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 Δ⁹-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 CB₁ and CB₂ receptors and involving the control of endogenous antioxidant defences. On the other hand, the activation of CB₂ 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 CB₂ 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 CB₂ receptor up-regulation has been found in many neurodegenerative disorders including HD and PD, which supports the beneficial effects found for CB₂ receptor agonists in both disorders. In conclusion, the evidence reported so far supports that those cannabinoids having antioxidant properties and/or capability to activate CB₂ 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; Δ⁹-THC, Δ⁹-tetrahydrocannabinol; Δ⁹-THCV, Δ⁹-tetrahydrocannabivarin
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, Mechoulan 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...
Cannabinoids and basal ganglia disorders

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 CB1 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 CB1 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 CB1 receptor down-regulation is consequence of an inhibitory effect of mutant huntingtin on CB1 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 CB1 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 CB1 receptors in HD concomitant with death of CB1 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 CB1 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, CB2, 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 CB2 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 CB1 and CB2 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).

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**Figure 2**

Comparison of CB1 and CB2 receptor changes during presymptomatic and symptomatic phases in experimental models of Huntington’s disease and Parkinson’s disease.
rather than derived from the activation of CB1 receptors, are endocannabinoids at the CB1 receptor, but we assumed that hyperkinesia in HD (Lastres-Becker et al., 2003) as 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 their inhibitory effects on subthalamoanigral glutamatergic neurons (Sañudo-Peña and Walker, 1997), whereas bradykinesia may be reduced with CB1 receptor agonists (Fernández-Espejo et al., 2005; González et al., 2005, 2010; and see also García-Arencibia et al., 2007). 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., 2006; Kelsey et al., 2004). A particular effect observed with cannabinoids in PD is the reduction of levodopa-induced dyskinesia because it was observed with CB1 receptor agonists but also with antagonists for this receptor, thus stressing the extreme complexity of the basal ganglia for cannabinoid effects (reviewed in Fabbriini 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 CB1 and CB2 receptors (Fernández-Ruiz et al., 2005; 2010; and see also Table 1 for a summary of neuroprotective effects described for CB1 agonists or antagonists attenuate dyskinesia in animal models (Lastres-Becker et al., 2003), whereas bradykinesia may be reduced with CB1 receptor agonists (Fernández-Espejo et al., 2005; González et al., 2005; Fernández-Ruiz, 2009, for review). In addition to hyperkinesia in HD, Parkinsonian tremor would be also susceptible to be reduced with CB1 receptor agonists given their inhibitory effects on subthalamoanigral glutamatergic neurons (Sañudo-Peña and Walker, 1997), whereas bradykinesia may be reduced with CB1 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 CB1 receptor agonists but also with antagonists for this receptor, thus stressing the extreme complexity of the basal ganglia for cannabinoid effects (reviewed in Fabbriini et al., 2007).

Table 1
Summary of effects observed with pharmacological manipulation of the cannabinoid system in basal ganglia disorders

<table>
<thead>
<tr>
<th>Neurological disorder</th>
<th>Symptom relieving effects</th>
<th>Effects on disease progression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huntington’s disease</td>
<td>– TRPV1 agonists reduce hyperkinesia in animal models (Lastres-Becker et al., 2003)</td>
<td>– CB2 agonists reduce inflammatory events and excitotoxicity in animal models (Palazuelos et al., 2009; Sagredo et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>– CB1 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)</td>
<td>– Cannabidiol and Δ2-THC reduce oxidative stress in animal models (Lastres-Becker et al., 2004; Sagredo et al., 2007)</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>– CB1 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)</td>
<td>– Antioxidant cannabinoids are neuroprotective in animal models (Lastres-Becker et al., 2005; García-Arencibia et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>– CB1 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)</td>
<td>– CB1 agonists may reduce inflammatory events in animal models (Price et al., 2009; García et al., 2011)</td>
</tr>
<tr>
<td>Tourette’s syndrome</td>
<td>– Plant-derived cannabinoids and analogues reduce tics in patients (reviewed in Müller-Vahl, 2009)</td>
<td></td>
</tr>
<tr>
<td>Dystonia</td>
<td>– Classic and non-classic cannabinoid agonists have antidyostonic effects in animals models and patients (reviewed in Fernández-Ruiz and González, 2005)</td>
<td></td>
</tr>
<tr>
<td>Dyskinesia</td>
<td>– CB1 agonists or antagonists attenuate levodopa-induced dyskinesia in animal models and patients (reviewed in Fabbriini et al., 2007)</td>
<td></td>
</tr>
</tbody>
</table>

Δ2-THC, Δ2-tetrahydrocannabinol.
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⁡₂-tetrahydrocannabivarin (Δ⁡₂-THCV) and cannabolin, or their analogues nabilone, levonantradol and dexamabinol, 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 CB₁ and CB₂ 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 particularly the case of CBD, a plant-derived cannabinoid with negligible activity at CB₁ and CB₂ 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
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; 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 CB1 or CB2 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 CB1 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 CB1 receptors (see below), but that do not activate CB1 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 Δ9-tetrahydrocannabivarin (Δ9-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 Δ9-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 Δ9-THCV (García et al., 2011). Similar effects were found with the synthetic CB1/CB2 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 CB1 or CB2 receptors, although selective activation of CB1 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

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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 CB1 and CB2 receptor-independent mechanisms. COX, cyclooxygenase; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; ER, endoplasmic reticulum; INOS, inducible nitric oxide synthase; LOX, lipoxigenase; MAO, monoamine oxidase; nrf-2, nuclear factor-erythroid 2-related factor 2; ROS, reactive oxygen species.
6-hydroxydopamine-lesioned rats (García-Arencibia et al., 2007). In addition, CB1 receptor-deficient mice display an increased vulnerability to 6-hydroxydopamine lesions (Pérez-Rial et al., 2011). However, selective CB1 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 CB1 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.

CB2 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 CB2 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 CB1 receptors, although a role for CB2 receptors can not be excluded (Fernández-Ruiz et al., 2007; see Figure 5). Therefore, CB2 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 CB2 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 CB2 receptors in different disorders or pathological conditions. Importantly, in most of these diseases, the activation of CB2 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 CB2 agonists, in comparison with CB1 agonists, are devoid of undesirable CNS side effects, like sedation and psychotomimetic effects.

The potential of CB2 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 enter 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 CB1 receptors during the course of this disease that occurs in concert with the death of striatal projection neurons where CB1 receptors are located (reviewed in Pazos et al., 2008). This explains the lack of efficacy of CB1 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 CB2 receptor activation afforded neuroprotection in other models, for example, in an excitotoxic model of HD (rats lesioned with quinolinolate; 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 CB2 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 CB1 receptors caused by mutant huntingtin is involved in HD pathogenesis, as we have recently reported (Blázquez et al., 2011). We propose that an early pharmacological correction of this reduced CB2 receptor signaling may be positive in presymptomatic phases of HD, but it does not appear that CB2 receptor agonists work at later symptomatic phases (Blázquez et al., 2011; see also Dowie et al., 2009). This places CB2 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 CB2 receptor agonists in these rats, whereas selective CB1 receptor agonists or antioxidant cannabinoids like CBD were not effective (Sagredo et al., 2009). The effects of CB1 receptor agonists were antagonized by selective CB2 receptor antagonists, and CB2 receptor-deficient mice were more vulnerable to malonate lesions (Sagredo et al., 2009), thus stressing the importance of CB2 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 CB2 receptor agonists in other models of HD such as R6/2 transgenic mice (Palazuelos et al., 2009) or mice lesioned with the excitotoxin quinolinolate (Palazuelos et al., 2009), or for the Sativex®-like combination of botanical extracts of A. sativa and CB2 receptors and CBD in malonate-lesioned mice (Sagredo et al., 2011).

On the other hand, the question of CB2 receptors in PD has remained elusive for a long time. The difficulty in generating an appropriate antibody against this receptor that selectively labels CB2 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 CB2-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 (García and Fernández-Ruiz, unpubl. results). This was concordant with the finding, mentioned in the previous section, that the neuroprotective effect of CB2 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 CB2 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 CB2.
<table>
<thead>
<tr>
<th>Insult/disease model</th>
<th>CB₂-positive cells</th>
<th>Observed effect</th>
<th>Technique</th>
<th>Animal species</th>
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<tr>
<td><strong>Huntington’s disease</strong></td>
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<td>IHC</td>
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EAE, experimental autoimmune encephalomyelitis; IF, immunofluorescence; IHC, immunohistochemistry; ISH, in situ hybridization; MDMA, 3,4-methylenedioxymethamphetamine; N.A., not analysed; WB, Western blotting.
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Conflict of interest

Authors declare that they have not any conflict of interest.

References


Cannabinoids and basal ganglia disorders


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