in CB₂R activity is associated with increased susceptibility [85], suggesting that an absent of CB₂R contributes to rising seizure susceptibility. The administration of caryophyllene, a CB₂R agonist, was found to improve an acutely epilepsy in mouse model[86]. Collectively, these lines of evidence support the idea that the activation of CB₂Rs exhibits the antiepileptic role. On the other hand, some studies reported different responses by CB₂R agonists. For instance, HU-308 [81] and JHW133 [85] show no significant effect on mice seizure occurrence. Moreover, CB₂R agonist AM1241 increases seizure intensity in PTZ model [87]. Additionally, CB₂R antagonists AM630 and SR144528 can increase seizure susceptibility[81]. Therefore, it is likely that the alternation of CB₂R activity is able to regulate seizure susceptibility although the underlying mechanisms are still unclear. The different effects of CB₂R-mediated modulations may come from the differences in using different types of CB₂R ligands, and also be based on different experimental designs including species, epileptic model types, and dosage. We recently found that the commercially available CB₂R agonists showed different effects on pancreatic acinar cell Ca²⁺ oscillations [88]. Nevertheless, numerous lines of evidence have demonstrated that CB₂R agonists ameliorate a variety of epileptic seizures, suggesting that CB₂R is a potential therapeutic target for treating epilepsy.

4.4. CB₂R-mediated anti-epileptic effects through a reduction of neural excitability and synchronization

Recently, CB₂ mRNA expression has been detected in diverse brain areas, exemplifying the cortex [89, 90], striatum [91], hippocampus [50, 89], amygdala [89], brainstem [84], and cerebellum [92]. In general, the activation of CB₂Rs decreases excitatory synaptic transmission in the CNS [93]. Furthermore, we have proved that
CB2 mRNA is highly expressed in VTA DA neurons, and the activation of CB2Rs gives rise to a reduction in DA neuron excitability mainly via CB2R-mediated decrease of endogenous cAMP, and in turn increases M-type K^+ currents [57].

As a crucial neuro-modulatory system of the brain, the midbrain dopaminergic system plays an important role in neuronal excitability. Temporal lobe epilepsy, involving pathological erethism of the hippocampus, is associated with VTA dopamine neuron activation [94]. The abnormally high synchronous activity of neuronal firing will cause phased impulse stimulation, causing more dopamine neurons to produce impulse firing, so that the dopaminergic system is in a super-reactive state during seizures occurrence [95]. This suggests that the dopaminergic system is vital in epileptic brain, and the evidence for a relationship between epilepsy and the dopaminergic system was previously described by Rezaei [2]. Pilocarpine-induced epileptic rats exhibit a significant enhancement in activity of dopaminergic neurons [96]. In the PTZ kindling model of epilepsy in mice, dopamine neurons within the VTA display hyperactivity when compared to saline-injected as controls [97]. Anti-epileptic treatments (antiepileptic drugs or brain stimulation) are applied to down-regulate the neuronal excitability for controlling epileptic seizures.

Adenylate cyclases (ACs) produced cAMP from ATP is upon stimulation of Gs-linked G protein-coupled receptors (GPCRs), and according to the expression features of AC subtypes, causing calcium influx through plasma membrane channels in a calmodulin-dependent manner [98]. Recent studies using transcriptomic analysis show that the transcription of a set of genes related to cAMP signaling is changed in patients who suffering from drug-resistant temporal lobe epilepsy [99, 100]. When seizures occur, levels of cAMP rise in the brain. The appearance of cAMP signaling can be divided into temporary influence on neuronal excitability, including ion channel or
receptor phosphorylation [101], and long-term effects on epileptogenesis, such as effects regulated by cAMP response factor binding protein CREB [102]. By coupling to G\textsubscript{i} proteins, the activation of CB\textsubscript{2}Rs causes inhibition of AC activity and down-regulation of cAMP release[103]. 5-HA\textsubscript{1A}-CB\textsubscript{2} heteroreceptors were characterized in cortical primary cultures of neurons and 5-HT\textsubscript{1A}R-CB2 heteroreceptor complex expression and functionality are significant enhanced in brain after cerebral ischemia, especially during the neonatal term, also suggesting this heteromer is associated with NHIBD pathophysiology [104]. Therefore, CB\textsubscript{2}R activation may suppress the occurrence of epilepsy via decreasing excitability of the CNS by reducing the level of cAMP.

Most K\textsuperscript{+} channels are controlled by various physiological mediators, such as transmembrane voltage, intracellular Ca\textsuperscript{2+} and G-proteins. The roles of K\textsuperscript{+} in membrane physiology have been extensively investigated in rodent models, and the basic electrophysiological properties and bursting patterns of primate central neurons are generally similar to those reported for the rodent [105]. K\textsuperscript{+} channels are very important in regulating the intrinsic excitability of neurons, and they are the main contributor to neuronal membrane repolarization [106]. K\textsuperscript{+} channels are on behalf of a promising target for the development of novel anti-epileptic drugs and activation of the K\textsuperscript{+} channels can be used for restoring control on neuronal excitability in patients with epilepsy [107]. Kv7 (M-) channels are out of the ordinary type of K\textsuperscript{+} channel which is different from those that repolarize an individual's action potential. The M-channels are partially activated within the resting membrane potential of neurons, and is further activated by membrane depolarization. The rate of M-channel open and close is more slowly than other types of K\textsuperscript{+} channels that contribute to the repolarization of action potentials. M-channels can inhibit the highly synchronized firing of neurons that may
cause hyper-excitation through suppressing muscarinic acetylcholine receptors, bring about a great extent in cellular excitability [108]. The K⁺ channel opener retigabine is a compelling and selective opener of M-type K⁺ channels and is approved for therapy of drug-resistant focal and focal to bilateral tonic-clonic seizures [109]. In VTA DA neurons, G-protein-coupled receptor signals regulate the excitability of neurons through a few ion channels, such as G protein-gated internally rectified K⁺ channels (GIRKs) [50-52]. The CB₂R agonist JWH133 has been confirmed to effectively modulate the excitability of neurons by regulating voltage-dependent M-type K⁺ channels[57].

4.5. Glia CB₂R-mediated anti-epileptic effects via inflammation and excitability

More and more lines of evidence show that CB₂Rs are relevant to both immune cell competence at peripheral region [110], and brain cells in the CNS. Actually, neurons, microglia and astrocytes cells express CB₂Rs [30, 111, 112], which are capable of modulating central neural-immune function and impact the related diseases [111, 113]. It is critical to accentuate that CB₂R is inducible expression in a number of immune cells under activated neuroinflammatory conditions. It means CB₂R level may be up-regulated in the CNS and increased in inflamed brain parenchyma due to the invasion of peripheral immune cells (such as peripheral T cells) that express CB₂Rs [111]. During activated process, microglia increase the expression of an array of membrane surface of CB₂Rs that may be essential in microglial production and/or degeneration within the brain. Both CB₁ and CB₂ receptors were expressed on microglia using in vitro assay, including immunoglobulin superfamily receptors, cell component receptors, toll-related receptors, opioid receptors, and cannabinoid receptors [13]. A notable example is that neuropathic pain upregulates CB₂Rs in microglia in rat spinal cord, while chronic inflammatory pain does not [114]. When there is inflammation response
in the body, microglia CB$_2$R is rapidly up-regulated and activated, which effectively inhibits the release of harmful factors, including TNF-$\alpha$ and free radicals [110].

In patients with epilepsy, medial temporal lobe sclerosis, cortical dysplasia, encephalitis, and glioma, the astrocytes show increased expression of CB$_2$Rs, which may change in K$^+$ currents during seizures. It may lead to overexcitation and changes in a series of enzymatic pathways, which means that astrocytes may change the M-type K$^+$ current by adjusting the expression of CB$_2$Rs to change the intensity of epilepsy. We believe that the role of astrocyte/astrocytic CB$_2$R-cAMP signaling pathway in control epileptogenesis is worthy of further exploration.

5. Conclusions and perspective

In early studies, CB$_2$Rs were found in the peripheral region, while CB$_1$Rs were comprehensive expressed in the CNS, which leads to a question of the existence of the CB$_2$Rs in the CNS. Nowadays, with the development of more sensitive detection technologies, CB$_2$Rs have been found in multiple brain regions in the CNS though at a low level of expression compared to CB$_1$Rs. However, CB$_2$R expression and function are rapidly and profoundly increased under pathological conditions in the CNS. This attractive feature makes CB$_2$R is considered as disease-associated target, suggesting that it will greatly reduce the occurrence of CB$_1$R’s side effects through modulating the activity of CB$_2$Rs to improve the neurological disorders. Drug-resistant epilepsy seriously affects the quality of life of patients, which highlights the need to invent more effective treatments. Although the underlying mechanisms of drug-resistant epilepsy are still unclear, several novel medicines to improve drug-resistant epilepsy have been developing. One example is the CB$_1$ agonist, THC, has been replace by CBD due to its psychoactive side-effect. Epidiolex has been recently approved for the treatment of
epileptic seizures by the USA’s Food and Drug Administration (FDA) [115]. Although this shows a promise of targeting the endocannabinoid system as a novel anti-seizure treatment, some harmful side-effects including somnolence, diarrhea, appetite inhibition, and increased the level of hepatic transaminase [116] and blood pressure [117], which limit its use. Considering CB₂Rs exhibit low expression levels in the brain under normal conditions, but are highly inducible during various disease states (including epilepsy), they appear to be an important substrate for neuroprotection [77]. Targeting CB₂Rs will likely offer a novel therapeutic strategy for treating epileptic seizures without the typical CB₁R-mediated side effects [78]. Figure 1 summarizes the potential mechanisms of CB₂R activation to inhibit seizures that includes reduced neuronal excitability by down-regulating cAMP, and consequently enhanced M-currents in both neurons and astrocytes. In addition, CB₂Rs can also regulate immune function and slow down neuroinflammatory responses. Together, it symbolizes that the CB₂R may be an important target for control epileptic seizures.

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Figure legend

**Figure 1** Diagram epitomizing the CB$_2$R-associated mechanisms in modulation of epileptic seizures.