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REVIEW ARTICLE

The impact of cannabinoid type 2 receptors (CB2Rs) in neuroprotection against neurological disorders

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Cannabinoids have long been used for their psychotropic and possible medical properties of symptom relief. In the past few years, a vast literature shows that cannabinoids are neuroprotective under different pathological situations. Most of the effects of cannabinoids are mediated by the well-characterized cannabinoid receptors, the cannabinoid type 1 receptor (CB1R) and cannabinoid type 2 receptor (CB2R). Even though CB1Rs are highly expressed in the central nervous system (CNS), the adverse central side effects and the development of tolerance resulting from CB1R activation may ultimately limit the clinical utility of CB1R agonists. In contrast to the ubiquitous presence of CB1Rs, CB2Rs are less commonly expressed in the healthy CNS but highly upregulated in glial cells under neuropathological conditions. Experimental studies have provided robust evidence that CB2Rs seem to be involved in the modulation of different neurological disorders. In this paper, we summarize the current knowledge regarding the protective effects of CB2R activation against the development of neurological diseases and provide a perspective on the future of this field. A better understanding of the fundamental pharmacology of CB2R activation is essential for the development of clinical applications and the design of novel therapeutic strategies.

Keywords: cannabinoid; cannabinoid type 2 receptor; neuroprotection; ischemic stroke; Alzheimer's disease; Parkinson disease

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INTRODUCTION

The endocannabinoid (eCB) system is defined as the ensemble of the two 7-transmembrane-domain G-protein-coupled receptors (CB1R and CB2R) for Δ(9)-tetrahydrocannabinol; their two most studied endogenous ligands, namely, the "endocannabinoids" N-arachidonoylethanolamine (anandamide) and 2-arachidonoylglycerol (2-AG); and the enzymes responsible for endocannabinoid metabolism [1]. The eCB system has recently attracted attention for its roles in various behavioral and brain functions and as a therapeutic target for neuropsychiatric, neurodegenerative, and neurological diseases [2–4]. However, these therapeutic efforts have been marked by disappointment, especially in relation to the serious psychiatric side effects, including anxiety, depression and even suicidal ideation from activation of CB1Rs [5, 6], which have limited the therapeutic use of drugs that activate or inactivate this receptor. Accumulating lines of evidence have shown the therapeutic potential of CB2R ligands and indicated new possibilities for safe targeting of this endocannabinoid system. CB2R is a G-protein-coupled receptor that was cloned in 1993 [7]. Since then, the expression and function of CB2Rs in the brain have been debated. Early studies suggested that CB2R was absent in the brain because mRNA transcripts of CB2Rs were not detected in brain tissues with various methods [8–11]. Based on these findings, CB2R has been considered a "peripheral" cannabinoid receptor. Recently, this concept was challenged by the identification of CB2Rs throughout the central nervous system (CNS). Interested readers are referred to the excellent reviews written by Atwood et al. [12], Jordan et al. [13], Cristino et al. [4], and Reddy et al. [14] for comprehensive overviews regarding the progress of research on the cannabinoid system, especially CB2Rs, in the CNS. Compared with CB1Rs, brain CB2Rs exhibit several unique features. (1) CB2R has lower expression levels than CB1R in the brain, suggesting that CB2Rs may not mediate the effect of cannabis under normal physiological conditions. (2) CB2R is highly inducible; thus, under some pathological conditions (e.g., addiction, inflammation, anxiety, etc.), CB2R expression is enhanced in the brain [15]. This suggests a close relationship between the alteration of CB2R expression/function and various psychiatric and neurological diseases. (3) CB2Rs have a unique distribution. Given that they are chiefly expressed in neuronal somatodendritic areas (postsynaptic) [16], the activation of CB2Rs may lead to opposing effects from those of CB1Rs, as CB1Rs are predominantly expressed on neuronal terminals, especially on GABAergic terminals (presynaptic) [17]. Considering these characteristics, CB2Rs appear to be an important substrate for neuroprotection [18], and targeting CB2Rs will likely offer a novel therapeutic strategy for treating neuropsychiatric and neurological diseases without typical CB1R-mediated side effects (including depression, anxiety, and suicidal thoughts) [19, 20]. Thus, an urgent need to understand the functional effects of CB2Rs in the brain has emerged. Extensive evidence supports the implication of the mesocorticolimbic

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dopamine (DA) system as a key brain circuit involved in a number of drug addictions. Alteration of the mesocorticolimbic DA circuit is the major cellular mechanism involved in promoting or preventing drug reward, dependence, and addiction. Emerging evidence demonstrates that CB2Rs mediate important modulations in drug-seeking behaviors in animals, including behaviors associated with cocaine, alcohol, and nicotine [21–23]. This suggests a significant impact of brain CB2Rs on animal drug reward, dependence, and addiction. Given the lack of psychoactivity demonstrated by selective CB2R agonists, CB2R ligands have been developed as new candidates for treating a variety of neurological and psychiatric disorders, including pain, neuroinflammation, stroke, Alzheimer's disease (AD), Parkinson's disease (PD), and Huntington's disease (HD). At the practical level, three medicines that activate the cannabinoid receptors CB1R/CB2R are now used in clinics: Cesamet (nabilone), Marinol (dronabinol; Δ^9-tetrahydrocannabinol [Δ^9-THC]), and Sativex (Δ^9-THC with cannabidiol). To date, there is no highly selective CB2R agonist available in clinical medicine. However, significant attention is currently being paid to the possibility of developing medicines from compounds that can activate CB2Rs at doses that induce little or no CB1R activation. This research was triggered by the evidence that many of the adverse effects induced by mixed CB1R/CB2R agonists result from CB1R activation, rather than from CB2R activation, and that CB2R-selective agonists have a number of important potential therapeutic applications [24]. Many highly selective compounds of various chemotypes have been identified, and several companies have initiated clinical trials. Therefore, we anticipate the emergence of new drugs from CB2R modulations once a better understanding of the cannabinoid receptors is gained. In this paper, we first provide a broad overview of CB2R expression and function in the brain, then discuss preclinical and clinical studies of CB2R-based drugs as potential therapeutic agents for a variety of neurological disorders in detail.

EXPRESSION OF CB2RS IN THE CENTRAL NERVOUS SYSTEM

CB2Rs were formerly considered to be exclusively peripheral cannabinoid receptors, restricted mainly to peripheral tissues and particularly immune cells, regulating immune responses and inflammation [8, 9]. Although initial studies were not able to detect the expression of CB2Rs in the brain, compelling evidence has demonstrated decentralized expression of CB2Rs, with their presence detected on neuronal, glial, and endothelial cells in various brain regions, including the cerebral cortex, hippocampus, thalamus, midbrain, pons, medulla, brain stem and cerebellum [25–31]. In neurons, CB2R's immunoreactivity was observed in somata, as well as large and medium-sized dendrites [32, 33]. Labeling of CB2Rs was also found to be associated with the plasma membrane in immunoreactive glial and endothelial cells [25, 26, 29].

Moreover, there are clear species-based differences in CB2Rs among humans, mice, and rats in terms of mRNA size in tissues and cell lines. Human CB2R (hCB2R) and mouse CB2R (mCB2R) genes are transcribed to yield two isoforms each, that is, hCB2A and hCB2B and mCB2A and mCB2B, respectively, while the rat CB2R (rCB2R) gene is transcribed to yield at least four isoforms, namely, rCB2A, rCB2B, rCB2C, and rCB2D [34]. The species-based differences in CB2R gene and receptor expression in the brain may partly explain why initial studies were not able to detect CB2R expression in both human and rodent brains. Human CB2A mRNA expression was observed predominantly in the testis, where the expression level was more than 100-fold lower that in the spleen and leukocytes. Human CB2A expression was also observed in the human caudate nucleus, amygdala, hippocampus, cerebellum, nucleus accumbens, putamen, and cortex of the brain, with similar levels of expression in peripheral tissues, such as muscle and spleen. In contrast, CB2B mRNA could not be detected in brain regions at a significant level and is predominantly expressed in spleen and leukocytes [35]. In mice, both mCB2A and mCB2B isoform transcripts were detected in brain regions such as the frontal cortex, striatum, and brain stem at ~1% of the spleen expression level [34, 35]. rCB2R mRNA was also present in some brain areas (retina, cerebellum, cortex, and brainstem) of rats [30, 36]. In situ hybridization RNAscope assays found higher levels of CB2R mRNA in different brain regions and cell types in mice than in rats. CB2R mRNA levels in tyrosine hydroxylase (TH)-positive dopamine (DA) neurons and TH-negative cells were very similar in mouse ventral tegmental areas (VTAs) but were significantly lower in dopamine transporter (DAT)-positive DA neurons than DAT-negative neurons in rat VTAs, suggesting species-based differences in CB2R mRNA expression in VTA DA neurons [37].

Generally, the expression of CB2Rs in the CNS has been found in many different types of cells, e.g., neurons [38], glial cells [39, 40], endothelial cells [26], retinal ganglion cells [36, 41] and neural progenitor cells [42]. The expression data of CB2R mRNA and protein in the CNS have been compiled in both Tables 1 and 2, from general distribution to cellular and subcellular localization.

NEUROPROTECTIVE ROLE OF CB2RS IN ISCHEMIC STROKE

Stroke is the second most common cause of death and the third leading cause of disability worldwide (see Tables 3 and 4), and ischemic stroke accounts for ~87% of all strokes [43]. The final consequence of stroke is patient death or disability characterized by multiple cognitive, motor and psychiatric problems associated with a major sanitary and socioeconomic burden. Despite significant advances in the development of neuroprotective compounds for ischemic stroke, recombinant tissue plasminogen activator (rt-PA) and endovascular thrombectomy are currently available to only a small subpopulation of stroke victims [44].

Considering the critical role of inflammation in the pathogenic progression of postischemic neuronal damage and the anti-inflammatory therapeutic potential of CB2Rs observed in several peripheral organs and CNS diseases, CB2R has drawn great attention as a potential therapeutic target for the treatment of ischemic stroke. Early activation of CB2Rs has been observed in animal models of stroke, and the binding levels of the CB2R tracer [^{11}C] NE40 were significantly higher in the cerebral cortical region on the lesion side than on the non-lesioned side in a photothrombotic stroke model [45]. Moreover, the selective CB2R agonists (O-3853, O-1966) caused a reduction in white blood cell rolling and adhesion along cerebral vascular endothelial cells, reduced infarct volumes and improved motor function in a mouse focal ischemia/reperfusion model [46]. Treatment with O-1966 contributed to protecting the brain through the attenuation of cerebral microcirculatory dysfunction, such as increased leukocyte/endothelial interactions, upregulation of adhesion molecule expression and disruption of the blood-brain barrier (BBB) [47]. In addition, it has been shown that the CB2R agonist JWH-133 protects against cerebral ischemia by inhibiting the recruitment of neutrophils to brain endothelial cells and the chemotaxis of neutrophils [48], ameliorating mitochondrial depolarization through modulation of AMPK/CREB signaling [49] and suppressing hypoxia-induced activation of the NF-κB-dependent neuroinflammatory pathway in microglial cells [50]. Recent research demonstrated, in a gerbil model of transient cerebral ischemia, that N-linoleyltyrosine, as an anandamide analog, could improve motor coordination, alleviate learning and memory impairments, attenuate ischemia-induced neural loss in the hippocampus and decrease inflammation in mice via the PI3K/Akt signaling pathway by activating CB2R [51].

Some studies have suggested that the effectiveness of CB2R agonists in animal models of stroke is affected by treatments. Delayed treatment with a CB2R agonist, AM1241, failed to

Table 1. Protein expression of CB2Rs in the CNS.

Brain region	Cell types	Subcellular localization	Species	Ref
Olfactory tubercle	Neurons		Healthy SD rats	[25]
Cerebral cortex: (layers III and v)	Pyramidal neurons	Cell body and apical dendrite	Healthy SD rats	[25]
Hippocampus: CA1	Neurons	Cell body and dendrite	Healthy SD rats	[26, 29]
CA2, CA3 and subiculum	Gila cells	Plasma membrane	Healthy SD rats	[26, 29]
	Endothelial cells	Plasma membrane	Healthy SD rats	[26, 29]
	Pyramidal neurons		Healthy SD rats	[25]
Thalamus		Cell body	Healthy SD rats	[25]
Hypothalamus		Cell body	Healthy SD rats	[25]
Midbrain: (periaqueductal gray, paratrochlear nucleus, paralemniscal nucleus, red nucleus, amygdala, geniculate nucleus and interpeduncular nucleus, inferior colliculus, substantia nigra)	Neurons	Cell body and dendrite	Healthy SD rats	[25, 26]
	Glial and endothelial cells	Plasma membrane and some unmyelinated axons	Healthy SD rats	[26]
Pons: (pontine nucleus)	Astrocytes or microglial cells	Cell processes	Healthy SD rats	[25]
Medulla:	Neurons	Cell body	Healthy SD rats	[25]
Brain stem:	Neurons		Healthy wistar rats, ferret	[28, 30]
Cerebellum: (molecular layers. Purkinje cell layers, the granule layers)	Neurons	Cell body and dendrite	Healthy rats, ferret	[25, 30]
	Endothelial cells		Healthy rats	[27]
	Perivascular microglial cells		Post-mortem brain	[31]
Retina: (segment of photoreceptor Layer, Outer nuclear layer, Henle fiber layer, outer plexiform layer, inner nuclear layer, inner plexiform layer, ganglion cell layer)	Cones	Cell body	Healthy mice	[150]
	Rods	Cell body	Healthy mice	[150]
	Müller cells	Cell fiber	Healthy SD rats and monkeys	[151, 152]
	Horizontal cells	Soma and dendrite	Healthy mice	[150]
	Rod bipolar cells	Soma and dendrite	Healthy mice	[150]
	Cone bipolar cells	Soma	Healthy mice	[150]
	Amacrine cells	Soma	Healthy mice	[150]
	Ganglion cells	Soma and nuclei	Healthy mice and monkey	[150, 152]

suppress brain damage after stroke. In contrast, pretreatment with AM1241 significantly reduced the area of infarction and neurological deficits [52]. Similar reports have also indicated that pretreatment with O-1966 was protective against cognitive impairments and neuronal tissue damage after permanent cerebral ischemia but may also influence the neuronal or glial function of learning and memory circuits in the uninjured brain [53]. CB2Rs in microglial cells following hypoxia-ischemia (HI) insult act as a neuroprotective mechanism to prevent inflammation mediated through modulation of the inflammation-related HIF-1α/TIM-3 signaling pathway [54]. Furthermore, CB2Rs were also found to be fundamental for driving neurogenesis by promoting neuroblast migration toward the boundary of the infarct area, increasing the number of new cortical neurons and improving functional outcome after stroke [55]. Regarding the underlying molecular mechanisms, CB2R activation has been shown to inhibit neuroinflammation, attenuate neuronal tissue damage, drive neurogenesis and improve motor function and memory impairment. Overall, the neuroprotective effects of CB2Rs in ischemic stroke pathogenesis present a novel promising therapeutic strategy that might overcome the limitations of current stroke treatment.

NEUROPROTECTIVE ROLE OF CB2RS IN ALZHEIMER'S DISEASE

Alzheimer's disease (AD) is the most common progressive neurodegenerative disease in the aged population and is characterized by abnormal accumulation of β-amyloid (Aβ) in senile plaques in the brain. The excessive deposition of Aβ triggers neurodegeneration, synaptic dysfunction, inflammation, and microvascular alterations, which can eventually lead to cognitive impairment, memory loss, and behavioral changes [56].

Emerging evidence indicates the involvement of CB2Rs in the pathological progression of AD. There is a great deal of experimental evidence demonstrating that CB2R is expressed at very low turnover rates in the healthy CNS, but the expression of CB2Rs is highly induced in plaque-associated microglial cells and astrocytes in brain tissues from AD patients and in genetic mouse models expressing pathogenic variants of amyloid precursor protein (APP) [57–60]. Interestingly, it seems that CB2R correlates with two relevant AD molecular markers, namely, $A\beta_{42}$ levels and senile plaque score, even though cognitive status shows no correlation [61]. Moreover, CB2Rs might be a suitable target for the development of PET radiotracers that could serve as a biomarker for neuroinflammation in the early preclinical stages of AD, when no significant neuronal loss has yet developed [62].

Previously, it was observed that a lack of CB2Rs enhanced the level of Iba1 staining and exacerbated soluble $A\beta_{42}$ and plaque deposition, which might confirm the constitutive role of CB2Rs in reducing amyloid plaque pathology in AD [63]. Pharmacological activation of CB2Rs with JWH-015 was also able to induce the removal of Aβ plaques from human AD tissue sections by human THP-1-derived macrophages via inhibition of the secretion of IL-1β and TNF-α [64]. The specific CB2R agonist JWH-133 could improve cognitive impairment, inhibit neuroinflammation and oxidative stress responses, and lower tau hyperphosphorylation in the vicinity of Aβ plaques when administered presymptomatically [65]. Moreover, JWH-133 could also improve the endothelial-dependent relaxations impaired by Aβ and exert vasodilatory effects that were maintained in Tg APP mice, thus being beneficial in the treatment of AD [66]. Similarly, pharmacological studies in rodents have also identified a crucial role of CB2Rs in AD-associated inflammatory processes, demonstrating that treatment with 1-((3-benzyl-3-methyl-2,3-dihydro-1-benzofuran-6-yl) carbonyl) piperidine (MDA7), a novel selective CB2R agonist, suppressed the activation of microglial cells and astrocytes, decreased the upsurge of CB2R, and promoted the clearance of Aβ, which eventually promoted recovery of neuronal synaptic plasticity and improved cognition and memory in AD models [67, 68]. In addition, both selective (JWH-133) and nonselective (WIN55212-2) CB2R agonists, but not a CB1R-selective agonist (ACEA), stimulated glucose uptake in the mouse brain, increasing the therapeutic interest in CB2R agonists as nootropic agents [69]. Furthermore, a recent study reported that CB2R activation by JWH-015 played a beneficial role in novel object recognition ability concomitant with region-specific regulation in microglia-mediated

Table 2. mRNA expression of CB2Rs in the CNS.

Brain region	Cell types	Subcellular location	Species	Ref
Prefrontal cortex			Healthy mice and rat	[35, 37]
Cerebral cortex: (layers III and V)	Neurons with pyramidal-like phenotype		Healthy mice, rat and *Macaca fascicularis*	[35, 37, 153]
Hippocampus: (CA1, CA3 and dentate gyrus)	Glutamatergic neurons	Nuclei	Healthy mice and *Macaca fascicularis*	[38, 153]
	GABAergic interneurons	Nuclei	Healthy mice and *Macaca fascicularis*	[38, 153]
	Pyramidal neurons	Cell body	Healthy mice	[38]
Hypothalamus			Healthy mice	[35]
Striatum			healthy rats, control mice and LPS-injected mice	[35, 37, 39]
Globus pallidus: (internal and external segments)	Pallidal neurons	Cytoplasm	Healthy *Macaca fascicularis* control, parkinsonian and dyskinetic monkeys	[153, 154]
	Pallidothalamic projection neurons	Cell body		
Nucleus accumbens			Human with developmental disorders, healthy rat and mice	[35, 37]
Amygdal			Human with developmental disorders,	[35]
Putaman			Human with developmental disorders,	[35]
Caudate			Human with developmental disorders,	[35]
Substantia nigra	Microglial cells		Post-mortem PD patients and control subjects	[39]
Ventral tegmental area	TH-positive DA neurons		Healthy mice,	[32, 37]
	DAT-positive DA neurons		Healthy rat	[37]
	TH-negative non-DA neurons		Healthy mice	[32]
Brain stem	Neurons of the dorsal motor nucleus of the vagus	Cell membrane and cytoplasm	Healthy Ferret, rat and mice	[30, 35]
Cerebellum	Cerebellar granule cells		Healthy Ferret, rat and mice	[30, 35]
Retina: (inner segments of photoreceptor cells, inner nuclear layer, retinal ganglion cell layer)	Retina ganglion cells	Soma	Healthy adult SD rats	[41]

neuroinflammation and dendritic complexity in AD model mice [70]. These results may constitute the basis of CB2R-based therapies or diagnostic approaches for AD.

Although CB2R has been thought to play an important role in neuroinflammatory responses and has been proposed as a therapeutic target for AD, the exact mechanism of CB2R signaling in AD remains elusive. Moreover, conflicting results have also been reported wherein CB2R is not required for ameliorating neuropathy or preventing cognitive decline by inhibiting 2-arachidonoylglycerol (2-AG, a full agonist of CB1R and CB2R) metabolism in AD model animals [71]. Similarly, the anti-inflammatory and neuroprotective effects of pharmacological and genetic inhibition of 2-AG metabolism were not mediated by CB1Rs or CB2Rs [72]. In another study, researchers suggested that CB2Rs participated in Aβ processing in a mouse model of AD and played a minor role in the therapeutic properties of a cannabis-based medicine, as CB2R deficiency did not affect the viability of AβPP/PS1 transgenic mice, did not accelerate memory impairment, did not modify tau hyperphosphorylation in dystrophic neurites associated with Aβ plaques, and did not attenuate the positive cognitive effect induced by cannabis-based medicine in these animals [73]. Interestingly, Schmöle et al. [74] found that microglial cells harvested from CB2R($^{-}/^{-}$) mice were less responsive to proinflammatory stimuli than CB2R($^{+}/^{+}$) microglial cells harvested from wild-type mice. Transgenic APP/PS1 mice lacking CB2Rs showed reduced percentages of microglial cells and infiltrating macrophages, lower expression levels of proinflammatory chemokines and cytokines in the brain, and diminished concentrations of soluble $A\beta_{40/42}$. Recently, the authors further reported that the genetic deletion of CB2Rs improved cognitive and learning deficits in APP/PS1*CB2R$^{-}/^{-}$ mice, which was accompanied by reduced neuronal loss and decreased plaque levels, which coincided with increased expression of Aβ-degrading enzymes. In addition, plaque-associated microglial cells in APP/PS1*CB2R$^{-}/^{-}$ mice showed a less activated morphology, while the plaques were smaller and more condensed than those in APP/PS1 mice [58]. These divergent results from previous studies reflected the complex roles of CB2Rs in the neuropathology and pathogenesis of AD. Nevertheless, CB2R might serve as a new potential therapeutic target for preventing, alleviating and treating AD through several mechanisms. CB2Rs appear to be part of a protective system that might be detrimental when engaged continuously. Further research is needed to elucidate the potential molecular mechanisms.

NEUROPROTECTIVE ROLE OF CB2RS IN PARKINSON'S DISEASE

Parkinson's disease (PD) is the second most common neurodegenerative disease and the most common motor disorder affecting millions of people worldwide [75]. Loss of the neurotransmitter dopamine has been regarded as the major pathological characteristic of PD, leading to motor dysfunction and cognitive impairment. However, until now, there has been no fully effective therapy developed to treat the clinical syndromes of PD, as current pharmacotherapies could only temporarily relieve PD symptoms but not prevent or slow down disease progression. There are several reports that describe the potential roles of CB2Rs as a viable target for anti-inflammatory therapy for PD. CB2R levels were significantly elevated in animal models of PD, and this increase correlated significantly with an increase in microglial activation [76]. Moreover, postmortem studies of human patients with PD have revealed that the expression of CB2R is elevated in microglial cells recruited and activated at lesioned sites in the substantia nigra of PD patients [39]. In short, the expression of CB2Rs in glial cells is upregulated in PD, but this receptor may also be located in certain neuronal subpopulations and serve as a marker of neuronal loss. For instance, García et al. observed that CB2R was located in TH-containing neurons in the substantia nigra

Table 3. The expression trend of CB2Rs in various neurological diseases.

Brain regions	Tendency	Detection methods	Species	Neurological disorders	Ref
Microglia in peri-infarct areas	Upregulated	Immunohistochemistry, positron emission tomography, real-time PCR	Rat (model)	Ischemic stroke	[45, 52]
Plaque-associated microglial cells and astrocytes	Upregulated	Immunofluorescence, immunohistochemistry, real-time PCR	Mice (model), patients	AD	[57, 59]
Glial cells in striatum and substantia nigra	Upregulated	Immunofluorescence, real-time PCR	Patients, Mice (model)	PD	[39, 76]
TH-neurons in substantia nigra	Downregulated	Immunofluorescence, immunohistochemistry, Western blot, real-time-PCR	Mice (model)	PD	[77, 78]
Hippocampus, cortex, striatum, cerebellum	Upregulated	Real-time PCR, Western blot, immunofluorescence, immunohistochemistry	Mice (model), patients	HD	[85, 86]
Activated microglial cells in CNS	Upregulated	Real-time PCR, immunofluorescence, immunohistochemistry	Mice (model), patients	MS	[93, 94]
Activated microglial cells in spinal cord	Upregulated	Real-time PCR, Western blot	Mice (model), patients	ALS	[94, 107]
DRG neurons and satellite glial cells.	Upregulated	Immunohistochemistry, Western blot, in situ hybridization,	Rat (model)	Pain	[134, 135]

at levels significantly lower in PD patients than in controls [77]. Similarly, mice with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced parkinsonian syndrome also showed a down-regulation of the CB2R protein in the substantia nigra and hippocampus three weeks after MPTP injection [78]. Additionally, CB2R-deficient mice displayed intense activation of microglial cells and much more intense deterioration of TH-containing nigral neurons in the case of the substantia nigra in models of PD, which supported the potential neuroprotective role of this receptor [39]. One in vivo study has also shown that pharmacological activation of CB2Rs with the nonselective cannabinoid receptor agonist WIN55,212–2 or the CB2R agonist JWH-015 protected against MPTP-induced nigrostriatal degeneration by inhibiting microglial activation/infiltration [79]. In addition, the selective CB2R agonist AM1241 has been shown to have a significant therapeutic effect on PD and regenerate DA neurons after the neurotoxic effect of MPTP treatment. The possible mechanisms underlying the neurogenic effect of AM1241 might be the induction of CB2R expression and an increase in phosphorylation of the PI3K/AKT signaling pathway [78]. Similar findings were observed in an LPS-induced animal model of PD after administration of Δ9-tetrahydrocannabivarin (THCV), which has antioxidant properties and the ability to activate CB2Rs and block CB1Rs. In another animal model of PD induced by rotenone (ROT), the authors reported that treatment with β-caryophyllene (BCP), a natural CB2R agonist, attenuated oxidative/nitrosative stress and neuroinflammation, inhibited gliosis and proinflammatory cytokine release, and decreased nigrostriatal degeneration [80]. Furthermore, it has been shown recently that the use of BCP offers significant protection against 1-methyl-4-phenylpyridinium (MPP)-induced neurotoxicity by activating a cellular redox enzyme system [81, 82]. In summary, agonists of CB2Rs that exert antioxidant and anti-inflammatory activities might have promising pharmacological profiles for ameliorating parkinsonian symptoms and delaying disease progression in PD.

NEUROPROTECTIVE ROLE OF CB2RS IN HUNTINGTON'S DISEASE

Huntington's disease (HD) is a genetic neurodegenerative disease caused by the expansion of a CAG triplet repeat in the gene encoding the protein huntingtin, which results in neuron degeneration mainly in the striatum. This leads to abnormal motor movements (chorea) and cognitive decline [83]. Currently, no successful treatment is known to prevent or slow the progression of HD. However, it is worth noting that CB2R is emerging as a new therapeutic target for the treatment and early diagnosis of different neurodegenerative disorders, including HD [84]. In the transgenic R6/2 Huntington chorea mouse model, the expression of CB2Rs was increased in the hippocampus, cortex, striatum and cerebellum, as shown by real-time polymerase chain reaction [85]. The upregulation of CB2Rs was also observed in striatal microglial cells of HD transgenic mouse models and in the caudate nucleus/putamen from HD patients. Notably, genetic ablation of CB2Rs in R6/2 mice enhanced microglial activation, aggravated disease symptomatology and reduced mouse lifespans. Likewise, microglial CB2Rs exerted neuroprotective effects against HD excitotoxicity by reducing neuroinflammation, brain edema, striatal neuronal loss and motor symptoms [86]. In line with these findings, a Sativex-like phytocannabinoid combination was capable of delaying signs of disease progression in a proinflammatory model of HD generated by intrastriatal injection of malonate in a CB1R- and CB2R-dependent manner. The role of CB2Rs was further confirmed by two observations: CB2R-deficient mice were more sensitive to malonate than wild-type animals [87], and genetic deletion of CB2Rs both accelerated the onset of motor deficits and increased their severity [88]. However, the authors found that treatment with GW405833, a high-affinity and

Table 4. Neuroprotective effects of selective CB2R ligands.

Agonists	Ki (nM) /CBR	Neuroprotective effects	Ref
O-3853	815 ± 127/CB1R 17.3 ± 2.5/CB2R	Inhibiting white blood cell rolling and adhesion, reducing infarct volumes, improving motor function in stroke.	[46]
O-1966	5055 ± 984/CB1R 23 ± 2.1/CB2R	Attenuating cerebral microcirculatory dysfunction, cognitive impairments and neuronal tissue damage in stroke; reducing white cell rolling and adhesion to cerebral microvessels, inhibiting the invasion of immune cells and improving neurologic function after insult.	[47, 53, 97]
JWH-133	677/CB1R 3.40/CB2R	Inhibiting nueroinflammation and mitochondrial depolarization after stroke; increasing the migration of neural progenitor cells in vitro stroke model; improving cognitive impairment, inhibiting neuroinflammation and oxidative stress responses, and lowering tau hyperphosphorylation in the vicinity of Aβ plaques; improving endothelial-dependent relaxations impaired by Aβ; stimulating glucose uptake in the mouse brain; suppressing both mechanical and cold hypersensitivity in an EAE mouse model; attenuating mechanical allodynia.	[48–50, 55, 65, 66, 69, 100, 139]
AM1241	280/CB1R 3.4/CB2R	Reducing cerebral infarction and neurological deficits; regenerating DA neurons after MPTP treatment; slowing motor neuron degeneration and preserving motor function and increasing survival interval in ALS model; attenuating mechanical allodynia.	[52, 104, 78, 105, 139]
JWH-015	383/CB1R 13.8/CB2R	Removing Aβ plaques from human AD tissue sections, attenuating novel object recognition ability in AD mouse model; protecting against MPTP-induced nigrostriatal degeneration, inhibiting microglial activation/infiltration, attenuating mechanical allodynia and thermal hyperalgesia.	[64, 70, 79, 139]
MDA7	2565 ± 695/CB1R 238 ± 143/CB2R	Suppressing activation of microglial cells and astrocytes, promoting Aβ clearance, promoting neuronal recovery, synaptic plasticity and improving cognition and memory in AD models, attenuating mechanical allodynia.	[67, 68, 139]
BCP	155 ± 4/CB2R	Attenuating oxidative/nitrosative stress, neuroinflammation, gliosis and nigrostriatal degeneration; diminishing axonal demyelination. and modulating Th1/Treg immune balance; attenuating mechanical allodynia	[80, 101, 139]
GW405833	4772/CB1R 3.92/ CB2 R	Extending life spans and suppressing neurodegeneration, synapse loss and motor deficits; attenuating mechanical allodynia	[88, 139]
HU308	>10,000/CB1R 22.7/CB2	Ameliorating EAE symptoms, reducing axonal loss, and inhibiting microglial activation and reactive astrogliosis	[96, 106],

highly selective partial CB2R agonist, extended life spans and suppressed neurodegeneration, synapse loss and motor deficits by CB2R signaling not in parenchymal microglial cells but rather in peripheral immune cells [88]. Moreover, Dowie et al. [89] demonstrated that CB2Rs were localized on the CD31-positive blood vessel endothelium and vascular smooth muscle but not expressed on microglial cells or astrocytes in the postmortem brains from HD patients. Although there is ambiguity about the cellular localization of CB2Rs, it could be speculated that selective CB2R agonists might have potential therapeutic value in the treatment of HD. Further mechanistic studies are still warranted to investigate the function of central CB2Rs in HD.

NEUROPROTECTIVE ROLE OF CB2RS IN MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is an autoimmune disorder of the nervous system characterized by inflammation, neurodegeneration, and demyelination of neurons, which are associated with symptoms such as sensory and motor impairment, ataxia and spasticity. Despite ongoing progress in the understanding of the pathogenesis of MS, new therapeutic approaches are still needed to overcome the lack of effective treatments for this disease, as there is no cure [90]. Among the new therapeutic strategies for the treatment of MS, the modulation of CB2Rs has recently emerged as a promising target for therapeutic intervention [84, 91]. Recently, a significant genetic association was observed between the CB2R rs35761398 (Q63R) polymorphism and MS, which implied the involvement of the CB2R gene in susceptibility to MS [92]. Moreover, several studies confirmed that CB2R expression was upregulated in brain tissues from patients or animal models of MS. Microglial activation was accompanied by the upregulation of CB2Rs at both the mRNA (100-fold) and protein (10-fold) levels compared to microglial cells in the resting state in an experimental autoimmune encephalomyelitis (EAE) mouse model of MS, suggesting that CB2Rs play an important role in the function of microglial cells in the CNS during autoimmune-induced inflammation [93]. Studies performed by Yiangou *et al*. indicated that human postmortem spinal cord specimens had a significantly greater density of CB2R-immunoreactive microglial cells/macrophages in the white matter in MS sections with lesions, appearing in clusters, usually within the edges of plaque-containing areas [94]. Furthermore, the expression of CB2Rs was found in T lymphocytes, astrocytes, and both perivascular and reactive microglial cells in postmortem brain tissues from donors with MS. Specifically, CB2R-positive microglial cells were evenly distributed within active plaques but were located in the periphery of chronically active plaques [95].

Experimental work has provided robust evidence of the immunomodulatory and neuroprotective properties by activation of CB2R in EAE animal models. CB2R knockout mice showed an exacerbated clinical score of the disease, which occurred in concert with extended axonal loss and microglial activation. In contrast, administration of the CB2R-selective agonist HU-308 markedly ameliorated EAE symptoms, reduced axonal loss, and inhibited microglial activation [96]. Investigations of the effects of O-1966 (a full CB2R agonist) on EAE progression demonstrated that administration of O-1966 resulted in reduced white cell rolling and adhesion to cerebral microvessels, inhibited the invasion of immune cells and improved neurologic function

after insult [97]. Exogenous administration of the endocannabinoid 2-AG significantly ameliorated the demyelinating and neurodegenerative processes partially through CB2Rs since it delayed disease onset, reduced relapse severity and chronic disability, and eliminated mortality in severe chronic EAE [98]. IL-12 and IL-23 are functionally related heterodimeric cytokines that play essential roles in the pathogenesis of MS, and the endocannabinoid anandamide inhibited the IL-12/IL-23 axis through the $ERK_{1/2}$ and JNK pathways in human and murine microglial cells, partially mediated by CB2R activation [99]. In addition, activation of CB2Rs with a CB2R-specific agonist (JWH-133) suppressed both mechanical and cold hypersensitivity without producing signs of sedation or ataxia in an EAE mouse model [100]. This was the first preclinical study to directly promote CB2Rs as a promising target for the treatment of central pain in an animal model of MS. Alberti *et al*. demonstrated that BCP significantly ameliorated both the clinical and pathological parameters of EAE, which seemed to be linked to the ability of BCP to inhibit microglial cells, $CD4^+$ and $CD8^+$ T lymphocytes, as well as the protein expression of proinflammatory cytokines. Furthermore, BCP diminished axonal demyelination and modulated Th1/Treg immune balance through the activation of CB2Rs [101].

Taken together, these observations indicate a neuroprotective role of CB2Rs in EAE pathology. We conclude that nonpsychoactive and selective CB2R agonists possess strong therapeutic potential for the treatment of both neurologic dysfunction and central pain in MS patients.

NEUROPROTECTIVE ROLE OF CB2RS IN AMYOTROPHIC LATERAL SCLEROSIS

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease of unknown etiology characterized by progressive deterioration of both upper and lower motor neurons. Approximately 90% of ALS cases are sporadic, and 10% are familial, due to genetic mutations [102]. Currently, there is no effective cure for this illness, with some evidence supporting an improvement in median survival by two to three months with the anti-excitotoxic agent riluzole, which remains the sole treatment option for ALS patients in the US and Europe. This drug has offered modest survival benefits since its approval in the 1990s [103]. Despite significant research efforts, an overwhelming majority of human clinical trials have failed to demonstrate clinical efficacy in the treatment of ALS. More effective drug therapies targeting disease progression are sorely needed.

As shown by several studies [104–106], cannabinoid CB2R-selective compounds may slow motor neuron degeneration, preserve motor function and represent a novel therapeutic modality for the treatment of ALS. Several observations have suggested that CB2Rs were markedly upregulated in activated microglial cells and astrocytes in both patients with ALS and experimental transgenic mouse models of ALS. The upregulation of CB2Rs appeared to occur in activated microglial cells in postmortem human spinal cord specimens from patients with ALS [94] and in spinal gray and white matter areas in TDP-43 transgenic mice at the postsymptomatic stage [107]. Additionally, the mRNA, receptor binding and function of CB2Rs were found to be dramatically and selectively upregulated in the spinal cords of G93A-SOD1 mice in a temporal pattern paralleling disease progression. More importantly, daily injections of the selective CB2R agonist AM1241 initiated at symptom onset increased the survival interval after disease onset by 56% [104]. CB2R expression was also found to be upregulated predominantly in reactive astrocytes in canine degenerative myelopathy [108] and the postmortem motor cortex of ALS patients [109]. Treatment with AM1241 was effective at slowing signs of disease progression when administered after the onset of these signs in G93A-SOD1 mutant mice [105]. Targeting glial cannabinoid CB2Rs delayed the progression of the pathological phenotype in TDP-43 transgenic mice by improving motor behavior, completely preserving motor neurons in the ventral horn, and attenuating reactive astrogliosis [106]. The CB2R is, therefore, considered a very promising target for therapeutic approaches as well as an imaging tool. To date, by applying in vitro autoradiography, the translational relevance of CB2R imaging was demonstrated with specific binding of [^{11}C]KD2 [110] and [^{11}C]RS-028 [85] to postmortem human ALS spinal cord tissues.

MODULATION OF CB2RS IN THE TREATMENT OF EPILEPSY

Epilepsy is a common chronic neurologic disorder that is characterized by recurrent spontaneous seizures that are associated with an imbalance between excitatory and inhibitory systems in various regions of the brain [111]. Treatment-resistant epilepsy affects 30% of epileptic patients and is associated with severe morbidity and increased mortality [112]. Accumulating data have demonstrated that cannabinoid systems, including endocannabinoids, anandamide, and 2-arachidonoyl glycerol, and their targets CB1Rs and CB2Rs appear to regulate seizure activity [113–120]. The rationale for the antiepileptic effects of the cannabinoid system contends that the CB1Rs (possibly also CB2Rs) are linked to inhibitory G-protein ($G_{i/o}$) signaling, which reduces neuronal excitability and/or neural synchronization. For example, the activation of brain CB1Rs modulates A-type K^+ channels and N- and P/Q-type voltage-gated Ca^{2+} currents, which stabilizes membrane potentials [121, 122] and modulates presynaptic neurotransmitter release [123–125]. Based on these concepts, numerous cannabinoid analogs have been examined in a variety of animal models [115, 116, 119, 126–129]. Although cannabinoid ligands and CB1R agonists possess some antiepileptic effects, nonspecific modulations of cannabinoid systems will limit their therapeutic use for the treatment of human epilepsy because of their severe adverse effects. Therefore, significant attention is currently being directed toward the possibility of developing medicines from compounds that can selectively activate CB2Rs and have important potential therapeutic applications at doses that induce little or no CB1R activity.

Emerging evidence has indicated that CB2Rs are involved in epileptic activity in animal models. In a rat model of acute pentylenetetrazole (PTZ)-induced seizure, pretreatment with palmitoylethanolamide (PEA) increased the latency of seizure initiation and reduced the duration of seizures. This antiepileptic effect was attenuated by either the CB1R (AM251) or CB2R (AM630) antagonists, suggesting that CB2R mediated PEA's effect [130]. In developing rats, Huizenga et al. examined the antiepileptic effects of a variety of cannabinoid ligands and found that CB1R/CB2R or selective CB1R agonists exhibited antiepileptic effects in 10-day-old rat models of either chemo-convulsing methyl-6,7-dimethoxy-4-ethyl-beta-carboline-3-carboxylate- or PTZ-induced seizure [131]. Although the CB2R-selective agonist HU308 did not show an antiepileptic effect, the CB2R-selective antagonist AM630 increased seizure severity [131]. In addition, a recent report showed that CB1R knockout mice did not have an epilepsy phenotype, but co-KO CB1Rs and CB2Rs caused epilepsy in animals [132], suggesting that CB2Rs play a role in stabilizing the neuronal system. Overall, while manipulation of CB2Rs is a reasonable and promising rationale, the current data are still very limited, and the effects of CB2R ligands on human epilepsy have not been tested. New insights into the exact mechanism by which CB2R agonists modulate neural networks and how they control human seizure activity are needed to determine the efficacy of CB2Rs as a therapeutic target for epilepsy and the associated seizure activity.

THERAPEUTIC POTENTIAL OF CB2RS IN THE TREATMENT OF PAIN

Pain is a ubiquitously unpleasant feeling among humans, as well as many animal species, often caused by actual and potential tissue damage. Opioids and nonsteroidal anti-inflammatory drugs have proven efficacious in the treatment of pain, but their use is significantly hampered due to serious adverse effects. A great need exists for the development of novel analgesics to control pain with long-term effectiveness [133]. Some studies have demonstrated that CB2Rs are redistributed in many important portions of pain pathways, such as DRG and afferent fibers in the dorsal horn of the spine, and may be upregulated under inflammatory and neuropathic pain conditions [134, 135]. In addition, it is being increasingly recognized that CB2Rs are located in neuronal circuits in the brain relevant for pain control and dopamine-mediated reward [32, 136]. These data provide an anatomical basis for the involvement of CB2Rs in the modulation of neuropathic pain.

Hyperalgesia and allodynia induced by sciatic nerve injury were enhanced in $CB2R^{-/-}$ mice, while a reduced manifestation of neuropathic pain was observed in transgenic mice overexpressing CB2Rs. Deletion of CB2Rs also contributed to the development of contralateral mirror image pain, associated with enhanced microglial and astrocytic activation in the contralateral spinal horn [137]. Recently, it has been shown that deletion of CB2Rs in DA neurons led to a significantly higher threshold for tail flick responses along with decreased, but insignificant, paw lick latency in the hot plate test [138]. There is an overwhelming body of convincing preclinical evidence demonstrating that CB2R agonists produce antinociceptive effects in laboratory animal models of pain [139–141]. Activation of CB2Rs by agonists inhibited sensory nerve activity, decreased the sensitization of the nerves and reduced these inflammatory mediators in animal models of acute and chronic pain, suggesting that CB2Rs are involved in the attenuation of inflammatory and neuropathic pain pathways [138, 139].

Tolerance and physical dependence are common complications of long-term treatment of pain with opioids. Glial cells became activated in response to opioids. This activation opposed opioid analgesia and enhanced both opioid tolerance and dependence. Conversely, the clinically relevant efficacy of opioids was improved in animal models by inhibition of glial activation or proinflammatory cytokine actions [142]. Previous studies have shown that CB2R expression is increased in microglia in different models of neuropathic pain [139]. CB2R activation stimulated the release of endogenous opioid beta-endorphin from keratinocytes, which in turn acted at opioid receptors on primary afferent neurons to inhibit nociception [143]. Treatment with CB2R agonists induced morphine analgesia and attenuated morphine tolerance, possibly via either decreasing proinflammatory mediators or inducing the expression of μ-opioid receptors [144–146]. Thus, microglial CB2Rs may be a new target for preventing the development of opioid tolerance and may be highly efficacious in neuropathic pain states that are responsive to opioid analgesics. Given that the combination of selective CB2R agonists with conventional analgesics may lead to enhanced antinociception and reduce untoward side effects, therapeutics targeting CB2Rs hold promise as novel analgesics.

CONCLUSIONS AND PERSPECTIVES

In summary, the present review reveals that CB2Rs are expressed at low levels in specific brain areas of healthy individuals but are significantly upregulated in glial elements during most neurodegenerative disorders. This inducible feature allows CB2Rs to serve as diagnostic markers of neuroinflammation in the context of pathological conditions. Although the detailed mechanisms underlying the inducibility of CB2Rs are still unclear, the disease-associated epigenetic modulations, CB2R promoter regulations, and transcription factors and their downstream cannabinoid receptor signaling, as well as cytokines, growth factors, hormones, and other factors released in response to tissue injury and inflammation, are rational starting points for further investigations of the inducible mechanisms. Moreover, a growing body of scientific literature has demonstrated that activation of CB2Rs suppresses neuroinflammation and prevents neuronal degeneration by a variety of mechanisms both in vitro and in vivo. At the same time, CB2R activation alters several ion channel functions, which, in turn, reduces neuronal excitability, leading to neuroprotection. Collectively, if proven therapeutic in clinical settings, selective CB2R activation may represent an avenue for the development of novel therapeutic agents that provide neuroprotection against a variety of neurological disorders (Fig. 1). On the other hand, the challenge of selectively targeting brain CB2Rs without affecting peripheral CB2Rs remains, as CB2R levels are much higher in peripheral tissues (e.g., T-cells in spleen) than in the brain. Thus, systemic exposure of CB2R ligands to activate brain CB2Rs will always activate peripheral CB2Rs. We have two thoughts regarding this challenge: (1) brain CB2Rs are strongly inducible, meaning that they are upregulated during disease conditions such as addiction, degeneration, and inflammation. This pathology-associated increase significantly enhances the benefit to side-effect ratio. (2) Activation of brain CB2Rs protects neurons against pathological conditions (e.g., addiction, anxiety, stroke, epilepsy), which additionally activates peripheral CB2Rs (e.g., T-cells), while peripheral CB2R activation will be beneficial to the central protective effect by reducing inflammation and immune responses. Therefore, the activation of peripheral CB2Rs may not

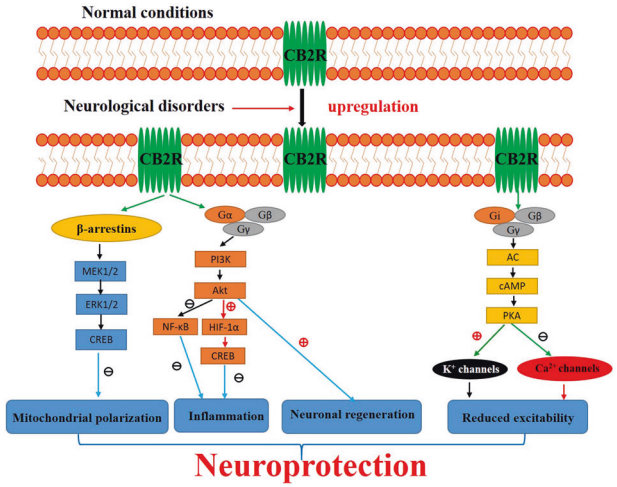

Fig. 1 Activation of CB2Rs after neurological disorders. Although brain CB2R levels are low in healthy individuals, they are significantly upregulated in glial elements in response to various neurological insults. Existing evidence demonstrates that signaling through CB2Rs can be mediated via G protein and β-arrestin, each with their own downstream effectors [147–149]. As shown in Fig. 1, CB2Rs are coupled to Gαi/o to inhibit adenylyl cyclase (AC) activity, leading to a decrease in cAMP levels. On the other hand, the Gβγ subunits, upon dissociation from Gαi/o, are known to activate G protein-gated inwardly rectifying K^+ channels and PI3K and inhibit voltage-gated Ca^{2+} channels. Alternatively, β-arrestin 2 recruitment to CB2Rs results in activation of the ERK pathway. Overall, activation of CB2Rs plays neuroprotective roles, including attenuation of neuroinflammation, amelioration of mitochondrial depolarization and stimulation of neurogenesis, which could eventually reduce deficits in cognition, memory and motor inhibition. In addition, the activation of CB2Rs also modulates ion channel functions, thereby altering neuronal excitability, and leads to neuroprotection.

induce side effects when brain CB2Rs are activated. Rather, both central and peripheral CB2Rs may work together to protect the brain's neurons against pathological alterations through neuronal and nonneuronal mechanisms.

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AUTHOR CONTRIBUTIONS

Both QX and FX equally contributed to writing the manuscript and sourcing references for the review. DHT and JFZ contributed to discussions and editing of the manuscript. JW conceived the outline of this paper and participated in critical review and further revision of the manuscript. All authors contributed to critical discussions and finalizing the manuscript before submission. They have all given approval to the final form of the manuscript.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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