Mechanisms of Control of Neuron Survival by the Endocannabinoid System

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Abstract: Endocannabinoids act as retrograde messengers that, by inhibiting neurotransmitter release via presynaptic CB1 cannabinoid receptors, regulate the functionality of many synapses. In addition, the endocannabinoid system participates in the control of neuron survival. Thus, CB1 receptor activation has been shown to protect neurons from acute brain injury as well as in neuroinflammatory conditions and neurodegenerative diseases. Nonetheless, some studies have reported that cannabinoids can also exert neurotoxic actions. Cannabinoid neuroprotective activity relies on the inhibition of glutamatergic neurotransmission and on other various mechanisms, and is supported by the observation that the brain overproduces endocannabinoids upon damage. Coupling of neuronal CB1 receptors to cell survival routes such as the phosphatidylinositol 3-kinase/Akt and extracellular signal-regulated kinase pathways may contribute to cannabinoid neuroprotective action. These pro-survival signals occur, at least in part, by the cross-talk between CB1 receptors and growth factor tyrosine kinase receptors. Besides promoting neuroprotection, a role for the endocannabinoid system in the control of neurogenesis from neural progenitors has been put forward. In addition, activation of CB1 cannabinoid receptors on glial cells may also participate in neuroprotection by limiting the extent of neuroinflammation. Altogether, these findings support that endocannabinoids constitute a new family of lipid mediators that act as instructive signals in the control of neuron survival.

Key Words: Cannabinoid, endocannabinoid system, neuron, neuroprotection, neurodegeneration, neurogenesis, neuroinflammation.

INTRODUCTION

Preparations from Cannabis sativa L. (marijuana) have been used for many centuries both medicinally and recreationally. However, the chemical structure of their active components - the cannabinoids - was not elucidated until the early 1960s. Among the approximately 70 cannabinoids produced by marijuana, Δ9-tetrahydrocannabinol (THC) is the most relevant owing to its high potency and abundance [1]. Since the early-mid 1990s it is widely accepted that THC acts in the organism by mimicking endogenous substances - the cannabinoids - was not elucidated until the early 1960s. Among the approximately 70 cannabinoids produced by marijuana, Δ9-tetrahydrocannabinol (THC) is the most relevant owing to its high potency and abundance [1].

Cannabis was shown to exert neuroprotective activity upon ischemic/excitotoxic injury both in vitro [9, 10] and in vivo [11]. These observations founded a new area of research focused on the study of the eCB system as an endogenous neuroprotective system and the potential of cannabinoid compounds as new pharmacological tools for the management of various neuropathologies [12]. A number of cannabinergic drugs, including synthetic and endogenous cannabinoid receptor agonists and inhibitors of eCB transport and degradation, have been used in those studies. Just to mention a few examples, THC administration reduces neuronal loss and brain damage in excitotoxicity and ischemia models [13]. Likewise, AEA exerts a neuroprotective action...
in excitotoxicity [14, 15] and 2AG protects neurons in traumatic brain injury [16]. In addition, administration of the synthetic cannabinoid receptor agonist WIN-55,212-2 confers neuroprotection in a model of neonatal hypoxic-ischemic encephalopathy [17].

A common approach to modulate eCB levels involves the use of inhibitors of their transport and degradation, which provides a means of elevating eCB levels and therefore of activating cannabinoid receptors in a more prolonged fashion and with higher sensitivity to physiological, on-demand regulation than acute administration of cannabinoid receptor agonists [18]. In agreement with the proposed neuroprotective action of the latter agents, administration of inhibitors of eCB transport or degrading enzymes [fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL)] can prevent behavioural alterations and memory impairment due to excitotoxic damage in a CB1 receptor-dependent manner [19, 20]. A similar strategy has proved useful in attenuating emotional symptoms of anxiety and depression [21].

In addition to the aforementioned pharmacological studies, the use of CB1 receptor knock-out mice has provided further evidence for a neuroprotective role of the eCB system as those animals are more sensitive to various brain insults and neurodegenerative disorders that their corresponding wild-type controls. Thus, for example, increased excitotoxic injury is observed in CB1 receptor-deficient mice after brain stroke or kainic acid administration [22, 23]. CB1 receptors can also favour hippocampal neuron survival in the hippocampus along life, as CB1 receptor-deficient mice show an enhanced loss of neurons in CA1 and CA3 with aging, which is accompanied by a decrease in cognitive functions [24].

The eCB system exerts an important control over excessive synaptic activity in different brain areas [7]. In the context of excitotoxicity, functional CB1 receptors are present at glutamatergic terminals [25] and become activated by eCBs upon excitatory synaptic transmission, thereby preventing massive glutamate release (Fig. 1). For example, the eCB system participates in the regulation of epileptogenic circuits of the hippocampus [25] and, in excitotoxicity models induced by ionotropic glutamate receptor agonist administration, cannabinoid treatment exerts a neuroprotective action that relies, at least mostly, on a depression of glutamatergic signalling [26, 27]. Likewise, dopaminergic neurons are protected by 2AG from ischemia-induced death [28]. In this context, increased levels of different molecular species of eCBs, including AEA, other acylethanolamides and 2AG, have been determined in various models of brain injury, and this has been widely related to the involvement of those compounds in neuroprotection [12, 29-32]. Likewise, CB1 receptors have been reported to be up-regulated in stroke and excitotoxicity [33, 34]. Nonetheless, FAAH-deficient mice, which have increased brain AEA levels, exhibit increased seizure severity [35], suggesting again that the notion of eCB system-mediated neuroprotection, although generally supported, is complex and may be context-dependent.

NEUROGENIC ROLE OF THE ENDOCANNABINOID SYSTEM

The mammalian brain shows very limited capacity for self-repair, but the occurrence of neurogenesis in certain
areas of the adult brain suggests that neural progenitor cells can participate in structural and functional neurorepair. Under normal conditions, neurogenesis in the adult brain appears to be restricted to discrete germinal areas - the subventricular zone and the hippocampal dentate gyrus [36]. While some reports indicate that neurogenesis in the adult central nervous system may be more widespread than previously thought, improved characterization of these observations is required [37]. Newly generated cells mature into functional neurons in the adult mammalian brain; in the hippocampus they can participate in memory processing [38], while in the olfactory bulb they can contribute to the control of olfactory inputs [36].

Within the many signalling systems involved in the instruction of neuronal generation, e.g. trophic factors and cytokines [39], the eCB system has been recently proposed to cooperate in the regulation of neural cell development and neurogenesis [40, 41]. Cannabinoid receptors are not only expressed in differentiated neurons of many brain areas [7, 42], but they are also present in neuroblasts and neural progenitors of the adult brain [40, 41], in which they modulate cell proliferation [43-45] and differentiation [46, 47]. Regarding proliferation, CB1 receptor-deficient mice show decreased progenitor proliferation in the hippocampus and the subventricular zone [43, 44]. Regarding differentiation, the situation seems more complex as, for example, in certain pharmacological paradigms cannabinoid administration inhibits synaptogenesis via CB1 receptors [48], while in others it does not affect the absolute number of newly born hippocampal neurons [49] and in others it promotes neurogenesis and exerts anxiolytic and antidepressant effects [46].

Some studies on neurogenesis have also been conducted in genetically-modified animals. Thus, in CB1-/- and FAAH-/- adult mice no alterations are evident in hippocampal neurogenesis under basal conditions, though hippocampal astrogliogenesis is depressed in the former and enhanced in the latter animals [47]. In contrast, upon excitotoxic damage CB1 receptor-deficient mice show a strongly depressed neurogenic response [34]. In that situation, activation of CB1 receptors in neural progenitors induce cell proliferation and contribute to the generation of new neurons by promoting the production of growth factors such as fibroblast growth factor-2 (FGF-2) [34]. CB2 receptor activation can also stimulate adult neural progenitor proliferation [45, 50] and, accordingly, CB2-/- mice show lower progenitor proliferation after excitotoxicity than their wild-type controls [45, 50]. Thus, the eCB system may participate in the control of brain structural plasticity and repair by modulating neural cell proliferation and commitment, although the precise role of CB1 and CB2 receptors in neuronal specification and differentiation remains to be elucidated.

**CB1 CANNABINOID RECEPTOR-DEPENDENT NEUROPROTECTION: SIGNAL TRANSDUCTION MECHANISMS**

Besides the various non-cell autonomous processes involved in cannabinoid receptor-mediated neuroprotection, such as the CB1 receptor-dependent reduction of glutamatergic secretion (see above) and the CB2 receptor-dependent inhibition of proinflammatory-mediator release from activated microglia (see below), it is likely that neuron-cell intrinsic, CB1 receptor-triggered intracellular signal transduction events also contribute to cannabinoid neuroprotective activity. The precise nature of those mechanisms is not fully understood, but there is accruing evidence that CB1 receptor activation can couple to at least two important cell survival signalling routes: the phosphatidylinositol 3-kinase (PI3K)/Akt pathway and the extracellular signal-regulated (ERK) pathway (Fig. 2).

CB1 receptor activation evokes PI3K/Akt stimulation in primary cortical neurons [51] and in the mouse hippocampus, striatum and cerebellum in vivo [52], and this event has been related with cannabinoid-mediated neuroprotection. CB1-mediated PI3K/Akt activation is also observed in cells heterologously expressing the receptor [53] and in primary glial cells [54, 55] and astrocytoma cells lines [56, 57], and is dependent on G_{i/o} protein dissociation [53]. The downstream targets by which CB1 receptors may signal neuroprotection via Akt are as yet unclear, but one of them could be glycogen synthase kinase-3, a potentially neurotoxic protein that is phosphorylated and inactivated by Akt [52, 53].

It is well documented that cannabinoid administration leads to CB1 receptor-mediated ERK pathway activation in the brain in vivo, for example in the hippocampus [58], the striatum [59], the frontal cortex [60] and the cerebellum [61]. This process is also observed in cells heterologously expressing the receptor [62] as well as in primary glial cells [63] and astrocytoma cells lines [56, 57]. CB1 receptor-dependent ERK activation relies on G_{i/o} protein dissociation [62] and may be dependent, at least in part, on the inhibition of the adenyl cyclase/protein kinase A/cAMP pathway [58, 64, 65]. In line with the notion that ERK actions are highly promiscuous, CB1 receptor coupling to this pathway most likely signals via different context-dependent effectors. For example, induction of various transcription factors such as the early-response genes c-fos [66, 67] and Krox-24 [58, 68] and phosphorylation/activation of transcription factors such as Elk-1 [59] or other targets such as p90 ribosomal S6 kinase [54] have been implicated in CB1 receptor-evoked ERK effects in neural cells.

G_{i/o} protein-coupled receptors can conceptually activate the PI3K/Akt and ERK pro-survival pathways through G protein βγ subunit release both “directly” - via activation of targets such as class Iα PI3K isoforms - and “indirectly” - via transactivation of growth factor tyrosine kinase receptors and subsequent stimulation of the downstream “canonical” class Iα PI3K/Akt and ERK pathways [69] (Fig. 2). Although for cannabinoid receptors the former process cannot be excluded, and indeed has received some experimental support [56, 70], the latter possibility has focused much more attention. The phylogenetically ancient eCB system must have evolved simultaneously with other signalling systems and in the process established multiple levels of interactions with cell surface and intracellular proteins at the ligand, receptor and post-receptor levels. Thus, accumulating evidence suggests that receptor cross-talk may be involved in coordinat-
tors [57, 67, 71]. This activates in turn tumor necrosis factor \( \alpha \)-converting enzyme (TACE/ADAM17), a member of the disintegrin-metalloprotease family, through cytoplasmic Src family member tyrosine kinases [57]. Proteolytic ectodomain shedding of EGF-like precursors by TACE would liberate active ligands at the cell surface, thereby inducing rapid EGF receptor tyrosine phosphorylation and downstream activation of mitogenic pathways. Other studies in cell lines indicate that CB1 receptors may also transactivate vascular endothelial growth factor receptors [72].

Another connection of cannabinoids with cell survival mediators is that of neuronal CB1 receptors with the brain-derived neurotrophic factor (BDNF) signalling system. Thus, CB1 receptors are involved in BDNF production, and this process plays a pivotal role in the neuroprotective response elicited by endocannabinoids upon excitotoxic damage in brain areas such as the hippocampus and the striatum [23, 73, 74]. In addition, studies on the cooperativity of cannabinoid- and BDNF-induced migration of cortical GABAergic interneurons have revealed that CB1 receptors promote neuronal differentiation through transactivation of BDNF TrkB receptors in a Src kinase-dependent fashion [75]. Likewise, CB1 receptors might cross-talk to the bFGF signalling system. Thus, for example, increased production of bFGF may be involved CB1 receptor-mediated hippocampal neuroregenerative response after excitotoxic injury [34], and the CB1 receptor antagonist rimonabant inhibits bFGF-stimulated axonal growth [76]. Taken together, these data support that promotion of signalling through transactivation of tyrosine kinase receptors may constitute a common mechanism involved in CB1 receptor-mediated neuroprotection.

CB2 CANNABINOID RECEPTOR-DEPENDENT NEUROPROTECTION: TARGETING OF MICROGLIAL CELLS

Cannabinoids are known to improve the symptoms of several models of neuroinflammatory disorders such as experimental autoimmune encephalomyelitis (EAE) at least in part by their ability to modulate microglial cell activation [77, 78]. Here we will focus on this topic, as the ability of cannabinoids to regulate other aspects of glial cell biology in various neuropathologies is discussed elsewhere in this Special Issue. Amelioration of EAE motor symptoms can occur at different levels and involves both CB1 and CB2 receptors [79-81]. Genetic and pharmacological studies support that neuronal CB1 receptors are majorly involved in preventing inflammation-induced neuron death [79]. Nonetheless, the absence of CB2 receptors significantly exacerbates EAE pathology, and the administration of CB2 receptor-selective agonists exerts beneficial symptomatic effects [80, 82]. In

![Diagram](image-url)
this context, CB2 receptor activation decreases leukocyte rolling, leukocyte/endothelial interactions and leukocyte infiltration into the nervous system [83], contributes to the attenuation of excitotoxic damage during demyelination and ischemic injury [84, 85] and inhibits microglial activation [86]. Microglial cells express CB2 receptors (Fig. 3), which are strongly up-regulated during neuroinflammation [87] and in plaques of multiple sclerosis [88] and Alzheimer’s disease patients [89]. Microglia also synthesize and degrade eCBs such as 2AG, the levels of which are negatively controlled by the IFN-γ released by primed T-cells invading the central nervous system during EAE, thus indicating that impaired 2AG production may be associated with neurodegeneration in this disorder [90]. In addition, cannabinoids down-regulate the production of proinflammatory cytokines as well as nitrogen and oxygen reactive species by microglial cells and autoreactive T cells [86]. Overall, this attenuation of microglial activation is considered to participate in cannabinoid-induced neuroprotection under neuroinflammatory conditions and in excitotoxicity [86, 91]. Additionally, brain microglia can be replenished from bone marrow-derived progenitors during inflammation and brain injury [92, 93], and myeloid progenitors mobilize through the bloodstream and target the inflamed central nervous system in a process that is under the negative control of CB2 receptors [82]. In summary, eCBs can contribute to neuron survival by their CB2 receptor-dependent central and peripheral immunomodulatory actions (Fig. 3).

**CANNABINOID RECEPTOR-INDEPENDENT NEUROPROTECTION**

In addition to the CB receptor-dependent promotion of neuron survival, some cannabinoids can also exert neuroprotective actions by receptor-independent mechanisms. On the one hand, the intrinsic antioxidant properties of phenol-containing cannabinoids as cannabidiol and THC are well known [94, 95] and, for example, cannabidiol administration protects neurons from ischemic damage [96] and in a Parkinson’s disease model [97] independently of CB receptors. On the other hand, eCBs are actively metabolized in the brain, and the resulting products constitute a variety of bioactive lipids that may control neuron survival. For example, cyclooxygenase-2-mediated metabolism of eCB substrates [98] generates different neuroactive prostaglandins [99] and prostanoids [100], and AEA hydrolysis by FAAH yields ethanolamine, that is protective for neuroblastoma cells [101]. During brain injury, alterations in eCBs levels are not restricted to AEA and 2AG species, and other fatty acid ethanolamides – including various putative eCBs - are also affected [12]. Among them, palmitoylethanolamine exerts anti-inflammatory effects that are most likely mediated by peroxisome proliferator-activated receptor-α [102]. Microglial cells produce palmitoylethanolamide and their motility is increased after focal cerebral ischemia, a situation in which palmitoylethanolamide levels raise [31]. Hence, under certain circumstances cannabinoid-mediated neuroprotection...
comprises various components that include CB receptor-dependent and independent actions. Nonetheless, it is plausible that at least some of those receptor-independent actions will be ascribed in the future to membrane receptors such as GPR55 - recently proposed as a new metabotropic cannabinoid receptor [103] - or intracellular targets such as peroxisome proliferator-activated receptors α and γ [104].

**CANNABINOID-INDUCED NEUROTOXICITY**

In contrast to the large number of reports showing cannabinoid-mediated neuroprotection, some other studies have shown that high eCB levels can be deleterious to neurons. Thus, for example, AEA can induce brain damage by a mechanism that depends, at least in part, on the activation of TRPV1 vanilloid receptors [105, 106]. It is therefore conceivable that, besides the intrinsic differences in the pharmacokinetics of synthetic, plant-derived and endogenous cannabinoid agonists, an important factor that determines the balance of protective versus toxic actions of these compounds is their distinct ability to engage (neuroprotective) metabotropic CB₁ receptors or (neurotoxic) ionotropic TRPV1 receptors [105, 107]. Nonetheless, the situation may be more complex as, under certain experimental conditions, administration of the CB₁ receptor antagonist SR141716 (rimonabant) has been shown to exert neuroprotection from excitotoxic/ischemic damage [108-110]. Various cell signaling mechanisms have been proposed for cannabinoid-evoked neuron killing, including prostanoid synthesis and generation of free radicals by cyclooxygenase [111], stimulation of the pro-apoptotic c-Jun N-terminal kinase [112] and p38 mitogen-activated protein kinase [113] cascades, activation of calpains [106, 114] and increase of p53-dependent lysosomal permeability [115]. Finally, different cannabinoid agonists are known to induce cyclooxygenase-2 expression [116, 117], which may also contribute to the final balance between neuronal protection and death.

**CONCLUDING REMARKS**

Research conducted during the last decade and summarized here provides support for a neuroprotective action of cannabinoids in which various cells types and both CB₁ and CB₂ receptors are most likely involved (Fig. 4). However, while in several animal models cannabinoids have shown promising symptomatic relief together with decreased neuron damage, data from human studies are still very scarce, except perhaps for the alleviation of multiple sclerosis-associated symptoms such as spasticity, tremor, neuropathic pain and nocturia. Besides exerting neuroprotection by acting on differentiated neural cells, the functionality of the eCB system in neurogenic areas prompts the study of its potential contribution to endogenous processes of neurorepair. This may be of importance not only in the adult brain but perhaps more in the younger, immature brain, which possesses higher plasticity and thus potential of repair. Much

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**Fig. (4). Summary of cannabinoid receptor-dependent processes involved in neuroprotection.** Cannabinoid receptors and their endogenous ligands are present in undifferentiated and differentiated neural cells. Neurons can be protected by cannabinoids through a combination of cell-autonomous and indirect actions. Cannabinoids also promote astrocyte and oligodendrocyte survival and inhibit microglial activation. In addition, the eCB system controls neural progenitor cell proliferation and lineage commitment. See text for further details.
more research is nonetheless required to define in detail the molecular mechanisms of the control of neuron generation and survival by the eCB system and the pathophysiological consequences of cannabinoid-evoked neuroprotection.

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ABBREVIATIONS

AEA = N-arachidonoylthelanolamine (anandamide)
2AG = 2-Arachidonoylglycerol
BDNF = Brain-derived neurotrophic factor
FGF-2 = Fibroblast growth factor-2
eCB = Endocannabinoid
EAE = Experimental autoimmune encephalomyelitis
EGF = Epidermal growth factor
ERK = Extracellular signal-regulated kinase
FAAH = Fatty acid amide hydrolase
MAGL = Monoacylglycerol lipase
PI3K = Phosphatidylinositol 3-kinase
THC = Δ9-Tetrahydrocannabinol

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