REVIEW

The endocannabinoid system as a target for the treatment of motor dysfunction

Javier Fernández-Ruiz

Departamento de Bioquímica y Biología Molecular and Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas (CIBERNED), Facultad de Medicina, Universidad Complutense, Madrid, Spain

There is evidence that cannabinoid-based medicines that are selective for different targets in the cannabinoid signalling system (e.g. receptors, inactivation mechanism, enzymes) might be beneficial in basal ganglia disorders, namely Parkinson's disease (PD) and Huntington's disease (HD). These benefits not only include the alleviation of specific motor symptoms [e.g. choreic movements with cannabinoid receptor type 1 (CB$_1$)/transient receptor potential vanilloid type 1 agonists in HD; bradykinesia with CB$_1$ antagonists and tremor with CB$_1$ agonists in PD], but also the delay of disease progression due to the neuroprotective properties demonstrated for cannabinoids (e.g. CB$_1$ agonists reduce excitotoxicity; CB$_2$ agonists limit the toxicity of reactive microglia; and antioxidant cannabinoids attenuate oxidative damage). In addition, extensive biochemical, anatomical, physiological and pharmacological studies have demonstrated that: (i) the different elements of the cannabinoid system are abundant in basal ganglia structures and they are affected by these disorders; (ii) the cannabinoid system plays a prominent role in basal ganglia function by modulating the neurotransmitters that operate in the basal ganglia circuits, both in healthy and pathological conditions; and (iii) the activation and/or inhibition of the cannabinoid system is associated with important motor responses that are maintained and even enhanced in conditions of malfunctioning and/or degeneration. In this article we will review the available data regarding the relationship between the cannabinoid system and basal ganglia activity, both in healthy and pathological conditions and will also try to identify future lines of research expected to increase current knowledge about the potential therapeutic benefits of targeting this system in PD, HD and other basal ganglia disorders.
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Function of the cannabinoid signalling system in the basal ganglia

Endocannabinoid ligands, their synthesizing and degrading enzymes, and their receptors are particularly abundant in basal ganglia structures (e.g. caudate-putamen, globus pallidus, substantia nigra) when compared with other brain regions (Herkenham *et al.*, 1991a; Mailleux and Vanderhaeghen, 1992; Tsou *et al.*, 1998a,b; Bisogno *et al.*, 1999; Dinh *et al.*, 2002; Egertova *et al.*, 2003; Köfalvi *et al.*, 2005). This is the logical consequence of the important modulatory function exerted by the cannabinoid signalling system on the control of movement, which is in turn the consequence of the location of specific elements of this signalling system in key groups of neurons and synapses within basal ganglia circuits (see Figure 1 and Gerdeman and Fernández-Ruiz, 2008, for review). Thus, the nuclei that receive striatal efferent outputs, namely the external globus pallidus and substantia nigra *pars reticulata*/internal globus pallidus, contain high levels of endocannabinoids, either anandamide or 2-arachidonoylglycerol (Bisogno *et al.*, 1999; Di Marzo *et al.*, 2000), as well as the cannabinoid receptor type 1 (CB$_1$) receptor, the cannabinoid receptor type predominantly involved in the control of movement in healthy circumstances (reviewed in Fernández-Ruiz and González, 2005). CB$_1$ receptor binding sites are located presynaptically in nerve terminals of striatal projection neurons, the so-called 'medium-spiny striatal neurons' (Herkenham *et al.*, 1991b; Tsou *et al.*, 1998a; Hohmann and Herkenham, 2000; Fusco *et al.*, 2004), which

Correspondence: Javier Fernández-Ruiz, Departamento de Bioquímica y Biología Molecular III, Facultad de Medicina, Universidad Complutense, Ciudad Universitaria s/n, Madrid 28040, Spain. E-mail: jjfr@med.ucm.es or J.fernandez-ruiz@cibernred.es
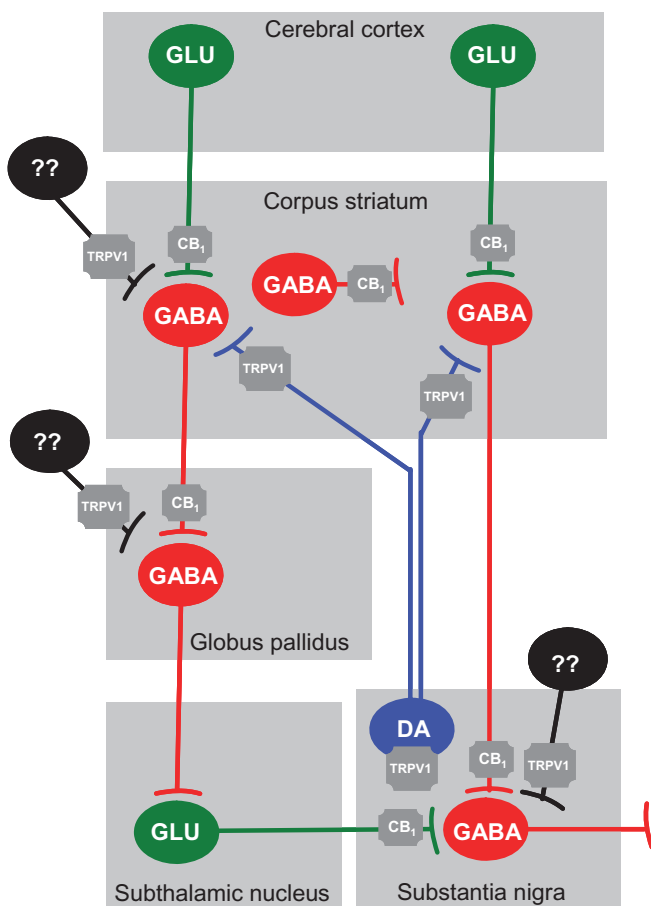
Figure 1 Location of CB_1 and TRPV1 receptors in specific neuronal subpopulations within basal ganglia circuits. Regulatory pathways are indicated in blue, whereas inhibitory and excitatory inputs are indicated in red and green respectively. Unknown neurons are shown in black. CB_1, cannabinoid receptor type 1; GABA, γ-aminobutiric acid; GLU, glutamate; TRPV1, transient receptor potential vanilloid type 1.

cell bodies are located in the subthalamic nucleus (Mailleux and Vanderhaeghen, 1992; Tsou *et al.*, 1998a). By contrast, nigrostriatal dopaminergic neurons do not contain CB_1 receptors, although they do contain the vanilloid TRPV1 receptor (Mezey *et al.*, 2000; de Lago *et al.*, 2004a; Lastres-Becker *et al.*, 2005; Tzavara *et al.*, 2006), a receptor functionally related to the cannabinoid signalling system, which is also evident in different basal ganglia structures [e.g. striatum (Maccarrone *et al.*, 2008), globus pallidus (Cristino *et al.*, 2006)]. Indeed, certain endocannabinoids, like anandamide and *N*-arachidonoyldopamine, but not 2-arachidonoylglycerol, can also act as endogenous ligands for this TRPV1 cation channel (reviewed in Starowicz *et al.*, 2007). On the other hand, glial cells, mainly astrocytes, might naturally express the cannabinoid receptor type 2 (CB_2) receptor type, although the levels of this receptor type would be very small in healthy conditions (Sheng *et al.*, 2005; Sagredo *et al.*, 2009). Interestingly, these receptors are strongly up-regulated in astrocytes and they are induced in microglial cells activated in response to different types of cytotoxic insults, such as those occurring in most basal ganglia disorders (Sagredo *et al.*, 2008).

Given the abundance of elements of the cannabinoid signalling system in the basal ganglia, it is likely that the activation or the inhibition of this signalling system might have an important influence on different motor responses (reviewed in Fernández-Ruiz and González, 2005). However, the complex localization of these elements at different sites in the basal ganglia circuits, particularly of the CB_1 receptor, may sometimes explain the paradoxical effects observed (reviewed in Fernández-Ruiz and González, 2005; Gerdeman and Fernández-Ruiz, 2008). In general, the substances that activate cannabinoid signalling (e.g. direct receptor agonists, inhibitors of endocannabinoid inactivation) have powerful effects on the control of movement, mostly of an inhibitory nature (Crawley *et al.*, 1993; Fride and Mechoulam, 1993; Wickens and Pertwee, 1993; Romero *et al.*, 1995a,b; Jarbe *et al.*, 1998; Giuffrida *et al.*, 1999; González *et al.*, 1999; Beltramo *et al.*, 2000; McLaughlin *et al.*, 2000; Sañudo-Peña *et al.*, 2000; de Lago *et al.*, 2002; 2004b). However, most studies published so far have found some variation in the magnitude and duration of the motor effects, depending on the characteristics of these compounds in terms of receptor affinity, potency and/or metabolic stability (reviewed in Gerdeman and Fernández-Ruiz, 2008). Most of these effects, if not all, originate through the activation of the CB_1 receptor as the motor effects of most cannabinoid agonists are usually prevented by rimonabant and other selective CB_1 receptor antagonists (Souilhac *et al.*, 1995; Di Marzo *et al.*, 2001; reviewed in Fernández-Ruiz and González, 2005). However, certain motor effects mediated by anandamide or its analogue AM404 (a compound initially developed as an inhibitor of the endocannabinoid uptake but that also exhibits other properties) were reversed by the TRPV1 receptor antagonist capsazepine, but not by rimonabant. Hence, it would appear that such effects are more closely related to the capacity of these compounds to directly bind to and activate vanilloid TRPV1 receptors rather than their ability to activate CB_1 receptors (Lastres-Becker *et al.*, 2002a; 2003a; de Lago *et al.*, 2004a). On the other hand, the inverse agonistic properties of most CB_1 receptor antagonists means that their *in vivo* administration

explain the high levels of mRNA transcripts for this receptor type found in the caudate-putamen (Mailleux and Vanderhaeghen, 1992). Indeed, CB_1 receptors are located in striatal neurons projecting to both the substantia nigra *pars reticulata*/internal globus pallidus (the so-called 'direct' striatal efferent pathway) and the external globus pallidus (the so-called 'indirect' striatal efferent pathway). Both groups of neurons use γ-aminobutiric acid (GABA) as a neurotransmitter, although also express selective phenotypical markers (e.g. enkephalin and D_2 receptors in striatopallidal neurons, and dynorphin/substance P and D_1 receptors in striatonigral neurons; see Hohmann and Herkenham, 2000; Julián *et al.*, 2003). Intrinsic striatal neurons do not generally contain CB_1 receptors, except most of the striatal GABAergic interneurons that are labelled with parvalbumin, and a few interneurons of the cholinergic subpopulation (Hohmann and Herkenham, 2000; Fusco *et al.*, 2004; Uchigashima *et al.*, 2007). CB_1 receptors are also present presynaptically in glutamatergic neurons within the basal ganglia circuits, including the afferences to the striatum coming from cortical structures (Köfalvi *et al.*, 2005; Uchigashima *et al.*, 2007) and, in particular from those arising preferentially in the substantia nigra and whose
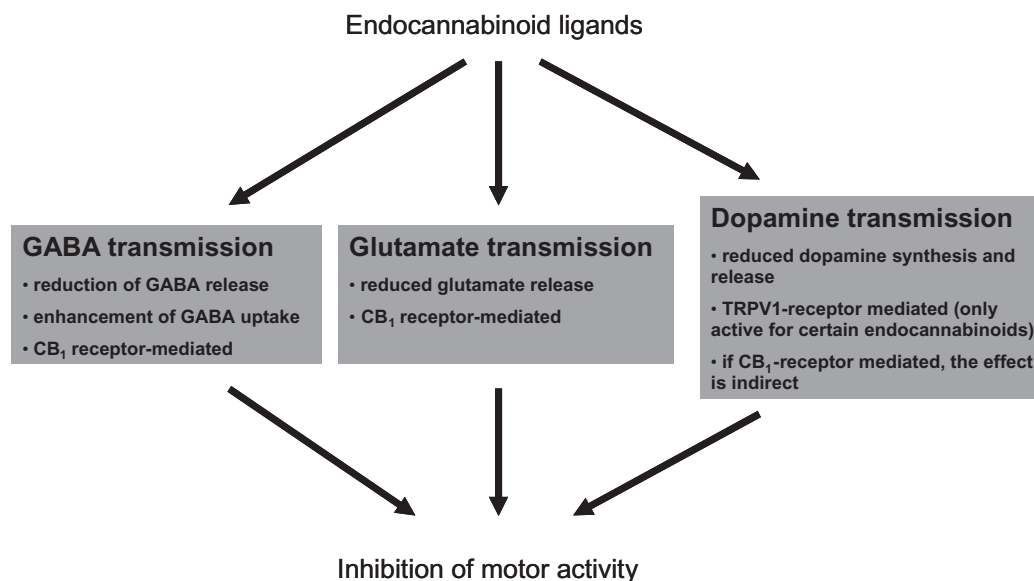
Figure 2 Schematic overview of the modulatory effects of endocannabinoid ligands and their receptors on the activity of the three major neurotransmitters operating in basal ganglia circuits. CB$_1$, cannabinoid receptor type 1; GABA, γ-aminobutiric acid; TRPV1, transient receptor potential vanilloid type 1.

frequently causes hyperlocomotion (Compton *et al.*, 1996), concordant with the motor activity observed in mice deficient in this receptor (Ledent *et al.*, 1999).

The motor effects resulting from the activation or blockade of cannabinoid signalling are related to the capacity of this system to modulate the activity of the neurotransmitters that participate in the control of basal ganglia function, namely glutamate and GABA (responsible for the excitatory and inhibitory inputs respectively), and dopamine (which plays a regulatory role; reviewed in Sañudo-Peña *et al.*, 1999; Romero *et al.*, 2002; Fernández-Ruiz and González, 2005; Gerdeman and Fernández-Ruiz, 2008). The identification of CB$_1$ receptors in several GABAergic and glutamatergic synapses within the basal ganglia, as well as the presence of TRPV1 receptors in nigrostriatal dopaminergic neurons, enables endocannabinoids to directly control the function of these three key neurotransmitters. In addition, the preferential presynaptic location of these receptors makes it likely that endocannabinoids mainly control presynaptic events, such as the synthesis, release or reuptake of these neurotransmitters (see Figure 2, and Gerdeman and Fernández-Ruiz, 2008, for review). In fact, different pharmacological (e.g. administration of cannabinoids combined with agonists or antagonists for receptors of these neurotransmitters; Romero *et al.*, 1996; Meschler *et al.*, 2000), electrophysiological (e.g. monitoring the inhibitory or excitatory currents after the application of cannabinoids *in vivo*; Huang *et al.*, 2001; Gerdeman *et al.*, 2002; Wallmichrath and Szabo, 2002) and neurochemical (e.g. analysis of neurotransmitter synthesis, release and reuptake *in vivo* or *in vitro*; Köfalvi *et al.*, 2005) studies support the idea that the main function of cannabinoid signalling within the basal ganglia circuitry is that of a retrograde signalling system in GABAergic and glutamatergic synapses (reviewed in Gerdeman and Fernández-Ruiz, 2008). Accordingly, endocannabinoids and CB$_1$ receptors seem to play a key role in the synaptic depolarization-induced suppression of

excitation or inhibition, and also in long-term depression operating at striatal synapses (reviewed in Gerdeman and Fernández-Ruiz, 2008). Anandamide might also be able to directly inhibit certain presynaptic events in dopaminergic synapses within the striatum by acting through TRPV1 receptors located in nigrostriatal dopaminergic neurons (de Lago *et al.*, 2004a). However, it is also possible to modulate dopamine transmission by interacting at the level of the G protein/ adenylyl cyclase signal transduction mechanisms shared by both CB$_1$ and D1/D2 dopamine receptors (Di Marzo *et al.*, 2000; Meschler and Howlett, 2001), especially as CB$_1$ receptors colocalize with D1 receptors in striatonigral neurons and with D2 receptors in the neurons projecting to the globus pallidus (reviewed in Fernández-Ruiz and González, 2005).

Therefore, the experimental evidence described above: (i) abundant endocannabinoid signalling elements in the basal ganglia; (ii) the important motor effects following the activation or inhibition of this signalling system; and (iii) the modulation of key neurotransmitters within the basal ganglia by endocannabinoids, supports the idea that the cannabinoid signalling system plays an important role in the activity of these brain circuits. Hence, the pharmacological management of this signalling system might serve as a novel therapy for the treatment of motor dysfunction in those disorders affecting basal ganglia structures. This concept is also supported by the fact that the levels of different elements of the cannabinoid system, in particular the CB$_1$ receptor, seem to be significantly altered in various basal ganglia disorders. Such alterations in the proportions of these proteins have been observed in samples obtained from patients (Glass *et al.*, 1993; 2000; Richfield and Herkenham, 1994; Lastres-Becker *et al.*, 2001a; Pisani *et al.*, 2005; Battista *et al.*, 2007) and in different experimental models of these diseases generated by neurotoxin lesion (Zeng *et al.*, 1999; Page *et al.*, 2000; Romero *et al.*, 2000; Lastres-Becker *et al.*, 2001a; 2002a,b; 2004; 2005), by mutation or deletion of specific genes (Lastres-Becker *et al.*, 2002c;

García-Arencibia *et al.*, 2008a; Pazos *et al.*, 2008), or by inducing apoptosis in cultured neurons (Lastres-Becker *et al.*, 2005). This cannabinoid-based therapy would not only be aimed at alleviating specific motor symptoms, such as bradykinesia, tremor, dystonia or choreic movements, but also at delaying/ arresting the degeneration of the basal ganglia given the well-known neuroprotective/neuroregenerative properties of cannabinoid agonists (reviewed in Fernández-Ruiz *et al.*, 2007; Gerdeman and Fernández-Ruiz, 2008). In general, Parkinson's disease (PD) and Huntington's disease (HD) are the two basal ganglia disorders that have attracted most attention with regards the potential clinical application of cannabinoids (reviewed in Fernández-Ruiz and González, 2005; Sagredo *et al.*, 2007a).

Parkinson's disease is the most prevalent neurodegenerative disorder affecting the basal ganglia. The disease is caused either by environmental factors (e.g. pesticides, metals, antidopaminergic agents) or anomalies affecting the genes encoding several proteins (e.g. α-synuclein, parkin, PINK1), although it is possible that the combination of both factors may also be important. These primary factors would elicit a series of co-operative cytotoxic events (e.g. abnormal proteolysis, protein aggregation, mitochondrial dysfunction, excitotoxicity, oxidative stress and glial activation) that would culminate in the progressive death of dopaminergic neurons located in the substantia nigra *pars compacta*. These alterations would in turn originate severe dopaminergic denervation of the striatum and the occurrence of different neurological symptoms, such as bradykinesia, rigidity and tremor (see Thomas and Beal, 2007, for a recent review). HD is an inherited (autosomal-dominant), progressive and fatal neurodegenerative disorder caused by an excess of CAG repeats in the gene encoding for huntingtin, which provokes a polyglutamine expansion in the amino-terminal portion of this protein. The expansion originates several conformational changes that result in altered protein–

protein interactions, abnormal protein aggregation and proteolysis, and they subsequently provoke transcriptional dysregulation, mitochondrial complex II deficiencies, excitotoxicity, oxidative damage and glial activation. This sequence of cytotoxic events damages specific neuronal subpopulations, particularly the striatal neurons that project to the globus pallidus and the substantia nigra, originating a biphasic pattern of motor abnormalities that evolves from an early hyperkinetic phase (choreic movements) to a late akinetic and more disabling phase (see Roze *et al.*, 2008, for a recent review). Although PD and HD are the two basal ganglia disorders for which the potential therapeutic application of cannabinoids has been best studied, the manipulation of cannabinoid signalling might also be useful in other disorders directly or indirectly affecting the function of the basal ganglia, for example, dystonia (Fox *et al.*, 2002a; Richter and Loscher, 2002), dyskinesias (reviewed in Brotchie, 2003), Gilles de la Tourette's syndrome (reviewed in Müller-Vahl, 2003), Alzheimer's disease (reviewed in Campbell and Gowran, 2007) or multiple sclerosis (reviewed in Baker *et al.*, 2007).

Changes to the cannabinoid signalling system in basal ganglia disorders

Given the hypokinetic profile of the cannabinoid agonists able to activate CB_1 receptors, one might expect the cannabinoid signalling system (and particularly that acting through this receptor type) would become hypoactive in the basal ganglia in HD and other hyperkinetic disorders, whereas it would be up-regulated in hypokinetic disorders like PD. If this hypothesis were correct, it would explain the type of motor symptoms that are predominant in these disorders: choreic movements and bradykinesia respectively (see Figure 3). This
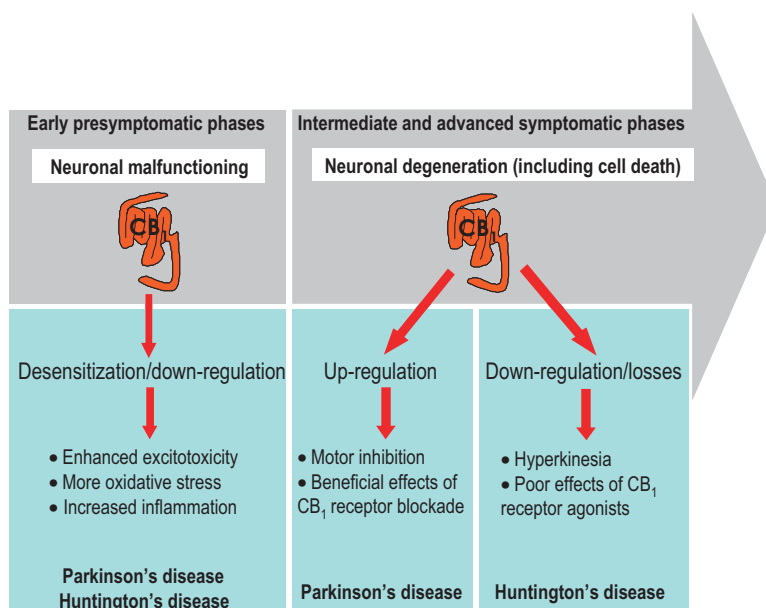
Figure 3 Changes to CB_1 receptors in the basal ganglia during the progression of Parkinson's and Huntington's diseases. CB_1, cannabinoid receptor type 1.

hypothesis has been confirmed in numerous studies conducted in recent years in which different endocannabinoid elements have been analysed in patients, in particular the CB_1 receptor type (Glass et al., 1993; 2000; Richfield and Herkenham, 1994; Lastres-Becker et al., 2001a; Pisani et al., 2005; Battista et al., 2007). Indeed, this system has also been studied in rodents or primates lesioned with neurotoxins (Page et al., 2000; Romero et al., 2000; Lastres-Becker et al., 2001b; 2002b; Gubellini et al., 2002; Fernández-Espejo et al., 2004), mice with mutations or deletion of specific genes (Denovan-Wright and Robertson, 2000; Lastres-Becker et al., 2002c; Centonze et al., 2005; Bisogno et al., 2008; García-Arencibia et al., 2008a), or animals subjected to different pharmacological treatments (Di Marzo et al., 2000), all animal models that totally or partially reproduce aspects of both diseases. However, in these studies it has also been emphasized that these responses are not the only changes experienced by the cannabinoid signalling system in both disorders (see Figure 3 for a schematic representation). Down-regulation in HD and overactivity in PD might be associated with degeneration in specific basal ganglia structures, although these changes or others might also be evident in early phases of these diseases when cell death does not occur or when it is sufficiently infrequent that it does not elicit motor symptoms. For example, fewer CB_1 receptors (down-regulation), or even their loss of function (desensitization), have already been documented in post-mortem samples of HD patients at very early stages of the disease (Glass et al., 2000). Likewise, similar events have been described in different transgenic mouse models of HD (e.g. R6/1, R6/2 and HD94) analysed at presymptomatic ages and in the absence of neuronal death (Denovan-Wright and Robertson, 2000; Lastres-Becker et al., 2002c; Naver et al., 2003; McCaw et al., 2004; Centonze et al., 2005), or in rats lesioned with the mitochondrial complex II inhibitor 3-nitropropionic acid at stages prior to the appearance of the first signs of neurodegeneration and neurological deterioration (Lastres-Becker et al., 2004). Similar observations have been made in the case of PD, where despite the up-regulation detected at intermediate and advanced phases of the disease (Romero et al., 2000; Lastres-Becker et al., 2001a), there is also evidence of a reduction in CB_1 receptors at early and presymptomatic ages in different knockout mouse models of three different genes linked to the development of PD (e.g. PARK1 encoding α-synuclein, PARK2 encoding parkin and PARK6 encoding the PINK1 protein; García-Arencibia et al., 2008a). Interestingly, at later symptomatic ages the same up-regulatory responses of CB_1 receptors were evident in these mice (García-Arencibia et al., 2008a), as observed in samples from patients (Lastres-Becker et al., 2001a) or in laboratory animals lesioned with neurotoxins (Romero et al., 2000). Therefore, down-regulation/desensitization of CB_1 receptors seems to be an early event in both HD and PD. Furthermore, this response appears to be associated with neuronal malfunctioning in the absence of cell death, presumably indicating that these losses might be involved in the initiation or early progression of striatal or nigral degeneration (reviewed in Sagredo et al., 2007a). It is possible that such early malfunctioning or loss of CB_1 receptors might render basal ganglia neurons more vulnerable to different cytotoxic stimuli that frequently operate in both

disorders. This might be so for excitotoxicity as CB_1 receptors are involved in the inhibition of glutamate release and thus, their loss or malfunction might enhance extracellular glutamate levels and aggravate the damage caused by this aminoacid (reviewed in van der Stelt et al., 2002; Centonze et al., 2007; Sagredo et al., 2007a; see Figure 3). This would explain why the activation of this receptor type might be used as a neuroprotectant strategy (see below). In addition, the loss and/or malfunctioning of CB_1 receptors might also aggravate other cytotoxic stimuli, such as oxidative stress or glial activation, although CB_1 receptors seem to be less important in these processes.

The loss of CB_1 receptors under conditions of neuronal malfunctioning in HD is much more marked when striatal degeneration is already evident. This was evident in post-mortem samples collected from patients at intermediate and advanced phases of the disease (Glass et al., 1993; Richfield and Herkenham, 1994), as well as in transgenic mice analysed at late and symptomatic ages (Pazos et al., 2008) and in rats with striatal atrophy generated by excitotoxins (Page et al., 2000) or mitochondrial toxins (Lastres-Becker et al., 2001b; 2002b). Similarly, the levels of endocannabinoids were also markedly reduced in the brain (Lastres-Becker et al., 2001b; Bisogno et al., 2008), even though some recent studies in patients identified an increase in endocannabinoid levels in the blood due to a reduction in fatty acid amide hydrolase (FAAH) activity (Battista et al., 2007). In conjunction with striatal degeneration, the loss of CB_1 receptors seems to be a mere side effect caused by the progressive destruction of CB_1 receptor-containing medium-spiny GABAergic neurons, which would explain why compounds targeting this receptor type are not effective against the hyperkinesia characteristic of HD (Lastres-Becker et al., 2003a; de Lago et al., 2006; see Figure 3). However, in conditions of striatal degeneration, the other major cannabinoid receptor type, the CB_2 receptor, is significantly up-regulated in glial elements that are recruited to and migrate to lesion sites (e.g. astrocytes and particularly reactive microglial cells; Sagredo et al., 2009), a response already described in other neurodegenerative or neuroinflammatory disorders (reviewed in Fernández-Ruiz et al., 2007; 2008). Interestingly, this response did not exist when animals were analysed at presymptomatic stages, when the major pathological hallmark is neuronal malfunction, supporting the idea that it is a phenomenon directly related to striatal degeneration and susceptible to pharmacological management (see below and Pazos et al., 2008).

The similarities found in the response of CB_1 receptors during early presymptomatic phases of HD and PD become inverted in intermediate and advanced phases of both these disorders. Thus, the progression of the nigral pathology and the appearance of major parkinsonian symptoms is associated with overactivity of the cannabinoid signalling system in most basal ganglia structures in patients (e.g. elevated endocannabinoid levels, increased CB_1 receptor density and function: Lastres-Becker et al., 2001a; Pisani et al., 2005), as well as in animal models of this disease (Di Marzo et al., 2000; Romero et al., 2000; Lastres-Becker et al., 2001a; Gubellini et al., 2002; Fernández-Espejo et al., 2004; van der Stelt et al., 2005). Such a situation would be in accordance with the

hypokinesia that characterizes this disease and with the possibility that CB_1 receptor antagonists might be useful for the alleviation of bradykinesia (see below and Figure 3). Interestingly, treatment with levodopa has been found to reverse both the elevation in endocannabinoid levels (Maccarrone et al., 2003; van der Stelt et al., 2005) and the increase in CB_1 receptor density (Lastres-Becker et al., 2001a). Another important difference between PD and HD is that CB_2 receptors do not appear to be up-regulated in glial elements recruited to the lesioned substantia nigra, either in patients or in animal models of PD. This might explain the failure of CB_2 receptor agonists to act as neuroprotectants in parkinsonian animals (García-Arencibia et al., 2007; see below), despite the fact that astrocytosis and reactive microgliosis have been documented in the substantia nigra in both patients and animal models of the disease (reviewed in Sagredo et al., 2007a).

Alleviation of motor symptoms by cannabinoid-related compounds in basal ganglia disorders

In the late 1990s, clinical trials examined whether plant-derived cannabinoids (reviewed in Consroe, 1998) or some of their synthetic analogues (Müller-Vahl et al., 1999) might be used to improve choreic movements in HD patients, in the light of the hypofunctionality of the cannabinoid system in this disease. However, rather than improving hyperkinesia, these studies reported an aggravation of choreic movements (Consroe, 1998; Müller-Vahl et al., 1999). It is possible that this is related to the lack of TRPV1 receptor activity of the cannabinoid agonists used in those clinical trials, as recent studies conducted in animal models have demonstrated that only TRPV1 receptor agonists (e.g. AM404, arvanil, capsaicin) alleviate hyperkinesia and restore neurochemical deficits (Lastres-Becker et al., 2002a; 2003a; de Lago et al., 2005). By contrast, CB_1 receptor agonists (e.g. CP55,940) were not particularly effective in combating these symptoms, particular when used at intermediate or advanced phases of the disease (grades 2–4), when there is a progressive loss of these receptors in association with the death of striatal projection neurons. Indeed, these observations were further confirmed in experiments with selective antagonists of both receptor types (Lastres-Becker et al., 2003a). Therefore, TRPV1 receptors appear to be a novel therapeutic target susceptible to clinical exploitation for the treatment of hyperkinesia in HD (see Figure 4). It is possible that the best clinical option might involve developing compounds with a dual capacity that can activate both TRPV1 and CB_1 receptors, at least for the first phases of HD (grades 0–2). However, the relative contribution of both targets is likely to vary during the course of the disease due to a progressive loss of CB_1 receptors with no concomitant change in TRPV1 receptors (reviewed in Lastres-Becker et al., 2003b).

The overactive cannabinoid transmission in PD and the hypokinetic profile of most cannabinoid agonists does not support the use of these compounds to alleviate the motor disruption in this disease, particularly in terms of bradykinesia, the major parkinsonian symptom. In this case, CB_1 receptor antagonists rather than agonists would seem to be a better option (see Figure 4). This issue has been addressed in a series of preclinical (Di Marzo et al., 2000; Meschler et al., 2001) and clinical studies (Mesnage et al., 2004). Although the initial results were controversial, it is possible that the blockade of CB_1 receptors might only be effective in special circumstances (e.g. with the use of low doses, at very advanced phases of the disease only and in patients with a poor levodopa response; Fernández-Espejo et al., 2005; González et al., 2006; García-Arencibia et al., 2008b). If this were the case, it would be possible to generate an antiparkinsonian agent for conditions in which classic therapy generally fails. The blockade of TRPV1 receptors might be also useful against the motor inhibition typical of PD, given the inhibition of dopamine activity caused by the activation of these receptors (de Lago et al., 2004a). However, in this case the death of nigrostriatal neurons in which the TRPV1 receptor is located (Lastres-Becker et al., 2005) might represent an important disadvantage. CB_1 receptor antagonists might also be useful for delaying and reducing the dyskinetic states caused by chronic levodopa treatment in PD patients and animal models (Brotchie, 2000; 2003; van der Stelt et al., 2005), even though this property has also paradoxically been attributed to CB_1 receptor agonists (Brotchie, 1998; Sieradzan et al., 2001; Fox et al., 2002b). Such effects would be consistent with the presence of CB_1 receptors in multiple sites (both in excitatory and inhibitory synapses) within basal ganglia circuits, which might facilitate these paradoxical effects (reviewed in Fernández-Ruiz and González, 2005; Gerdeman and Fernández-Ruiz, 2008). Along similar lines, CB_1 receptor agonists (or inhibitors of endocannabinoid inactivation) have also been attributed beneficial effects in PD (see Figure 4): (i) in reducing the tremor associated with the overactivity of the subthalamic nucleus (Sañudo-Peña et al., 1998; 1999), although the only clinical trial developed so far produced negative results (Frankel et al., 1990); (ii) by interacting with dopamine agonists to improve certain motor impairments (Brotchie, 1998; Sañudo-Peña et al., 1998; Kreitzer and Malenka, 2007); and (iii) by delaying the progression of nigral degeneration, although this effect may not be caused by their hypokinetic profile, but rather by their neuroprotective properties (see below).

Neuroprotection/neuroregeneration with cannabinoids in basal ganglia disorders

Cannabinoids have also been proposed to offer a novel type of therapy for HD and PD, delaying, arresting or repairing the striatal and nigral damage due to their capacity to buffer excitotoxicity, calcium influx, oxidative injury, cerebrovasoconstriction, body temperature and/or inflammation in conditions of acute or chronic neurodegeneration (reviewed in Fernández-Ruiz et al., 2005; van der Stelt and Di Marzo, 2005), as well as through their influence on the survival, proliferation and differentiation of neural progenitors (reviewed in Galve-Roperh et al., 2007). Unfortunately, this possibility has not yet progressed beyond the preclinical stage, although it is sufficiently robust to justify an urgent clinical evaluation with some of the already available cannabinoid-based medicines.
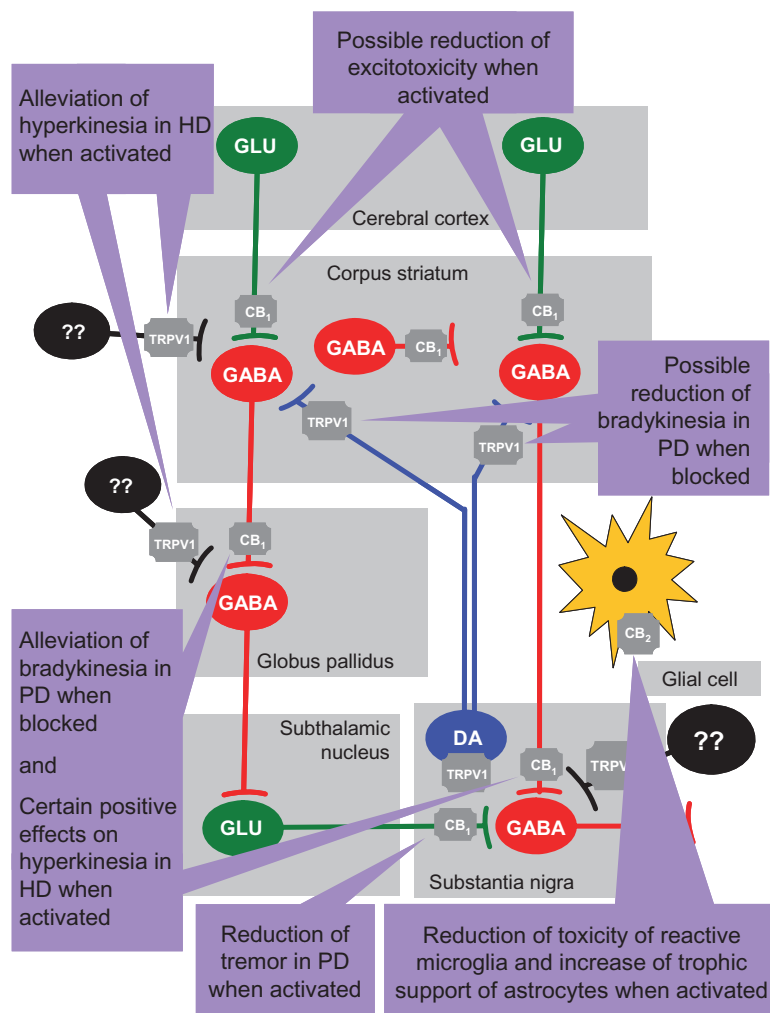
Figure 4 On the scheme shown in Figure 1, a diagram has been superimposed to show the different targets (CB$_1$, CB$_2$ and TRPV1 receptors) that might mediate the ability of cannabinoid-based medicines to alleviate specific symptoms, or to delay/arrest the progression of the disease in basal ganglia disorders. CB$_1$, cannabinoid receptor type 1; DA, dopamine; GABA, γ-aminobutiric acid; GLU, glutamate; HD, Huntington's disease; PD, Parkinson's disease; TRPV1, transient receptor potential vanilloid type 1.

In HD, both the early desensitization of CB$_1$ receptors located in certain neuronal subpopulations and the late up-regulation of CB$_2$ receptors in glial elements suggest that compounds selectively targeting both receptor types might be able to attenuate striatal degeneration, as mentioned above. The importance of CB$_1$ receptors has been demonstrated in conditions of excitotoxicity, such as those observed in rats after the lesion of the striatum with the excitotoxin quinolinate (Pintor et al., 2006). By contrast, selective CB$_2$ receptor agonists are effective in conditions of inflammation and glial activation, such as those occurring after the intrastriatal application of the reversible mitochondrial complex II inhibitor malonate in rats (Fernández-Ruiz et al., 2007; 2008; Sagredo et al., 2009). In addition, some plant-derived antioxidant cannabinoids can protect striatal projection neurons from death in conditions of mitochondrial dysfunction and oxidative stress, such as those provoked by the administration of the irreversible mitochondrial complex II inhibitor 3-nitropropionic acid in rats. These cannabinoids include Δ⁹-tetrahydrocannabinol (Δ⁹-THC) (Lastres-Becker et al.,

2004), which binds to both CB$_1$ and CB$_2$ receptors, and cannabidiol (Sagredo et al., 2007b), which has negligible activity at both receptor types. Given the fact that HD is a neurodegenerative disorder in which the death of striatal neurons is caused by cooperation between different cytotoxic mechanisms, including excitotoxicity, inflammation and oxidative damage, any neuroprotective cannabinoid compound(s) suitable for clinical evaluation in HD should be broad-spectrum cannabinoids, or alternatively combinations of specific existing compounds could be assessed. The objective would be to activate both CB$_1$ and CB$_2$ receptors (e.g. to normalize glutamate homeostasis and to reduce local inflammatory events caused by glial activation), but also to decrease oxidative damage with an antioxidant cannabinoid. Hence, the cannabinoid-based preparation Sativex, a combination of Δ⁹-THC and cannabidiol (reviewed in Russo and Guy, 2006), might be a good candidate for clinical evaluation in HD.

Despite the fact that the hypokinetic profile of most cannabinoid agonists may represent a disadvantage in the treatment of PD, there is also preclinical evidence indicating that certain

cannabinoid agonists may offer neuroprotection in this disease (reviewed in Fernández-Ruiz *et al.*, 2005). This has been studied in rats with hemiparkinsonism generated by unilateral injection of the neurotoxin 6-hydroxydopamine (Lastres-Becker *et al.*, 2005; García-Arencibia *et al.*, 2007). Δ^9-THC and cannabidiol were the first cannabinoids shown to be capable of attenuating the damage to nigrostriatal dopaminergic neurons caused by this neurotoxin (Lastres-Becker *et al.*, 2005). As mentioned above, Δ^9-THC can bind to and activate both CB_1 and CB_2 receptors, although cannabidiol has negligible activity at both receptor types. Hence, the neuroprotective effect of both compounds is probably exerted through their cannabinoid receptor-independent antioxidant properties (Lastres-Becker *et al.*, 2005). This was further confirmed by examining the antioxidant properties of additional compounds that are more selective for both receptor types (de Lago *et al.*, 2006; García-Arencibia *et al.*, 2007). Similar findings were recently obtained in an invertebrate model of Parkinsonism (Jiménez-del Río *et al.*, 2008) and in an *in vitro* model of 6-hydroxydopamine toxicity (Lastres-Becker *et al.*, 2005). Both studies used synthetic cannabinoid agonists (e.g. CP55,940 and HU-210 respectively), which also have well-demonstrated cannabinoid receptor-independent antioxidant properties (Marsicano *et al.*, 2002). Therefore, the cannabinoid receptor-independent antioxidant profile of certain cannabinoids seems to be a key mechanism in the protection of nigrostriatal neurons from death in PD. This observation predisposes the cannabinoid-based medicine, Sativex, to clinical evaluation. By contrast, the studies conducted on the role of CB_2 receptors in PD indicate that this receptor is not a relevant target in this disease (García-Arencibia *et al.*, 2007), unlike in HD.

Alternatively, the pharmacological manipulation of the cannabinoid system might also serve to repair the injured tissue in these and other neurodegenerative disorders (reviewed in Galve-Roperh *et al.*, 2007; Maccarrone *et al.*, 2007), particularly given the recent identification of a cannabinoid-sensitive mechanism that participates in the control of adult neurogenesis (Aguado *et al.*, 2005). A compound with this ability would mean that it complied with the aforementioned requirement of these molecules to preserve striatal or nigral neurons from death, permitting injured neurons to be replaced if treatments were initiated after degeneration had already begun, or if the treatment were only effective in delaying but not arresting the progression of the disease. Hence, cannabinoid compounds could be used to activate neurogenic structures (the subventricular zone and the hippocampal dentate gyrus in the adult brain), thereby encouraging the proliferation of cell progenitors, their differentiation to neurons and/or their migration to the damaged structures (striatum or substantia nigra) where they could acquire the phenotype corresponding to striatal medium-spiny neurons or nigrostriatal neurons that are lost in both diseases respectively. Unfortunately, the experimental evidence accumulated so far regarding the possible reparative actions of cannabinoids in both disorders is still minimal. Nevertheless, recent data have demonstrated the presence of a cannabinoid-sensitive population of progenitor cells in the subependymal layer of the normal and HD human brain (Curtis *et al.*, 2006), possibly representing a suitable source for the replacement of the cells lost in this disease.

Concluding remarks and future perspectives

The studies reviewed here support the view that the cannabinoid signalling system is a key modulatory element in the activity of the basal ganglia. This concept is supported by different anatomical, electrophysiological, pharmacological and biochemical data. Indeed, we have shown that the cannabinoid system is impaired in different disorders that directly or indirectly affect the basal ganglia, which supports the idea of developing novel pharmacotherapies with compounds that selectively target specific elements of the cannabinoid system. The development of such compounds would not only provide novel therapeutic agents capable of minimizing the frequent side effects observed when classic cannabinoids are used in patients, but also help to elucidate the exact role played by the cannabinoid system in the pathogenesis of these disorders. In the light of the neuroprotectant/neuroregenerative properties demonstrated for certain cannabinoids, these treatments should not only aim to alleviate specific symptoms (e.g. hyperkinesia in HD, tremor and bradykinesia in PD), but also attempt to delay/arrest disease progression and to repair the damaged structures. However, most of the studies that have examined the therapeutic potential of these compounds in basal ganglia disorders have been conducted in animal models, with very few clinical trials carried out to date. Therefore, the importance of this intercellular signalling system needs further clinical research to be carried out in patients with the aim of validating the results found in animal and cellular models. Such studies should examine the potential of hybrid vanilloid/cannabinoid agonists to reduce hyperkinesia in HD patients. Another challenge in HD is to develop a broad-spectrum cannabinoid, or a combination of selective compounds, which might reduce excitotoxicity while limiting the activation of microglia and the generation of reactive oxygen intermediates. In this respect, given what is already known about the nature of their location and pharmacological properties, it will be important to establish in greater detail both the part that CB_2 receptors play in HD and the potential therapeutic benefits of targeting them with a medicine. This receptor type might also be important in PD, although novel tools will have to be developed to determine whether this receptor is up-regulated or not in this disease (e.g. more selective anti-CB_2 antibodies, selective ligands useful for *in vivo* analysis). In the absence of such information, it might be reasonable to test combinations of antioxidant cannabinoids as novel neuroprotectant therapies in patients. Alternatively, the capacity of CB_1 and TRPV1 receptor antagonists to alleviate bradykinesia should be considered in patients who do not benefit from classical levodopa therapy.

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Conflict of interest

The author has not any conflict of interest. All agencies that supported the studies included in this review had no further role in study design, the collection, analysis and interpretation of data, in the writing of the report, or in the decision to submit the article for publication.

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