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Abstract: Abstract
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UNIVERSITA' Di ROMA CAMPUS BIOMEDICO

Professor Mauro Maccarrone
Department of Medicine
Rome, 14th July 2016

To **Dr. Livio Luongo**
Lead Guest Editor
Pharmacological Research

Dear Dr. Luongo,

Please find enclosed a revised version of the manuscript "Type-2 Cannabinoid Receptors in Neurodegeneration", by Tiziana Bisogno, Sergio Oddi, Alessandra Piccoli, Domenico Fazio and myself. It has been amended in order to take into full account all minor criticism raised by the handling Editor and Reviewer 1, whom we thank very much indeed for their constructive suggestions. The changes made to the manuscript have been highlighted in red, and are detailed in the point-by-point replies below. We hope that now our revised paper can be considered acceptable for publication.

Looking forward to hearing from you, I remain

Yours sincerely,
Mauro Maccarrone

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*Response to Reviewers

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Point-by-point replies to the criticism raised

To the Editor

1) We have added the section "chemical compounds studied in the article", as suggested (see page 3).

2) We have provided the graphical abstract.

3) We have rephrased the following sentences, as requested:

and remote cell death is thought to be related to several factors, including the type and extent of the primary insult, the type of connectivity, and the intrinsic vulnerability of the circuits that are involved.

changes in neurotransmitter release, synthesis or generation of endogenous autodestructive neurochemicals, and/or inflammatory mediators, or even alterations in endogenous protective and/or trophic factors, just to list a few.

To Reviewer 1

Minor comments

1) We thank the Reviewer for her/his observation, and have revised the text accordingly (see page 7).

2) We have added a final sentence to each chapter, as kindly suggested (see pages 10, 13 and 15).

Type-2 Cannabinoid Receptors in Neurodegeneration

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Abstract

Based on its wide expression in immune cells, type 2 cannabinoid (CB_2) receptors were traditionally thought to act as "peripheral receptors" with an almost exclusively immunomodulatory function. However, their recent identification in mammalian brain areas, as well as in distinct neuronal cells, has opened the way to a re-consideration of CB_2 signaling in the context of brain pathophysiology, synaptic plasticity and neuroprotection. To date, accumulated evidence from several independent preclinical studies has offered new perspectives on the possible involvement of CB_2 signaling in brain and spinal cord traumatic injury, as well as in the most relevant neurodegenerative disorders like Alzheimer's disease, Parkinson's disease and Huntington's chorea. Here, we will review available information on CB_2 in these disease conditions, along with data that support also its therapeutic potential to treat them.

Chemical compounds studied in this article

JWH-015 (PubChem CID: 4273754); JWH-133 (PubChem CID: 6918505); HU-308 (PubChem CID: 101014676); AM630 (PubChem CID: 4302963); SR144528 (PubChem CID: 3081355); WIN 55,212-2 (PubChem CID: 6604176); PF3845 (PubChem CID: 25154867).

1. Introduction

Over the last two decades, the endocannabinoid (eCB) system has emerged as a prominent lipid signaling network widely expressed in the body, and involved in multiple adaptive responses to stressful internal and/or environmental insults [1]. The eCB system comprises endogenous lipid transmitters, *N*-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) — known as endocannabinoids, eCBs (Figure 1) —, their G-protein-coupled receptors (GPCRs), namely, type-1 (CB_1) and type-2 (CB_2) cannabinoid receptors, and the proteins responsible for eCB biosynthesis, inactivation, transport and accumulation [2-4].

Within the central nervous system (CNS), the eCB system is considered one of the major players in regulating the activity of various neurotransmitters, thus participating in synaptic plasticity [5]. In addition, eCBs and their receptors are involved in neuroprotective pathways, whereby they modulate neuronal, glial and endothelial cell functions to produce neuromodulatory, anti-excitotoxic, anti-inflammatory and anti-oxidative effects [6,8]. Indeed, several preclinical studies have opened new perspectives on the possible therapeutic potential of eCB signaling in neurological disorders, and notably in neurotraumatic and neurodegenerative diseases [9-16]. Most of the beneficial effects documented for cannabinoid-based drugs under these pathological conditions have been ascribed to CB_1, the most abundant GPCR in the brain; instead, an immunomodulatory effect has been traditionally attributed to CB_2, that is clearly present in

immune cells [17-18]. However, recent evidence suggests that CB_2 is much more widely distributed in the CNS than originally thought, where it plays an unexpected neuroprotectant role.

Here, we will review available information supporting the therapeutic potential of CB_2 in different neurological disorders. In the first part of the paper, current knowledge on CB_2 structure, expression and signaling will be briefly summarized. Next, we will focus on the involvement of this receptor in brain and spinal cord injury, as well as in three of the most important chronic neurodegenerative disorders in humans: Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD), where protective effects and therapeutic potential of CB_2 have been studied more in depth.

2. Type-2 cannabinoid receptor

CB_2 was identified and cloned in 1993 (3 years after CB_1) from the marginal zones of the spleen and HL60 promyelocytic leukemic cell line [19]. Its cloning, structure and distribution in human tissues have been recently described in comprehensive reviews [20-21].

Briefly, CB_2 gene *CNR2* is located on chromosome 1p36 in humans and the comparison among species revealed that this gene is less divergent than CB_1, with a sequence identity of 82% between human and mouse, and 81% between human and rat. Structurally CB_2 shares 44% amino acid identity with CB_1, and is constituted by a single polypeptide chain with a 7-transmembrane domain, an extracellular glycosilated N-terminus and an internal C-terminus domain [20-21].

In healthy brain, CB_2 is barely detectable, although recent evidence suggests that it is expressed in some neuronal populations [22-24]. Remarkably, CB_2 expression can increase in neuronal and glial cells (e.g., astrocytes and microglial cells) following brain trauma or under other pathological conditions (see below).

Similarly to CB_1, CB_2 has an extremely complex signaling activity that is cell-type-specific, agonist-specific and/or dose-dependent [25]. In particular, CB_2 is coupled to

heterotrimeric $G_{i/o}$ proteins through which it triggers multiple signal transduction pathways that are involved in cell proliferation, differentiation and survival, and include: *i*) adenylyl cyclase and cyclic AMP-protein kinase A (PKA); *ii*) extracellular signal-regulated kinase 1 (ERK1) and ERK2; and *iii*) p38 mitogen-activated protein kinase and JUN N-terminal kinases (JNKs) [20-21, 26]. In addition, it has been also demonstrated that CB_2 is coupled, via inositol trisphosphate receptor, to Ca^{2+}-activated Cl^- channels in pyramidal neurons of the rodent medial prefrontal cortex, suggesting that also this receptor may regulate ion homeostasis and neuronal excitability [27].

Based on its ability to modulate neuronal and glial functions, generally in a pro-homeostatic manner, CB_2 is currently regarded as a promising target for multimodal drug approaches to treat neurological conditions. In particular, CB_2 activation can promote neuronal homeostasis and survival by acting at multiple levels: *i*) on neurons, by inhibiting excitotoxicity, oxidative stress and apoptosis [10, 27-29]; *ii*) on astrocytes, by promoting the release of pro-survival (e.g., transforming growth factor-β) and anti-inflammatory (e.g., interleukin-10 and interleukin-1 receptor antagonist) mediators [30-31]; and *iii*) on microglial cells and other invading immune cells, by controlling their inflammatory response in terms of cell migration and cytokine production [20-21, 26, 32].

In the next sections we will summarize data on the pivotal role of CB_2 in neurotraumatic injuries and in AD, PD and HD.

3. CNS injury

Spinal cord injury (SCI) and traumatic brain injury (TBI) are the leading causes of invalidity and mortality for young people worldwide [33]. Pathologic sequelae after neurotrauma can be divided into primary and secondary injuries. Primary or direct injury encompasses the immediate damages to the CNS, including mechanical processes like shearing, laceration and stretching of nerve

fibers, which occur at the time of the impact [34-35]. These initial events are irreversible and only amenable to preventative measures. Secondary or delayed injury is made up of the delayed pathophysiological events that are initiated by the impact in the site of lesion, and evolves over subsequent days or months, accounting for much of the morbidity following CNS injury [36]. The pathophysiology of secondary injury is very complex and involves multiple injury mechanisms that are spatially and temporally specific. Indeed, after focal CNS injury neurodegeneration is not restricted to the primary lesion site, nor in its adjacent areas, but appears also in regions that are functionally connected − but not contiguous − to the focal lesion [37]. This phenomenon, also known as "remote damage", may contribute to neurological deficits associated with the secondary lesion [38]. Different mechanisms have been proposed to account for neurodegeneration upon brain damage associated with both TBI and SCI, including exacerbated inflammatory response, dysregulated neurotransmitter release and changes in the pro-survival signaling pathways [37].

In this context, it is noteworthy that a substantial increase in the production of AEA and 2-AG has been observed in brain parenchyma after different type of TBI and SCI [39-42]. Increased eCB concentration, and thus enhanced eCB signaling, is thought to prevent neurodegeneration by regulating key-processes known to be engaged in neuronal homeostasis and survival, overall triggering anti-excitotoxic, anti-apoptotic, anti-oxidative and anti-inflammatory mechanisms [7, 32, 40, 43].

Accumulated evidence of constitutive and/or inducible expression of CB_2 in non-immune cells within the CNS, like neurons and endothelial cells [10, 22, 24, 44-45], along with the efficacy of genetic and pharmacological deletion of CB_2 in ameliorating paradigms of brain injury, have recently highlighted a direct involvement of this receptor subtype in neurovascular and neuronal protection. The pathophysiological relevance of CB_2 in the context of brain injury is sustained by several lines of evidence. Firstly, CB_2 expression is markedly up-regulated upon tissue damage in different models of focal brain injury and SCI [10, 29, 41, 46]. Notably, CB_2

appear upregulated in inflammatory, neuronal and endothelial cells, presumably as part of an endogenous pro-homeostatic response that allows them to limit altogether neuroinflammation and blood-brain barrier disruption, and to promote neuronal survival and neurogenesis [9, 47]. Secondly, CB_2 agonists (Tables 1 and 2) exerted beneficial effects, generally reverted by CB_2 antagonists, in pre-clinical models of TBI [10, 29, 48-51] and SCI [52-56]. Of note, in one report it was also reported an exacerbation of neurotraumatic damages by AM630 used alone in a model of SCI [55], suggesting that CB_2 antagonism could be detrimental *per se*. The beneficial effects of CB_2 agonists in these models include improvement in motor function, attenuation of neuroinflammation, neurodegeneration and blood-brain barrier disruption (Table 2). Thirdly, transgenic mice where CB_2-encoding gene *CNR2* was ablated ($CB_2^{-/-}$) exhibited larger cerebral injury and inflammation upon craniotomy [57]. Finally, in two different experimental models of remote neurodegeneration, we found that stimulation of CB_2 signaling by the selective agonist JWH-015 (Table 1) triggered a cascade of molecular and cellular events engaging Akt/phosphatidylinositide 3-kinase signaling pathway, which limits severity of neurodegeneration associated with the late stages of axotomy [10, 29, 56]. In particular, we demonstrated that after axotomy CB_2 agonism was able to: *i*) increase neuronal nitric oxide synthase (NOS) expression and activity in neurons; *ii*) reduce inducible NOS expression and activity in astrocytes; iii) attenuate oxidative/nitrative stress in damaged neurons; *iv*) increase the levels of proteins that mediate anti-oxidative (heat skock protein 70) and anti-apoptotic (Bcl-2) mechanisms; and *v*) reduce neurodegeneration and neuroinflammation [29]. These findings are schematically depicted in Figure 2.

Collectively, most of the available evidence supports the notion that selective targeting of CB_2 may by an useful strategy to treat SCI and TBI by eliciting multiple cell-specific responses,

from anti-excitotoxicity and antioxidant defense to vasodilatory, neuromodulatory and immunomodulatory actions, which help to restore brain homeostasis following neurotrauma.

4. Alzheimer's disease

AD is a progressive, degenerative, and irreversible neurological disorder, which kills brain neurons, thus causing loss of intellectual and social skills [58]. From the pathological point of view, AD is characterized by the accumulation in the brain of senile plaques and neurofibrillary tangles, that are insoluble aggregates of amyloid-β (Aβ) peptides and hyperphosphorylated tau, respectively [59]. These neuropathological characteristics are linked to synapse dysfunctions and progressive loss of specific neuronal populations, especially in brain regions serving memory and other cognitive functions. Noteworthy, other invariant hallmarks of AD are exacerbated neuroinflammation (due to hyperactivation of both resident and infiltrated innate and adaptive immune cells), excitotoxicity and oxidative stress. Despite decades of intensive research, AD remains incurable. Current therapies mainly target cholinergic and *N*-methyl-*D*-aspartate receptor pathways, and provide only symptomatic relief. Unfortunately, different drugs that target Aβ and tau have failed in Phase III clinical trials [60]. Therefore, identification of novel therapeutic targets that may prevent or delay disease progression is dramatically needed.

In line with its inducible nature, CB_2 has been found to be upregulated in the hippocampus of AD patients [61]. In particular, in different areas (i.e., hippocampus, entorhinal and frontal cortex) of postmortem brains from patients with AD, CB_2 was found to be upregulated in neuritic plaque-associated astrocytes and microglia, whereas CB_1 expression remained unchanged [62-64]. Consistently, marked increase in the expression and activity of CB_2 in glial elements (i.e., astrocytes and microglial cells) has been found also in different AD-like animal models, including transgenic mice and Aβ-injected rats [24, 65-68] (Table 2).

Much alike other pathological conditions, it is believed that up-regulation of CB_2 signaling in AD represents an adaptive response aimed at reducing associated inflammatory processes [9]. Indeed, *in vitro* activation of CB_2 reduced the production of neurotoxic factors (e.g., nitric oxide and reactive oxygen species) and of proinflammatory mediators (e.g., tumor necrosis factor, TNF-α, and interleuchin-6, IL-6) by reactive astrocytes and microglial cells [63, 69-71] (Table 1 and Table 3). Moreover, activation of CB_2 was found to stimulate other relevant biological activities of microglial cells, most notably phagocytic activity [71], proliferation [72], and migration at lesioned sites [73]. Altogether, these activities could contribute to protect neurons against Aβ-toxicity.

Also the therapeutic potential of CB_2 has been adequately documented in animal models of AD. For example, pharmacological activation of this receptor was found to reduce Aβ-toxicity, by mitigating neuroinflammation, neurodegeneration and cognitive decline in Aβ-injected rodents [63, 66, 74-75]. Consistently, stimulation of CB_2 induced beneficial effects in Tg2576 mice, a transgenic model of AD that expresses high levels of human mutant (K670N/M671L) amyloid precursor protein (APPSwe) [76]. In these animals, chronic administration of JWH-133, a selective CB_2 agonist (Table 1), markedly lowered Aβ production, reducing reactive microglia and expression of COX-2 and TNF-α, and improving cognitive performance in old mice [76].

Similar results were reported in APPSwe/PS1ΔE9 mice, another transgenic model of AD that co-expresses human mutated forms of APP (APPSwe) and presenilin 1 (PS1ΔE9); as a consequence, numerous amyloid deposits were developed much earlier than age-matched Tg2576 mice. APPSwe/PS1ΔE9 animals treated with JWH-133, during both pre-symptomatic and early symptomatic stage, significantly improved their learning and memory performances compared to vehicle-treated mice [68]. Although long-term stimulation of CB_2 did not influence Aβ production and deposition in the brain cortex, it did produce remarkable anti-inflammatory effects, and reduced the number of neuritic-associated reactive microglial cells, as well as the expression

of several proinflammatory cytokines, such as IL-1β, IL-6, TNF-α and IFN-γ. Notably, these anti-inflammatory effects were associated with a reduction of oxidative stress damage and tau hyperphosphorylation in neuritic plaques [68]. The key role of CB_2 in modulating microglial activity has been further confirmed in J20APP mice, an AD-like model expressing mutated APP (K670N/M671L/V717F) in cerebral neurons, especially of neocortex and hippocampus [77]. In these animals, deletion of CB_2 led to increased Aβ production and deposition, confirming the likely constitutive role of CB_2 in reducing amyloid plaque pathology in AD. Interestingly, although CB_2 deletion did not influence whole brain microgliosis, it did cause enhanced plaque-associated microglia, suggesting that this receptor subtype is essential for microglial phagocytic activity, even though it is not strictly necessary for chemotaxis of microglia to regions of plaque deposition [77]. Apparently, conflicting results have been obtained in an independent study on APPSwe/PS1ΔE mice that lacked CB_2 [78]. This study showed that CB_2 deficiency affected both recruitment of macrophages into the brains of AD mice, and ability to activate microglia. Furthermore, $CB_2^{-/-}$ mice showed lower expression of pro-inflammatory chemokines and cytokines in the brain, as well as reduced amounts of soluble Aβ 40/42. Notably, the reduction in neuroinflammation did not affect spatial learning and memory in APPSwe/PS1ΔE/$CB_2^{-/-}$ mice.

Collectively, all these findings show remarkable changes in expression and/or activity of CB_2 receptor in different paradigms of AD, encouraging further research on the therapeutic potential of manipulation of CB_2 signaling in context of this neurodegenerative disorder (Table 3).

5. Parkinson's disease

PD is the second most common neurodegenerative disorder after AD, and is the most prevalent affecting the basal ganglia. The motor pathological symptoms include bradykinesia, rigidity, tremor as well as postural instability, typical in the late stages of the disease. They are due to a

progressive degeneration of dopaminergic neurons of the *substantia nigra pars compacta* (SNpc), that produces dopaminergic denervation of the striatum, thus altering motor function and the whole basal ganglia circuit.

Overactivity of eCB system has been observed in PD patients and in animal models of this disease [79-84]. Moreover, several evidences support the role of CB_1 in reducing motor inhibition typical of PD patients [85-87], as well as in enhancing the therapeutic effect of moderate doses of levodopa [11, 84, 87]. Neuroinflammation has emerged as a key component of PD pathogenesis in 1998, with the assessment of activated microglia in the SN of patients at post-mortem [88]. Thus, CB_2 expressed in glial elements may participate in modulating homeostasis and survival. The involvement of CB_2 in nigrostriatal cell loss was detected in a classic rodent model of PD, induced by injection of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), known to induce neurotoxicity and neuroinflammation in mice [89] (Table 3). It was found that protection by non-selective CB_1/CB_2 agonists against MPTP-induced loss of tyrosine hydroxylase (TH) positive neurons in the SNpc was mediated by CB_2, but not by CB_1. Moreover, activation of CB_2 by selective CB_2 agonists reduced microglial activation and/or infiltration, in the ventral midbrain of MPTP-treated mice; moreover, pharmacological inactivation of CB_2 reverted this effect [89]. The beneficial action of CB_2 in PD was evaluated in a well-established model of pronounced nigrostriatal inflammation associated with PD, leading to nigrostriatal dopaminergic neuron loss and motor impairment [90-91], induced in mice by intrastriatal injection of endotoxin lipopolysaccharides (LPS) [92]. In particular, up-regulation of CB_2 in the SN of LPS-treated mice compared with contralateral non-lesioned structures, and the protective effect of the selective CB_2 agonist HU-308 (Table 1) on TH positive neurons in these animals, both supported CB_2 as promising target to alleviate symptoms and to delay neurodegeneration typical of PD [92]. In order to facilitate preclinical development of drugs targeting CB_2, changes that occur in the eCB system at large, and in CB_2 expression in particular, were investigated and compared in two

widely-used animal models of AD: LPS-lesioned and 6-hydroxydopamine-lesioned rats [93]. The model induced by injection of catecholaminergic neurotoxin is associated with selective nigrostriatal dopaminergic neuron loss and stable motor deficits, as well as with nigrostriatal neuroinflammation [94-96]. Intra-striatal injections of either 6-hydroxydopamine or LPS caused contralateral motor dysfunction and striatal neuroinflammation, that where associated with increased expression of *CNR2* [93]. Up-regulation of CB_2, more pronounced in LPS than in 6-hydroxydopamine model, correlated with elevations in AEA and 2-AG levels, and with the expression of the microglial marker CD11b, a β-integrin [93]. Experimental evidence revealed a different dysregulation of eCB system in different models of PD, and highlighted the prominent role of microglial CB_2 in the inflammatory environment in PD [93]. In addition, CB_2 was found in the human post-mortem SN, and its expression in TH positive neurons decreased in PD patients compared with controls [97]. Importantly, up-regulation of CB_2 observed in MPTP- [89] and LPS-lesioned rats [92] has been recently assessed also in post-mortem SN of PD patients compared with healthy subjects [98]. CB_2 enhancement was associated with increased levels of Iba-1, a marker of microglial cells, and of CD68, a marker of activated microglia, macrophages and monocytes that was probably recruited at lesioned sites [98]. Moreover, by using LPS-lesioned mice administration of the selective CB_2 agonist HU-308 was shown to reverse LPS-induced striatal elevation of both CD68 and proinflammatory inducible NOS, paralleled by a reduction in TH immunostaining [98]. Collectively, these findings suggest that targeting CB_2 might represent a novel therapeutic opportunity to combat PD. In this context, it should be recalled that also plant-derived (phyto-) cannabinoids (Figure 3) have been investigated against neurodegeneration, due to their ability to activate CB_2 but not CB_1 (that may even be blocked by them), and to their remarkable antioxidant properties. To this aim, cannabidiol (CBD) and Δ^9-tetrahydrocannabivarin (THCV) exhibited neuroprotective effects along with the ability to ease symptoms in different

animal models of PD [13, 92, 99], showing a promising pharmacological profile that might be useful to design novel anti-parkinsonian therapeutics.

In conclusion, up-regulation of CB_2 and subsequent neuroprotection against local inflammation and gliosis due to receptor activation, clearly encourage the therapeutic exploitation of CB_2 agonists against PD. Indeed, the ability to reduce inflammation through CB_2 activation, together with the ability to reduce motor disturbance by blocking CB_1 and to exert antioxidant activity, could be the right combination required for novel anti-parkinsonian drugs.

6. Huntington's disease

HD is a neurodegenerative genetic disorder caused by a mutation (CAG trinucleotide expansion) in exon 1 of the *IT15* gene coding for huntingtin on chromosome 4 [100]. HD is characterized by abnormal involuntary movements collectively called chorea, which are produced by a neuronal dysfunction in the striatum, and dementia caused by neuronal decline in the cortical structures. Several studies have supported the relevance of both CB_1 and CB_2 as potential targets for neuroprotective therapies of HD. Several independent studies have documented down-regulation of CB_1 in post-mortem HD patients and in mouse models of HD [101-104]. CB_1 loss affected medium spiny GABA-ergic neuron, and occurred prior to damage of other receptors. Conversely, a recent study suggested a restricted population of CB_1 receptors located on cortical glutamatergic neurons, preserved during HD, as first players in mediating neuroprotection against HD [105]. The involvement of CB_2 in HD has been investigated in different animal models and in post-mortem patients, and a role in neuroinflammatory processes has been highlighted (Table 3). In particular, the selective CB_2 agonist HU-308 (Table 1) reduces the magnitude of striatal lesions induced in mice by the mitochondrial complex II inhibitor toxin malonate [106]. The effect of this CB_2 agonist was reverted by the selective CB_2 antagonist SR144528, and toxin-induced lesions were more pronounced upon genetic CB_2 ablation. Moreover, striatal CB_2 expression increased in

reactive microglial cells in response to neurodegeneration produced by the toxin, and its activation attenuated malonate-induced increase in TNF-α levels [106]. The ability of CB_2 to decrease microglial activation and to prevent neurodegeneration was reported also in different transgenic mice models [107-108] (Table 3). CB_2 transcripts were increased in the striatal microglia of R6/2 transgenic mice model, that expresses exon 1 of human mutant huntingtin [109], both at pre-symptomatic and at symptomatic stages. In addition, genetic CB_2 ablation enhanced microglial activation in R6/2 mice [107], and exacerbated disease symptomatology [107]. In keeping with these data, deterioration of motor deficits due to deletion of CB_2 was also confirmed in a slowly progressing model of HD, the so called BACHD mice [108]. In addition, two studies have investigated CB_2 expression in post-mortem human tissues, reporting apparently divergent results [107, 110]. Increased expression of CB_2 was reported in CD68-positive microglia but not in cells positive to the astrocyte marker GFAP in caudate putamen of HD patients [107]. Instead, CB_2 staining was found to be expressed in human post-mortem striatum of HD patients, but it did not co-localize with GFAP-positive astrocytes nor with Iba1-positive microglia [110]. Even though the reason of this apparent discrepancy remains unclear, it may be simply due to the reagents used (e.g., antibodies specificity). On a final note, also the beneficial effect of the phytocannabinoids Δ^9-THC and CBD, alone or in combination in the form of Sativex®, has been investigated in several animal models of HD. Their action was reported to be mediated by multiple mechanisms including CB_1, CB_2, additional eCB-binding receptors like peroxisome proliferator-activated receptor, or even non-eCB targets [106, 111-112]. In addition, controversial results were obtained by using phytocannabinoids in clinical trials of HD [113], and unfortunately a recent phase II clinical trial with Sativex® failed [114]. More recently, also the effects of cannabigerol (CBG), a non-psychotropic phytocannabinoid, was investigated in both R6/2 and 3NP-lesioned mice models of HD [115]. CBG preserved striatal neurons death and neurological deterioration, although these effects were much more evident in 3NP-lesioned mice than in R6/2 mice. Although the

mechanisms responsible for the beneficial effects of CBG in HD were not further interrogated, it was ruled out that they could engage CB_1 and/or CB_2, given the poor affinity of CBG for both receptors [115].

In conclusion, pharmacological activation of CB_2 might represent a frontier that needs to be explored to develop novel drugs able to counteract motor and neurological deterioration in HD.

7. Concluding remarks

Traditionally, the neuroprotective effects of cannabinoid-based drugs have been attributed to stimulation of CB_1, the most abundant GPCR in the brain. Instead for decades CB_2, the non-psychotropic cannabinoid receptor, has been associated almost exclusively to immunomodulatory effects. This view is rapidly changing after the recent discovery of the functional expression of CB_2 also in non-immune cells of the brain, such as neurons and endothelial cells, under physiological and most often pathological conditions. Here, we have discussed recent studies on the pathophysiological relevance of CB_2 signaling in the context of different neurodegenerative disorders, including brain and spinal neurotrauma, AD, PD and HD. These studies support the intriguing possibility that CB_2 may be part of a protective mechanism that is both acutely and chronically expressed and/or activated upon brain damage, and operates at once and at multiple levels to orchestrate a series of pro-homeostatic responses. However, the widespread expression of CB_2 and the complexity of its signal transduction pathways make it extremely difficult to decipher its distinct role in pathogenic events related to neurodegeneration. Future research is deemed necessary to identify the precise mechanisms triggered by CB_2 in order to regulate key pro-homeostatic pathways in the brain. In addition, since both CB_1 and CB_2 seem to co-exist in the same cell, further research is also required to elucidate what type of interaction exists between these two receptor subtypes, and what is its physiological and pharmacological relevance. Unsurprisingly, as yet only a few clinical data exist on the potential therapeutic exploitation of

CB_2 in neurodegeneration, though it can be anticipated that targeting it to reduce neuroinflammation and blood-brain barrier disruption, while promoting neurosurvival and neurogenesis, is likely to be of particular interest in treating neurodegenerative disorders. An added value of this potential therapeutic strategy appears the reduced risk of psychoactive effects associated with CB_2 manipulation.

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REFERENCES

[1] M. Maccarrone, I. Bab, T. Bíró, G.A. Cabral, S.K. Dey, V. Di Marzo, J.C. Konje, G. Kunos, R. Mechoulam, P. Pacher, K.A. Sharkey, A. Zimmer, Endocannabinoid signaling at the periphery: 50 years after THC, Trends in Pharmacol Sci. 36 (2015) 1-20.

[2] M. Maccarrone, E. Dainese, S. Oddi S, Intracellular trafficking of anandamide: new concepts for signaling, Trends Biochem Sci. 35 (2010) 601-618.

[3] F. Fezza, M. Bari, R. Florio, E. Talamonti, M. Feole, M. Maccarrone, Endocannabinoids, related compounds and their metabolic routes, Molecules. 19 (2014) 17078-17106.

[4] H.C. Lu, K. Mackie, An Introduction to the Endogenous Cannabinoid System, Biol Psychiatry. 79 (2016) 516-525.

[5] V. Chevaleyre, K.A. Takahashi, P.E. Castillo, Endocannabinoid-mediated synaptic plasticity in the CNS, Annu Rev Neurosci. 29 (2006) 37-76.

[6] A. Paradisi, S. Oddi, M. Maccarrone, The endocannabinoid system in ageing: a new target for drug development, Curr Drug Targets. 7 (2006) 1539-1552.

[7] R. Mechoulam, L.A. Parker, The endocannabinoid system and the brain, Annu Rev Psychol. 64 (2013) 21-47.

[8] A.E. Bonnet, Y. Marchalant, Potential Therapeutical Contributions of the Endocannabinoid System towards Aging and Alzheimer's Disease, Aging Dis. 1 (2015) 400-405.

[9] J. Fernández-Ruiz, J. Romero, G. Velasco, R.M. Tolón, J.A. Ramos, M. Guzmán, Cannabinoid CB_2 receptor: a new target for controlling neural cell survival?, Trends Pharmacol Sci. 28 (2007) 39–45.

[10] M.T. Viscomi, S. Oddi, L. Latini, N. Pasquariello, F. Florenzano, G. Bernardi, M. Molinari, M. Maccarrone, Selective CB_2 receptor agonism protects central neurons from remote axotomy-induced apoptosis through the PI3K/Akt pathway, J Neurosci Off J Soc Neurosci. 29 (2009) 4564–4570.

[11] T. Bisogno, V. Di Marzo, Cannabinoid receptors and endocannabinoids: role in neuroinflammatory and neurodegenerative disorders, CNS Neurol Disord Drug Targets. 9 (2010) 564-573.

[12] J. Fernández-Ruiz, M. Hernández, J.A. Ramos, Cannabinoid-dopamine interaction in the pathophysiology and treatment of CNS disorders, CNS Neurosci Ther. 16 (2010) e72-91.

[13] J. Fernández-Ruiz, M.A. Moro, J. Martínez-Orgado, Cannabinoids in Neurodegenerative Disorders and Stroke/Brain Trauma: From Preclinical Models to Clinical Applications, Neurotherapeutics. 12 (2015) 793-806.

[14] G. Pryce, D.R. Riddall, D.L. Selwood, G. Giovannoni, D. Baker, Neuroprotection in Experimental Autoimmune Encephalomyelitis and Progressive Multiple Sclerosis by Cannabis-Based Cannabinoids, J Neuroimmune Pharmacol. 10 (2015) 281-292.

[15] G. Bedse, A. Romano, A.M. Lavecchia, T. Cassano, S. Gaetani, The role of endocannabinoid signaling in the molecular mechanisms of neurodegeneration in Alzheimer's disease, J Alzheimers Dis. 43 (2015) 1115-1136.

[16] F.A. Iannotti, V. Di Marzo, S. Petrosino, Endocannabinoids and Endocannabinoid-Related Mediators: Targets, Metabolism and Role In Neurological Disorders, Prog Lipid Res. 62 (2016) 107-128.

[17] T.W. Klein, Cannabinoid-based drugs as anti-inflammatory therapeutics, Nat Rev Immunol. 5 (2005) 400–411.

[18] V. Chiurchiù, L. Battistini, M. Maccarrone, Endocannabinoid signaling in innate and adaptive immunity, Immunology 144 (2015) 352-364.

[19] S. Munro, K.L. Thomas, M. Abu-Shaar, Molecular characterization of a peripheral receptor for cannabinoids, Nature 365 (1993) 61-65.

[20] B.K. Atwood, K. Mackie, CB_2: a cannabinoid receptor with an identity crisis, Br J Pharmacol. 160 (2010) 467-479.

[21] A.M. Malfitano, S. Basu, K. Maresz, M. Bifulco, B.N. Dittel, What we know and do not know about the cannabinoid receptor 2 (CB_2), Semin. Immunol. 26 (2014) 369-379.

[22] E.S. Onaivi, H. Ishiguro, J.P. Gong, S. Patel, A. Perchuk, P.A. Meozzi, L. Myers, Z. Mora, P. Tagliaferro, E. Gardner, A. Brusco, B.E. Akinshola, Q.R. Liu, B. Hope, S. Iwasaki, T. Arinami, L. Teasenfitz, G.R. Uhl, Discovery of the presence and functional expression of cannabinoid CB_2 receptors in brain, Ann N Y Acad Sci. 1074 (2006) 514–536.

[23] Y. Li, J. Kim J,Neuronal expression of CB_2 cannabinoid receptor mRNAs in the mouse hippocampus, Neuroscience. 311 (2015) 253-267.

[24] A.V. Savonenko, T. Melnikova, Y. Wang, H. Ravert, Y. Gao, J. Koppel, D. Lee, O. Pletnikova, E. Cho, N. Sayyida, A. Hiatt, J. Troncoso, P. Davies, R.F. Dannals, M.G. Pomper, A.G. Horti, Cannabinoid CB_2 Receptors in a Mouse Model of Aβ Amyloidosis: Immunohistochemical Analysis and Suitability as a PET Biomarker of Neuroinflammation, PLoS One. 10 (2015) e0129618.

[25] A.C. Howlett, Cannabinoid receptor signaling, Handb Exp Pharmacol. 168 (2005) 53-79.

[26] M. Aghazadeh Tabrizi, P.G. Baraldi, P.A. Borea, K. Varani, Medical chemistry, pharmacology and potential therapeutic benefits of cannabinoid CB_2 receptor agonists, Chem Rev. 116 (2016) 519-560.

[27] F.S. den Boon, P. Chameau, Q. Schaafsma-Zhao, W. van Aken, M. Bari, S. Oddi, C.G. Kruse, M. Maccarrone, W.J. Wadman, T.R. Werkman, Excitability of prefrontal cortical pyramidal neurons is modulated by activation of intracellular type-2 cannabinoid receptors, Proc Natl Acad Sci U S A. 109 (2012) 3534-3539.

[28] F.S. den Boon, P. Chameau, K. Houthuijs, S. Bolijn, N. Mastrangelo, C.G. Kruse, M. Maccarrone, W.J. Wadman, T.R. Werkman, Endocannabinoids produced upon action potential firing evoke a Cl(-) current via type-2 cannabinoid receptors in the medial prefrontal cortex. Pflugers Arch. 466 (2014) 2257-2268.

[29] S. Oddi, L. Latini, M.T. Viscomi, E. Bisicchia, M. Molinari, M. Maccarrone, Distinct regulation of nNOS and iNOS by CB_2 receptor in remote delayed neurodegeneration, J Mol Med Berl Ger. 90 (2012) 371–387.

[30] S.R. Smith, C. Terminelli, G. Denhardt, Effects of cannabinoid receptor agonist and antagonist ligands on production of inflammatory cytokines and anti-inflammatory interleukin-10 in endotoxemic mice, J. Pharmacol. Exp. Ther. 293 (2000) 136–150.

[31] F. Molina-Holgado, E. Pinteaux, J.D. Moore, E. Molina-Holgado, C. Guaza, R.M. Gibson, N.J. Rothwell, Endogenous interleukin-1 receptor antagonist mediates anti-inflammatory and neuroprotective actions of cannabinoids in neurons and glia, J. Neurosci. 23 (2003) 6470–6474.

[32] A. Franklin, S. Parmentier-Batteur, L. Walter, D.A. Greenberg, N. Stella, Palmitoylethanolamide increases after focal cerebral ischemia and potentiates microglial cell motility, J Neurosci. 23 (2003) 7767-7775.

[33] P. Reilly, The impact of neurotrauma on society: an international perspective, Prog Brain Res. 161 (2007) 3-9.

[34] D.I. Graham, J.C. Clark, J.H. Adam, T.A. Gennarelli, Diffuse axonal injury caused by assault, J Clin Pathol. 45 (1992) 840-841.

[35] D.I. Graham, S.M. Gentleman, J.A. Nicoll, M.C. Royston, J.E. McKenzie, G.W. Roberts, W.S. Griffin, Altered beta-APP metabolism after head injury and its relationship to the aetiology of Alzheimer's disease, Acta Neurochir Suppl. 66 (1996) 96-102.

[36] T.K. McIntosh, D.H. Smith, D.F. Meaney, M.J. Kotapka, T.A. Gennarelli, D.I. Graham, Neuropathological sequelae of traumatic brain injury: relationship to neurochemical and biomechanical mechanisms, Lab Invest. 74 (1996) 315-342.

[37] F. Block, M. Dihné, M. Loos, Inflammation in areas of remote changes following focal brain lesion, Prog Neurobiol. 75 (2005) 342-365.

[38] F. Binkofski, R.J. Seitz, S. Arnold, J. Classen, R. Benecke, H.J. Freund, Thalamic metabolism and corticospinal tract integrity determine motor recovery in stroke, Ann Neurol. 39 (1996) 460-470.

[39] D. Panikashvili, C. Simeonidou, S. Ben-Shabat, L. Hanus, A. Breuer, R. Mechoulam, E. Shohami, An endogenous cannabinoid (2-AG) is neuroprotective after brain injury, Nature 413 (2001) 527-531.

[40] D. Baker, G. Pryce, J.L. Croxford, P. Brown, R.G. Pertwee, A. Makriyannis, A. Khanolkar, L. Layward, F. Fezza, T. Bisogno, V. Di Marzo, Endocannabinoids control spasticity in a multiple sclerosis model, FASEB J. 15 (2001) 300-302.

[41] D. Garcia-Ovejero, A. Arevalo-Martin, S. Petrosino, F. Docagne, C. Hagen, T. Bisogno, M. Watanabe, C. Guaza, V. Di Marzo, E. Molina-Holgado, The endocannabinoid system is modulated in response to spinal cord injury in rats, Neurobiol Dis. 33 (2009) 57–71.

[42] H.H. Hansen, P.C. Schmid, P. Bittigau, I. Lastres-Becker, F. Berrendero, J. Manzanares, C. Ikonomidou, H.H. Schmid, J.J. Fernández-Ruiz, H.S. Hansen, Anandamide, but not 2-arachidonoylglycerol, accumulates during in vivo neurodegeneration, J Neurochem. 78 (2001) 1415-1427.

[43] G. Marsicano, S. Goodenough, K. Monory, H. Hermann, M. Eder, A. Cannich, S.C. Azad, M.G. Cascio, S.O. Gutiérrez, M. van der Stelt, M.L. López-Rodriguez, E. Casanova, G. Schütz, W. Zieglgänsberger, V. Di Marzo, C. Behl, B. Lutz, CB1 cannabinoid receptors and on-demand defense against excitotoxicity, Science 302 (2003) 84-88.

[44] S.A. Golech, R.M. McCarron, Y. Chen, J. Bembry, F. Lenz, R. Mechoulam, E. Shohami, M. Spatz, Human brain endothelium: coexpression and function of vanilloid and endocannabinoid receptors, Brain Res Mol Brain Res. 132 (2004) 87–92.

[45] A. Brusco, P.A. Tagliaferro, T. Saez, E.S. Onaivi, Ultrastructural localization of neuronal brain CB_2 cannabinoid receptors, Ann N Y Acad Sci. 1139 (2008) 450–457.

[46] G. Wotherspoon, A. Fox, P. McIntyre, S. Colley, S. Bevan, J. Winter, Peripheral nerve injury induces cannabinoid receptor 2 protein expression in rat sensory neurons, Neuroscience 135 (2005) 235–245.

[47] P. Pacher, R. Mechoulam, Is lipid signaling through cannabinoid 2 receptors part of a protective system?, Prog Lipid Res. 50 (2011) 193–211.

[48] M.B. Elliott, R.F. Tuma, P.S. Amenta, M.F. Barbe, J.I. Jallo, Acute Effects of a Selective Cannabinoid-2 Receptor Agonist on Neuroinflammation in a Model of Traumatic Brain Injury, J Neurotrauma. 28 (2011) 973–981.

[49] P.S. Amenta, J.I. Jallo, R.F. Tuma, M.B. Elliott, A cannabinoid type 2 receptor agonist attenuates blood-brain barrier damage and neurodegeneration in a murine model of traumatic brain injury, J Neurosci Res. 90 (2012) 2293–2305.

[50] F. Tchantchou, L.B. Tucker, A.H. Fu, R.J. Bluett, J.T. McCabe, S. Patel, Y. Zhang, The fatty acid amide hydrolase inhibitor PF-3845 promotes neuronal survival, attenuates inflammation and improves functional recovery in mice with traumatic brain injury, Neuropharmacology 85 (2014) 427–439.

[51] A.B. Lopez-Rodriguez, E. Siopi, D.P. Finn, C. Marchand-Leroux, L.M. Garcia-Segura, M. Jafarian-Tehrani, M-P. Viveros, CB_1 and CB_2 Cannabinoid Receptor Antagonists Prevent Minocycline-Induced Neuroprotection Following Traumatic Brain Injury in Mice, Cereb Cortex. 25 (2015) 35–45.

[52] D.E. Baty, M. Zhang, H. Li, C.J. Erb, M.W. Adler, D. Ganea, C.M. Loftus, J.I. Jallo, R.F. Tuma, Cannabinoid CB_2 receptor activation attenuates motor and autonomic function deficits in a mouse model of spinal cord injury, Clin Neurosurg. 55 (2008) 172–177.

[53] J.E. Heller, D.E. Baty, M. Zhang, H. Li, M. Adler, D. Ganea, J. Gaughan, C.M. Loftus, J.I. Jallo, R.F. Tuma, The combination of selective inhibition of the cannabinoid CB_1 receptor and

activation of the cannabinoid CB_2 receptor yields improved attenuation of motor and autonomic deficits in a mouse model of spinal cord injury, Clin Neurosurg. 56 (2009) 84–92.

[54] S. Adhikary, H. Li, J. Heller, M. Skarica, M. Zhang, D. Ganea, R.F. Tuma, Modulation of Inflammatory Responses by a Cannabinoid-2–Selective Agonist after Spinal Cord Injury, J Neurotrauma. 28 (2011) 2417–2427.

[55] A. Arevalo-Martin, D. Garcia-Ovejero, Y. Sierra-Palomares, B. Paniagua-Torija, I. Gonzalez-Gil, S. Ortega-Gutierrez, E. Molina-Holgado, Early Endogenous Activation of CB_1 and CB_2 Receptors after Spinal Cord Injury Is a Protective Response Involved in Spontaneous Recovery, PLoS One. 7 (2012) e49057.

[56] L. Latini, E. Bisicchia, V. Sasso, V. Chiurchiù, V. Cavallucci, M. Molinari, M. Maccarrone, M.T. Viscomi, Cannabinoid CB_2 receptor (CB2R) stimulation delays rubrospinal mitochondrial-dependent degeneration and improves functional recovery after spinal cord hemisection by ERK1/2 inactivation, Cell Death Dis. 5 (2014) e1404.

[57] P.S. Amenta, J.I. Jallo, R.F. Tuma, D.C. Hooper, M.B. Elliott, Cannabinoid receptor type-2 stimulation, blockade, and deletion alter the vascular inflammatory responses to traumatic brain injury, J Neuroinflammation 11 (2014) 191.

[58] D.J. Selkoe, Alzheimer's disease: genes, proteins, and therapy, Physiol. Rev. 81 (2001) 741-766.

[59] E. Giacobini & G. Gold, Alzheimer disease therapy-moving from amyloid-β to tau, Nat Rev Neurol. 9 (2013) 677-686.

[60] M.S. Rafii, P.S. Aisen, Advances in Alzheimer's disease drug development, BMC Med. 13 (2015) 62-68.

[61] E. Grünblatt, N. Zander, J. Bartl, L. Jie, C-M. Monoranu, T. Arzberger, R. Ravid, W. Roggendorf, M. Gerlach, P. Riederer, Comparison analysis of gene expression patterns between sporadic Alzheimer’s and Parkinson’s disease, J Alzheimers Dis. 12 (2007) 291–311.

[62] C. Benito, E. Núñez, R.M. Tolón, E.J. Carrier, A. Rábano, C.J. Hillard, J. Romero, Cannabinoid CB_2 Receptors and Fatty Acid Amide Hydrolase Are Selectively Overexpressed in Neuritic Plaque-Associated Glia in Alzheimer's Disease Brains, J Neurosci. 23 (2003) 11136–11141.

[63] B.G. Ramírez, C. Blázquez, T. Gómez del Pulgar, M. Guzmán, M.L.de Ceballos, Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation, J Neurosci. 23 (2005) 1904-1913.

[64] M. Solas, P.T. Francis, R. Franco, M.J. Ramirez, CB_2 receptor and amyloid pathology in frontal cortex of Alzheimer's disease patients, Neurobiol. Aging 34 (2013) 805–808.

[65] M. van der Stelt, C. Mazzola, G. Esposito, I. Matias, S. Petrosino, D.D. Filippis, V. Micale, L. Steardo, F. Drago, T. Iuvone, V. Di Marzo, Endocannabinoids and β-amyloid-induced neurotoxicity in vivo: effect of pharmacological elevation of endocannabinoid levels, Cell Mol Life Sci CMLS. 63 (2006) 1410–1424.

[66] G. Esposito, T. Iuvone, C. Savani, C. Scuderi, D.D. Filippis, M. Papa, V.D. Marzo , L. Steardo, Opposing Control of Cannabinoid Receptor Stimulation on Amyloid-β-Induced Reactive Gliosis: In Vitro and in Vivo Evidence, J Pharmacol Exp Ther. 322 (2007)1144–1152.

[67] A.G. Horti, Y. Gao, H.T. Ravert, P. Finley, H. Valentine, D.F. Wong, C.J. Endres, A.V. Savonenko, R.F. Dannals, Synthesis and biodistribution of [^{11}C]A-836339, a new potential radioligand for PET imaging of cannabinoid type 2 receptors (CB_2), Bioorg Med Chem. 18 (2010) 5202–5207.

[68] E. Aso, S. Juvés, R. Maldonado, I. Ferrer, CB_2 cannabinoid receptor agonist ameliorates Alzheimer-like phenotype in AβPP/PS1 mice, J Alzheimers Dis. 35 (2013) 847–858.

[69] F. Molina-Holgado, E. Molina-Holgado, C. Guaza, N.J. Rothwell, Role of CB_1 and CB_2 receptors in the inhibitory effects of cannabinoids on lipopolysaccharide-induced nitric oxide release in astrocyte cultures, J Neurosci Res. 67 (2002) 829–836.

[70] W.S. Sheng, S. Hu, X. Min, G.A. Cabral, J.R. Lokensgard, P.K. Peterson, Synthetic cannabinoid WIN55,212-2 inhibits generation of inflammatory mediators by IL-1beta-stimulated human astrocytes, Glia 49 (2005) 211–219.

[71] J. Ehrhart, D. Obregon, T. Mori, H. Hou, N. Sun, Y. Bai, T. Klein, F. Fernandez, J. Tan, R.D. Shytle, Stimulation of cannabinoid receptor 2 (CB_2) suppresses microglial activation, J Neuroinflammation 2 (2005) 2-29.

[72] E.J. Carrier, C.S. Kearn, A.J. Barkmeier, N.M. Breese, W. Yang, K. Nithipatikom, S.L. Pfister, W.B. Campbell, C.J. Hillard, Cultured Rat Microglial Cells Synthesize the Endocannabinoid 2-Arachidonylglycerol, Which Increases Proliferation via a CB_2 Receptor-Dependent Mechanism, Mol Pharmacol. 65 (2004) 999–1007.

[73] L. Walter, A. Franklin, A. Witting, C. Wade, Y. Xie, G. Kunos, K. Mackie, N. Stella, Nonpsychotropic Cannabinoid Receptors Regulate Microglial Cell Migration, J Neurosci. 23 (2003) 1398–1405.

[74] A.M. Martín-Moreno, D. Reigada, B.G. Ramírez, R. Mechoulam, N. Innamorato, A. Cuadrado, M.L. de Ceballos, Cannabidiol and Other Cannabinoids Reduce Microglial Activation In Vitro and In Vivo: Relevance to Alzheimer's Disease, Mol Pharmacol. 79 (2011) 964–973.

[75] J. Wu, B. Bie, H. Yang, J.J. Xu, D.L. Brown, M. Naguib, Activation of the CB_2 receptor system reverses amyloid-induced memory deficiency, Neurobiol. Aging 34 (2013) 791–804.

[76] A.M. Martín-Moreno, B. Brera, C. Spuch, E. Carro, L. García-García, M. Delgado, M.A. Pozo, N.G. Innamorato, A. Cuadrado, M.L.de Ceballos, Prolonged oral cannabinoid

administration prevents neuroinflammation, lowers β-amyloid levels and improves cognitive performance in Tg APP 2576 mice, J Neuroinflammation 9 (2012) 8-12.

[77] J. Koppel, V. Vingtdeux, P. Marambaud, C. d'Abramo, H. Jimenez, M. Stauber, R. Friedman, P. Davies, CB_2 Receptor Deficiency Increases Amyloid Pathology and Alters Tau Processing in a Transgenic Mouse Model of Alzheimer's Disease, Mol Med. 19 (2014) 357–364.

[78] A-C. Schmöle, R. Lundt, S. Ternes, Ö. Albayram, T. Ulas, J.L. Schultze, D. Bano, P. Nicotera, J. Alferink, A. Zimmer, Cannabinoid receptor 2 deficiency results in reduced neuroinflammation in an Alzheimer's disease mouse model, Neurobiol. Aging 36 (2015) 710–719.

[79] V. Di Marzo, M.P. Hill, T. Bisogno, A.R. Crossman, J.M. Brotchie, Enhanced levels of endogenous cannabinoids in the globus pallidus are associated with a reduction in movement in an animal model of Parkinson's disease, FASEB J. 14 (2000) 1432-1438.

[80] J. Romero, F. Berrendero, A. Pérez-Rosado, J. Manzanares, A Rojo, J.J. Fernández-Ruiz, J.G de Yebenes, J.A. Ramos JA, Unilateral 6-hydroxydopamine lesions of nigrostriatal dopaminergic neurons increased CB_1 receptor mRNA levels in the caudate-putamen, Life Sci. 66 (2000) 485-494.

[81] I. Lastres-Becker, M. Cebeira, M.L. de Ceballos, B.Y. Zeng, P. Jenner, J.A. Ramos, J.J. Fernández-Ruiz, Increased cannabinoid CB_1 receptor binding and activation of GTP-binding proteins in the basal ganglia of patients with Parkinson's syndrome and of MPTP-treated marmosets, Eur J Neurosci. 14 (2001) 1827-1832.

[82] P. Gubellini, B. Picconi, M. Bari, N. Battista, P. Calabresi, D. Centonze, G. Bernardi, A. Finazzi-Agrò, M. Maccarrone, Experimental parkinsonism alters endocannabinoid degradation: implications for striatal glutamatergic transmission, J Neurosci. 22 (2002) 6900-6907.

[83] A. Pisani, F. Fezza, S. Galati, N. Battista, S. Napolitano, A. Finazzi-Agrò, G. Bernardi, L. Brusa, M. Pierantozzi, P. Stanzione, M. Maccarrone, High endogenous cannabinoid levels in the cerebrospinal fluid of untreated Parkinson's disease patients, Ann Neurol. 57 (2005) 777-779.

[84] J.J. Fernández-Ruiz, The endocannabinoid system as a target for the treatment of motor dysfunction, Br J Pharmacol. 156 (2009) 1029-1040.

[85] E. Fernandez-Espejo, I. Caraballo, F.R. de Fonseca, F. El Banoua, B. Ferrer, J.A. Flores, B. Galan-Rodriguez, Cannabinoid CB_1 antagonists possess antiparkinsonian efficacy only in rats with very severe nigral lesion in experimental parkinsonism, Neurobiol Dis. 18 (2005) 591-601.

[86] S. González, C. Scorticati, M. García-Arencibia, R. de Miguel, J.A. Ramos, J.J. Fernández-Ruiz, Effects of rimonabant, a selective cannabinoid CB_1 receptor antagonist, in a rat model of Parkinson's disease, Brain Res. 1073-1074 (2006) 209-219.

[87] J.E. Kelsey, O. Harris, J. Cassin, The CB(1) antagonist rimonabant is adjunctively therapeutic as well as monotherapeutic in an animal model of Parkinson's disease, Behav Brain Res. (2009) 304-307.

[88] P.L. McGeer, E.G. McGeer, Glial cell reactions in neurodegenerative diseases: pathophysiology and therapeutic interventions, Alzheimer Dis Assoc Disord. 12 (1998) Suppl 2:S1-6.

[89] D.A. Price, A.A. Martinez, A. Seillier, W. Koek, Y. Acosta, E. Fernandez, R. Strong, B. Lutz, G. Marsicano, J.L. Roberts, A. Giuffrida, WIN55,212-2, a cannabinoid receptor agonist, protects against nigrostriatal cell loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease, Eur J Neurosci. 29 (2009) 2177-2186.

[90] T. Hoang, D.K. Choi, M. Nagai, D.C. Wu, T. Nagata, D. Prou, G.L. Wilson, M. Vila, V. Jackson-Lewis, V.L. Dawson, T.M. Dawson, M.F. Chesselet, S. Przedborski, Neuronal NOS

and cyclooxygenase-2 contribute to DNA damage in a mouse model of Parkinson disease, Free Radic Biol Med. 47 (2009) 1049-1056.

[91] D.B. Hoban, E. Connaughton, C. Connaughton, G. Hogan, C. Thornton, P. Mulcahy, T.C. Moloney, E. Dowd, Further characterisation of the LPS model of Parkinson's disease: a comparison of intra-nigral and intra-striatal lipopolysaccharide administration on motor function, microgliosis and nigrostriatal neurodegeneration in the rat, Brain Behav Immun. 27 (2013) 91-100.

[92] C. García, C. Palomo-Garo, M. García-Arencibia, J. Ramos, R. Pertwee, J.J. Fernández-Ruiz, Symptom-relieving and neuroprotective effects of the phytocannabinoid Δ^9-THCV in animal models of Parkinson's disease, Br J Pharmacol. 163 (2011) 1495-1506.

[93] R.M. Concannon, B.N. Okine, D.P. Finn, E. Dowd, Differential upregulation of the cannabinoid CB_2 receptor in neurotoxic and inflammation-driven rat models of Parkinson's disease, Exp Neurol. 269 (2015) 133-141.

[94] F. Cicchetti, A.L. Brownell, K. Williams, Y.I. Chen, E. Livni, O. Isacson, Neuroinflammation of the nigrostriatal pathway during progressive 6-OHDA dopamine degeneration in rats monitored by immunohistochemistry and PET imaging, Eur J Neurosci. 15 (2002) 991-998.

[95] A.M. Depino, C. Earl, E. Kaczmarczyk, C. Ferrari, H. Besedovsky, A. del Rey, F.J. Pitossi, W.H. Oertel, Microglial activation with atypical proinflammatory cytokine expression in a rat model of Parkinson's disease, Eur J Neurosci. 18 (2003) 2731-2742.

[96] S. Walsh, D.P. Finn, E. Dowd, Time-course of nigrostriatal neurodegeneration and neuroinflammation in the 6-hydroxydopamine-induced axonal and terminal lesion models of Parkinson's disease in the rat, Neuroscience. 175 (2011) 251-261.

[97] M.C. García, V. Cinquina, C. Palomo-Garo, A. Rábano, J. Fernández-Ruiz, Identification of CB_2 receptors in human nigral neurons that degenerate in Parkinson's disease, Neurosci Lett. 587 (2015) 1-4.

[98] Y. Gómez-Gálvez, C. Palomo-Garo, J.J. Fernández-Ruiz, C. García, Potential of the cannabinoid CB(2) receptor as a pharmacological target against inflammation in Parkinson's disease, Prog Neuropsychopharmacol Biol Psychiatry. 64 (2016) 200-208.

[99] S.V. More, D.K. Choi, Promising cannabinoid-based therapies for Parkinson's disease: motor symptoms to neuroprotection, Mol Neurodegener. 10 (2015) 17-31.

[100] The Huntington's Disease Collaborative Research Group, A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. The Huntington's Disease Collaborative Research Group, Cell 72 (1993) 971-983.

[101] M. Glass, R.L. Faull, M. Dragunow, Loss of cannabinoid receptors in the substantia nigra in Huntington's disease, Neuroscience. 56 (1993) 523-527.

[102] M. Glass, M. Dragunow, R.L. Faull, The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA(A) receptor alterations in the human basal ganglia in Huntington's disease, Neuroscience. 97 (2000) 505-519.

[103] I. Lastres-Becker, M. Gómez, R. De Miguel, J.A. Ramos, J.J. Fernández-Ruiz, Loss of cannabinoid CB(1) receptors in the basal ganglia in the late akinetic phase of rats with experimental Huntington's disease, Neurotox Res. 4 (2002) 601-608.

[104] M. Maccarrone, N. Battista, D. Centonze, The endocannabinoid pathway in Huntington's disease: a comparison with other neurodegenerative diseases, Prog Neurobiol. 81 (2007) 349-379.

[105] A. Chiarlone, L. Bellocchio, C. Blázquez, E. Resel, E. Soria-Gómez, A. Cannich, J.J. Ferrero, O. Sagredo, C. Benito, J. Romero, J. Sánchez-Prieto, B. Lutz, J.J. Fernández-Ruiz, I. Galve-Roperh, M. Guzmán, A restricted population of CB_1 cannabinoid receptors with neuroprotective activity, Proc Natl Acad Sci U S A. 111 (2014) 8257-8262.

[106] O. Sagredo, S. González, I. Aroyo, M.R. Pazos, C. Benito, I. Lastres-Becker, J.P. Romero, R.M. Tolón, R. Mechoulam, E. Brouillet, J. Romero, J.J. Fernández-Ruiz, Cannabinoid CB_2 receptor agonists protect the striatum against malonate toxicity: relevance for Huntington's disease, Glia. 57 (2009) 1154-1167.

[107] J. Palazuelos, T. Aguado, M.R. Pazos, B. Julien, C. Carrasco, E. Resel, O. Sagredo, C. Benito, J. Romero, I. Azcoitia, J. Fernández-Ruiz, M. Guzmán, I. Galve-Roperh, Microglial CB_2 cannabinoid receptors are neuroprotective in Huntington's disease excitotoxicity, Brain 132 (2009) 3152–3164.

[108] Bouchard, J. Truong, K. Bouchard, D. Dunkelberger, S. Desrayaud, S. Moussaoui, S.J. Tabrizi, N. Stella, P.J. Muchowski, Cannabinoid receptor 2 signaling in peripheral immune cells modulates disease onset and severity in mouse models of Huntington's disease, J Neurosci. 32 (2012) 18259-18268.

[109] L. Mangiarini, K. Sathasivam, M. Seller, B. Cozens, A. Harper, C. Hetherington, M. Lawton, Y. Trottier, H. Lehrach, S.W. Davies, G.P. Bates, Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice, Cell. 87 (1996) 493-506.

[110] M.J. Dowie, N.L. Grimsey, T. Hoffman, R.L. Faull, M. Glass, Cannabinoid receptor CB_2 is expressed on vascular cells, but not astroglial cells in the post-mortem human Huntington's disease brain, J Chem Neuroanat. 59-60 (2014) 62-71.

[111] O. Sagredo, M. García-Arencibia, E. de Lago, S. Finetti, A. Decio, J. Fernández-Ruiz, Cannabinoids and neuroprotection in basal ganglia disorders, Mol Neurobiol. 36 (2007) 82-91.

[112] O. Sagredo, M.R. Pazos, S. Valdeolivas, J. Fernandez-Ruiz, Cannabinoids: novel medicines for the treatment of Huntington's disease, Recent Pat CNS Drug Discov. 7 (2012) 41-48.

[113] J. Fernández-Ruiz, J. Romer, J.A. Ramos, Endocannabinoids and neurodegenerative disorders: Parkinson's disease, Huntington's chorea, Alzheimer's disease and others. In: Pertwee RG (ed.) Handbook of experimental pharmacology: endocannabinoids. Germany, Dortmund: Springer, 231 (2015) 233-259.

[114] J.L. López-Sendón Moreno, J. García Caldentey, P. Trigo Cubillo, C. Ruiz Romero, G. García Ribas, M.A. Alonso Arias, M.J. García de Yébenes, R.M. Tolón, I. Galve-Roperh, O. Sagredo, S. Valdeolivas, E. Resel, S. Ortega-Gutierrez, M.L. García-Bermejo, J. Fernández Ruiz, M. Guzmán, J. García de Yébenes Prous. A double-blind, randomized, cross-over, placebo-controlled, pilot trial with Sativex in Huntington's disease, J Neurol. 263 (2016) 1390-400.

[115] S. Valdeolivas, C. Navarrete, I. Cantarero, M.L. Bellido, E. Muñoz, O. Sagredo, Neuroprotective properties of cannabigerol in Huntington's disease: studies in R6/2 mice and 3-nitropropionate-lesioned mice, Neurotherapeutics. 12 (2015) 185-199.

[116] A. Arevalo-Martin, D. Garcia-Ovejero, E. Molina-Holgado E, The endocannabinoid 2-arachidonoylglycerol reduces lesion expansion and white matter damage after spinal cord injury, Neurobiol Dis. 38 (2010) 304-312.

[117] E. Janefjord, J. L. Mååg, B.S. Harvey, S.D. Smid SD, Cannabinoid effects on β amyloid fibril and aggregate formation, neuronal and microglial-activated neurotoxicity in vitro, Cell Mol Neurobiol. 34 (2014) 31-42.

Legend to Figures

Figure 1. Chemical structures of the major endocannabinoids. AEA, *N*-arachidonoylethanolamine; 2-AG, 2-arachidonoylglycerol.

Figure 2. Schematic representation of CB_2 impact on axotomy-induced neurodegeneration. Axonal injury leads to a *de novo* synthesis of CB_2 in damaged neurons, as well as to activation of glial cells which produce nitric oxide (NO) via induction of inducible nitric oxide synthase (iNOS). Released NO readily diffuses to neurons, where it reacts with reactive oxygen species (ROS) produced from dysfunctional mitochondria, thus forming the more reactive oxidant peroxynitrite (ONOO−). The latter eventually triggers mitochondrial and other cell death pathways. CB_2 stimulation decreases axonal injury by attenuating glial cell activation, as well as by promoting neuronal nitric oxide synthase (nNOS)-dependent protective mechanisms, via Akt-dependent signals that include expression of antioxidant (Hsp70) and antiapoptotic (Bcl-2) systems.

Figure 3. Chemical structures of pharmacologically active plant-derived (phyto-) cannabinoids. THC, Δ^9-tetrahydrocannabinol; CBG, cannabigerol; CBD, cannabidiol; THCV, Δ^9-tetrahydrocannabivarin.

Legend to Tables

Table 1. K_i values of CB_2 agonists and their doses in preclinical studies.

Table 2. Potential therapeutic use of CB_2 in SCI and TBI.

Table 3. Potential therapeutic use of CB_2 in AD, PD and HD.

Table 1. K_i values of CB_2 agonists and their doses in preclinical studies.

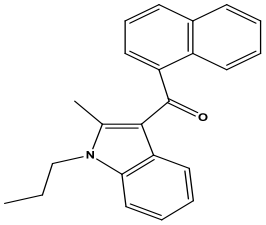
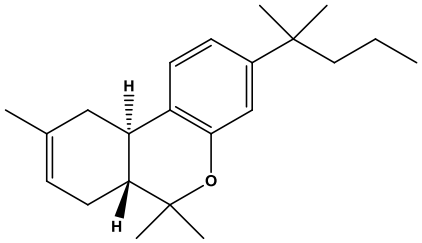
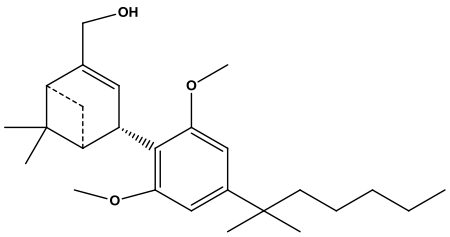
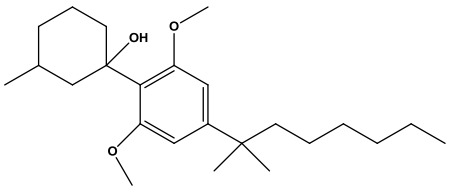
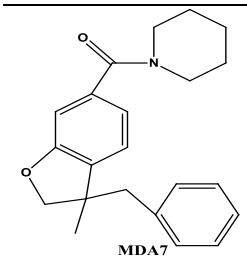
Compound	K_i for CB_2 (nM)	K_i for CB_1 (nM)	Dose rate	Reference
 JWH-015	13.8	383	10^{-6}-10^{-8} M	[66]
			5 mg/Kg	[66]
			3 mg/Kg	[10, 56]
 JWH-133	3.4	677	1 mg/Kg	[57]
			100 nM	[63]
			0.2 mg/Kg	[68]
			200 nM	[74]
 HU-308	22.7	>10000	5 mg/Kg	[92, 106-107]
 O-1966	23	5055	5 mg/Kg	[54, 57]
 MDA7	422	>10000	15 mg/Kg	[75]

Table 2. Potential therapeutic use of CB_2 in SCI and TBI.

Condition	Model	Change	CB_2 manipulation	Effect	Reference
SCI	Spinal cord contusion (mice)	↑ in activated microglia	O-1966	Improvement in motor function	[54]
				Attenuation of neuroinflammation	[52]
				Increase of autonomic function recovery	[53]
	Spinal cord moderate contusion (rats)	nd	2-AG	Reduction of lesion expansion	[116]
				Preservation of white matter	[116]
		↑ in immune infiltrates and astrocytes	AM630	Impairment of spontaneous locomotor recovery	[55]
				Worsening of secondary damage	
	Sciatic nerve section (rats)	↑ in damaged neurons			[46]
	Spinal cord dorsal hemisection (rats)	↑ in axotomized neurons	JWH-015	Attenuation of atrophy and neurodegeneration; Improvement in functional recovery; Attenuation of neuroinflammation	[56]

			SR144528	Reversion of CB_2 agonist-mediated protective effects	
TBI	CCI	↑ in activated microglia	O-1966	Attenuation of neuroinflammation	[48]
				Reduction of cerebral edema	[14]
				Attenuation of blood-brain barrier disruption; Attenuation of neuronal degeneration	[57]
			PF3845	Improvement in motor function, working memory and anxiety behavior Attenuation of neuroinflammation Attenuation of neurodegeneration	[50]
			SR144528	Reversion of PF3845-mediated protective effects	
	Craniotomy	nd	O-1966	Reduced neuroinflammation	[14]
			JWH-133	Improvement of cerebral infarction	
			CB_2-/-	Worsened inflammation	
			SR144528	Reversion of CB_2 agonist-mediated protective effects	

Weight-drop	nd	AM630	Prevention of protective actions of minocycline	[51]
Hemicerebellectomy	↑ in axotomized neurons	JWH-015	Attenuation of neurodegeneration Improvement in motor function and behavior Attenuation of microgliosis Attenuation of oxidative/nitrosative damage	[10, 29]
		SR144528	Reversion of CB_2 agonist-mediated protective effects	

Agonists: JWH-015, JWH-133, O-1966, 2-AG; antagonists: AM630, SR144528; FAAH inhibitor: PF3845. nd, not determined.

Table 3. Potential therapeutic use of CB_2 in AD, PD and HD.

Condition	Model	Change	CB_2 manipulation	Effect	Reference
AD	**Aβ neurotoxicity *in vitro***				
	Mouse microglial cells	↑ in activated microglia	JWH-133	Reduction of Aβ-induced microglia activation	[63, 74]
	Human neuroblastoma cells		2-AG	Reduction of Aβ mediated neurotoxicity	[117]
	Aβ neurotoxicity *in vivo*				
	Rats	↑ in activated microglia	MDA7	Attenuation of neuroinflammation Reduction of pro-inflammatory cytokine production Promotion of Aβ clearance Improvement in synaptic plasticity, cognition, and memory	[75]
		↑ in astrocytes	JWH-015 SR144528	Increase of astrogliosis Reduction of astrogliosis	[66]
		↑ in hippocampus			[65]

	Mice	↑ in activated microglia	WIN 55,212-2	Reduction of pro-inflammatory cytokine production	[63, 74]
	Transgenic mice				
	Tg2576	↔ in brain	JWH-133	Reduction of Aβ production Reduction of microgliosis Improvement in cognitive performance	[55]
	APPSwe/PS1ΔE9	↑ in cortex	JWH-133	Improvement in learning and memory performance Reduction of neuroinflammation	[68]
			CB_2-/-	Impairment in immune cell recruitment in amyloid plaques Reduction of pro-inflammatory cytokine production Reduction of soluble Aβ 40/42 levels	[78]
	J20APP		CB_2-/-	Increase of Aβ production	[77]
PD	MPTP-treated mice	↑ in the ventral midbrain	WIN55,212-2, JWH015 WIN55,212-2 CB2-/-	Reduced MPTP-induced microglial activation Reversed MPTP-associated motor deficits Exacerbated MPTP toxicity	[89]
	LPS-treated mice	↑ in the substantia nigra ↑ in the striatum	HU-308	Protected TH positive neurons	[92]

				Exacerbated LPS lesions	[98]
			CB_2-/-	Protected TH positive neurons and reduces LPS-induced proinflammatory responses	[92, 98]
				Exacerbated LPS lesions and inflammation	[93]
	LPS-treated rats	↑ in the striatum			[98]
	6-OHDA-treated rats	↑ in the striatum			
	Malonate-treated mice	↑ in the striatum	HU-308	Reduced striatal lesions	[106]
			SR144528	Reverted HU-308 protective actions	
			CB_2-/-	Exacerbated malonate lesions	
HD	R6/2 transgenic mice	↑ in the striatum at pre-symptomatic and symptomatic stage	HU-308	Reduced brain oedema and microglial activation induced by quinolinic acid	[107]
			CB_2-/-	Enhanced microglial activation and exacerbated motor symptoms	
	BACHD mice			Accelerated disease onset and exacerbated severity	[108]

Agonists: JWH-015, JWH-133, O-1966, 2-AG, WIN55,212-2, HU-308, MDA7; antagonist: SR144528.

Figure(s)

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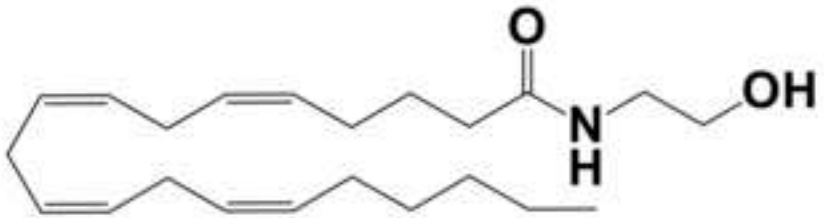

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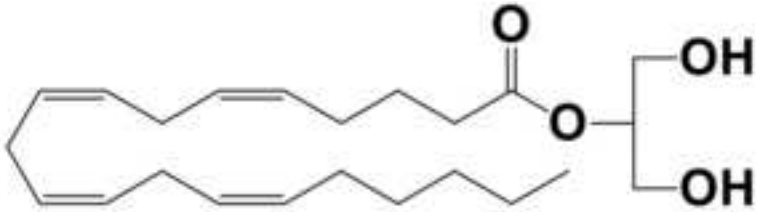

2-Arachidonoylglycerol (2-AG)

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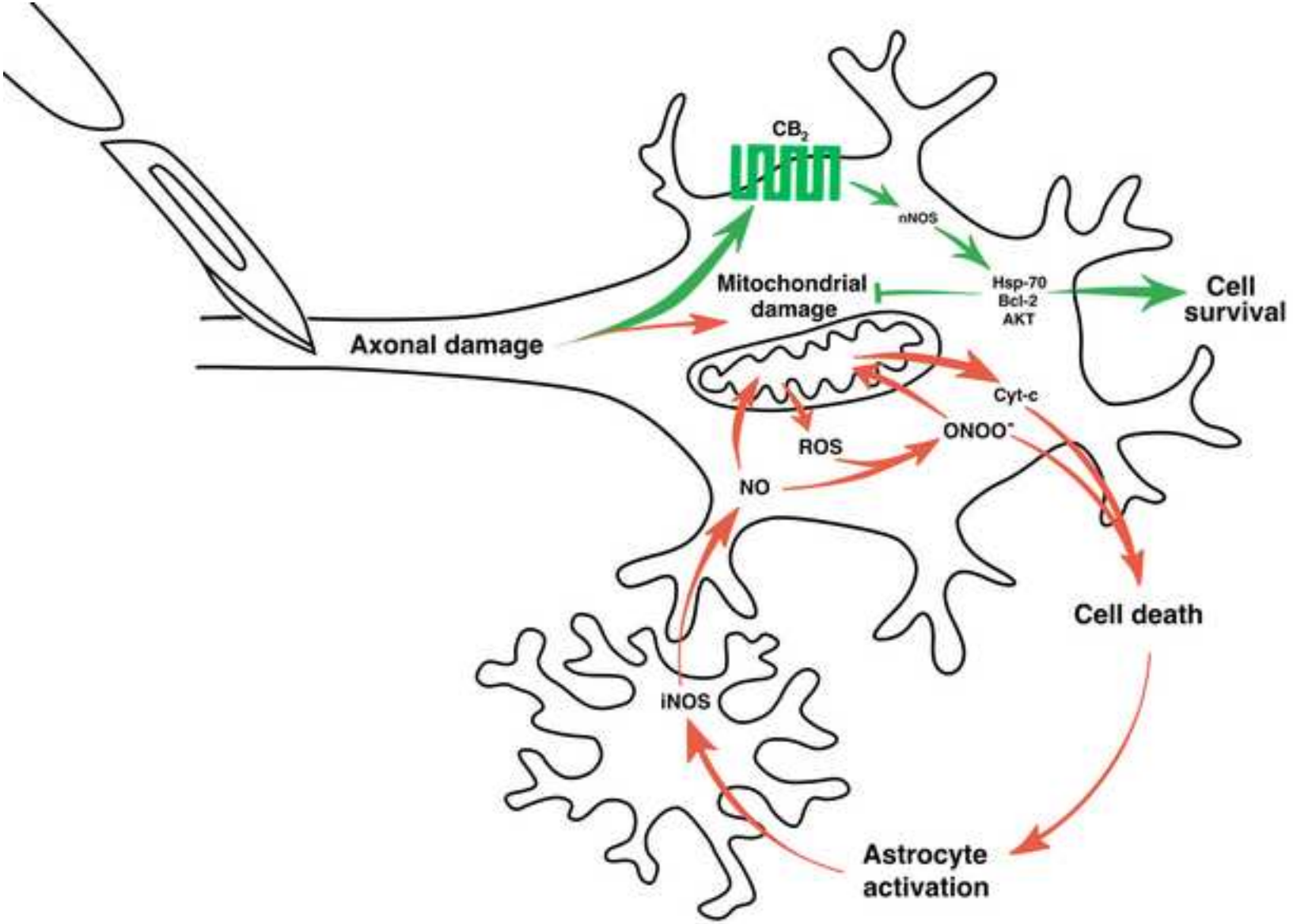

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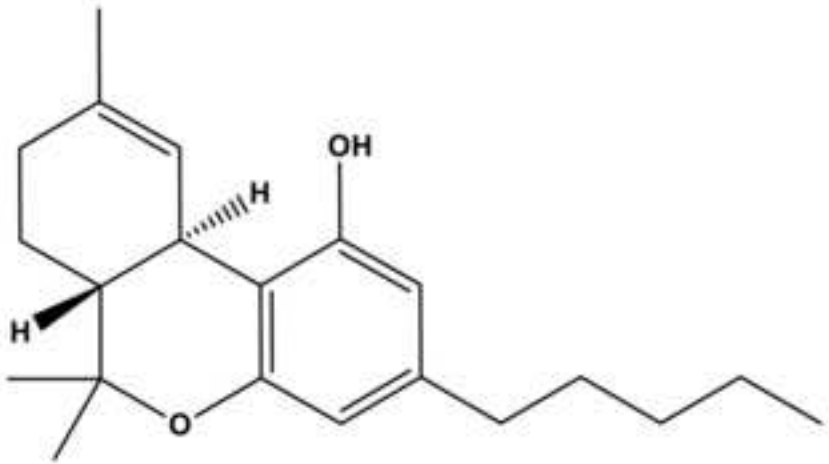

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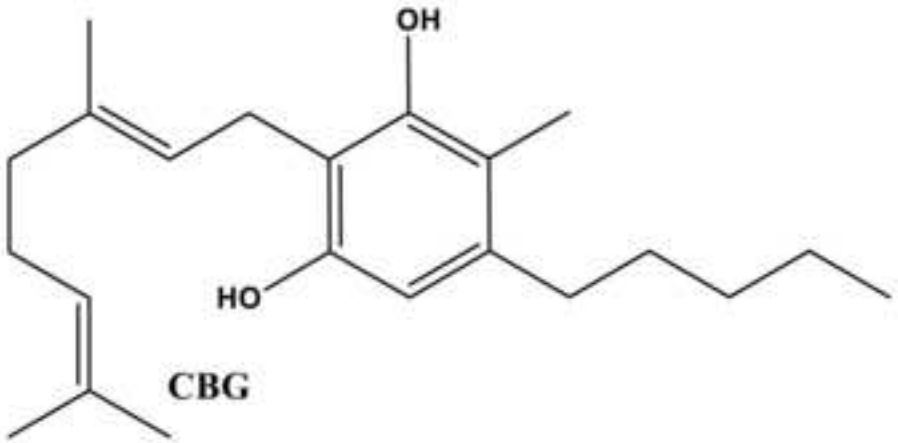

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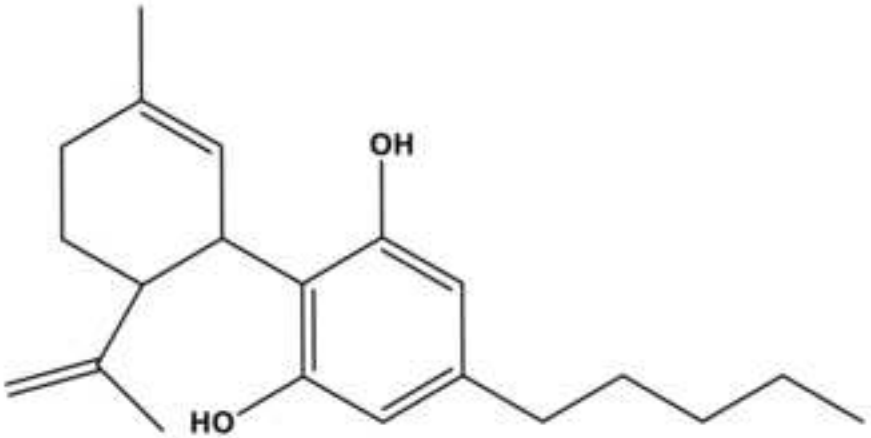

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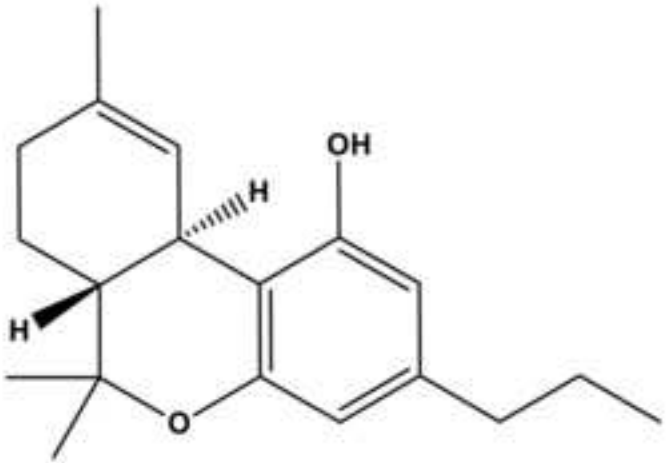

THCV

*Graphical Abstract

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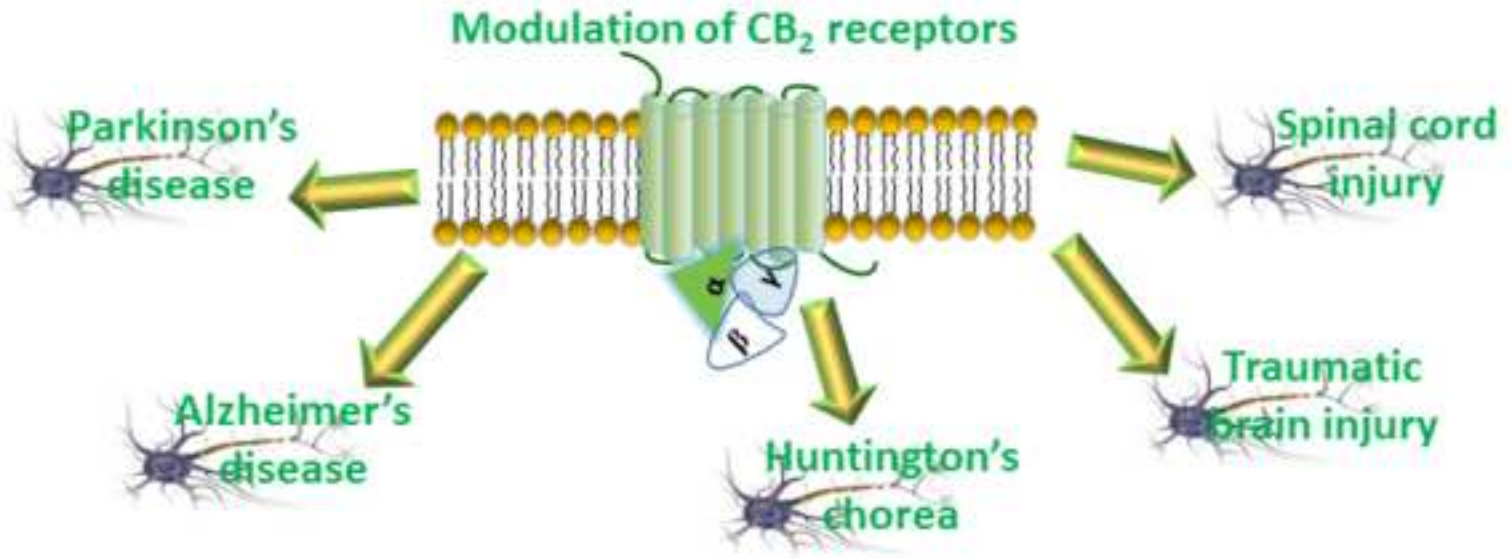

***Supplementary Figure**

***Conflict Of Interest**

All the authors declare no competing financial interest.