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Cannabis and cannabinoid biology in stroke: controversies, risks, and promises

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Stroke is the fifth leading cause of death and affects approximately 0.8 million Americans annually¹. The causes, duration, localization, and injury severity, as well as the presence of comorbidity factors, impact the overall outcome and likelihood of survival. The current therapeutical approach for acute ischemic stroke includes thrombolysis and mechanical thrombectomy. Reperfusion with intravenous Alteplase (recombinant tissue plasminogen activator; Activase® and Actilyse®) remains the mainstay treatment for ischemic stroke, and a recent pooled analysis of 9 clinical trials showed that this therapy is beneficial regardless of patient age and stroke severity, as long as it can be administered within 4.5 hours of onset². Although Alteplase increases the risk of early hemorrhage, the number of patients with a good outcome exceeds the ones with a fatal intracranial hemorrhage².

Nevertheless, immediate reperfusion therapy does not directly address the secondary neurological sequelae that lead to continued brain injury after stroke. Depending on injury severity, complex cascades of biochemical events are activated, generating deleterious responses, such as excitotoxicity, oxidative stress, and inflammation that affect both the central nervous system (CNS) and the overall stroke outcome. Homeostatic cellular functions governing ATP-dependent ion gradients, calcium influx, endoplasmic reticulum, and mitochondrial function, membrane stability, redox balance, and blood-brain barrier permeability all become dysfunctional after ischemic stroke³. Pharmacological interventions targeting one or more steps of this cascade have led to the development of many neuroprotective drugs over the last two decades⁴. While some of the drugs produced encouraging results, particularly when combined with Alteplase therapy, many proved ineffective in clinical trials⁵. Consequently, no neuroprotective treatment options currently

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exist to improve neurological outcome after ischemic stroke, making it imperative to conduct new research for novel therapies.

The Endocannabinoid System

In recent years, the endocannabinoid system (ECS) has become subject of great interest in neurobiology and neuropharmacology, mainly due to its predominant distribution in the CNS⁶. The ECS is composed of endocannabinoids, endogenous lipid-based retrograde neurotransmitters that bind to the cannabinoid receptors CB1 and CB2, as well as cannabinoid receptor proteins expressed throughout the CNS and peripheral nervous system (Fig. 1). Importantly, modulating the activity of the ECS turned out to hold therapeutic promise in a wide range of pathological conditions and neurological disorders⁶.

The ECS has emerged as a new therapeutic target in a variety of neurological disorders with a robust neuroinflammatory component that leads to brain tissue injury⁷. There is convincing evidence that the components of the endocannabinoid system are altered during ischemic stroke in both animals and humans, indicating that this system may contribute to the consequences of ischemic stroke⁸. Although numerous studies have examined the effects of cannabinoids and the role of the ECS in experimental models of ischemic stroke, results have been somewhat conflicting in support of either a beneficial or detrimental role. Nevertheless, a recent systematic review and meta-analysis of the currently available preclinical studies reported neuroprotective effects from a range of approaches to use of cannabinoids⁹. The main advantage of cannabinoids in neuroprotection is their broad-spectrum activity at multiple cellular and molecular mechanisms that involve not only the ECS itself but also the immune system¹⁰. Cannabinoids can limit excitotoxicity, oxidative stress, and neuroinflammation, and to enhance the trophic and metabolic support of neurons by acting through either specific cannabinoid receptor-mediated signaling pathways or via direct interactions with transcription factors. There are no previous or current clinical trials using cannabinoids in stroke, but their pleiotropic effects on the ischemic penumbra and cerebral vasculature after stroke, combined with their excellent tolerability, make them promising candidates for future treatment development.

Neurological benefits of cannabis and cannabinoid use

Cannabis (*Cannabis sativa*), also known as marijuana, has been used for centuries as a medicinal and recreational drug. It contains more than 120 different cannabinoids that have been identified, but the role and importance of most of them are yet to be fully understood¹¹. Broadly, cannabinoids are defined as phytocannabinoids, synthetic cannabinoids, and endogenous cannabinoids. The two most notable and thoroughly investigated phytocannabinoids are the delta-9-tetrahydrocannabinol (Δ^9 -THC) and cannabidiol (CBD). Studies of Δ^9 -THC and CBD led to the discovery of the two endogenous cannabinoid receptors type 1 (CB1R) and type 2 (CB2R)^{12, 13}. THC exerts its well-known psychoactive effects, including relaxation, euphoria, dreaminess, feelings of anxiety and paranoia, through the CB1R. THC has also been demonstrated to impair cognition and psychomotor performance^{14, 15}. However, recent studies have shown that THC promotes hippocampal

neurogenesis and restores memory and cognitive function in aged animals^{16, 17}. Despite the beneficial effects of THC, its psychoactive effects have limited the medical use of cannabis.

On the other hand, CBD is known as the main non-psychoactive component of cannabis and has shown anti-inflammatory, immunosuppressive, analgesic, and anxiolytic effects. In particular, CBD has a very low affinity in the micromolar range for the cannabinoid receptors and was found to be an anticonvulsant in animal models and humans with epilepsy¹⁸. Although cannabis is still listed by the U.S. Drug Enforcement Administration (DEA) as a Schedule I substance of the Controlled Substances Act, the U.S. Food and Drug Administration (FDA) has approved on June 25, 2018, the first prescription drug derived from cannabis for treating two rare and severe forms of epilepsy¹⁹. The Epidiolex® oral solution contains highly purified plant-derived CBD. In September 2018, the DEA classified Epidiolex as a Schedule V substance, clearing the final hurdle for its legal prescription in the USA. While Epidiolex is the first FDA-approved drug directly derived from the cannabis plant, it is not the first cannabinoid-based drug approved in the USA. The FDA approved three synthetic cannabinoid-based drugs: Marinol®, Syndros®, and Cesamet®. Marinol and Syndros include the active component dronabinol, a synthetic form of THC. Cesamet includes the active ingredient nabilone, which is synthetically derived and has a chemical structure similar to THC. All three synthetic compounds are prescribed to adults for the treatment of nausea and vomiting associated with chemotherapy, while Marinol and Syndros are also indicated for the treatment of anorexia associated with weight loss in AIDS patients. Although Epidiolex is currently only approved for two rare forms of epilepsy, this sets a precedent that may benefit the world of cannabinoid research.

Accumulating preclinical studies suggest that cannabinoids have significant therapeutic value in stroke. A recent systemic review and meta-analysis by England et al. demonstrated that all subclasses of cannabinoids, cannabis-derived, synthetic, specific CB1R, and CB2R agonists, significantly reduced infarct volume in transient and permanent ischemia and improve both early and late functional outcome in experimental stroke when given after stroke onset⁹. CBD showed trends to infarct reduction with delayed administration up to 6 hours after stroke onset⁹. Repeated treatment with CBD from day 1 or day 3 to day 14 improved functional outcome and survival rates, which suggest that CBD may have neuroprotective effects not only at the early phase but also at the late time point²⁰. Multiple targets have been proposed to mediate the neuroprotective effects of CBD such as a combination of a potent antioxidant, immunosuppression, and anti-inflammatory actions²¹. CBD also counteracts the cerebral hemodynamic impairment and produces beneficial cardiac effects. Sultan *et al.* conducted a systemic review and meta-analysis with 25 studies and concluded that CBD is associated with changes in hemodynamics *in vivo*²². Acute and chronic administration of CBD did not affect blood pressure or heart rate under control conditions, but reduced blood pressure and heart rate in stressful conditions. Jadoon *et al.* found that acute administration of CBD reduced resting systolic blood pressure and blunted the blood pressure response to stress in humans²³. In mouse and piglet models of stroke, CBD significantly increased cerebral blood flow^{24, 25}.

Moreover, CBD reduced brain edema and blood-brain barrier permeability associated with ischemic condition²⁶. CBD has negligible activity on cannabinoid receptors but may

interfere with the endocannabinoid system and directly or indirectly stimulate 5-hydroxytryptamine 1A receptors, adenosine receptors, transient receptor potential vanilloid subtype 1, and nuclear receptors of the peroxisome proliferator-activated receptor family²¹. THC also showed trends to infarct reduction with delayed administration up to 4 hours⁹. The neuroprotective effect of THC is related to the CB1R-mediated inhibition of voltage-sensitive calcium channels, which reduces calcium influx, excessive glutamate release, hypothermia, and enhances cerebral blood flow^{27, 28}. Also, THC, when acting on the CB2R found in the immune system, decreased the severity of stroke. Oral treatment with a low dose of THC inhibited atherosclerosis progression in a murine model of established atherosclerosis, through pleiotropic immunomodulatory effects on lymphoid and myeloid cells. CBD was also effective in diabetic complications and related atherosclerosis²⁹. Administration of the CB1R agonist HU-210 significantly reduced motor disability and infarct volume in a dose-dependent manner and was useful 4 hours after stroke onset³⁰. This neuroprotection is associated with the indirect protective effects of hypothermia.

Despite a large number of preclinical studies, there are no previous clinical trials using cannabinoids in stroke, while relevant clinical trials using cannabinoids in other neurological disorders already exist³¹. THC/CBD oromucosal spray (Sativex®) is the first cannabis-based medicine to be licensed in the U.K. and currently available in numerous countries worldwide for the treatment of multiple sclerosis-related spasticities not responded adequately to other medication³². Spasticity is common after stroke and other neurological conditions and causes significant limitations on activities of daily living³³. Importantly, a clinical trial investigating THC:CBD oromucosal spray efficacy and safety for post-stroke spasticity has been registered³⁴. Also, another relevant clinical trial was efficacy and safety evaluation of a single intravenous dose of dexanabinol (HU-211), a synthetic and non-psychoactive cannabinoid derivate, in patients suffering from severe traumatic brain injury³⁵. This phase III trial concluded that dexanabinol was safe but was not efficacious for the treatment of traumatic brain injury.

Neurological complications of cannabis and cannabinoid use

Cannabis is the most commonly produced and consumed illicit substance in the world and the contents of THC and CBD varied widely in street cannabis. As THC is the primary psychoactive component cannabinoid, cannabis users prefer the strains of the plant with higher THC content. Potter *et al.* carried out the potency of THC and CBD seized by police in England in 2005 and found that the median content of THC was significantly higher than the one recorded ten years before³⁶. In contrast, CBD content was found to be extremely low in more recent cannabis. Cascini *et al.* also performed a meta-analysis to assess the potency of THC from 1970 to 2009 and reported a temporal trend of increasing potency worldwide³⁷. These findings indicate that trends for preferring higher THC content variants carry significant health risks, particularly to those who are susceptible to its harmful effects.

The growing popularity of recreational consumption of cannabis, especially among the young population, raises immediate concerns regarding its safety. Recent case-control studies and systemic reviews have shown that cannabis use can significantly affect physical and mental health and lead to substance dependence³⁸. Mainly, ischemic stroke is the most

commonly reported adverse neurovascular effect of cannabis use in the young population. Kalla *et al.* conducted a study in patients aged 18–55 years and found that cannabis use was independently associated with a 26% increase in the risk of stroke after corrected for known risk factors, such as obesity, hypertension, smoking, and alcohol use³⁹. This study drew data from the Nationwide Inpatient Sample, which includes the health records of patients admitted over 1,000 hospitals comprising about 20% of U.S. medical centers.

Similarly, Jouanjus *et al.* conducted a systemic review with 116 cases published between January 2011 to March 2016 in patients aged 18–44 years and concluded that although cannabis use is linked to several adverse cardiovascular events, the evidence is most persuasive for ischemic stroke³⁸. Similar results were also reported from the U.S. nationwide inpatient sample, showing that recreational use of cannabis was independently associated with a 2.25-fold increase in the risk of acute ischemic stroke among aged 25–34 years⁴⁰. On the contrary, a recent population-based cohort and long-term follow-up study of 45,000 Swedish men carried out by Falkstedt *et al.* analyzed the effects of cannabis, tobacco, and alcohol use on the risk of early stroke⁴¹. They found no apparent association between cannabis use in young adult and stroke, including strokes occurring before 45 years of age. However, tobacco smoking showed a clear association with stroke. Since most studies were based on hospital discharge records, the findings may not be reflective of the general population regarding cannabis use and early stroke. Furthermore, epidemiological studies have not performed an individual analysis of patients without other cardiovascular risk factors, which may limit the estimation of the risk of stroke associated with cannabis use alone. Nevertheless, there are currently limited data, inadequate assessment of cannabis use, and few population-based studies to confirm or reject the hypothesis of the effects of cannabis use and early stroke.

It is critically important to identify all factors that may play a role in the recent increase in the incidence of stroke among the young population. One striking element reported in the majority of the case studies was a temporal relationship between cannabis use and the occurrence of stroke. However, a temporal correlation does not mean causation, and other factors may be involved. Currently, reversible cerebral vasoconstriction triggered by cannabis use may be a possible mechanism of stroke. Chronic cannabis use leads to impairment in cerebrovascular function, which has been associated with an increased risk for stroke⁴². Cannabis-related angiopathy has also been linked with ischemic stroke in heavy users⁴³. Another possible mechanism of acute ischemic stroke due to cannabis use could be an increase in pro-coagulant effects, as THC increases the expression of glycoprotein IIb-IIIa and P-selectin on human platelets in a concentration-dependent manner⁴⁴. Platelet aggregation is a significant risk factor for acute ischemic stroke⁴⁵, and it is relatively more important in younger than in older stroke patients⁴⁶. Earlier research has indicated that THC induces tachycardia⁴⁷. It has been suggested that CBD reduces THC-induced adverse cardiovascular effects⁴⁸. Jamil *et al.* reported a case of possible concurrent use of cannabis and other drugs on the development of stroke⁴⁹. Cannabis often precedes or is used along with other substances.

Besides this vascular role of cannabis in the occurrence of stroke, a cellular effect of cannabis on brain mitochondrial respiratory chain dysfunction and oxidative stress was

recently suggested as a potential mechanism involved in cannabis-related stroke⁵⁰. Indeed, despite the widespread use of cannabis, the low frequency of neurovascular complications after their use may be due to a genetic predisposition to neurovascular toxicity in some individuals.

There is still debate about the possible behavioral and pathological consequences of cannabis use. Since several questions remain unsolved, further research is still needed to assess the pathophysiological mechanisms involved in young cannabis users with stroke.

New approaches to targeting the endocannabinoid system with selective inhibitors of monoacylglycerol lipase

In recent decades, multiple lines of research have provided insights into the biochemical regulation, pathophysiological roles, and therapeutic potential of the endogenous cannabinoid 2-arachidonoylglycerol (2-AG)⁵¹. 2-AG is synthesized “on-demand” and acts as a full agonist of cannabinoid receptors, but its rapid degradation by monoacylglycerol lipase (MAGL) results in short-lived actions. Many physiological and pathological processes involve 2-AG, which behaves as a retrograde signaling lipid that inhibits neurotransmitter release at both excitatory and inhibitory synapse. Several neurological disorders with an inflammatory involvement, including ischemic stroke, show elevated levels of 2-AG⁵². Although the exact role of 2-AG is not clear, the neuroprotection exerted by exogenous 2-AG suggests that 2-AG contributes to neuroprotection not only by reducing neuronal excitotoxicity and inhibiting the production of proinflammatory cytokines and reactive oxygen species but also by lowering cerebral vasoconstriction⁵³.

Inactivation of MAGL leads to the elevation of the level of 2-AG, thus resulting in an enhancement of the endocannabinoid signaling. Beyond this critical role, the study of Nomura et al. provides compelling evidence linking both the endocannabinoid and eicosanoid signaling pathways through MAGL, which hydrolyzes 2-AG to arachidonic acid (AA)⁵⁴. AA is of particular interest as the precursor of the eicosanoid family that includes proinflammatory prostaglandins and leukotrienes. It is possible that pharmacological inhibition of MAGL might be a promising therapeutic target not only by enhancing anti-inflammatory and neuroprotective 2-AG signaling through cannabinoid receptor-dependent mechanisms but also by reducing proinflammatory eicosanoid production. Although early MAGL inhibitors had poor selectivity and low potency, they contributed significantly to advancing the understanding of the pathophysiological roles of MAGL⁵⁵. Inhibition of MAGL activity with JZL184 reduced 2-AG hydrolysis by ~85% in the mouse brain and led to dramatic elevations in brain 2-AG levels. A single dose of JZL184 was capable of inhibiting MAGL for up to 24 hours, with a maximal 8-fold elevation of brain 2-AG levels for at least 8 hours⁵⁵.

Most importantly, acute MAGL blockade with JZL184 has been shown to exhibit a wide range of beneficial effects in neurodegenerative diseases⁵⁴. Recent studies demonstrated that the MAGL inhibitor JZL184 significantly reduces infarct volume in both transient and permanent models of ischemic stroke, and improve the functional outcome when administered it after stroke onset⁵⁶. CPD-4645, a newly characterized MAGL inhibitor,

modified the transcription profile of brain vasculature and restored its functional homeostasis in the photothrombotic model of ischemic stroke⁵⁷. These results highlight a bidirectional mechanism of action – simultaneous enhancement of cannabinoid signaling through elevation of 2-AG and reduction of AA and downstream eicosanoids - that can achieve therapeutic efficacy through either cannabinoid receptor-dependent or independent mechanisms.

Over the past decades, considerable efforts were made for developing novel MAGL inhibitors with improved selectivity and cross-species activity compared to JZL184. KML29, an analog of JZL184, was the most selective for MAGL over other serine hydrolase family of enzymes⁵⁸. MJN110, another recently developed selective MAGL inhibitor, showed markedly increased potency and no significant cross-reactivity with fatty acid amide hydrolase⁵⁹. Interestingly, those MAGL inhibitors do not cause cannabimimetic activity such as catalepsy and hypothermia. Clinical research in the field of MAGL inhibitors is still at early stages. During the past five years, several academic groups and pharmaceutical companies have patented about 20 MAGL inhibitors for a large number of therapeutic uses, such as pain, inflammation, metabolic, and neurodegenerative diseases, as well as the treatment of cancer, anxiety, and epilepsy⁶⁰. Recently, a potent human MAGL inhibitor ABX1431 (Abide Therapeutics) has completed a placebo-controlled phase I for safety and tolerability⁶¹. ABX-1431 is currently subjected to phase II trials in patients with Tourette Syndrome and neuropathic pain. Although the translational potential of MAGL inhibitors still needs basic and preclinical studies, the results of ongoing and future clinical trials will hopefully unveil the MAGL inhibitors as a new drug class for the treatment of human diseases, including stroke.

Conclusions

Neurovascular complications, such as reversible cerebral vasoconstriction syndrome, intracranial hemorrhages, and ischemic strokes, and their association with cannabis and cannabinoids are an emerging area of research as more states legalize cannabis for medical and recreational use. However, several questions remain unanswered about the pathophysiological role of cannabis and cannabinoids in such complications. Epidemiological studies must provide detailed information concerning not only the quantity and the frequency of cannabis use but also the type of cannabis used. Longitudinal studies are needed in order to clarify the impact of cannabis on the severe consequences associated with their use. Despite the controversy of cannabis use, the first cannabis-derived Epidiolex® received the U.S. FDA approval as a treatment for some types of epilepsy and opened new horizons for medical use of cannabis. This approval highlights the importance of critical benefit-risk analysis and careful evaluation in the drug development process.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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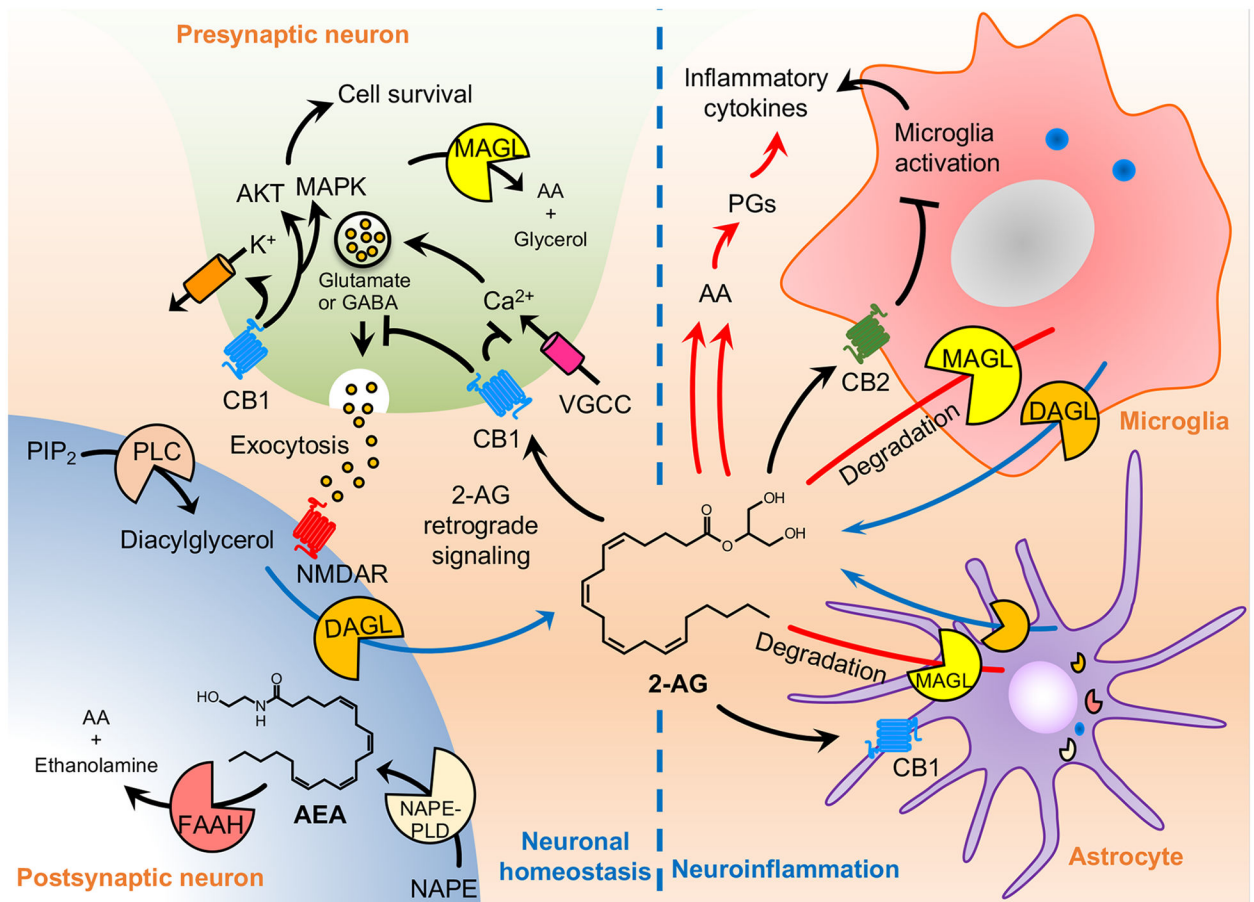


Figure 1.

Overview of biosynthesis and degradation of endocannabinoids and its multiple cellular actions in the brain. The endocannabinoid system is composed of cannabinoid receptors (CB1 and CB2), endocannabinoids (2-AG and AEA), and its synthesizing (DAGL and NAPE-PLD) and degrading enzymes (MAGL, and FAAH). CB1 receptors are abundant in the central nervous system, particularly in the cortex, basal ganglia, hippocampus, hypothalamus, and cerebellum. CB2 receptors are primarily present in microglia and immune system and are highly inducible following tissue injury or during neuroinflammation. As a lipid signaling molecule, 2-AG readily cross the membrane and activate CB1 receptors located in the presynaptic neurons. Activated CB1 receptor inhibits neurotransmitter release through the suppression of calcium influx and activates potassium (K^+) channels, as well as induces the activation of AKT and MAPK survival pathway. Although 2-AG mainly mediates retrograde endocannabinoid signaling, AEA also activates presynaptic CB1 receptors and intracellular CB1 receptors as well. 2-AG functions as the primary cannabinoid receptor signaling molecule. 2-AG is also a significant precursor for AA and therefore plays a substantial role in pro-inflammatory pathways. 2-AG is synthesized from DAG by DAGL, while AEA is synthesized from NAPE by NAPE-PLD. MAGL is the rate-limiting enzyme in the degradation of 2-AG. 2-AG also modulates the activity of microglia and astrocytes. These glial cells also are a source for brain endocannabinoids. Increased number of microglia and astrocytes are typically found in the

ischemic brain and result in increased production of proinflammatory cytokines. This process leads to a change towards a more proinflammatory milieu in the brain.

Abbreviations: 2-AG, 2-arachidonoyl glycerol; AA, arachidonic acid; AEA, *N*-aradhidonoylethanolamine; AKT, protein kinase B; CB1, cannabinoid receptor type 1; CB2, cannabinoid receptor type 2; DAGL, diacylglycerol lipase; FAAH, fatty acid amide hydrolase; GABA, gamma aminobutyric acid; MAGL, monoacylglycerol lipase; MAPK, mitogen-activated protein kinase; NAPE-PLD, *N*-arachidonoyl phosphatidyl ethanol-preferring phospholipase D; NMDAR, *N*-methyl-D-aspartate receptor; PI3K, phosphoinositide 3-kinase; PLC, phospholipase C; PIP₂, phosphatidylinositol 4,5-bisphosphate; VGCC, voltage-gated calcium channel.