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MINI-REVIEWS

Endocannabinoid System of the Blood–Brain Barrier: Current Understandings and Therapeutic Potentials

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Abstract

The endocannabinoid system (ECS) has been found at the blood–brain barrier (BBB), as Cannabinoid receptors were characterized in human brain microvascular endothelial cells and astrocytes. In several *in vitro* and *in vivo* studies, cannabinoids decreased BBB permeability and enhanced membrane integrity, which may be achieved through endothelial tight junctions and other mechanisms. These permeability regulation effects of cannabinoids suggested that the ECS may protect the brain by enhancing barrier integrity. Related questions about cannabinoid–drug interaction and drug distribution across the BBB are also raised. Specifically, can cannabinoids significantly reduce drug bioavailability to the brain? More in-depth and systematic investigations are needed to characterize and quantify these effects of cannabinoids on brain microvasculature physiopathology. Therefore, this review summarizes literatures from different disciplines to promote more research on assessing the therapeutic benefits and risks of using cannabinoids to protect BBB from dysfunctions or breakdown, and to avoid consequent brain damages due to inflammation, neurodegenerations, hemorrhage, ischemia, or other causes.

Keywords: blood–brain barrier; human brain microvascular cells; stroke; ischemia; permeability; drug interaction

Introduction

At the interface between the central nervous system (CNS) and peripheral circulation, the blood–brain barrier (BBB) not only protects the CNS from hostile chemicals and pathogens, but also transports and regulates essential nutrients, signaling molecules and immune factors. Along the complex brain capillary network, the BBB is constituted by the neurovascular unit (NVU) of microvascular endothelial cells, pericytes, and astrocytes, as well as associated microglia and neurons. NVU is the essential cellular building block that contributes to the stability and functions of vascular BBB, as a critical interface of substance exchanges and communications across CNS, cardiovascular, and immune systems. Studying the molecular mechanisms of NVU cells can reveal potential or novel therapeutic strategies.

Cannabinoid receptors have been found in brain microvascular cells and astrocytes of the NVU.[1] The endocannabinoid system at the BBB (BBB ECS) may have important functions, such as to maintain barrier integrity and regulate the transportation of important molecules under both physiological or pathological conditions.[1] Several recent reviews implied the significances of the BBB ECS.[2,3] The structures and functions of BBB ECS can be further characterized, as the recent advancements of cannabinoid research brought more insightful mechanisms, such as structure–activity relationship of cannabinoids, and more powerful methodologies, such as specific imaging probes and crystallography and cryoelectron microscopy that revealed high-resolution structures of cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2).[4,5]

This review integrates current publications and understandings of the BBB ECS, focusing on the cellular functions and molecular mechanisms of NVU, to discuss potential avenues of translating these mechanistic findings from *in vitro* and *in vivo* models to successful clinical applications.

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The BBB

Blood vessels that supply the CNS have unique structures and mechanisms to control the movement of ions, molecules, and even cells between peripheral circulation and the cerebral environment.[6] This interface, traditionally called the BBB, is essential in maintaining CNS homeostasis, which protects the CNS against infections, toxins, and the continually changing environment in the bloodstream.[7]

Most transportations of molecules across the BBB cells are strictly protected and regulated by protein transporters, and many are ATP-binding cassette transporters. For example, efflux pumps such as p-glycoprotein are highly expressed on the brain endothelial cells, actively recognizing many drug molecules, and transporting them back to the blood vessel without entering the brain through transcellular mechanisms. The paracellular movement of fluid and molecules across the BBB is restricted by adherens and tight junction (TJ) complexes between the cerebral endothelial cells.[8,9]

The NVU contributes to barrier integrity by comprehensive structures and interactions among endothelial cells, pericytes, and the end feet of surrounding astrocytes and neurons. Interactions between astrocytes and endothelial cells enhance the formation and development of the TJ complex.[10] Pericytes regulate survival, migration, differentiation, and vascular branching of the endothelial cells.[11] The interactions and coordination among these cells are important for the development and maintenance of the BBB integrity (Fig. 1).[9]

Endothelial Junctions at the BBB

Endothelial junctions, including adherens junctions (AJs) and TJs, are responsible for the highly restrictive and selective molecular permeation through BBB paracellular route.[12–14] The BBB is characterized by high expression levels of integral TJ proteins and low expression level of AJs,[15–18] but stable AJs are still needed for the existence of TJs. For example, vascular endothelial cadherin (VE-cadherin) causes phosphorylation of the transcription factor FoxO1, allowing it to activate the expression of TJ protein claudin-5.[19] AJs comprise a cluster of cadherins and associated molecules, including p120-catenin, β-catenin, and α-catenin.[20] AJ mediate endothelial cell–cell adhesion through homotypic interactions between VE-cadherins.[21] They also provide a link between transmembrane proteins and the actin cytoskeleton.[16–18]

TJs consist of integral membrane proteins, such as occludin and claudin, together with the cytoplasmic accessory proteins, such as zonula occludin ZO-1 and ZO-2.[9,22] Endothelial TJ proteins interact with the same proteins of adjacent endothelial cells to control paracellular diffusion of solutes and ions, and limit the free movement of lipids and proteins from the apical and basolateral cell surfaces, thus, contributing to the polarity of the BBB.[23]

BBB Dysfunctions or Breakdown and the Endothelial Junctions

BBB dysfunctions can be observed in neurological disorders or brain injuries, such as epilepsy, multiple sclerosis, Alzheimer's disease, hemorrhagic or ischemic stroke, and ischemia by various causes. Pathological conditions of BBB can undermine its function of protection, selective transportation, and clearance, thus can be the direct or indirect cause for these diseases.

BBB dysfunction could be complete barrier breakdown or subtle barrier impairments without manifesting end-organ damage.[7] Due to the constitution of the BBB, it is imperative to understand that BBB dysfunction involves myriad signaling cascades that affect the different components during a pathological disorder. BBB dysfunction can also be termed acute or chronic, considering the molecular mechanisms and clinical presentation during a pathological condition.[24] Acute BBB dysfunction results in cytotoxic and vasogenic edema formation, ionic imbalance that may promote seizures and inflammation. Chronic effects of BBB dysfunction are directed toward epilepsy or neuronal damages such as altered synaptic connectivity.[25,26]

BBB dysfunctions are often related to alterations in BBB structures and properties such as redistribution of TJs or leukocyte adhesion molecules.[9] *In vivo* and *in vitro* studies have indicated that endothelial junction disruption is central for the BBB breakdown. Inflammation induced by TNF-α could alter cerebral endothelial permeability in cultured human brain endothelial cells.[27] TNF-α and IL-6 downregulated ZO-1 expression and occludin/ZO-1 association, which correlates with ZO-1 phosphorylation tyrosine and threonine sites.[28]

In animal models, ischemia/reperfusion stimulates actin polymerization in brain endothelial cells through phosphorylation of myosin light chain (MLC) and Rho-associated protein kinase-enhanced postischemia MLC phosphorylation that lead to the formation of F-actin-enriched stress fibers that increase cellular tension.[29] These cytoskeletal alterations induce redistribution of junctional transmembrane proteins to the cytosol,

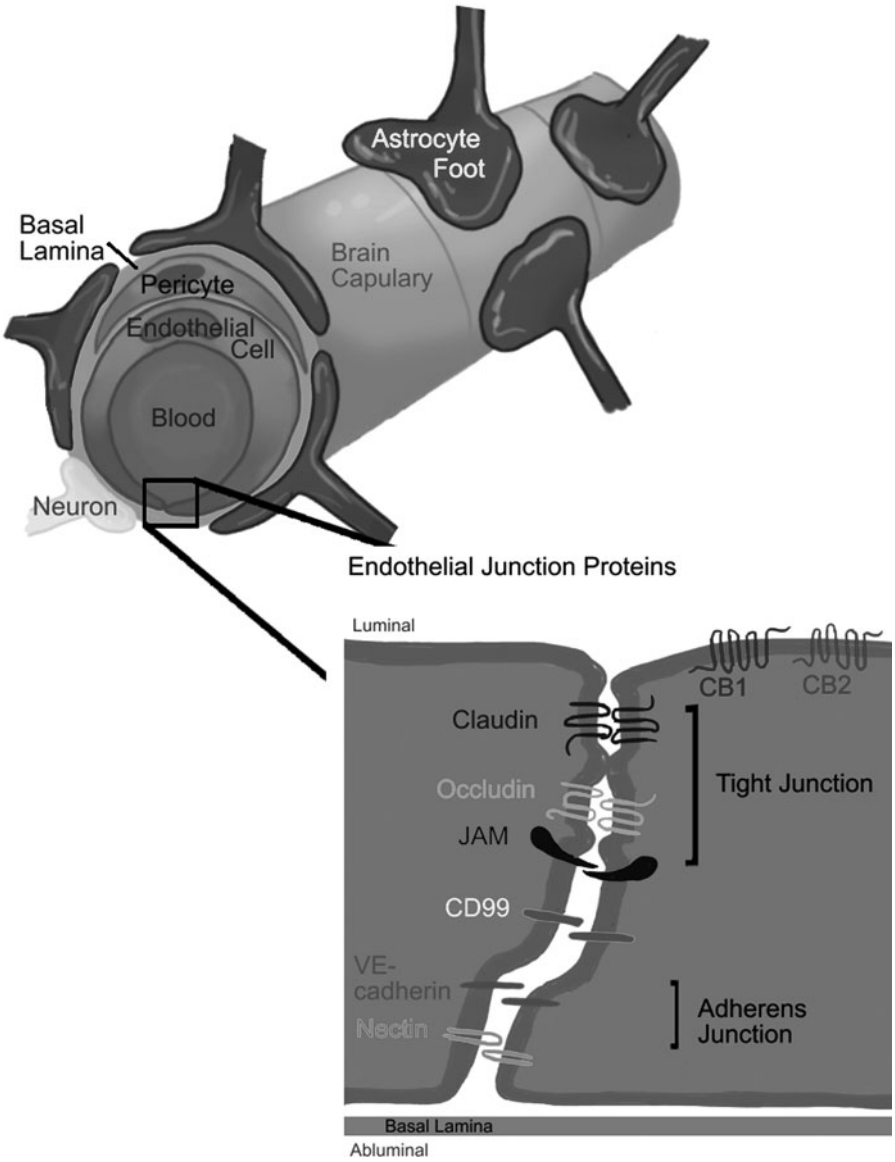

FIG. 1. NVU are comprised of endothelial cells, pericytes, and astrocytes, and associated neurons and microglia. Adherens junctions (VE-cadherin and nectin) and tight junctions (claudin, occludin, JAM), and another transmembrane protein CD99 connected to the actin cytoskeleton are located at the border of the brain microvascular endothelial cells of the BBB (as illustrated). Association of these transmembrane junctions and cytoplasmic proteins result in the high resistance across the BBB. We hypothesize that some of these tight junction molecules may be regulated through the endocannabinoid system. BBB, blood–brain barrier; JAM, junction adhesion molecule; NVU, neurovascular units; VE-cadherin, vascular endothelial cadherin.

loosening the paracellular pathway. These reports confirmed that the changing permeability of endothelial cells in the brain can be caused by redistribution of TJ that interacts between the actin cytoskeleton and other scaffolding proteins.[30]

The ECS

The identification of psychoactive Δ^9-tetrahydrocannabinol (Δ^9-THC) from cannabis[31] further led to the discovery of cannabinoid receptors and the ECS in the 1990s.

The ECS comprises cannabinoid receptor proteins, endocannabinoid (eCB) ligands, such as *N*-arachidonoylethanolamine (AEA, also called anandamide) and 2-arachidonylglycerol (2-AG), and their synthesis and degradation enzymes.[32,33] AEA is synthesized *in vivo* through hydrolysis by phospholipase D from a membrane phospholipid precursor, *N*-arachidonoyl phosphatidylethanolamine. Another important eCB in the brain, 2-AG, is synthesized mainly by diacylglycerol lipase from diacylglycerol. AEA is degraded mainly by fatty acid amide hydrolase (FAAH), and 2-AG by monoacylglycerol lipase (MAGL).[34] FAAH is a membrane-bound homodimer, and MAGL a 303-amino-acid protein ($\sim$33 kDa), both belong to the enzyme family of serine hydrolase.

MAGL has no identifiable transmembrane domain and is associated with cytosolic and particulate compartments.[35,36] These enzymes of biosynthesis and degradation may regulate ECS signaling and tone throughout the human body.[37] How these enzymes influence homeostasis and functions under pathological conditions are still an important subject of ongoing basic and clinical research.

The cannabinoid receptor family has at least two G protein-coupled receptors: CB1 and CB2.[38,39] The CB1 receptor was discovered in the CNS and is particularly abundant in specific brain areas such as basal ganglia, cerebellum, and hippocampus.[38] It is also expressed in human retina, peripheral neurons, testis, sperm cells, colonic tissues, adipocytes, and other organs such as the adrenal gland, heart, lungs, prostate, uterus, and ovary.[40–42] The CB2 receptor is abundantly expressed on immune cells, macrophages, monocytes, $CD4^+$ and $CD8^+$ T cells, and B cells.[43]

CB1 and CB2 are expressed in the brain on presynaptic and postsynaptic neuronal terminals.[44] In the brain, CB1 is also found in hippocampal astrocytes.[45] CB2 receptors are expressed at low levels in the brain under physiological conditions. However, their expression is upregulated in pathological conditions, such as traumatic brain injury (TBI), neurological diseases, stroke, or ischemia, caused by cardiovascular or pulmonary failures.[46,47]

The expressions of CB1 and CB2 in microglia change depending on the phenotype and activation profile.[48] Healthy microglia cells usually do not express CB2 receptors.[49] Studies on most primary cell cultures have demonstrated that CB2 expression are typically activated during the preparation of the cultures, hence detecting trace amounts of CB2 mRNA.[50,51] At the BBB, both CB1 and CB2 are expressed in the human brain microvascular endothelial cells (HBMECs).[1,52,53] CB1 and CB2 were also found in astrocytes.[1] Their *in vivo* expression level and distribution in other NVU cells have not been published to our knowledge. Their mechanisms of regulation and coordination need to be better characterized for a complete picture of BBB ECS, so that related functions can be further revealed.

BBB Permeability and the ECS

The presence of ECS on the cerebral microvascular endothelia provides the basis to hypothesize that ECS may influence and regulate BBB permeability.[14,16,54] A few studies have reported effects of cannabinoids on the BBB, but more systematic investigations focusing on the BBB ECS are still needed.

Table 1 summarized the cannabinoid effects on the BBB, from published *in vitro* and *in vivo* studies, and clinical trials. In 2008, Lu et al. reported that Δ^9-THC could prevent the downregulation of ZO-1, claudin-5, and junction adhesion molecule 1 in HBMEC *in vitro* model.[1] In mice models, activating CB2 receptor suppressed inflammation caused by TBI, prevented BBB damage, and attenuated expression increase of intercellular adhesion molecule 1 (ICAM-1). ICAM-1 promotes immune cell adherence to the endothelium and the transmigration caused by injury and inflammation.[55–58] CB2 selective agonist JWH133 was shown to extenuate the increasing expression of ICAM-1, thus protect the BBB integrity.[57] When another CB2 receptor agonist, JWH015, was injected to a rat model 20 min before transient spinal cord ischemia–reperfusion injury, occludin, and ZO-1 expression was upregulated at the blood–spinal cord barrier.[59]

These findings indicated that cannabinoids can regulate BBB permeability through junction protein complexes, and CB2 selective agonist could prevent TJ breakdown induced by ischemia–reperfusion injury.[59] To evaluate the barrier protection potentials of cannabinoids, as well as their impacts on the brain bioavailability of CNS drugs, these BBB regulation effects on BBB permeability need to be further characterized and quantified. Then, the questions about cannabinoid–drug interactions at the BBB also can be addressed, as both medical and recreational cannabinoid-based products become increasingly prevalent.

Discussions: ECS as Therapeutic Target to Protect BBB Integrity

Physiological processes regulated by the ECS include homeostasis, energy balance, gastrointestinal motility,

Table 1. Cannabinoid Effects on the Blood–Brain Barrier by *in vitro* and *in vivo* Models and a Published Clinical Trial

Cannabinoids	Models	Effects and mechanism
Nonselective agonist, CP 55940; selective CB1 ACEA vs. inverse agonist AM251	BBB coculture model of HBMEC and human astrocyte	Inhibition of HIV-1 Gp120-induced calcium influx mediated by substance P to decrease permeability of HBMEC and preventing downregulation of ZO-1, claudin-5, and JAM-1 in HBMEC.[1]
CBD	BBB cellular coculture model of HBMEC and human astrocyte	Preventing increase in permeability caused by 4 h OGD; most effective when administered before the OGD.[58]
Endocannabinoids of anandamide, oleoylethanolamide, PEA	BBB Cellular coculture model of HBMEC and human astrocyte	OEA, PEA decreased the OGD-induced increase in permeability during reperfusion.[15]
Selective CB2 agonist JWH133 vs. selective CB2 antagonist SR144528	Rat model of SAH	Reducing leukocyte infiltration improved neuro score, reduced water contents, and increased the ZO-1 expression, when SAH decreased.[59]
Selective CB2 agonist JWH015	Rat model of transient spinal cord ischemia	Downregulation of the expression of ICAM-1, upregulation of the expression of TJ proteins to decrease the permeability of BBB.[57]
Agonist WIN55,212-2	Mouse model of virus induced multiple sclerosis	Suppression of ICAM-1 and VCAM-1 in brain endothelium, together with a reduction in perivascular $CD4^+$ T lymphocyte infiltrates and microglial responses.[53]
Selective CB2 agonists (0-1966 and JWH133) vs. a selective CB2 antagonist	Wild-type C57BL/6 vs. CB2 knockout mice of CCI and craniotomy	Attenuation of TNF-α protein, ICAM-1 mRNA was increased at 6 h, and at 1 to 2 days after CCI, reduced in mice treated with a CB2 agonist, and increased in CB2 knockout mice with CCI.[55]
Selective CB2 agonist, 0-1966	C57BL/6 mice model of CCI	Decrease in permeability shown by reduction in NaF uptake and number of degenerating neurons. Prolonged reduction in macrophage/microglia cell counts.[56]
Selective CB2 agonist AM1241	Rat MCAo	Pretreatment with AM1241 significantly reduced brain infarction and neurological deficits.[60]
Endocannabinoid 2-AG	Mice models of closed head injury	Decreased BBB permeability and inhibited the acute expression of the main proinflammatory cytokines: TNF-a, IL-1B, and IL-6.[61]
PEA, an endocannabinoid, with Luteolin	Ischemic stroke patients; rat models	Significant improvement in neurological status, impairment of cognitive abilities, the degree of spasticity, pain, and independence in daily living.[62]

2-AG, 2-arachidonylglycerol; BBB, blood–brain barrier; CB1, cannabinoid receptor 1; CB2, cannabinoid receptor 2; CBD, cannabidiol; CCI, controlled cortical impact; HBMEC, human brain microvascular endothelial cells; ICAM-1, intercellular adhesion molecule 1; JAM-1, junction adhesion molecule 1; MCAo, model of middle cerebral artery occlusion; NaF, sodium fluorescein; OGD, oxygen and glucose deprivation; PEA, palmitoylethanolamide; SAH, subarachnoid hemorrhage; TJ, tight junction.

musculoskeletal development, cardiovascular regulation, fertility, immune functions, and CNS functions (such as synaptic plasticity, mood, memory, analgesia, pain transmission, movement, and food intake).[60] As generally recognized, the ECS regulates through two molecular signaling pathways: neurotransmitters from neurons and cytokines from the immune cells.[61] Clinically, cannabinoids have been approved as important therapeutic agents for epilepsy, vomiting, pain, and other potential CNS applications. If ECS directly regulates physical BBB permeability and protects its barrier functions, this can be another promising therapeutic paradigm of cannabinoid-based therapy for brain injuries, strokes, epilepsy, and neurodegenerative diseases.

In vitro studies using cocultures of HBMEC and astrocytes demonstrated cannabidiol's (CBDs) protective effects.[62] Besides CB1 and CB2, it was suggested that these effects of cannabinoids may also exert through other targets such as PPARγ (peroxisome proliferator-activated receptor gamma) and 5-HT_{1A} receptors, which are also expressed in brain endothelial cells.[62] Studies using animal models have shown that enhancing eCB tone offers therapeutic benefits, by either adding exogenous cannabinoids or using FAAH inhibitors that prolong the eCB half-life.[63,64] The effect of 2-AG for TBI recovery was demonstrated in mouse models. An increased level of 2-AG was observed in mouse models in the ipsilateral brain 1 and 24 h after TBI. In the same experiment, administration of additional 2-AG also resulted in reduced inflammation and edema, and improved clinical recovery through CB1-mediated mechanisms.[63] In another *in vitro* model of ischemic stroke, rats exposed to 20-min oxygen and glucose deprivation had reduced brain hippocampal injury when treated with 2-AG.[64]

Before middle cerebral artery occlusion, pretreatment of mice with CB2 agonist AM1241 significantly reduced brain infarction and neurological deficits, while delayed treatment with AM1241 offered no protective benefit.[65] In a rat model of subarachnoid hemorrhage, CB2 selective agonist JWH133 reduced leukocyte infiltration of the BBB, improved neuro score, and reduced edema, while ZO-1 expression also increased.[66] These preclinical findings point to the putative BBB protection effect through ECS under pathological conditions.

Several clinical studies have reported promising results about cannabinoids' therapeutic potential of neuroprotection from TBI or stroke. As early as 1989, a nonpsychotropic cannabinoid HU210 demonstrated neuroprotective effects in animal models, but the latter did not find significant improvements through a clinical trial that enrolled 846 patients.[67] eCB palmitoylethanolamide was tested on stroke rehabilitation patients in combination with luteolin, and reported improvements on spasticity and cognitive impairment.[68] Also, oral mucosal spray of THC/CBD is under investigation for poststroke spasticity conditions.[69] These studies focused on the neuroprotective mechanisms of cannabinoids, which may be related to the BBB ECS. Although these clinical studies focused on clinical endpoints of stroke without monitoring BBB, BBB integrity and function could be an important outcome indicator to demonstrate the clinical benefits of cannabinoids.

Also, osmotic substances, such as mannitol or hypertonic sodium chloride solutions, are used to reduce the cerebral edema and intracranial pressure during brain injury treatments. The intact BBB ensures osmotic gradient between the brain and the blood. These lifesaving therapeutics are administered intravenously. As the BBB integrity is critical to withdraw water from the intra- and extracellular brain compartment to the endovascular compartment, compromised or leaky BBB is a frequent complication, leading to cytotoxic, ionic, or vasogenic cerebral edema in overlapping phases.[70] Multiple intervention targets to protect the BBB have been proposed such as vascular endothelial growth factor, aquaporins, or ion channels.[71–73] Direct protection of the BBB through ECS may be a promising and new therapeutic strategy to support osmotic treatment.

Challenges and Opportunities

Translational research and drug development to protect BBB are challenging, partially because of the BBB differences between human and animal models. Also, the BBB is a complex system between the CNS and the vascular networks, and the immune system; therefore, result interpretation from *in vivo* models and clinical data can be challenging. In addition, due to the ubiquitous and complex nature of the ECS throughout the body, modifying ECS balance may result in multidirectional or unpredictable clinical effects. To harvest the beneficial effects without interfering other ECS-containing organs, smart drug delivery and targeting strategies are important. The *in vivo* spatial distribution and regulation of human BBB ECS should be characterized and quantified to understand their physiology and pathology.

Nevertheless, translational and clinical research has been exciting to reveal the complex ECS as "endocannabinoidome," which provides more comprehensive view that involves more endogenous cannabinoid ligands and targeting receptors, such as GPR55 and PPARs.[74] With recent methodology advancements, characterizing and investigating the whole BBB ECS is becoming feasible. Investigations focusing on NVU are foundational and essential, and should be continued to reveal ECS mechanisms in whole.

Conclusion

Pioneering studies have confirmed the presence of ECS at the BBB. Endogenous, botanical, or synthetic cannabinoids have been found to enhance BBB barrier integrity by *in vivo* and *in vitro* models. Published evidence suggested that ECS may protect and regulate the BBB integrity, probably through modifying TJ protein complexes and other mechanisms. Future human clinical trials with proper treatment strategies and BBB assessments are needed to confirm these benefits. Continuing investigations at molecular and cellular mechanisms of BBB ECS are essential to provide practical guidance on cannabinoid selection, drug delivery strategies, dosing regimen design, sex-based difference monitoring, and other details, to develop and optimize pharmacotherapy protocols through clinical trials. We believe BBB ECS research can unlock the therapeutics potentials of cannabinoids for neurodegeneration, brain injuries, strokes, and ischemia caused by cardiorespiratory arrests or other diseases in the near future.

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Abbreviations Used

Δ^9-THC = Δ^9-tetrahydrocannabinol
2-AG = 2-arachidonylglycerol
AEA = N-arachidonoylethanolamine
AJs = adherens junctions
BBB = blood–brain barrier
CB1 = cannabinoid receptor 1
CB2 = cannabinoid receptor 2
CBD = cannabidiol
CCI = controlled cortical impact
CNS = central nervous system
eCBs = endocannabinoids
ECS = endocannabinoid system
FAAH = fatty acid amide hydrolase
FoxO1 = fork head box factor 1
HBMEC = human brain microvascular endothelial cells
ICAM-1 = intercellular adhesion molecule 1
JAM-1 = junction adhesion molecule 1
MAGL = monoacylglycerol lipase
MCAo = model of middle cerebral artery occlusion
MLC = myosin light chain
NaF = sodium fluorescein
NVU = neurovascular unit
OGD = oxygen and glucose deprivation
PEA = palmitoylethanolamide
PPARγ = peroxisome proliferator-activated receptor gamma
SAH = subarachnoid hemorrhage
TBI = traumatic brain injury
TJs = tight junctions
VE-cadherin = vascular endothelial cadherins