Chapter 68

Cannabis, Cannabinoid Receptors, and Stress-Induced Excitotoxicity

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Abbreviations

2-AG 2-Arachidonoylglycerol
7TM Seven transmembrane domains
Δ^9-THC Δ^9-Tetrahydrocannabinol
AEA Anandamide
AP-1 Activator protein 1
BDNF Brain-derived neurotrophin receptor
CNS Central nervous system
COX-2 Cyclooxygenase 2
DAG-L Diacylglycerol lipase
EAAT-2 Excitatory amino acid transporter 2
ECS Endocannabinoid system
FAAH Fatty acid amide hydrolase
GPR55 G-protein-coupled receptor 55
GR Glucocorticoid receptor
HPA axis Hypothalamus–pituitary–adrenal axis
IL-1β Interleukin 1β
iNOS Inducible NO synthase
MAG-L Monoacylglycerol lipase
MAPK Mitogen-activated protein kinases
MCP-1 Monocyte chemotactic protein 1
NAPE-PLD *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D
NF-κB Nuclear factor κB
NMDA receptor *N*-methyl-D-aspartic acid receptor
nNOS Neuronal NO synthase
NO· Nitric oxide
OEA *N*-oleoylethanolamine
$ONOO^-$ Peroxynitrite anion
PEA *N*-palmitoylethanolamine
PGE_2 Prostaglandin E_2
PPARα Peroxisome proliferator-activated receptor α
PPARγ Peroxisome proliferator-activated receptor γ
PPI Prepulse inhibition test
PVN Paraventricular nucleus of hypothalamus
ROS Reactive oxygen species
TNF-α Tumor necrosis factor α
TPVR1 Transient receptor potential vanilloid 1
VCAM-1 Vascular cell adhesion protein 1

INTRODUCTION

Cannabis

"Cannabinoids" are a family of psychoactive compounds present in the plant *Cannabis sativa*, both in the leaf/flowered sprout and in the resin. Preparations of this plant have been much used throughout history, for therapeutic reasons or as part of social/religious rituals in various civilizations. However, the use of cannabis in Western societies began at the start of the nineteenth century.

C. sativa contains more that 460 different chemical compounds, and more than 70 of them are grouped under the term cannabinoids (with a common carbocyclic structure of 21 atoms). In 1964, Δ^9-tetrahydrocannabinol, the main psychoactive component of the plant, was isolated and determined.

The Endocannabinoid System

The endocannabinoid system (ECS) consists of the cannabinoid receptors, their endogenous ligands (known as endocannabinoids; eCB), the enzymes involved in the synthesis and degradation of these eCBs, and their uptake/reuptake mechanisms. The two main eCB $G_{i/o}$-protein-coupled receptors CB1R and CB2R were characterized in the early 1990s. CB1R is predominantly located in the central nervous system (CNS) but is also expressed in several peripheral tissues. CB2R is primarily expressed in peripheral immune cells, but also in some brain areas, predominantly in microglia and neural stem cells. Other receptors related to the ECS are the nuclear receptor peroxisome proliferator-activated receptor α (PPARα), which interacts with the eCB-like molecules *N*-palmitoylethanolamine (PEA) and *N*-oleoylethanolamine (OEA), regulating ingestion, pain, and inflammation; the transient receptor potential vanilloid 1 (TPVR1); and the GPR55 orphan receptor. eCBs belong to a family of polyunsaturated fatty acid derivatives that function as lipid signaling molecules released "on demand." The main eCBs are *N*-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG). AEA is produced by immune cells and neurons and it is more selective for CB1R than CB2R, whereas 2-AG acts as a full agonist for both receptors. AEA has several possible routes of synthesis, the most important

being constituted by a two-step enzyme reaction catalyzed by *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D, and the major degradation enzyme for AEA is fatty acid amide hydrolase. On the other hand, the synthesis of 2-AG is catalyzed by diacylglycerol lipase. Several enzymes can hydrolyze 2-AG, but this reaction appears to be principally catalyzed by monoacylglycerol lipase.

Cannabinoid Receptors

CB1R is predominantly located in the CNS, but it is also expressed in several peripheral tissues (e.g., adrenal glands, heart, liver, spleen, gut, and testis). The main function of CB1R is the regulation of neurotransmission, and its stimulation is responsible of the psychoactive effects derived from *C. sativa* consumed in its various preparations. At the CNS level, CB1R is mostly expressed in the basal ganglia, cerebral cortex, cerebellum, and hippocampus, but also in the brainstem, thalamic nuclei, amygdalar complex, and dorsal horn of the medulla. This distribution suggests a fundamental function for CB1R in the regulation of movement, memory, and pain. CB1R is mainly expressed in neurons and also in astrocytes, microglia, and oligodendroglia, although to a lesser extent. Neuronal CB1R expression is mainly presynaptic, in axons and nerve endings, where it regulates the release of neurotransmitters.

CB2R is primarily expressed in peripheral immune cells and organs, but also in other organic systems, such as muscle, liver, gut, testis, and adipose tissue. CB2R is also present in some types of cells of the brain, predominantly in microglia and neural stem cells. In general the expression of CB2R in the CNS is low but it is increased in glial cells in response to various pathological stimuli and conditions (e.g., dopaminergic neurotoxins, stroke, β-amyloid deposition, glioma, etc.).

The main intracellular pathways activated by CB1/CB2R lead to the inhibition of the enzyme adenylate cyclase, the control of different ionic channels (K^+ and Ca^{2+} dependent), the activation of the mitogen-activated protein kinases, and the production of nitric oxide (NO·; mainly through the regulation of the enzyme neuronal NO· synthase) and arachidonic acid (by means of the stimulation of the enzymes phospholipase D and A_2).

Other receptors related to the ECS are the nuclear receptor PPARα, which interacts with the eCB-like molecules PEA and OEA, regulating ingestion, pain, and inflammation; the TPVR1; and the GPR55 orphan receptor.

Functions of Cannabinoids

A brief description of the more relevant functions of cannabinoids, in terms of neuroprotection, follows:

Antioxidant

Mainly because of their chemical structure, some cannabinoids afford neuroprotection through the regulation of the oxidative status of a cell in potential neuropathological situations of oxidative stress (i.e., neurodegenerative pathologies). Oxidative stress could be produced by a marked increase in reactive oxygen species and/or a deficiency in the antioxidant endogenous systems, and some cannabinoids act at both levels. The cannabinoids with a higher antioxidant potential are the phytocannabinoids cannabidiol and cannabinol, or their analogues nabilone, levonantradol, and dexanabinol.

Anti-inflammatory Profile

Some cannabinoid agonists present an anti-inflammatory profile in diverse neurologic/neurodegenerative pathologies such as stroke, Parkinson, Alzheimer, and Huntington disease, mainly by the regulation of activated microglia, astroglia, and macrophages. Specifically, some CB1R and CB2R agonists such as arachidonyl-2'-chloroethylamide (ACEA), JWH-133, or WIN 55,212-2, among others, are capable of downregulating proinflammatory molecules such as cytokines (interleukin 1β (IL-1β) or tumor necrosis factor-α (TNF-α)), chemokines (monocyte chemotactic protein 1), the adhesion molecule vascular cell adhesion protein 1, nuclear factors (nuclear factor κB (NF-κB), activator protein 1), enzymes (inducible NO synthase (iNOS), cyclooxygenase-2 (COX-2)), etc.

Regulation of the Intracellular Concentration of Ca^{2+}

The rapid and massive increase in intracellular Ca^{2+} levels is a key event in the neuronal damage observed in some chronic neurodegenerative pathologies. This increase produces the depolarization of the cellular and mitochondrial membranes, precipitating their failure and potentiating the oxidative stress. These events trigger programmed neuronal death, or apoptosis. The cannabinoid agonists, acting on postsynaptic CB1R, are able to block voltage-dependent Ca^{2+} channels, inhibiting the activation of multiple intracellular cascades correlated with cellular damage/death.

Regulation of Brain Vasculature

Some CB1R agonists improve neuronal degeneration by increasing the blood supply in an episode of cerebral stroke. This vasodilatation is mediated by the inhibition of some endothelin-derived molecules, such as endothelin-1 or NO. This effect is mainly mediated by CB1R, but other authors speculate about the existence of a specific "endothelial cannabinoid receptor."

Finally, two of the most relevant functions of cannabinoids are the regulation of the activation of the hypothalamus–pituitary–adrenal (HPA) axis after stress exposure and the control of neurotransmitter metabolism (specifically glutamate). These functions will be discussed in detail in the following subsections.

ENDOCANNABINOID SYSTEM AND STRESS: A BIDIRECTIONAL RELATIONSHIP

Since 2005, increasing evidence has supported the existence of a decisive role for the ECS as a regulator of the stress response. The ECS controls the release of stress-related neurotransmitters (catecholamines, dopamine, glutamate, and γ-aminobutyric acid (GABA)) in various neuronal populations associated with stress and emotional responses. Several alterations in the elements of

the ECS have been demonstrated after stress and, in contrast, there are also changes in the stress response following modulation of eCB signaling. As an illustrative example, it has been shown that acute stress produces transient changes in the content of eCBs in the amygdala and the prefrontal cortex and, on the other hand, the activation of cannabinoid receptors prevents the activation of the HPA stress axis, as occurs in the case of the administration of the dual CB1/CB2R agonist WIN 55,212-2 in the basolateral amygdala.

CB1R and Stress

The relationship between cannabis consumption, the ECS, and the mechanisms governing the stress response are complex.

Cannabis abuse is classically described as an acute stressor, able to activate the HPA stress axis. In fact, several epidemiological studies have identified that cannabinoids affect anxiety and stress responsivity.

On the other hand, there is growing evidence showing that the activation of the HPA axis by exposure to physical, psychological, or mixed stress produces the immediate release of eCBs in a mechanism dependent on the activation of the glucocorticoid receptors. This release of eCBs activates the CB1R expressed in glutamatergic and GABAergic neurons in specific brain areas such as the prefrontal cortex, amygdala, striatum, and hypothalamus, in an attempt to restore the homeostasis of the HPA axis after stress.

Alterations in HPA axis activity under basal conditions and after stress exposure in CB1R-knockout (KO) mice have been also shown. CB1R-KO mice present increased levels of corticotrophin-releasing factor mRNA in the hypothalamic paraventricular nucleus (PVN) and an overactivity of the HPA axis negative feedback mechanism. In addition to HPA axis dysregulation, chronic excitotoxicity, and neuroinflammation, CB1R-KO mice present defective adult neurogenesis, increased anxiety, and deficiency in neuronal plasticity from decreasing brain-derived neurotrophin receptor (BDNF) levels in the hippocampus, diminished locomotor activity, reduced expression of the serotonin receptor 5-HT2C, and reactivity of raphe nuclei to stress, suggesting a possible role for CB1R in the pathophysiology of stress-related depression and its pharmacological manipulation as a potential therapeutic strategy. Based on all these alterations, CB1R-KO mice have been proposed as a suitable animal model to study depression. From a pharmacological point of view, CB1R seems to be implicated in the regulation of GABAergic neurotransmission and in the anxiolytic profile of benzodiazepines. CB1R-KO mice are also less responsive to treatment with antidepressant drugs.

CB2R and Stress

The role of CB2R in the regulation of the stress response is more controversial. Using a protocol of immobilization and acoustic stress, the increase in plasma corticosterone induced by stress is not modified by genetic (transgenic CB2xP mice overexpressing CB2R in neurons and CB2R-KO mice) or pharmacological manipulations (CB2R agonist JWH-133) of CB2R. In agreement with this, increased plasma corticosterone content elicited by systemic endotoxin administration did not change after the pharmacological modulation of CB2R.

The expression of these receptors in stress-responsive neural circuits, however, such as the hippocampus, amygdala, and hypothalamus, indirectly suggests that CB2R activation could regulate the neuroendocrine response. In fact, CB2xP mice submitted to 30 min of restraint stress presented lower levels of pro-opiomelanocortin mRNA in the arcuate nuclei than their wild-type (WT) counterparts, as well as a complete block of the stress-induced increase in the mRNA for corticotropin-releasing factor in the PVN of the hypothalamus.

Thus, more detailed neuroendocrine studies regarding the time course of synthesis and release of corticosterone and other stress hormones in stress models of different nature and duration are needed.

CB2R-KO mice presented an enhanced neuroinflammatory response in the frontal cortex after stress exposure, and deletion of the CB2R also induced schizophrenia (deficits in the prepulse inhibition test) and depression-like behaviors in mice. In addition, transgenic mice overexpressing CB2R (CB2xP) have increased levels of the neurotrophin BDNF in the hippocampus and a better response to depression behavioral tests.

STRESS AND CELLULAR DAMAGE/DEATH BY EXCITOTOXICITY

One of the initial processes that take place in the stress response is the release of excitatory amino acids (glutamate and aspartate) in some brain areas. As early as 20 min after the onset of immobilization (used as an experimental model of psychological stress in rodents), there is an immediate and sustained release of glutamate and aspartate into the synaptic cleft that reaches excitotoxic levels, indicating a role for excitatory amino acids in excitotoxic necrotic brain injury. Interestingly, in contrast to apoptotic cell death, necrotic cells swell and burst, releasing proinflammatory mediators. The increased extracellular glutamate binds to its ionotropic *N*-methyl-D-aspartic acid (NMDA) receptor, whose overactivation causes a continuous excitation of neurons. This overactivation induces further glutamate release, ATP depletion, and a dramatic increase in intracellular Ca^{2+} levels, which then activates Ca^{2+}-dependent enzymes (e.g., proteases, lipases, peroxidases) and eventually leads to neuronal death. In the particular case of restraint/immobilization stress exposure, this massive release of glutamate induces the release of proinflammatory cytokines such as TNF-α or IL-1β. Stress also activates the NF-κB pathway in a TNF-α-dependent mechanism. NF-κB activation elicits the expression and activity of proinflammatory enzymatic sources, such as iNOS and COX-2, among others. The result of this sequence of events is the accumulation of oxidative and nitrosative mediators, which can attack membrane phospholipids and cause cell damage in a process known as lipid peroxidation. Despite stress-induced production and accumulation of potentially cytotoxic and/or proinflammatory mediators like glutamate, $NO^{\bullet}$, peroxynitrite anion ($ONOO^-$), or prostaglandin E_2, various authors have discussed the possibility that some of the many changes caused

by stress response effectors are not damaging to the neurons, but in fact are predominantly beneficial to their structure and function.

This process is especially relevant, considering that uncontrolled excitotoxicity and neuroinflammation contribute to cell death and damage in neurological and neuropsychiatric diseases, including some that are related to stress exposure (neurodegenerative diseases, depression, posttraumatic stress disorder, and schizophrenia).

An important issue to consider when excitotoxicity is analyzed is the contribution of uptake mechanisms. The excitatory amino acid transporters (EAATs) are responsible for the uptake of glutamate from the synaptic cleft and, specifically, astrocyte glutamate uptake is a high-affinity process regulated by EAAT-2. Immobilization stress in rats induces a decrease in the expression of EAAT-2 in the cortex and it is plausible that stress-induced regulation of the expression of EAAT-2 may be involved in the excitotoxic effects of stress in the brain. Interestingly, this change seems to be dependent on the duration of the stress exposure and the brain area studied. Using a repeated-stress (21–40 days) model, an increase in EAAT-2 expression has been found in the hippocampus, which could be considered a counterregulatory mechanism.

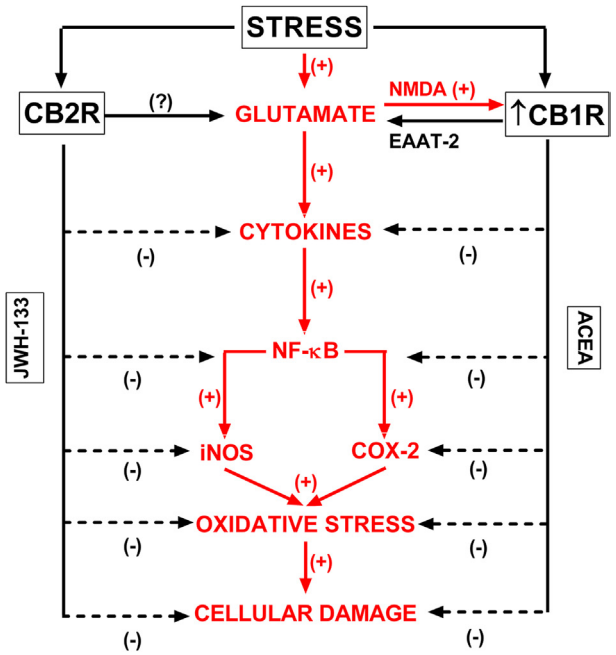

FIGURE 1 Schematic view of the regulatory role of CB1R and CB2R on stress-induced excitotoxicity, neuroinflammation, and oxidative stress damage. Stress exposure induces excitotoxicity through the activation of proinflammatory cytokines, nuclear factors, proinflammatory enzymes, and oxidative/nitrosative stress mediators in the rat prefrontal cortex. This excitotoxic/proinflammatory/oxidant pathway could be regulated by the endogenous/exogenous activation of CB1R and CB2R. NMDA, *N*-methyl-D-aspartic acid ionotropic receptor; EAAT-2, excitatory amino acid transporter 2; NF-κB, nuclear factor κB; iNOS, inducible nitric oxide synthase; COX-2, cyclooxygenase-2.

CANNABINOID RECEPTOR ACTIVATION AND STRESS-INDUCED EXCITOTOXICITY

CB1R and Excitotoxicity

A CB1R upregulation in the mouse prefrontal cortex exposed to subchronic restraint/acoustic stress in a mechanism dependent on NMDA glutamate receptors has been demonstrated. In addition, the daily administration of the potent and highly selective CB1R agonist ACEA prevented stress-induced upregulation of CB1R mRNA and protein and presented an antiexcitotoxic profile, regulating glutamate transport and uptake after stress exposure, at least in part by restoring protein expression of EAAT-2. This effect could explain, to some extent, the anti-inflammatory profile of ACEA against stress-induced NF-κB-dependent neuroinflammation (see Figure 1).

Stress-induced CB1R upregulation could represent a compensatory response aimed at controlling the massive release of glutamate into the synaptic cleft that might produce cellular damage and even death by excitotoxicity after stress exposure. CB1R activation with ACEA enhances the main glutamate uptake mechanism, by restoring protein expression of the glutamate transporter EAAT-2. Astrocyte uptake of glutamate is a high-affinity process regulated by EAAT-2; the fact that stress decreases EAAT-2 expression in the cerebral cortex indicates a specific damaging effect at this level and makes the effect of ACEA more interesting. CB1R effects on EAAT-2 have also been demonstrated in other experimental settings, such as an in vitro model of multiple sclerosis or after prenatal exposure to the dual CB1R/CB2R agonist WIN 55,212-2. It has been widely demonstrated that presynaptic CB1R inhibits glutamate release, but glutamatergic neurotransmission is also regulated by CB1R at the level of postsynaptic reuptake clearance mechanisms.

How neuronal CB1R activation enhances glutamate glial uptake may be explained by neuron–astrocyte communication that regulates the PPARγ activity in astrocytes. Notably, the EAAT-2 glutamate transporter is a target gene of PPARγ in excitotoxic-related models, such as stress exposure or stroke. As well, a study identified eCBs as PPARγ agonists affording neuroprotection targeting EAAT-2 in an in vitro model of multiple sclerosis.

In addition, several in vivo and in vitro studies have demonstrated that some cannabinoids can block neuronal death by excitotoxicity through a decrease in the release of glutamate in the presynaptic neuron. This mechanism is CB1R-dependent and affects several processes, such as motor activity, memory, and the stress response.

CB2R and Excitotoxicity

The other cannabinoid receptor type, CB2R, has been recognized as a major regulator of the immune system in the periphery, as it is highly expressed in a wide range of immune cells. However, it is now accepted that CB2R is also expressed in

the CNS, by microglia, astrocytes, and subpopulations of neurons present in brain areas related to the HPA axis, although the extent of CB2R expression in neurons remains controversial. Furthermore, CB2R is inducible in microglia under neuroinflammatory conditions, suggesting that such upregulation could be a common pattern of response against various types of chronic human neurodegenerative and neurological pathologies. Although there is still debate regarding the role of CB2R in the brain, specific functions for CB2R in neuropsychiatric conditions are emerging.

In synaptosomes, samples from the prefrontal cortex of mice exposed to subchronic restraint/acoustic stress, the administration of the selective CB2R agonist JWH-133, or the overexpression of CB2R in the transgenic mice CB2xP showed increased control levels of glutamate uptake, which were reduced by stress back to control levels. The absence of CB2R (CB2R-KO mice) did not modify the glutamate uptake by synaptosomes, compared with WT mice, under either control or stress conditions (see Figure 1). These effects were not due to changes in the protein expression of EAAT-2. However, other authors have demonstrated a role for CB2R in the regulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) excitotoxicity in in vivo and in vitro models of multiple sclerosis, through the upregulation of EAAT-2. Under stress conditions, other approaches, such as the determination of glutamate levels in the tissue, will help to show whether the effects of CB2R manipulation on glutamate uptake are due to inflammation-related actions on EAAT-2 activity or to a direct effect of the CB2R in neurons.

THERAPEUTIC POTENTIAL IN STRESS-RELATED NEUROPSYCHOPATHOLOGIES

There is increasing evidence supporting the idea that cellular damage/death produced by excitotoxicity/neuroinflammation is a main factor in the pathophysiology of neurologic/neurodegenerative diseases such as stroke, Alzheimer, Parkinson's, and Huntington's diseases. Thus, the use of cannabinoid-based compounds (i.e., Sativex©) is now in the clinical area in diverse ongoing clinical trials.

On the other hand, it is worth mentioning that the use of stress-based animal models is relevant to study possible mechanisms involved in the pathophysiology of schizophrenia and depression, bearing in mind that stress is a major contributor to the etiology and progression of psychiatric illness in its multiple clinical and subclinical manifestations. Thus, the most used animal models for depression are based on exposure to chronic stress. It has been suggested that excitotoxicity/neuroinflammation could be a cause of the pathophysiological alterations observed in particular brain structures of patients diagnosed with psychotic disease, bipolar disorder, and schizophrenia. If this is finally the case, the therapeutic use of cannabinoid-based compounds in the treatment of psychiatric diseases is promising and deserves further investigation.

Finally, "cannabinoid" treatment affords neuroprotection in various stress- and non-stress-related pathological experimental settings (see a detailed summary in Table 1).

DEFINITION OF TERMS

Excitotoxicity This is the process, by means of glutamate or related excitatory amino acids, which mediates the death of central neurons under specific conditions (e.g., stress exposure).

Neuroinflammation This is the specific immune response of the CNS against endogenous/exogenous threatening stimuli.

Oxidative stress This is a deregulation of the balance between the production of reactive oxygen species (free radicals) and the endogenous cellular antioxidant defenses

Hypothalamic–pituitary–adrenal axis This is a multifaceted set of homeostatic interactions between the hypothalamus, the pituitary gland, and the suprarenal glands aimed at regulating the response of an organism against stress exposure.

***N*-Methyl-D-aspartic acid ionotropic receptor** This is a glutamate-gated ion channel widely expressed in the CNS.

Excitatory amino acid transporter 2 This is a membrane-bound protein that is mainly localized in astroglial cells placed in presynaptic glutamatergic nerve endings, whose function is the termination of the glutamatergic neurotransmission.

Synaptosome This is the isolated synaptic terminal from a neuron that allows ex vivo studies of neurotransmitter transport.

KEY FACTS OF EXCITOTOXICITY

- Excitotoxicity is a key event in the pathophysiology of neurologic/neurodegenerative diseases such as amyotrophic lateral sclerosis, Huntington disease, or stroke.
- The "excitotoxic hypothesis" of schizophrenia has received an increasing interest in past years.
- The use of drugs capable of regulating glutamate transport and metabolism is emerging as a putative therapeutic strategy for the treatment of depression and other stress-related psychopathologies.
- In January 2015, the term "excitotoxicity" retrieved 6196 entries in PubMed and "neuroinflammation," a hot topic in biomedical sciences, a very similar number, 6770.
- In the US National Institutes of Health Web page, www.ClinicalTrials.gov, the term "glutamate" retrieved 306 clinical trials.

SUMMARY POINTS

- Excitotoxicity and neuroinflammation contribute to the pathophysiology of both neurological/neurodegenerative and neuropsychiatric diseases.
- Stress exposure elicits excitotoxicity and neuroinflammation in limbic brain areas such as the prefrontal cortex.
- The ECS is a homeostatic system that regulates the organic stress response.
- CB1R and CB2R agonists present a multifaceted neuroprotective profile regulating stress-induced excitotoxicity, neuroinflammation, and oxidative stress.
- The pharmacological manipulation of the cannabinoid receptors is a promising therapeutic strategy for the treatment of CNS pathologies that deserves further elucidation.

TABLE 1 Effects of "Cannabinoid" Treatment on Excitotoxicity

Main Effect	"Cannabinoid" Treatment	Model	References
Prevention of mitochondrial dysfunction and oxidative stress	Dual CB1R/CB2R agonist WIN 55,212-2	Quinolinic acid-induced excitotoxicity in rat striatal cultured cells and rat brain synaptosomes	Rangel-López et al. (2015)
More susceptibility to cell lost due to excitotoxic damage	–	Mutant mice lacking CB1R in glutamatergic neurons injected in the striatum with quinolinic acid	Chiarlone et al. (2014)
Increased control levels of glutamate uptake	CB2R agonist JWH-133	Prefrontal cortex synaptosomes of rats exposed to immobilization and acoustic stress	Zoppi et al. (2014)
Protected dentate gyrus granule cells and reduced the number of activated microglia	G-protein-coupled receptor 55 ligand L-α-lysophosphatidylinositol	Rat organotypic hippocampal slice cultures exposed to NMDA	Kallendrusch et al. (2013)
Decreased excitotoxicity (glutamate/*N*-acetylaspartate ratio)	CB2R activation with cannabidiol	Hypoxic–ischemic newborn pigs	Pazos et al. (2013)
Protected neuronal cells from excitotoxicity	Cannabigerol quinone (VCE-003)	Theiler murine encephalomyelitis virus (TMEV) model of multiple sclerosis (MS)	Granja et al. (2012)
Protected neuronal cells from NMDA excitotoxicity	Cannabinoid agonist HU-210	Ethanol withdrawal increased NMDA-evoked neuronal death by excitotoxicity	Rubio et al. (2011)
Prevented stress-induced decrease in glutamate uptake and EAAT-2 protein levels	CB1R agonist ACEA	Prefrontal cortex synaptosomes of rats exposed to immobilization and acoustic stress	Zoppi et al. (2011)
Reduced AMPA-induced excitotoxicity both in vivo and in vitro	Cannabinoid agonist HU-210 through CB1R and CB2R	TMEV model of MS	Docagne et al. (2007)
Increased neuronal viability	Δ^9-tetrahydrocannabinol	Hippocampal neurons in culture exposed to reduced extracellular concentrations of Mg^{2+}	Gilbert, Kim, Waataja, and Thayer (2007)
Reduced lactate dehydrogenase efflux and increased glutamate extracellular levels	ACEA, JWH-133, WIN 55,212-2	Brain slices from 7-day-old Wistar rats were exposed to oxygen–glucose deprivation for 30 min	Fernández-Lopez et al. (2006)
Increased neuronal viability	WIN 55,212-2	Murine cerebrocortical neurons in vitro and mouse cerebral cortex in vivo exposed to NMDA toxicity	Kim, Won, Mao, Jin, and Greenberg (2006)
More susceptibility to cell lost due to excitotoxicity in hippocampal pyramidal neurons	–	CB1R-KO mice exposed to kainic acid	Marsicano et al. (2003)
Attenuated cytotoxic edema	Anandamide, arvanil acting at both CB1R and VR1 receptor	Ouabain-induced in vivo excitotoxicity in rats	Veldhuis et al. (2003)
Reducing the infarct area and the number of cortical degenerating neurons	WIN 55,212-2	Unilateral intrastriatal microinjection of NMDA	Hansen et al. (2002)
Increased cellular viability	Palmitoylethanolamide	Excessive NMDA receptor stimulation of cultured mouse cerebellar granule cells	Skaper et al. (1996)

The pharmacological modulation of CB1R/CB2R reduces excitotoxic cellular damage/death in various in vivo and in vitro models of neuropathologies.

REFERENCES

Chiarlone, A., Bellocchio, L., Blázquez, C., Resel, E., Soria-Gómez, E., Cannich, A., … Guzmán, M. (2014). A restricted population of CB1 cannabinoid receptors with neuroprotective activity. *Proceedings of the National Academy of Sciences of the United States of America*, *111*(22), 8257–8262.

Docagne, F., Muñetón, V., Clemente, D., Ali, C., Loría, F., Correa, F., … Guaza, C. (2007). Excitotoxicity in a chronic model of multiple sclerosis: neuroprotective effects of cannabinoidsthrough CB1 and CB2 receptor activation. *Molecular and Cellular Neuroscience*, *34*(4), 551–561.

Fernández-López, D., Martínez-Orgado, J., Nuñez, E., Romero, J., Lorenzo, P., Moro, M. A., & Lizasoain, I. (2006). Characterization of the neuroprotective effect of the cannabinoid agonist WIN-55212 in an in vitro model of hypoxic-ischemic brain damage in newborn rats. *Pediatric Research*, *60*(2), 169–173.

Gilbert, G. L., Kim, H. J., Waataja, J. J., & Thayer, S. A. (2007). Delta9-tetrahydrocannabinol protects hippocampal neurons from excitotoxicity. *Brain Research*, *1128*(1), 61–69.

Granja, A. G., Carrillo-Salinas, F., Pagani, A., Gómez-Cañas, M., Negri, R., Navarrete, C., … Muñoz, E. (2012). A cannabigerol quinone alleviates neuroinflammation in a chronic model of multiple sclerosis. *Journal of Neuroimmune Pharmacology*, *7*(4), 1002–1016.

Hansen, H. H., Azcoitia, I., Pons, S., Romero, J., García-Segura, L. M., Ramos, J. A., … Fernández-Ruiz, J. (2002). Blockade of cannabinoid CB(1) receptor function protects against in vivo disseminating brain damage following NMDA-induced excitotoxicity. *Journal of Neurochemistry*, *82*(1), 154–158.

Kallendrusch, S., Kremzow, S., Nowicki, M., Grabiec, U., Winkelmann, R., Benz, A., … Koch, M. (2013). The G protein-coupled receptor 55 ligand l-α-lysophosphatidylinositol exerts microglia-dependent neuroprotection after excitotoxic lesion. *Glia*, *61*(11), 1822–1831.

Kim, S. H., Won, S. J., Mao, X. O., Jin, K., & Greenberg, D. A. (2006). Molecular mechanisms of cannabinoid protection from neuronal excitotoxicity. *Molecular Pharmacology*, *69*(3), 691–696.

Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., & Lutz, B. (2003). CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science*, *302*(5642), 84–88.

Pazos, M. R., Mohammed, N., Lafuente, H., Santos, M., Martínez-Pinilla, E., Moreno, E., … Martínez-Orgado, J. (2013). Mechanisms of cannabidiol neuroprotection in hypoxic-ischemic newborn pigs: role of 5HT(1A) and CB2 receptors. *Neuropharmacology*, *71*, 282–291.

Rangel-López, E., Colín-González, A. L., Paz-Loyola, A. L., Pinzón, E., Torres, I., Serratos, I. N., … Santamaría, A. (2015). Cannabinoid receptor agonists reduce the short-term mitochondrial dysfunction and oxidative stress linked to excitotoxicity in the rat brain. *Neuroscience*, *285*, 97–106.

Rubio, M., Villain, H., Docagne, F., Roussel, B. D., Ramos, J. A., Vivien, D., … Ali, C. (2011). Pharmacological activation/inhibition of the cannabinoid system affects alcohol withdrawal-induced neuronal hypersensitivity to excitotoxic insults. *PLoS One*, *6*(8), e23690.

Skaper, S. D., Buriani, A., Dal Toso, R., Petrelli, L., Romanello, S., Facci, L., & Leon, A. (1996). The ALIAmide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons. *Proceedings of the National Academy of Sciences of the United States*, *93*(9), 3984–3989.

Veldhuis, W. B., van der Stelt, M., Wadman, M. W., van Zadelhoff, G., Maccarrone, M., Fezza, F., … Di Marzo, V. (2003). Neuroprotection by the endogenous cannabinoid anandamide and arvanil against in vivo excitotoxicity in the rat: role of vanilloid receptors and lipoxygenases. *Journal of Neuroscience*, *23*(10), 4127–4133.

Zoppi, S., Pérez Nievas, B. G., Madrigal, J. L., Manzanares, J., Leza, J. C., & García-Bueno, B. (2011). Regulatory role of cannabinoid receptor 1 in stress-induced excitotoxicity and neuroinflammation. *Neuropsychopharmacology*, *36*(4), 805–818.

Zoppi, S., Madrigal, J. L., Caso, J. R., García-Gutiérrez, M. S., Manzanares, J., Leza, J. C., & García-Bueno, B. (2014). Regulatory role of the cannabinoid CB2 receptor in stress-induced neuroinflammation in mice. *British Journal of Pharmacology*, *171*(11), 2814–2826.