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Themed Issue: Cannabinoids in Biology and Medicine, Part I

REVIEW

Prospects for cannabinoid therapies in basal ganglia disorders

Javier Fernández-Ruiz[1,2,3], Miguel Moreno-Martet[1,2,3], Carmen Rodríguez-Cueto[1,2,3], Cristina Palomo-Garo[1,2,3], María Gómez-Cañas[1,2,3], Sara Valdeolivas[1,2,3], Carmen Guaza[4], Julián Romero[2,5], Manuel Guzmán[2,3,6], Raphael Mechoulam[7] and José A Ramos[1,2,3]

[1]*Departamento de Bioquímica y Biología Molecular III, Instituto Universitario de Investigación en Neuroquímica, Facultad de Medicina, Universidad Complutense, Madrid, Spain,* [2]*Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Madrid, Spain,* [3]*Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain,* [4]*Neuroimmunology Group, Functional and Systems Neurobiology Department, Cajal Institute (CSIC), Madrid, Spain,* [5]*Laboratorio de Investigación, Fundación Hospital Alcorcón, Madrid, Spain,* [6]*Departamento de Bioquímica y Biología Molecular I, Instituto Universitario de Investigación en Neuroquímica, Facultad de Biología, Universidad Complutense, Madrid, Spain, and* [7]*Department of Medicinal Chemistry and Natural Products, Medical Faculty, Hebrew University, Jerusalem, Israel*

Correspondence
Javier Fernández-Ruiz, Departamento de Bioquímica y Biología Molecular III, Facultad de Medicina, Universidad Complutense, Ciudad Universitaria s/n, 28040 Madrid, Spain. E-mail: jjfr@med.ucm.es

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Cannabinoids are promising medicines to slow down disease progression in neurodegenerative disorders including Parkinson's disease (PD) and Huntington's disease (HD), two of the most important disorders affecting the basal ganglia. Two pharmacological profiles have been proposed for cannabinoids being effective in these disorders. On the one hand, cannabinoids like Δ^9-tetrahydrocannabinol or cannabidiol protect nigral or striatal neurons in experimental models of both disorders, in which oxidative injury is a prominent cytotoxic mechanism. This effect could be exerted, at least in part, through mechanisms independent of CB_1 and CB_2 receptors and involving the control of endogenous antioxidant defences. On the other hand, the activation of CB_2 receptors leads to a slower progression of neurodegeneration in both disorders. This effect would be exerted by limiting the toxicity of microglial cells for neurons and, in particular, by reducing the generation of proinflammatory factors. It is important to mention that CB_2 receptors have been identified in the healthy brain, mainly in glial elements and, to a lesser extent, in certain subpopulations of neurons, and that they are dramatically up-regulated in response to damaging stimuli, which supports the idea that the cannabinoid system behaves as an endogenous neuroprotective system. This CB_2 receptor up-regulation has been found in many neurodegenerative disorders including HD and PD, which supports the beneficial effects found for CB_2 receptor agonists in both disorders. In conclusion, the evidence reported so far supports that those cannabinoids having antioxidant properties and/or capability to activate CB_2 receptors may represent promising therapeutic agents in HD and PD, thus deserving a prompt clinical evaluation.

LINKED ARTICLES

This article is part of a themed issue on Cannabinoids in Biology and Medicine. To view the other articles in this issue visit http://dx.doi.org/10.1111/bph.2011.163.issue-7

Abbreviations

CBD, cannabidiol; CNS, central nervous system; FAAH, fatty acid amide hydrolase; HD, Huntington's disease; Nrf-2, nuclear factor-erythroid 2-related factor 2; PD, Parkinson's disease; ROS, reactive oxygen species; Δ^9-THC, Δ^9-tetrahydrocannabinol; Δ^9-THCV, Δ^9-tetrahydrocannabivarin

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The cannabinoid signalling system and the pathophysiology of the basal ganglia

Trying to elucidate the mechanisms of action of cannabinoids, the active constituents of the plant *Cannabis sativa*, Mechoulam and many other colleagues discovered in late 1980s and early 1990s the so-called cannabinoid system, a novel intercellular signalling system particularly active in the central nervous system (CNS) (see Chevaleyre *et al.*, 2006; Kano *et al.*, 2009 for reviews). Most of the elements that constitute this signalling system have been already identified and characterized (see Di Marzo, 2009; Pertwee *et al.*, 2010, for review), and, more importantly, they have been found to be altered in numerous pathologies, either in the CNS or in the periphery (Di Marzo, 2008; Martínez-Orgado *et al.*, 2009), which explains the proposed therapeutic potential of certain cannabinoid compounds in these disorders (Janero and Makriyannis, 2009; Pertwee, 2009). Presently, the cannabinoid signalling system represents an important field of study for the development of novel therapeutic agents with properties for symptom relief or control of disease progression in numerous CNS pathologies including chronic pain, feeding disorders, addictive states, movement disorders, brain tumours and others (Bahr *et al.*, 2006). Novel cannabinoid-based medicines have been recently approved for specific pathologies such as multiple sclerosis (Wright, 2007; Pertwee, 2009), whereas various clinical studies with these preparations are presently underway and should lead to novel indications over the next few years.

Basal ganglia disorders, mainly Parkinson's disease (PD) and Huntington's disease (HD) (an overview on the basal ganglia circuitry and its main pathologies can be seen in Figure 1), are included in the group of illnesses that may benefit from the use of cannabinoid-based medicines. HD is an inherited neurodegenerative disorder caused by a mutation in the gene encoding the protein huntingtin. The mutation consists of a CAG triplet repeat expansion translated into an abnormal polyglutamine tract in the amino-terminal portion of huntingtin, which due to a gain of function becomes toxic for specific striatal and cortical neuronal subpopulations, although a loss of function in mutant huntingtin has been also related to HD pathogenesis (see Zuccato *et al.*, 2010 for review). Major symptoms include hyperkinesia (chorea) and cognitive deficits (see Roze *et al.*, 2010 for review). PD is also a progressive neurodegenerative disorder whose aetiology has been, however, associated with environmental insults, genetic susceptibility or interactions between both causes (Thomas and Beal, 2007). The major clinical symptoms in PD are tremor, bradykinesia, postural instability and rigidity, symptoms that result from the severe dopaminergic denervation of the striatum caused by the progressive death of dopaminergic neurons of the substantia nigra *pars compacta* (Nagatsu and Sawada, 2007).

As mentioned above, both disorders could potentially receive significant benefits from the use of novel cannabinoid-based medicines. This is supported by the changes experienced during the progression of PD and HD by cannabinoid receptors, and also by other elements of the cannabinoid signalling system, all of them already identified in basal ganglia structures (reviewed in Fernández-Ruiz and

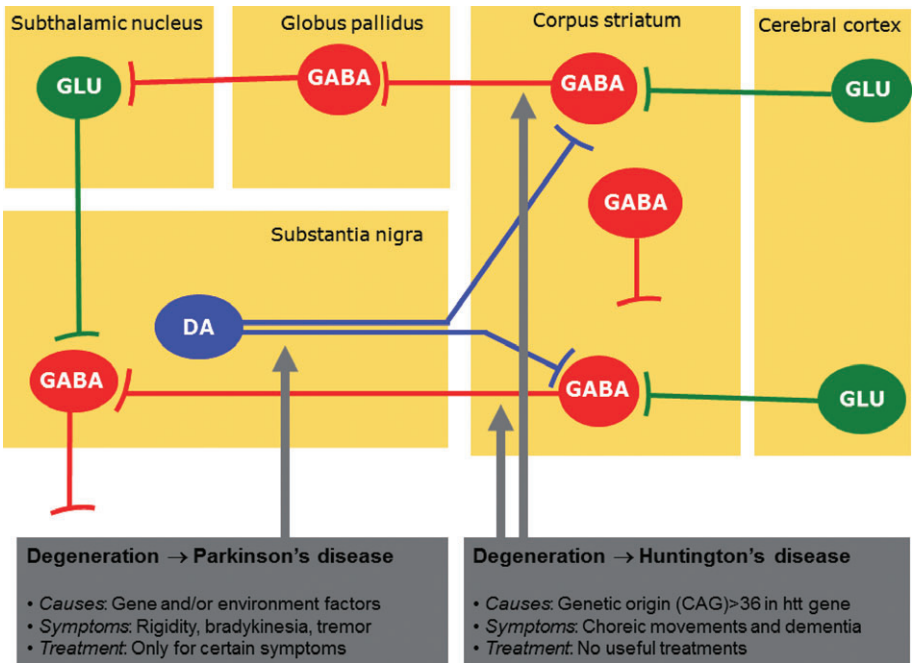

Figure 1

Diagram showing the most important neuronal pathways involved in the basal ganglia function. The neuronal subpopulations that are affected in the two pathologies reviewed in this article, Huntington's disease and Parkinson's disease, are indicated by arrows. DA, dopamine; GABA, γ-aminobutiric acid; GLU, glutamate.

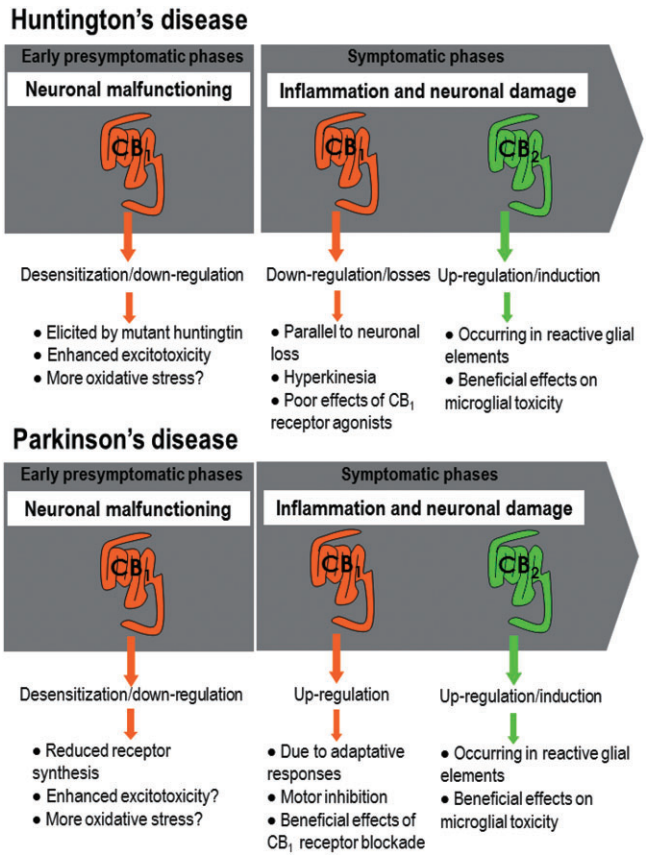

Figure 2

Comparison of CB_1 and CB_2 receptor changes during presymptomatic and symptomatic phases in experimental models of Huntington's disease and Parkinson's disease.

González, 2005; Gerdeman and Fernández-Ruiz, 2008). These changes are summarized in Figure 2, and, in general, are compatible with the following three ideas:

(a) Early presymptomatic phases in both disorders characterized by neuronal malfunctioning rather than neuronal death, particularly in HD and also in PD, are associated with down-regulation/desensitization of CB_1 receptors (Denovan-Wright and Robertson, 2000; Glass *et al.*, 2000; Lastres-Becker *et al.*, 2002a; Dowie *et al.*, 2009; García-Arencibia *et al.*, 2009a; Ferrer *et al.*, 2010; Blázquez *et al.*, 2011). Given that the activation of CB_1 receptors inhibits glutamate release, one may expect that the down-regulation/desensitization of these receptors observed in both disorders is associated with enhanced glutamate levels and excitotoxicity, then playing an instrumental role and contributing to disease progression (Maccarrone *et al.*, 2007; García-Arencibia *et al.*, 2009b). In the case of HD, we recently demonstrated that CB_1 receptor down-regulation is consequence of an inhibitory effect of mutant huntingtin on CB_1 receptor gene promoter exerted through the repressor element 1 silencing transcription factor (Blázquez *et al.*, 2011). On the other hand, some authors found that the enzyme that metabolizes endocannabinoids (mainly anandamide) called fatty acid amide hydrolase (FAAH), was also defective in the cortices of presymptomatic HD patients (Battista *et al.*, 2007). A reduction of FAAH activity is concordant with increased levels of endocannabinoids. However, the issue is controversial because FAAH mRNA expression was found to be increased in the striata of symptomatic R6/2 and R6/1 mice as well as in caudate-putamen samples from symptomatic HD patients (Blázquez *et al.*, 2011), resulting in enhanced endocannabinoid metabolism and low levels of these endogenous compounds. This fact would be concordant with the reduction in CB_1 receptors and would support the idea of a low endocannabinoid activity in HD.

(b) Intermediate and advanced symptomatic phases, when neuronal death is the key event, are characterized by opposite changes in both disorders, with a profound loss of CB_1 receptors in HD concomitant with death of CB_1 receptor-containing striatal neurons, which is compatible with the hyperkinetic symptoms typical of these patients (reviewed in Pazos *et al.*, 2008) and which has also been demonstrated in patients using *in vivo* imaging procedures (Van Laere *et al.*, 2010). By contrast, a significant up-regulation of CB_1 receptors was found in PD, which is caused by adaptive responses and is also compatible with the akinetic profile of these patients (García-Arencibia *et al.*, 2009b, for review), although a few studies also described reductions (Hurley *et al.*, 2003; Walsh *et al.*, 2010).

(c) Recent studies have also addressed the possible presence of the second cannabinoid receptor type, CB_2, in the basal ganglia structures (reviewed in Fernández-Ruiz *et al.*, 2007). This receptor, which is typical of immune tissues, has been found in the basal ganglia in a few neuronal subpopulations (Lanciego *et al.*, 2011) but, in particular, in glial elements that become active during pathologies (Fernández-Ruiz *et al.*, 2007). Thus, the activation of astrocytes and/or microglia, linked to neuronal injury in lesioned structures in HD and PD, has been associated with up-regulatory responses of CB_2 receptors that are located in these cells and that would play protective roles by enhancing astrocyte-mediated positive effects and/or by reducing microglia-dependent toxic influences (Fernández-Ruiz *et al.*, 2007, for review).

Therefore, these observations support the idea that both CB_1 and CB_2 receptors, as well as other elements of the cannabinoid signalling system, represent attractive targets for developing novel pharmacotherapies useful in PD and HD (and also other basal ganglia disorders as has been summarized in Table 1). Benefits that patients may receive from cannabinoid-based medicines would include first to be used as symptom-relieving substances, but also to serve as neuroprotective molecules able to slow down disease progression. The first of these two properties will be addressed only marginally in this review (see Table 1 for a summary of the most relevant effects), as this potential is based on the well-known motor effects of these compounds, for example, cannabinoid agonists inhibit motor activity, then they may be useful for HD, whereas cannabinoid antagonists produced the opposite effects, then they may be useful in PD (reviewed in Fernández-Ruiz and González, 2005; Fernández-Ruiz, 2009).

Table 1

Summary of effects observed with pharmacological manipulation of the cannabinoid system in basal ganglia disorders

Neurological disorder	Symptom relieving effects	Effects on disease progression
Huntington's disease	– $TRPV_1$ agonists reduce hyperkinesia in animal models (Lastres-Becker *et al.*, 2003) – CB_1 agonists produce only modest effects in animal models (Lastres-Becker *et al.*, 2003), whereas the data in patients are controversial (Müller-Vahl *et al.*, 1999; Curtis and Rickards, 2006; Curtis *et al.*, 2009)	– CB_2 agonists reduce inflammatory events and excitotoxicity in animal models (Palazuelos *et al.*, 2009; Sagredo *et al.*, 2009) – Cannabidiol and Δ^9-THC reduce oxidative stress in animal models (Lastres-Becker *et al.*, 2004; Sagredo *et al.*, 2007) – CB_1 agonists may also reduce excitotoxicity in animal models (Pintor *et al.*, 2006; Blázquez *et al.*, 2011), but they are lost during the progression of the disease
Parkinson's disease	– CB_1 antagonists reduce bradykinesia in animal models (Fernández-Espejo *et al.*, 2005; González *et al.*, 2006; Kelsey *et al.*, 2009) but not in patients (Mesnage *et al.*, 2004) – CB_1 agonists may reduce tremor in animal models (Sañudo-Peña and Walker, 1997) but the issue is not clear in patients (Consroe, 1998; Sieradzan *et al.*, 2001; Carroll *et al.*, 2004)	– Antioxidant cannabinoids are neuroprotective in animal models (Lastres-Becker *et al.*, 2005; García-Arencibia *et al.*, 2007) – CB_2 agonists may reduce inflammatory events in animal models (Price *et al.*, 2009; García *et al.*, 2011)
Tourette's syndrome	– Plant-derived cannabinoids and analogues reduce tics in patients (reviewed in Müller-Vahl, 2009)	
Dystonia	– Classic and non-classic cannabinoid agonists have antidystonic effects in animals models and patients (reviewed in Fernández-Ruiz and González, 2005)	
Dyskinesia	– CB_1 agonists or antagonists attenuate levodopa-induced dyskinesia in animal models and patients (reviewed in Fabbrini *et al.*, 2007)	

Δ^9-THC, Δ^9-tetrahydrocannabinol.

These effects are the normal consequence of the important role exerted by the cannabinoid signalling system in regulating motor activity and the neurotransmitters involved in this function (Fernández-Ruiz and González, 2005; Marsicano and Lutz, 2006). Specific motor effects have been related to activation or blockade of CB_1 receptors that are critically located in glutamatergic and GABAergic synapses within the basal ganglia circuitry (Gerdeman and Fernández-Ruiz, 2008). Based on these properties, several studies conducted in animal models addressed, for example, the potential of inhibitors of the endocannabinoid transporter to reduce hyperkinesia in HD (Lastres-Becker *et al.*, 2002b; 2003). A priori these compounds would act by enhancing the action of endocannabinoids at the CB_1 receptor, but we assumed that these benefits would progressively disappear as soon as striatal projection neurons that contain CB_1 receptors degenerate. In fact, Müller-Vahl *et al.* (1999) demonstrated that the CB_1 receptor agonist nabilone, rather than improving hyperkinesia, enhanced choreic movements in patients. However, in our studies with animal models, we surprisingly observed that the effect of endocannabinoid transporter inhibitors was maintained even in cases of profound striatal degeneration (Lastres-Becker *et al.*, 2003), and we found that these benefits, rather than derived from the activation of CB_1 receptors, are related to the capability of these inhibitors to directly or indirectly (by elevating anandamide levels) activate the vanilloid $TRPV_1$ receptors, which have been recently related to the control of basal ganglia function (see Fernández-Ruiz and González, 2005; Fernández-Ruiz, 2009, for review). In addition to hyperkinesia in HD, Parkinsonian tremor would be also susceptible to be reduced with CB_1 receptor agonists given their inhibitory effects on subthalamonigral glutamatergic neurons (Sañudo-Peña and Walker, 1997), whereas bradykinesia may be reduced with CB_1 receptor antagonists (Fernández-Espejo *et al.*, 2005; González *et al.*, 2006; Kelsey *et al.*, 2009). However, these effects were not reproduced in most of studies conducted in patients (Consroe, 1998; Sieradzan *et al.*, 2001; Carroll *et al.*, 2004; Mesnage *et al.*, 2004). A particular effect observed with cannabinoids in PD is the reduction of levodopa-induced dyskinesia because it was observed with CB_1 receptor agonists but also with antagonists for this receptor, thus stressing the extreme complexity of the basal ganglia for cannabinoid effects (reviewed in Fabbrini *et al.*, 2007).

As mentioned above, the potential of cannabinoids as symptom-relieving agents in basal ganglia disorders is addressed here only marginally, and we will put the major emphasis on the potential of cannabinoids to control disease progression in PD and HD, given the important neuroprotective properties described for agonists of both CB_1 and CB_2 receptors (Fernández-Ruiz *et al.*, 2005; 2010; and see also Table 1 for a summary of neuroprotective effects described for

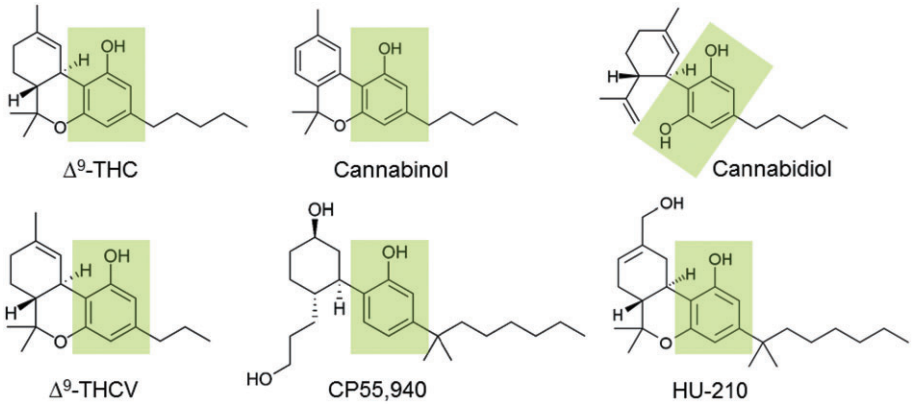

Figure 3

Chemical structures of representative cannabinoid compounds having cannabinoid receptor-independent antioxidant properties. The phenolic moiety responsible of this antioxidant effect is indicated with a green square. Δ^9-THC, Δ^9-tetrahydrocannabinol; Δ^9-THCV, Δ^9-tetrahydrocannabivarin.

cannabinoid compounds in basal ganglia disorders). In this respect, it is important to remark that the molecular mechanisms underlying the neuroprotective properties of cannabinoids are quite diverse and include also some events not mediated by cannabinoid receptors, such as the blockade of NMDA receptors or the reduction of oxidative injury exerted by some specific groups of cannabinoids with particular chemical characteristics (Fernández-Ruiz *et al.*, 2005; 2010). Other neuroprotective actions of cannabinoids are definitively mediated by either CB_1 (Fernández-Ruiz *et al.*, 2005; 2010) or CB_2 receptors (Fernández-Ruiz *et al.*, 2007; 2010), and even through the activation of the endocannabinoid-related receptor $TRPV_1$ (Veldhuis *et al.*, 2003). These receptor-mediated events would be involved in the inhibition of glutamate release, reduction of calcium influx, improvement of blood supply to the injured brain and/or decrease of local inflammatory events exerted by cannabinoids (for review, see Fernández-Ruiz *et al.*, 2005; 2007; 2010). The present review will focus on these neuroprotective properties, particularly in two that have been demonstrated to be of major interest for basal ganglia disorders: their antioxidant properties and their activity at the CB_2 receptors.

Antioxidant cannabinoids for the treatment of oxidative injury in basal ganglia disorders

The normal balance between oxidative events and antioxidant endogenous mechanisms is frequently disrupted [by an excessive production of reactive oxygen species (ROS), by a deficiency in antioxidant endogenous mechanisms, or by both causes] in neurodegenerative disorders, including PD and HD (reviewed in Wang and Michaelis, 2010). Certain cannabinoids are able to restore this balance, thereby enhancing neuronal survival (Fernández-Ruiz *et al.*, 2010, for review). A priori this capability seems to be inherent to compounds such as the plant-derived cannabinoids cannabidiol (CBD), Δ^9-tetrahydrocannabinol (Δ^9-THC) and cannabinol, or their analogues nabilone, levonantradol and dexanabinol, whose chemical structure with phenolic groups enables them to act as ROS scavengers (see Figure 3 and Marsicano *et al.*, 2002, for details on those compounds that may serve for this function). This would be a cannabinoid receptor-independent effect (Eshhar *et al.*, 1995; Hampson *et al.*, 1998; Chen and Buck, 2000; Marsicano *et al.*, 2002). However, additional mechanisms involving a direct improvement of endogenous antioxidant enzymes through the modulation of the signalling triggered by the transcription factor nuclear factor-erythroid 2-related factor 2 (nrf-2), as found for other classic antioxidants (see below), have been also proposed and are presently under investigation (reviewed in Fernández-Ruiz *et al.*, 2010; see Figure 4).

The antioxidant potential of certain cannabinoids, particularly the case of CBD, a plant-derived cannabinoid with negligible activity at CB_1 and CB_2 receptors but significant antioxidant properties, has been already evaluated in experimental models of HD. Most of the studies have focused on the model of rats lesioned with 3-nitropropionic acid (reviewed in Pazos *et al.*, 2008), a mitochondrial toxin that replicates the complex II deficiency characteristic of HD patients and that provokes striatal injury by mechanisms that mainly involve the Ca^{++}-regulated protein calpain and generation of ROS (reviewed in Brouillet *et al.*, 2005). Neuroprotective effects in this experimental model have been described for Δ^9-THC (Lastres-Becker *et al.*, 2004), CBD (Sagredo *et al.*, 2007) or the Sativex®-like combination of botanical extracts of both phytocannabinoids (Sagredo *et al.*, 2011). By contrast, selective CB_1 receptor agonists, such as ACEA, or CB_2 receptor agonists, such as HU-308, both devoid of antioxidant properties, failed to provide neuroprotection in this model (Sagredo *et al.*, 2007). The effects of CBD (Sagredo *et al.*, 2007) or the Sativex®-like combination of botanical extracts of Δ^9-THC and CBD (Sagredo *et al.*, 2011) in this HD model were not blocked by selective antagonists of either CB_1 or CB_2 receptors, thus supporting the idea that these effects are caused by the antioxidant and cannabinoid receptor-independent properties of these phytocannabinoids. These properties would be comparable, or even superior in

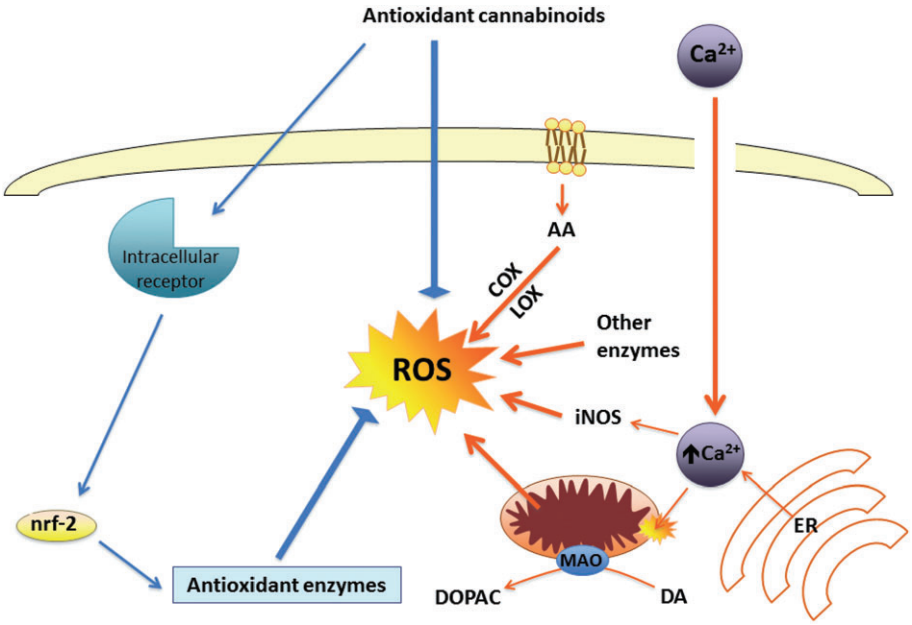

Figure 4

Mechanisms proposed for the neuroprotective effects exerted by cannabinoids against oxidative injury that occurs in most neurodegenerative disorders, including HD and PD. These neuroprotective effects involve mainly CB_1 and CB_2 receptor-independent mechanisms. COX, cyclooxygenase; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; ER, endoplasmic reticulum; iNOS, inducible nitric oxide synthase; LOX, lipoxygenase; MAO, monoamine oxidase; nrf-2, nuclear factor-erythroid 2-related factor 2; ROS, reactive oxygen species.

some case, to those reported for other known antioxidant compounds, such as N-acetylcysteine, S-allylcysteine, coenzyme Q10, taurine, the flavonoid kaempferol, ascorbate, α-tocopherol, ginseng components, melatonin or dehydroepiandrosterone, all of which are highly effective at protecting the brain against 3-nitropropionate-induced neurotoxicity or in similar HD models (Fontaine *et al.*, 2000; Nam *et al.*, 2005; Tadros *et al.*, 2005; Túnez *et al.*, 2005; Herrera-Mundo *et al.*, 2006; Lagoa *et al.*, 2009; Yang *et al.*, 2009; Kalonia *et al.*, 2010). It is possible, however, that this antioxidant/neuroprotective effect of phytocannabinoids involves the activation of signalling pathways implicated in the control of redox balance (e.g. nrf-2/antioxidant response element 7), as suggested recently for cystamine (Calkins *et al.*, 2010). It is well-known that nrf-2 activation is neuroprotective against a variety of cytotoxic stimuli including 3-nitropropionate (Calkins *et al.*, 2005), and indeed such activation may constitute a common mechanism of action for a range of different antioxidants, including phytocannabinoids. If this was the case, it could be that there was a cannabinoid receptor/target, other than CB_1 or CB_2 receptors, that might be coupled to the activation of nrf-2 signalling (see Figure 4). We are presently working in this direction.

Antioxidant cannabinoids have been also found highly effective as neuroprotective compounds in experimental models of PD and also by acting through cannabinoid receptor-independent mechanisms (reviewed in García-Arencibia *et al.*, 2009b). This observation is particularly important in the case of PD due to two reasons: (i) PD is a degenerative disorder in which oxidative injury is particularly relevant (Wang and Michaelis, 2010); and (ii) the hypokinetic profile of most of the cannabinoids able to activate CB_1 receptors represents a disadvantage for this disease because, in long-term treatments, agonists of this receptor can acutely enhance rather than reduce motor disability, as a few clinical data have already revealed (reviewed in Fernández-Ruiz and González, 2005; Fernández-Ruiz, 2009). Therefore, major efforts are in the direction to find cannabinoid molecules that may provide neuroprotection based on their antioxidant properties and that may also activate CB_2 receptors (see below), but that do not activate CB_1 receptors, or even, they are able to block them, which may provide additional benefits in the relief of specific symptoms as bradykinesia. An interesting case with this profile is the phytocannabinoid Δ^9-tetrahydrocannabivarin (Δ^9-THCV), which is presently under investigation in PD (see below). Most of the studies to determine the antioxidant properties of certain cannabinoids in PD have been conducted in rats with unilateral lesions of the nigrostriatal neurons caused by 6-hydroxydopamine (reviewed in García-Arencibia *et al.*, 2009b). Neuroprotective effects in this experimental model have been described for Δ^9-THC (Lastres-Becker *et al.*, 2005), CBD (Lastres-Becker *et al.*, 2005; García-Arencibia *et al.*, 2007), the antioxidant anandamide analogue AM404 (García-Arencibia *et al.*, 2007) and Δ^9-THCV (García *et al.*, 2011). Similar effects were found with the synthetic CB_1/CB_2 receptor agonist CP55,940 in an invertebrate model of PD (Jiménez-Del-Rio *et al.*, 2008). A priori these compounds acted through antioxidant mechanisms that seem to be independent of CB_1 or CB_2 receptors, although selective activation of CB_2 receptors showed efficacy in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned mice (Price *et al.*, 2009; see below), but not in

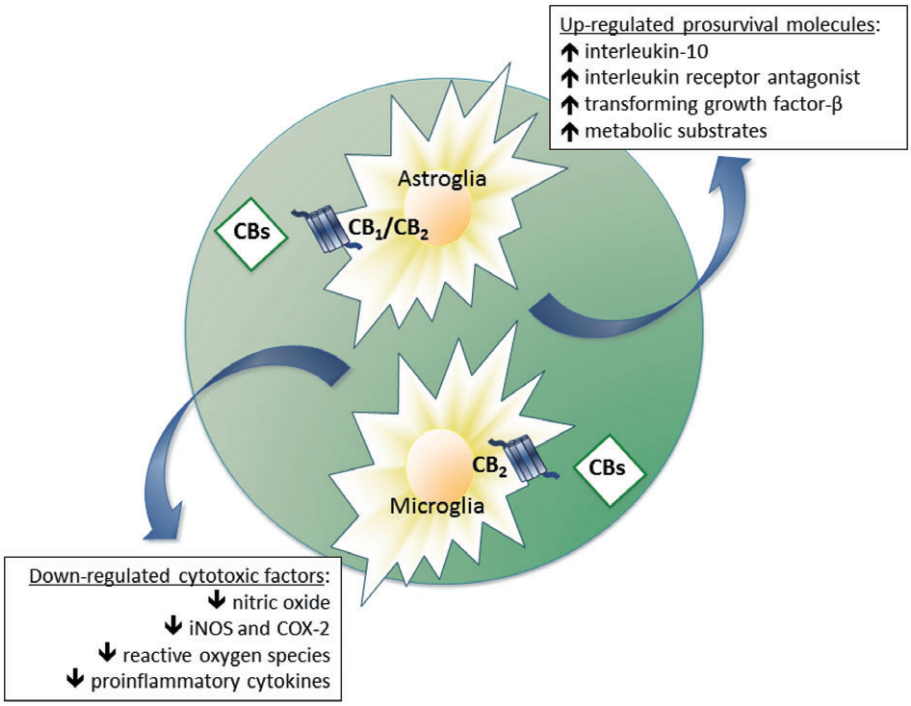

Figure 5

Mechanisms proposed for the neuroprotective effects exerted by cannabinoids against inflammatory events that occur in most neurodegenerative disorders, including Huntington's disease and Parkinson's disease. These neuroprotective effects involve mainly the activation of CB_2 receptors located in glial cells (reactive microglia and/or astrocytes). COX-2: cyclooxygenase type-2; iNOS, inducible nitric oxide.

6-hydroxydopamine-lesioned rats (García-Arencibia *et al.*, 2007). In addition, CB_1 receptor-deficient mice display an increased vulnerability to 6-hydroxydopamine lesions (Pérez-Rial *et al.*, 2011). However, selective CB_1 receptor agonists, such as ACEA, have been found not to protect against 6-hydroxydopamine-induced damage (García-Arencibia *et al.*, 2007) and they may aggravate major Parkinsonian symptoms, given the hypokinetic effects associated with the activation of CB_1 receptors (García-Arencibia *et al.*, 2009b). Therefore, these data support the idea that antioxidant and cannabinoid receptor-independent cannabinoids may serve as potential neuroprotective agents against oxidative injury frequently observed in PD.

CB_2 receptor agonists for the treatment of inflammatory events in basal ganglia disorders

The pathogenesis of PD, HD and other neurodegenerative disorders also includes the development of local inflammatory events that are caused by the recruitment and activation of astrocytes and microglial cells at the lesioned structures (Amor *et al.*, 2010, for review). These responses, in particular in the case of microglial cells, although initially aimed at eliminating dead neurons and repairing the brain parenchyma, may become negative when they are permanently activated as happens in chronic neurodegenerative disorders, then aggravating neuronal damage (Heneka *et al.*, 2010, for review). In the case of reactive microglial cells, this toxicity is due to the generation and release of different factors, such as nitric oxide, proinflammatory cytokines (e.g. tumour necrosis factor-α, interleukin-1β) and ROS, all able to deteriorate neuronal homeostasis (Lull and Block, 2010, for review). Numerous studies have demonstrated that various cannabinoid agonists also have important anti-inflammatory properties exerted, for example, by reducing the generation of these cytotoxic factors (reviewed in Fernández-Ruiz *et al.*, 2007; Stella, 2009), an effect preferentially mediated by the activation of CB_2 receptors (see Figure 5). By contrast, cannabinoid agonists might also increase the production of prosurvival molecules, such as several trophic factors (e.g. transforming growth factor-β) or anti-inflammatory cytokines (e.g. interleukin-10, interleukin-1 receptor antagonist) (Smith *et al.*, 2000; Molina-Holgado *et al.*, 2003; Correa *et al.*, 2010), or improve the trophic support exerted by astrocytes on neurons (Guzmán and Sánchez, 1999), an effect possibly mediated by the activation of CB_1 receptors, although a role for CB_2 receptors can not be excluded (Fernández-Ruiz *et al.*, 2007; see Figure 5). Therefore, CB_2 receptors appear to be the key target for these glial-mediated effects of cannabinoids, but the presence of this receptor type in the healthy brain is very weak and restricted to specific subpopulations of astrocytes, microglial cells and, to a lesser extent, neurons (reviewed in Benito *et al.*, 2008). However, numerous studies developed from the pioneering study by Benito *et al.* (2003) using post-mortem brain samples from Alzheimer's disease patients, have provided solid evidence that CB_2 receptors experience a marked up-regulation in glial elements in those structures

undergoing neuronal damage in different pathological conditions, including HD and PD. Table 2 contains a summary of major characteristics of all *in vivo* studies showing up-regulation of CB_2 receptors in different disorders or pathological conditions. Importantly, in most of these diseases, the activation of CB_2 receptors has been associated with reduced proinflammatory events and enhanced neuronal survival, thereby supporting the importance of this receptor as a potential therapeutic target in neuroinflammatory/neurodegenerative conditions (reviewed in Fernández-Ruiz *et al.*, 2007; 2010). In addition, it should be remarked that CB_2 agonists, in comparison with CB_1 agonists, are devoid of undesirable CNS side effects, like sedation and psychotomimetic effects.

The potential of CB_2 receptor agonists has been also studied in basal ganglia disorders, particularly in HD, in which these agonists combined with antioxidant cannabinoids have been proposed as promising neuroprotective agents and might entry in clinical testing very soon (Fernández-Ruiz *et al.*, 2010). An important aspect of HD pathology is that, as mentioned above, the brain of HD patients experiences a progressive decrease of CB_1 receptors during the course of this disease that occurs in concert with the death of striatal projection neurons where CB_1 receptors are located (reviewed in Pazos *et al.*, 2008). This explains the lack of efficacy of CB_1 agonists for the treatment of HD symptoms (e.g. chorea) in experimental models (Lastres-Becker *et al.*, 2002b; 2003) and the controversial data obtained in patients (Müller-Vahl *et al.*, 1999; Curtis and Rickards, 2006; Curtis *et al.*, 2009), as well as their poor activity as neuroprotective agents in models of HD generated by mitochondrial neurotoxins (Sagredo *et al.*, 2007; 2009). However, it should be noted that CB_1 receptor activation afforded neuroprotection in other models, for example, in an excitotoxic model of HD (rats lesioned with quinolinate; Pintor *et al.*, 2006), in a PC12 cell model expressing exon 1 mutant huntingtin (Scotter *et al.*, 2010), and also in R6/2 mice, a transgenic model of HD (Blázquez *et al.*, 2011). However, in the latter model, the activation of CB_1 receptors was effective only when the treatment was initiated before the onset of symptoms and not later, in concordance with the idea that an early reduction of CB_1 receptors caused by mutant huntingtin is involved in HD pathogenesis, as we have recently reported (Blázquez *et al.*, 2011). We assume that an early pharmacological correction of this reduced CB_1 receptor signalling may be positive in presymptomatic phases of HD, but it does not appear that CB_1 receptor agonists work at later symptomatic phases (Blázquez *et al.*, 2011; see also Dowie *et al.*, 2009). This places CB_2 receptors, and also antioxidant cannabinoid receptor-independent mechanisms described in the previous section, as the key targets within the cannabinoid system for a long-term cannabinoid-based neuroprotective treatment in HD. As mentioned above, the presence of this receptor type in the healthy striatum is relatively modest, but it is, however, markedly up-regulated in reactive microglial cells, and also in astrocytes, when striatal degeneration progresses, a process observed both in HD patients (Palazuelos *et al.*, 2009) and in rats lesioned with malonate (Sagredo *et al.*, 2009) or in R6/2 mice (Palazuelos *et al.*, 2009). In this context, it is likely that compounds targeting selectively this receptor type may be effective in attenuating striatal degeneration in HD, a notion that has been demonstrated recently in various studies using different animal models in which inflammatory events associated with glial activation are predominant over other cytotoxic events that cooperatively contribute to HD pathogenesis in patients (Borrell-Pages *et al.*, 2006). This is the case of striatal injury in rats generated by unilateral injections of malonate, another complex II inhibitor that, in contrast with 3-nitropropionic acid, produces cell death through the activation of apoptotic pathways and enhancement of proinflammatory factors (Sagredo *et al.*, 2009). We found neuroprotection with selective CB_2 receptor agonists in these rats, whereas selective CB_1 receptor agonists or antioxidant cannabinoids like CBD were not effective (Sagredo *et al.*, 2009). The effects of CB_2 receptor agonists were antagonized by selective CB_2 receptor antagonists, and CB_2 receptor-deficient mice were more vulnerable to malonate lesions (Sagredo *et al.*, 2009), thus stressing the importance of CB_2 receptors in this model. We also demonstrated that the activation of this receptor type located in glial cells, particularly in reactive microglial cells within the striatal parenchyma, reduced the proinflammatory scenario caused by the malonate lesion, with a reduction in the generation of TNF-α and other proinflammatory factors (e.g. cyclooxygenase-2, inducible nitric oxide synthase) (Sagredo *et al.*, 2009). Similar results have been recently found for CB_2 receptor agonists in other models of HD such as R6/2 transgenic mice (Palazuelos *et al.*, 2009) or mice lesioned with the excitotoxin quinolinate (Palazuelos *et al.*, 2009), or for the Sativex®-like combination of botanical extracts of Δ^9-THC (active at CB_1 and CB_2 receptors) and CBD in malonate-lesioned mice (Sagredo *et al.*, 2011).

On the other hand, the question of CB_2 receptors in PD has remained elusive for a long time. The difficulty in generating an appropriate antibody against this receptor that selectively labels CB_2 receptor-containing cells, as well as the scarcity of alternative experimental tools, has delayed the identification of this receptor in lesioned structures, for example, substantia nigra and striatum, in Parkinsonian models. Price *et al.* (2009) were the first to demonstrate CB_2-positive immunostaining in a classic model of PD in rodents, namely MPTP-lesioned mice, in which they identified the receptor in reactive microglial cells (Price *et al.*, 2009). We also explored the issue in 6-hydroxydopamine-lesioned rats and mice, but our data did not reveal a significant up-regulatory response of these receptors in lesioned substantia nigra, showing poor response in rats (García *et al.*, 2011) or equivalent immunostaining levels between lesioned and non-lesioned sides in mice (Garcia and Fernández-Ruiz, unpubl. results). This was concordant with the finding, mentioned in the previous section, that the neuroprotective effect of CB_2 receptor agonists was very modest in this PD model (García-Arencibia *et al.*, 2007), in which only antioxidant cannabinoids protected nigral neurons (Lastres-Becker *et al.*, 2005; García-Arencibia *et al.*, 2007), and also with the observation that the vulnerability to 6-hydroxydopamine was similar in CB_2 receptor-deficient mice and wild-type animals (García *et al.*, 2011). We assumed that this might be related to the poor inflammatory responses frequently found in models of PD generated with 6-hydroxydopamine and therefore went to a more proinflammatory model in which nigral lesions were caused by local application of lipopolysaccharide (LPS). Mice-lesioned with LPS showed a profound up-regulation of CB_2

Table 2

Major characteristics of those studies showing CB_2 receptor up-regulation in Huntington's disease, Parkinson's disease and other pathological conditions

Insult/disease model	CB_2-positive cells	Observed effect	Technique	Animal species	Reference
Huntington's disease					
Malonate lesion	Astrocytes Microglia	↑ CB_2 protein ↑ CB_2 mRNA	IHC qRT-PCR	Rat	Sagredo *et al.*, 2009
Mutant huntingtin	Microglia	↑ CB_2 protein ↑ CB_2 mRNA	IHC qRT-PCR	Mouse and human	Palazuelos *et al.*, 2009
Parkinson's disease					
MPTP lesion	Microglia	↑ CB_2 protein	IHC	Mouse	Price *et al.*, 2009
LPS lesion	Microglia?	↑ CB_2 protein	IHC	Mouse	García *et al.*, 2011
Other pathological conditions					
Chronic constriction injury Freund's complete adjuvant injection Spinal nerve ligation	Microglia	↑ CB_2 mRNA	ISH	Rat	Zhang *et al.*, 2003
Spinal nerve ligation	Neurons	↑ CB_2 protein	IHC	Rat	Wotherspoon *et al.*, 2005
Spinal cord ligation	N.A.	↑ CB_2	qRT-PCR	Mouse	Beltramo *et al.*, 2006
Peripheral nerve transection	Microglia Perivascular microglia	↑ CB_2 protein	IHC	Rat	Romero-Sandoval *et al.*, 2008
Neuropathic pain	Microglia Astrocytes	↑ CB_2 protein	IHC	Mouse	Luongo *et al.*, 2009
Paw incision	Microglia	↑ CB_2 protein	IHC	Rat	Alkaitis *et al.*, 2010
Lipopolysaccharide	Microglia	↑ CB_2 protein	WB	Rat	Mukhopadhyay *et al.*, 2006
Multiple sclerosis	Microglia Astrocytes	↑ CB_2 protein	IHC	Human	Benito *et al.*, 2007
Experimental autoimmune enchepalomyelitis	Microglia	↑ CB_2 mRNA	qRT-PCR	Mouse	Maresz *et al.*, 2005
EAE, multiple sclerosis	Myeloid progenitors Microglia	↑ CB_2 protein ↑ CB_2 mRNA	IHC qRT-PCR	Mouse and Human	Palazuelos *et al.*, 2008
Theiler's virus	N.A.	↑ CB_2 mRNA	qRT-PCR	Mouse	Loría *et al.*, 2008
Multiple sclerosis Amyotrophic lateral sclerosis	Microglia	↑ CB_2 protein	IHC WB	Human	Yiangou *et al.*, 2006
Hemicerebellectomy	Neurons	↑ CB_2 protein ↑ CB_2 mRNA	IHC qRT-PCR	Rat	Viscomi *et al.*, 2009
Middle cerebral artery occlusion Hypoxia-ischaemia	Macrophages/ microglia	↑ CB_2 protein	IF WB	Rat	Ashton *et al.*, 2007
Middle cerebral artery occlusion and reperfusion	N.A.	↑ CB_2 mRNA	qRT-PCR	Mouse	Zhang *et al.*, 2008
Alzheimer's disease	Microglia	↑ CB_2 protein	IHC	Human	Benito *et al.*, 2003
Alzheimer's disease	Microglia	↑ CB_2 protein	IHC	Human	Ramirez *et al.*, 2005
Alzheimer's disease	N.A.	↑ CB_2 mRNA	Gene-chip microarrays qRT-PCR	Human	Grünblatt *et al.*, 2007
β-Amyloid	N.A.	↑ CB_2 protein ↑ CB_2 mRNA	IHC qRT-PCR	Rat	Esposito *et al.*, 2007
Down's syndrome	Microglia	↑ CB_2 protein	IHC	Human	Núñez *et al.*, 2008
Simian immunodeficiency virus	Microglia	↑ CB_2 protein	IHC	Macaque	Benito *et al.*, 2005
MDMA neurotoxicity	Microglia	↑ CB_2 protein	IHC	Rat	Torres *et al.*, 2010

EAE, experimental autoimmune encephalomyelitis; IF, immunofluorescence; IHC, immunohistochemistry; ISH, in situ hybridization; MDMA, 3,4-methylenedioxymethamphetamine; N.A., not analysed; WB, Western blotting.

receptors in the nigral parenchyma (García *et al*., 2011) and, in this case, the activation of CB_2 receptors with the selective agonist HU-308 or with the phytocannabinoid Δ^9-THCV preserved tyrosine hydroxylase-positive neurons in the LPS-lesioned substantia nigra, whereas CB_2 receptor-deficient mice were more vulnerable to LPS than wild-type animals (García *et al*., 2011). Similar findings were obtained by Price *et al*. (2009) after the activation of CB_2 receptors in MPTP-lesioned mice, and also in *in vitro* studies in which neuronal cells were incubated with conditioned media generated by exposing glial cells to the non-selective cannabinoid agonist HU-210, which showed high rates of survival compared with the poor effects found upon the direct exposure of neuronal cells to HU-210 (Lastres-Becker *et al*., 2005). All these data support the possibility that CB_2 receptors may be a relevant cannabinoid target also in PD, serving to control local inflammatory events and, particularly, the generation of glial-derived cytotoxic factors that play a key role in PD pathogenesis (reviewed in Lee *et al*., 2009).

Concluding remarks and futures perspectives

Over the last decade, a considerable volume of preclinical work has allowed the accumulation of solid evidence to assume that the endocannabinoid system may serve as a target to develop potential neuroprotective agents for the treatment of basal ganglia diseases and also other neurodegenerative disorders. In this article, we have reviewed all this preclinical work and have discussed the cellular and molecular mechanisms underlying the neuroprotective effects of cannabinoids, putting emphasis on two aspects: (i) their capability to decrease oxidative injury by acting as scavengers of ROS or by enhancing endogenous antioxidant defences, a property independent of CB_1 and CB_2 receptors and restricted to specific cannabinoids; and (ii) their anti-inflammatory activity, that is exerted predominantly through the activation of CB_2 receptors located on glial elements, in which cannabinoids would enhance neuronal survival by inhibiting microglia-mediated cytotoxic influences and/or by increasing astroglia-mediated positive effects. However, as has been mentioned, most of the studies that have examined the therapeutic potential of cannabinoids in these disorders have been conducted in animal or cellular models, whereas the number of clinical trials is still very limited. Therefore, it should be expected that the number of studies examining this potential increases in next years, as soon as the promising expectations generated for these molecules progress from the present preclinical evidence to a true clinical application. In this respect, given the capability of cannabinoids to serve as neuroprotective agents against oxidative injury, inflammation and also other cytotoxic insults, as well as the current belief that these cytotoxic processes occur in a synergistic manner during the pathogenesis of HD and PD in humans, it would be important that the type of cannabinoid compound(s) that might be subjected to clinical evaluation in HD or PD would be a broad-spectrum, non-selective or hybrid compound, or alternatively a combination of compounds, which act on a range of targets known to mediate a neuroprotective effect.

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Conflict of interest

Authors declare that they have not any conflict of interest.

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