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5 Role of endogenous cannabinoids in the control of basal ganglia activity

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Abstract

The cannabinoid system is a novel intercellular signaling system that plays a prominent role, among others, in the control of basal ganglia function. This finding can be concluded from the data obtained in different series of anatomical, biochemical, electrophysiological and pharmacological studies. These data demonstrated: (i) that the basal ganglia contain high levels of endocannabinoids and their receptors, mainly including the cannabinoid CB_1 receptor subtype but also a related receptor type, the

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vanilloid $TRPV_1$ receptor; (ii) that the activation or the blockade of this system produces important changes in motor behavior, changes that are originated as a consequence of interactions of the cannabinoid system with various classic neurotransmitters such as GABA, dopamine or glutamate; and (iii) the occurrence of marked changes in specific elements of the cannabinoid signaling system in various basal ganglia disorders, with emphasis in the induction/upregulation of the cannabinoid CB_2 receptor subtype. This large evidence relating endocannabinoids and their receptors to the function of the basal ganglia, both in the healthy and the pathological brain, has provided support for the idea that cannabinoid-based medicines, with selectivity for different targets of the cannabinoid signaling system (synthetic enzymes, receptors, inactivation system), might have therapeutic potential to alleviate symptoms and/or provide neuroprotection in basal ganglia disorders, in particular Parkinson´s disease and Huntington´s chorea. The present chapter will review the knowledge on this issue trying to establish the future lines for the research on the therapeutic potential of the cannabinoid signaling system in basal ganglia disorders.

Introduction: Brief summary of the cannabinoid signaling system

The great increase experienced in the research on cannabinoids and related-molecules during the last years can be directly related to the discovery of the so-called "endogenous cannabinoid system", a new intercellular communication system that is mostly active in the central and peripheral nervous system [1]. The cannabinoid system is made up of a family of endogenous arachidonic acid derivatives called "endocannabinoids" that act through at least two types of G protein-coupled receptors, termed cannabinoid CB_1 and CB_2 receptors [2]. These receptors are also activated by different compounds present in the plant *Cannabis sativa* that are called phytocannabinoids. The action of endocannabinoids at their receptors terminates in an endogenous mechanism of inactivation that involves a membrane transport system and at least two degradative enzymes [2]. From a physiological point of view and despite several efforts to define endocannabinoid ligands as emerging novel neurotransmitters, there is a general consensus that cannabinoid receptors and their ligands, rather than transmitters, function as neuromodulators [3]. Thus, by acting, for example, as retrograde messengers at various synapses, endocannabinoids participate in the control of processes such as memory and learning, appetite, emesis, nociception, certain motivational responses, and also in the control of movement [1-3]. The latter is exerted mainly by modulating the function of the basal ganglia, but also acting at the cerebellum [4-6].

Function of the cannabinoid signaling system in the basal ganglia

Endocannabinoids and their receptors, in particular the CB_1 receptor subtype, are abundant in the basal ganglia in comparison with other brain structures [7-10]. Two basal ganglia structures, the globus pallidus and the substantia nigra which are strongly innervated by striatal projection neurons, deserve to be mentioned because of their abundancy in elements of the cannabinoid system [7-10]. The activation or blockade of CB_1 receptors located in these structures produce important effects on the function controlled by the basal ganglia which are reflected in marked changes in different motor responses (for review, see [4,5]). However, the location of these receptors at different sites in the basal ganglia circuit may sometimes produce paradoxical effects (see [6] for a recent review). In general, cannabinoid agonists have powerful actions, mostly of inhibitory nature, on motor activity ([11-15]; for reviews see [5,6,16]), although there are differences in the magnitude and duration of their effects depending on their differences in receptor affinity, potency, and/or metabolic stability (see [6] for review). The behavioral consequences following the activation or blockade of CB_1 receptors are certainly related to the capability of these receptors to influence the activity of a series of neurotransmitters that have been currently shown to be involved in the basal ganglia function (for reviews, see [4-6,16]). Finally, it is also important to note that cannabinoid receptors or other key proteins of the cannabinoid system appear to be significantly altered in different basal ganglia disorders, a phenomenon proved in human patients [5,6,17-21] or in different animal models for these diseases [21-26]. This opens the possibility for cannabinoid-based medicines to be used for the treatment of these disorders (see [6] for a recent review). The present chapter will address all of this previous pharmacological, biochemical, anatomical, and pathological evidence, trying to establish the bases that support the therapeutic potential of the cannabinoid system in basal ganglia disorders.

Presence of elements of the cannabinoid system in the basal ganglia

As mentioned above, the identification and quantification of diverse elements of the cannabinoid signaling system in the basal ganglia have served as a way to demonstrate the importance of the role played by this system in the control of motor function (for a review see [5,6]). Thus, the use of autoradiographic techniques during the 90s demonstrated that the basal ganglia are among the brain structures containing the highest levels of both binding sites and mRNA expression for the CB_1 receptor (for details, see [5,6]). In particular, the three nuclei recipient of striatal efferent outputs (globus pallidus,

entopeduncular nucleus, and substantia nigra pars reticulata in rodents; see details in Figure 1) contain high levels of receptor binding sites [7]. By contrast, CB_1 receptor-mRNA transcripts were not detectable in these three nuclei, but they could be measured in the caudate-putamen ([8] and Figure 1). This particular distribution of binding sites and mRNA transcripts for the CB_1 receptor [7,8] is compatible with a possible presynaptic location of these receptors in striatal projection neurons [6], a fact that was corroborated by studying the changes in CB_1 receptors produced by selective lesions of different subpopulations of striatal neurons [27]. More recently, the availability of selective CB_1 receptor antibodies allowed a more precise analysis of the cellular distribution of this receptor subtype in the basal ganglia [9,28,29]. Thus, CB_1 receptors are located in both striatonigral (the so-called "direct" striatal efferent pathway) and striatopallidal (the so-called "indirect" striatal efferent pathway) projection neurons, which are GABA-containing neurons. In

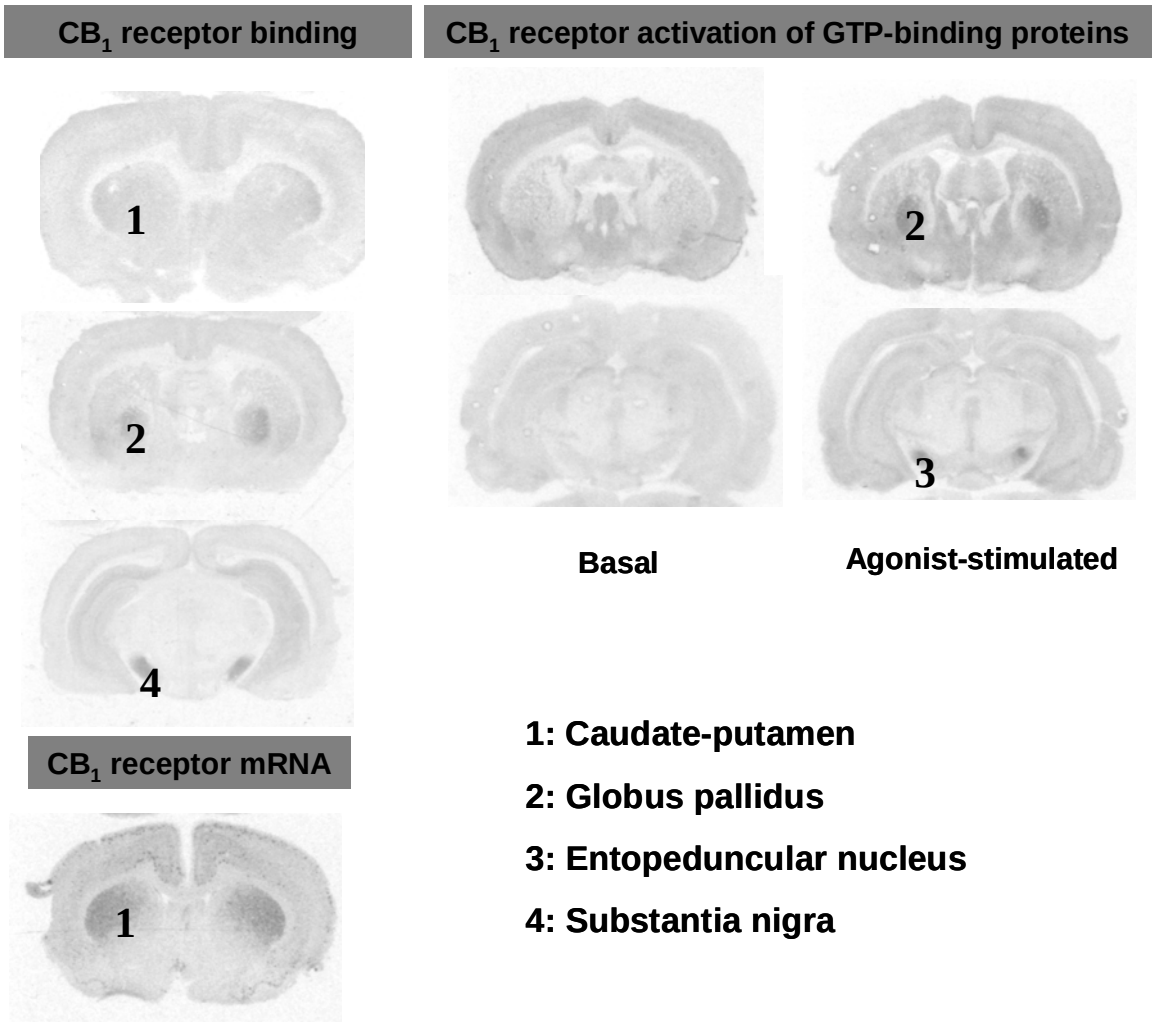

Figure 1. Autoradiograms demonstrating the high concentrations of cannabinoid CB_1 receptors (binding, mRNA expression and activation of GTP-binding proteins) in the rat basal ganglia.

both pathways, CB_1 receptors are co-expressed with other markers, such as glutamic acid decarboxylase, prodynorphin, substance P, or proenkephalin, as well as D_1 or D_2 dopaminergic receptors [29]. In contrast, intrinsic striatal neurons, that contain somatostatin or acetylcholine, do not appear to contain CB_1 receptors [29], although a recent study by Fusco and coworkers [28] has demonstrated immunoreactivity for this receptor subtype in some subclasses of striatal interneurons. Another subpopulation of CB_1 receptors in the basal ganglia is located on subthalamopallidal and/or subthalamonigral glutamatergic terminals, as revealed by the presence of measurable levels of mRNA for this receptor in the subthalamic nucleus, together with the absence of detectable levels of cannabinoid receptor binding in that structure [8].

The studies performed during the 90s indicated that CB_2 receptors do not appear to be present in the healthy brain [30]. However, some recent studies have provided direct evidence of their presence in the healthy cerebellum [31,32], suggesting that this receptor subtype might also play a role in various cerebellar processes in normal conditions. These data have been recently corroborated by the identification of CB_2 receptors in other brain regions of different species without neurological pathology [33,34], including unpublished data from our group that proved CB_2 receptor immunoreactivity and mRNA expression in the rat striatum although possibly located in astrocytes rather than in neurons. This obviously points to a role for this receptor subtype in normal brain functions, including the control of basal ganglia activity. However, CB_2 receptor function seems to be more important in response to different types of insults, including injury or inflammation, when they are significantly induced/upregulated in activated astrocytes or reactive microglia to control several events related to the protective and/or cytotoxic influences that the different glial cells exert on neuronal survival [35,36].

The vanilloid $TRPV_1$ receptor has been functionally related to the cannabinoid signaling system since certain endocannabinoids are able to act as endogenous ligands for this cation channel. Although $TRPV_1$ receptors were primarily identified in pain-related areas, there is evidence indicating that $TRPV_1$ receptors are also present in the basal ganglia circuitry colocalizing with tyrosine hydroxylase, which suggests that they are located in nigrostriatal dopaminergic neurons [37]. In fact, they are lowered by treatment with 6-hydroxydopamine (6-OHDA) which degenerates dopaminergic neurons [38]. By contrast, $TRPV_1$ receptor binding increased in the striatum of mice deficient in dopamine (DA) transporter [39]. This upregulation may be aimed at compensating the spontaneous hyperactivity exhibited by these mice and the low anandamide levels found in their striatum. In this respect, the administration of several inhibitors of endocannabinoid inactivation decreased the spontaneous hyperlocomotion by acting preferentially through $TRPV_1$ receptors [39]. As will be detailed below, the location of $TRPV_1$ receptors in

nigrostriatal dopaminergic neurons provides an alternative for certain endocannabinoids to act through these receptors directly controlling DA synthesis and release [40], which would complete their classic action mediated by CB_1 receptors directly controlling GABA and/or glutamate (GLU) function in the basal ganglia circuitry [5,6]. However, a recent double immunohistochemical study has shown that CB_1 and $TRPV_1$ receptors might colocalize in the caudate-putamen, the globus pallidus and the substantia nigra [41].

Anandamide and 2-arachidonoylglycerol, the two major endocannabinoid ligands, are also present in the basal ganglia in concentrations that are in general higher than those measured in the whole brain [10,42]. As for the density of CB_1 receptors, the globus pallidus and the substantia nigra are the two basal ganglia that contain the highest levels of endocannabinoids [42]. Both endocannabinoids seem to be synthesized in the basal ganglia [6] although the phenotype of the nerve cells that produce these endocannabinoids is presently unknown. It is important to mention that, in the case of anandamide, the synthesis seems sensitive to DA, so that D_2 receptors activated by DA released by nigrostriatal neurons would be associated with an enhancement of anandamide synthesis aimed at serving as an inhibitory feedback mechanism that counteracts DA-induced facilitation of psychomotor activity [43].

Other elements of the cannabinoid signaling system that have been identified, in some cases at high concentrations, in the basal ganglia are: (i) the endocannabinoid transporter, although the evidence for this protein is indirect and obtained mainly from pharmacological studies ([44-47]; for review see [6]), (ii) the enzyme fatty acid amide hydrolase (FAAH), which is involved in the degradation of anandamide [48,49], and (iii) the enzyme monoacylglycerol-lipase, which is involved in the degradation of 2-arachidonoylglycerol (see [50] for a review). However, these two enzymes accept as substrates various *N*-acylethanolamines or mono-acylglycerols, respectively, so lacking the necessary specificity to be used as selective markers of cannabinoid transmission.

Control of motor activity by cannabinoids

The abundancy of elements of the cannabinoid signaling system in the basal ganglia suggests an important role for this system in the control of neurobiological function(s) that reside(s) in these structures, namely, the control of motor function. This seems to be the case since the evidence published so far indicates that: (i) the consumption by humans of psychoactive cannabinoids, such as those present in marijuana or other *Cannabis sativa* preparations, which act by activating CB_1 receptors, produce a series of motor effects, most of them of inhibitory nature (for review see [51]), (ii) the direct activation of CB_1 receptors with plant-derived, synthetic, or endogenous agonists produced dose-dependent impairments in a variety of motor tests (open-field, ring test, actimeter, rotarod) in laboratory animals [11-15,52-59],

and (iii) the administration of inhibitors of endocannabinoid inactivation, so-called indirect agonists, which act by prolonging the action of endocannabinoids at their receptors, mainly the CB_1 receptor subtype, also inhibited movement in laboratory animals ([44-47], see [6] for a recent review). By contrast, a few studies that used administration of very low doses of anandamide, Δ^9-tetrahydrocannabinol (Δ^9-THC), or other cannabinoids reported hyperkinetic effects of these agonists in mice [60] and rats [61] in comparison with the hypokinetic effects reported when higher doses were used (see [5,6] for review).

It is important to note at this point that the above evidence was obtained with compounds that act both, directly or indirectly, and selectively or non-selectively, at the CB_1 receptor, thus stressing the relevance of this cannabinoid receptor subtype in the control of basal ganglia function in concordance with its abundant presence in this circuitry. In support of this notion, the motor effects of most cannabinoid agonists were usually prevented by SR141716, a selective CB_1 receptor antagonist ([60,62], for a review, see [4]), which, due to its inverse agonist properties, was also able to cause hyperlocomotion by itself [63]. However, there is pharmacological evidence that certain cannabinoids might have additional targets which affect motor function. Thus, the motor effects of anandamide or other eicosanoid-based cannabinoid agonists, such as AM404, might be originated by their capability to bind and activate vanilloid $TRPV_1$ receptors, since these effects were reversed by capsazepine but not by SR141716 [25,40,64]. In concordance with this observation, the activation of $TRPV_1$ receptor by agonists, such as capsaicin, produced motor inhibition [62]. The recent detection of $TRPV_1$ receptors in the basal ganglia [37] supports this hypothesis.

Interactions with classic transmitters acting at the basal ganglia

The motor effects following the activation or the inhibition of the cannabinoid signaling system likely depend on the control exerted by this system on the activity of those neurotransmitters that have been involved in the control of basal ganglia function, in particular, DA, GABA and GLU (Figure 2). The location of CB_1 receptors in several GABA- or GLU-containing neuronal subpopulations in the basal ganglia, as well as the recent identification of $TRPV_1$ receptors in nigrostriatal dopaminergic neurons (see [6] for a recent review), enables endocannabinoids to directly control the function of GABAergic, glutamatergic or dopaminergic transmissions. Due to the preferent presynaptic location of the receptors, they seem to participate mainly in the control of presynaptic events, such as synthesis, release, or reuptake for these neurotransmitters (see [6] for a recent review).

The supporting evidence that GABAergic transmission in the basal ganglia mediates the motor effects of cannabinoids is based on (i) anatomical (i.e. location

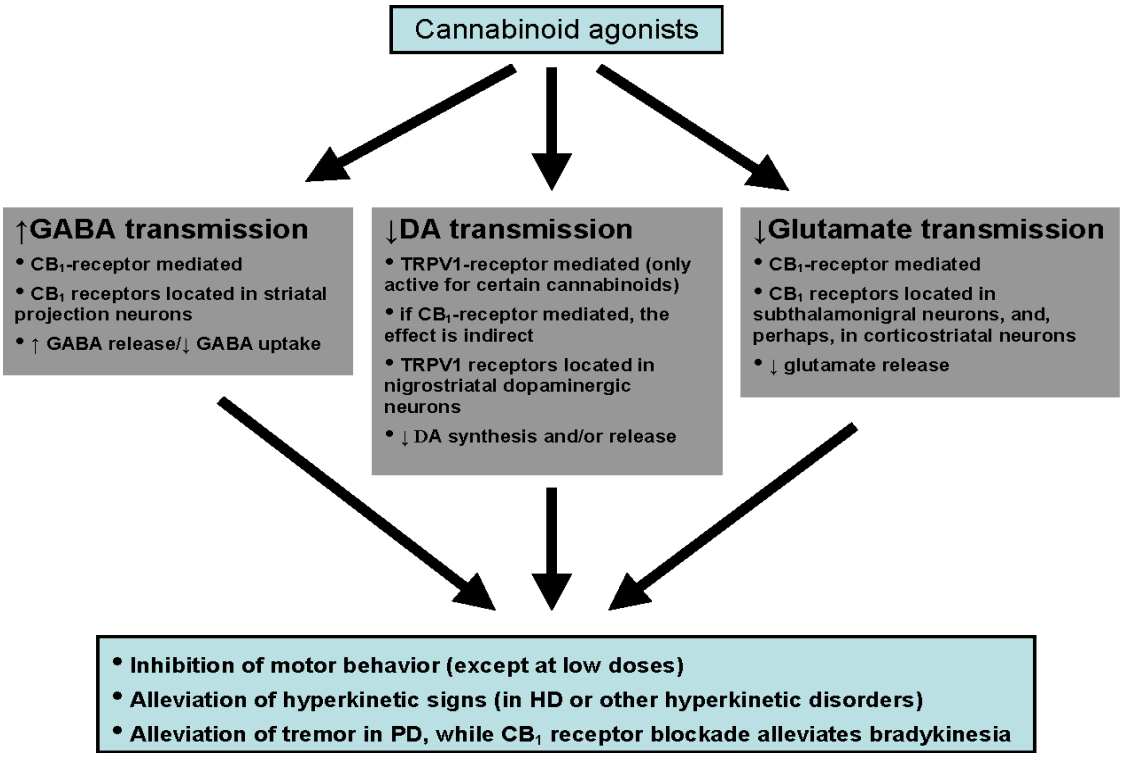

Figure 2. Diagram summarizing the different interactions proposed for the cannabinoid system with classic neurotransmitters acting at the basal ganglia.

location of CB_1 receptors in striatal projection neurons [8,27]), (ii) pharmacological (i.e. administration of cannabinoids combined with agonists or antagonists for GABA-A or –B receptors [13,52,65]), (iii) electrophysiological (i.e. monitoring the inhibitory currents after the application of cannabinoids *in vivo* [66,67]), and (iv) neurochemical (i.e. analysis of neurotransmitter synthesis, release and reuptake *in vivo* or in GABA-containing synaptic preparations [25,68,69]) data. All this evidence is compatible with an enhancement of GABA-mediated transmission by endocannabinoids in the globus pallidus and the substantia nigra, which concurs with the hypokinetic action of these compounds (see [6] for a recent review). However, there are some studies showing inhibition rather than stimulation of GABAergic inputs by cannabinoid agonists [70-72].

In a similar way to GABA, there is also evidence to show that cannabinoids also exert a direct action on GLU-releasing neurons in the basal ganglia. This is based on the anatomical demonstration that CB_1 receptors are located in subthalamonigral glutamatergic neurons [8] and their activation inhibits movement [73]. Also, electrophysiological evidence shows that cannabinoid agonists modify the activity of pallidal and nigral neurons through inhibiting GLU release from the terminals projecting from the subthalamic nucleus [74,75]. There is also increasing evidence indicating that CB_1 receptors may be also located in cortical afferents projecting to the caudate-putamen which are glutamatergic, since

the activation of these receptors results in an inhibition of GLU release from these terminals, this effect being also blocked by SR141716 [76].

DA transmission in the basal ganglia is also inhibited by cannabinoid agonists, as has been revealed by several neurochemical analyses ([14,15,44,77]; see [78] for a recent review). This effect is consistent with the ability of cannabinoid agonists to produce hypokinesia (see above), and also with the observations that cannabinoids potentiated reserpine induced hypokinesia [55], while reducing amphetamine-induced hyperactivity [56]. However, the effects of cannabinoid agonists on DA transmission were frequently small and transient. This is possibly due to the fact that, contrary to the case of GABA- and GLU-containing neurons, CB_1 receptors are not located on DA-containing neurons in the basal ganglia in the adult brain [6], so that the DA effects of classic cannabinoids (plant-derived and analogs) would be originated indirectly through their effects on GABAergic transmission [68,69]. However, it is also possible that the effects of cannabinoids on DA transmission may be produced by an interaction at the level of G protein/adenylyl cyclase signal transduction mechanisms shared by both CB_1 and D_1/D_2 dopaminergic receptors [42,79]. The colocalization of CB_1 receptors with D_1 receptors in striatal neurons projecting to the substantia nigra, or with D_2 receptors in those projecting to the globus pallidus (see [6] for review), makes this type of postreceptor interaction possible. In addition and in contrast with classic cannabinoids, certain agonists, such as anandamide and some analogs, would also be able to directly influence dopaminergic transmission through the activation of vanilloid $TRPV_1$ receptors. These have been identified in DA-containing neurons in the basal ganglia [37] and their activation by anandamide decreased motor activity via a DA-lowering effect exerted directly on these neurons [40]. Classic cannabinoids, such as Δ^9-THC, that do not bind to vanilloid-like receptors were not able to produce this effect [40].

Therapeutic potential of the cannabinoid system in basal ganglia disorders

The above data relate the cannabinoid system to the control of basal ganglia function. Studies that will be presented below demonstrate the occurrence of changes in different elements of this system, in particular the CB_1 receptor, in the postmortem basal ganglia of humans affected by several basal ganglia disorders and also in animal models of these disorders (for review, see [6]). Based on these two ideas, one may hypothesize that compounds active in cannabinoid transmission might be useful in alleviating motor symptoms [6,78] and delaying/arresting the degeneration of basal ganglia [36,80] in both hyper- and hypokinetic disorders. Among these disorders, Parkinson's disease (PD) and Huntington's disease (HD) are the two diseases directly related to the control of movement that have attracted most

interest in terms of a potential therapeutic application of cannabinoids (for review see [6,36]). Another interesting related disorder in which cannabinoids might be effective is Gilles de la Tourette's syndrome (TS) ([81] for a review). Finally, other diseases not directly related to a degeneration of basal ganglia structures but exhibiting strong motor symptoms, such as Alzheimer's disease (AD) (see [82] for a review) or multiple sclerosis (MS) (see [83] for a review), have also been examined for a potential therapeutic application of cannabinoid-based compounds. Most of the data presented in this review will concentrate on HD and PD as the most representative examples of therapeutic application of cannabinoids in basal ganglia degeneration, although some additional comments on other diseases are also included.

Changes in the cannabinoid signaling system in basal ganglia disorders

Assuming that the activation of the cannabinoid system increases GABA transmission in the basal ganglia, consequently reducing movement, it would be expected for this system to become progressively hypofunctional in HD, the most representative hyperkinetic disorder [84]. This was confirmed by analyzing postmortem tissue from patients where an almost complete disappearance of CB_1 receptors could be observed in different structures of the basal ganglia [17,18,20]. Similar results were obtained in different types of animal models for this disease, where the reductions affected CB_1 receptors [24-26,85,86] but also endocannabinoid levels [87]. This loss of CB_1 receptors is representative of the pattern of neuronal loss observed in HD that predominantly affects CB_1 receptor-containing medium-spiny GABAergic neurons [27,29], so *a priori* it could be a mere side effect caused by the progressive and selective destruction of striatal GABAergic projection neurons where these receptors are located. However, an interesting aspect is that the reduction of CB_1 receptors seems to occur in advance of other receptor losses and even before the appearance of major HD symptomatology, when the incidence of cell death is still low [18], thus indicating that these losses might be involved in the pathogenesis itself or in the progression of striatal degeneration (see [84] for review). This concurs with the data obtained in various transgenic mouse models that express mutated forms of the human huntingtin gene, where the reductions of CB_1 receptors in the basal ganglia already occur with neuronal malfunctioning but in absence of cell death [85,86]. In the same line, reductions in CB_1 receptor function (G-protein activation by WIN55,212-2), in the absence of changes in binding and mRNA levels for this receptor, have been documented at very early stages of striatal degeneration caused by inhibition of mitochondrial complex II [88]. Collectively, these observations seem to indicate that the losses or the malfunctioning of CB_1 receptors in specific neuronal subpopulations of the basal

ganglia might render these neurons more vulnerable to different cytotoxic stimuli that frequently operate in HD (oxidative stress, excitotoxicity, inflammation), suggesting that the activation of these receptors may be used as a neuroprotectant strategy in this disease (see below). In support of this hypothesis, van der Stelt and coworkers recently suggested that the malfunctioning of the cannabinoid system may be a signal to trigger an unbalance in GLU homeostasis and initiate excitotoxicity (see [89] for review), although this remains to be demonstrated.

By contrast, hypokinetic disorders, such as PD, should be characterized by an overactivity of the cannabinoid signaling system in the basal ganglia, a fact already proved in patients [19,21] and animal models of this disease [21-23,41,90-92], and compatible with the hypokinesia that characterizes this disease. Compared with HD, much less data exist on the status of CB_1 receptors in the postmortem basal ganglia of humans affected by PD. We recently found that CB_1 receptor binding and signaling were significantly increased in the basal ganglia of patients [21]. The same happens with endocannabinoid levels in the cerebrospinal fluid (CSF) of patients [19] and in different basal ganglia in animal models of PD [41,90,92]. Interestingly, these increases were reversed by treatment with levodopa [91], thus suggesting the existence of a marked imbalance between DA and endocannabinoids in the basal ganglia in PD (see [6] for a recent review). As in HD, the upregulation of CB_1 receptors might also be an early event related to the pathogenesis of PD. This is supported by data obtained from individuals affected by incidental Lewy body disease, an early and presymptomatic phase of PD [21], and also in *PARK2* knockout mice that only develop neuronal dysfunction but not neuronal death [93].

There is no information on the status of the cannabinoid signaling system in other diseases where alterations in the basal ganglia function have been documented. This is the case in tardive dyskinesia, TS, dystonia, and others. However, as will be described below, cannabinoid-related medicines may be useful as symptom-alleviating agents or as neuroprotectant substances in these disorders (see [6,36] for review). Little information exists for other disorders such as MS and AD. Both diseases are not originated by a primary degeneration of the basal ganglia, but in both cases, the primary cause of these diseases originates secondarily in the malfunctioning of the basal ganglia circuitry and the appearance of motor extrapyramidal signs. For instance, some studies have recently examined the status of brain cannabinoid transmission in animal models of multiple sclerosis [94-97] and found alterations restricted mainly to the basal ganglia structures [95-97], in particular in the case of CB_1 receptors [95,97], which is consistent with the fact that motor deterioration is one of the most prominent neurological signs in this disease. By contrast, to date there is no published data on CB_1 and CB_2 receptors or endocannabinoid levels in the postmortem brain or in biological fluids of patients with MS, although several laboratories have

presented the first evidence in abstract form (see 2006 annual meeting of ICRS). The case of AD, however, is possibly the opposite to that of MS because most of the data on the status of cannabinoid transmission in this disease come from studies in postmortem human tissue, whereas confirmatory studies in animal models are still pending. Thus, the analysis of CB_1 receptors in postmortem brain regions of patients affected by this disease revealed a significant loss in the basal ganglia but not in other regions [98]. However, it is important to mention that the authors considered that their results reflected more an influence of aging than an effect selectively associated with the pathology characteristic of AD [98]. Also using postmortem tissue from Alzheimer's patients, Benito et al. [35] reported the induction of CB_2 receptors in activated microglia that surround senile plaques. This would suggest a role for this receptor subtype in the pathogenesis of this disease and a therapeutic potential for compounds that selectively target this receptor (see [36] for details).

Alleviation of motor symptoms by cannabinoids in basal ganglia disorders

As mentioned above, the hypofunctionality of the cannabinoid system reported for HD might contribute to some extent to the hyperkinesia typical of this disorder. This would also support a therapeutic usefulness of cannabinoid agonists for alleviating hyperkinetic symptoms (for review see [6,84]), which might be particularly relevant in a disorder where the therapeutic outcome is still poor. However, the few clinical trials developed so far indicate that the administration of plant-derived cannabinoids [4], or some of their synthetic analogs [99], increase choreic movements in HD patients. It is possible that this is related to the lack of $TRPV_1$ receptor activity of the cannabinoid agonists used in those clinical trials, since recent studies carried out in our laboratory in a rat model of HD revealed that only those cannabinoid-based compounds having an additional profile as $TRPV_1$ receptor agonists were really effective in alleviating hyperkinetic signs [25,64]. This was the case for AM404, which, in addition to its ability to block the endocannabinoid transporter, also exhibited direct activity at the $TRPV_1$ receptor. This compound was able to reduce hyperkinesia and to induce recovery from GABAergic and dopaminergic deficits in a rat model of HD [25,64], while direct agonists of CB_1 receptors, such as CP 55,940, only produced very modest effects [64]. Other inhibitors of the endocannabinoid inactivation, that are not active at the $TRPV_1$ receptor, such as VDM11 or AM374, did not have any antihyperkinetic action in a rat model of HD [64], whereas UCM707, the most potent inhibitor to date, only produced modest effects [100]. Therefore, our data suggest that $TRPV_1$ receptors alone, or better in combination with CB_1 receptors, might represent novel targets through which the hyperkinetic symptoms of HD could be alleviated. Possibly, the best option might be to develop "hybrid" compounds with the dual capability of activating both $TRPV_1$ and CB_1 receptors, although the relative

contribution made by each of these targets is likely to change during the course of the disease due to a progressive loss of CB_1 receptors without any concomitant loss of $TRPV_1$ receptors (see [6,84] for a review).

Cannabinoid-based compounds might also be useful as symptom-alleviating agents in PD. In this case, the overactivity found in the cannabinoid transmission in the basal ganglia in PD supports the idea that CB_1 receptor antagonists, rather than agonists, might be the most valuable compounds for alleviating bradykinesia in PD [42,101,102] or for reducing the development of dyskinesia caused by prolonged replacement therapy with levodopa [103]. However, CB_1 receptor agonists have also been reported to have therapeutic value in PD in certain circumstances, for instance: (i) by interacting with dopaminergic agonists to improve motor impairments [104,105], (ii) by reducing tremor associated with an overactivity of the subthalamic nucleus [16,105] although the only clinical trial developed so far led to negative results [106], (iii) by delaying levodopa-induced dyskinesia [107], and (iv) by delaying/arresting the progression of nigral degeneration (see [36] and below). Because of the hypokinetic profile of cannabinoid agonists, it is unlikely that these compounds might be, however, useful for alleviating bradykinesia, which is the major symptom in most PD patients, being the blockade of CB_1 receptors a better strategy for reducing this symptom (see [6] for review) and levodopa-induced dyskinesia [92,103]. In theory, CB_1 receptor blockade would prevent the excessive inhibition of GABA uptake produced by the increased activation of CB_1 receptors in striatal projection neurons [68,69], thus allowing a faster removal of this inhibitory neurotransmitter from the synaptic cleft, which would reduce hypokinesia. Despite this evidence, the first pharmacological studies that have examined the capability of rimonabant (SR141716) to reduce hypokinesia in animal models of PD have yielded conflicting results [42,108] and no response was found in patients in the only clinical trial developed so far [109]. It is possible that the blockade of CB_1 receptors might be effective only in special circumstances, such as: (i) low doses of rimonabant, (ii) very advanced phases of the disease, or (iii) patients with a poor response to classic levodopa treatment, as recent studies have claimed [101,102]. If this were the case, it would be possible to have an antiparkinsonian agent for conditions in which classic therapy generally fails.

Cannabinoids might be also of interest for the alleviation of motor symptoms in other disorders affecting the basal ganglia directly or indirectly. For example, cannabinoids have antidystonic effects demonstrated in humans [110] and animal models [111,112]. Plant-derived cannabinoids have also been reported to reduce tics and also to improve behavioral problems in patients with TS [4,81,113-116]. Studies in laboratory animals have convincingly demonstrated that both direct and indirect cannabinoid receptor agonists are useful in alleviating motor-related symptoms in multiple sclerosis, such as spasticity, tremor, dystonia, and others (for

reviews see [83,117]). These effects seem to be preferentially mediated by CB_1 receptors [118]. In line with these data, a clinical trial, recently completed in the UK, has explored the efficacy of cannabinoid-based medicines in MS, showing some beneficial effects on specific symptoms [119,120].

Neuroprotection with cannabinoids in basal ganglia disorders

In addition to their capability to alleviate specific symptoms in basal ganglia disorders, cannabinoids might have an additional therapeutic value because of their neuroprotective properties [36]. Cannabinoids are capable of reducing excitotoxicity, calcium influx and oxidative injury (see [36,80] for review). They are also able to decrease inflammation by acting on glial processes that regulate neuronal survival, and to restore blood supply to the injured area by reducing the vasoconstriction produced by several endothelium-derived factors [36]. Through one or more of these processes, cannabinoids may provide neuroprotection in conditions of acute neurodegeneration, such as that occurring in traumatic injury or ischemic episodes [121], but also in chronic diseases, such as those initially caused by inflammatory processes, like MS [122], or those related to cognitive deterioration, such as AD [36,81], or those affecting motor control or performance, such as amyotrophic lateral sclerosis, PD and HD [36]. Here, we will concentrate on these two last neurodegenerative diseases.

As regards to HD, we and other laboratories have recently provided preclinical supporting evidence (see above sections) that cannabinoids might be used as neuroprotectant agents in patients (see [36] for review). The rationale for this hypothesis is based on the idea that the cannabinoid system experiences important changes during the onset and progression of HD [84]. Thus, as mentioned above, CB_1 receptor losses and/or dysfunction in the basal ganglia would be very early events that would take place before the appearance of major neuropathological signs and when cell death has not occurred yet or is minimal (see above sections). In addition, CB_2 receptors are induced/upregulated when cell death progresses (see [123] for review). In this context, compounds targeting selectively CB_1 and/or CB_2 receptors would be expected to be effective in attenuating striatal degeneration in HD (see above sections). Thus, Pintor et al. [124] reported that CB_1 receptor agonists were able to reduce the striatal damage in rats lesioned with quinolinic acid, a neurotoxin able to reproduce an excitotoxic episode. Therefore, one may assume that the activation of CB_1 receptors may provide neuroprotection against the excitotoxic death that occurs in HD. For these authors [124], HD patients would experience a decrease in CB_1 receptor-mediated inhibition of GLU release, thus resulting in excitotoxicity (see above sections). This can be hypothesized from the data that indicate that HD patients have low levels of both CB_1 receptors and their ligands in the striatum, so that a recovery of CB_1

receptor function might be effective in reducing striatal damage [124]. However, there are other studies that indicate that cannabinoids may also be effective against other types of neurotoxic events that also operate in HD. Thus, we observed that the administration of the agonist phytocannabinoid Δ^9-THC reduced the degeneration of striatal GABA projection neurons caused by mitochondrial complex II inhibition with 3-nitropropionic acid in rats, a HD model where neuronal death progresses through non-apoptotic mechanisms [88]. The basis for this neuroprotective effect might be the capability of Δ^9-THC to elevate cannabinoid receptor signaling in the basal ganglia, which, as mentioned in the study conducted by Pintor et al. [124], is concordant with the observation that cannabinoid CB_1 receptors and their ligands are impaired in several structures of the basal ganglia in HD patients [17,18,20] and also in animal models of this disease [24-26,88]. Therefore, it might be *a priori* postulated that the neuroprotective effect of Δ^9-THC in this HD model is also mediated by the activation of CB_1 receptors. However, Δ^9-THC is a partial agonist of CB_1 receptors, so the implication of other different mechanisms (i.e., antioxidant, vascular and/or antiinflammatory effects; see [36] for review) might also be expected. In fact, we have preliminary and unpublished data indicating that another phytocannabinoid, cannabidiol, which does not bind to cannabinoid receptors but that exhibits an important antioxidant potential, was neuroprotectant to the same extent as Δ^9-THC in this rat model of HD (Sagredo et al., unpublished results). This suggests that antioxidant properties of phytocannabinoids would possibly be the key mechanism allowing for the neuroprotective potential of these compounds against the non-apoptotic death of striatal neurons. By contrast, we have also unpublished data indicating that the activation of CB_2 receptors, presumably located on reactive microglia recruited at the lesioned striatum, might reduce the cytotoxicity exerted by these cells (i.e. generation of nitric oxide, proinflammatory cytokines, and reactive oxygen species) on striatal neurons in rats lesioned with malonate, a model of HD where neuronal death progresses by activation of the apoptotic machinery (Sagredo et al., unpublished results). Therefore, the three key mechanisms that enable cannabinoid compounds to provide neuroprotection in HD would be: (i) their capability to reduce GLU-toxicity mediated by CB_1 receptors, (ii) their cannabinoid receptor-independent antioxidant properties, and/or (iii) their activity at the CB_2 receptor to control microglial influence on neurons. Clinical trials to validate these options are going to be conducted soon.

We have also provided preclinical evidence that certain cannabinoid agonists may also be neuroprotectant in PD (see [36] for review), although the hypokinetic profile of most cannabinoid agonists may represent a disadvantage, since they might acutely enhance rather than reduce motor symptoms in this disease, as proved in several clinical trials (see [4,36] for review). We found that several plant-derived cannabinoids were able to reduce the degeneration of nigrostriatal dopaminergic

neurons generated by 6-OHDA [38]. The fact that this effect was observed for Δ^9-THC, which is able to bind both CB_1 and CB_2 receptors, but also for cannabidiol, which does not bind either cannabinoid receptor subtypes suggested that it is likely originated by the antioxidant and cannabinoid-receptor independent properties of both plant-derived cannabinoids. This has been confirmed by examining the potential of other compounds with antioxidant properties such as AM404, in the same PD model [125]. However, other additional mechanisms seem also to be active in PD. Thus, we also found that HU-210, a non-selective cannabinoid receptor agonist, increased neuronal survival against the *in vitro* neurotoxicity of 6-OHDA [38]. HU-210 is also antioxidant, but, in our study [38], it acted through increasing the trophic support exerted by glial cells on neurons, an effect that might be presumably CB_1 or CB_2 receptor-mediated. Recent evidence indicate that, it is most likely to be CB_2 receptor-mediated, since HU-308, a selective CB_2 receptor agonist, was also able to provide some degree of neuroprotection in hemiparkinsonian rats [125]. Therefore, the two key mechanisms that enable cannabinoids to provide neuroprotection in PD would be their cannabinoid receptor-independent antioxidant properties and their affinity at the CB_2 receptors. Clinical trials should also validate these two options in PD patients.

Concluding remarks and future perspectives

The studies reviewed here are all concordant with the view that the cannabinoid signaling system plays a key function in the control of movement. This view is supported by large anatomical, electrophysiological, pharmacological and biochemical evidence. We have also shown that the cannabinoid system becomes impaired in different disorders that affect directly or indirectly the basal ganglia. This provides the basis for the development of novel pharmacotherapies with compounds that selectively target specific elements of the cannabinoid system. These treatments might cover not only the alleviation of specific symptoms (i.e. hyperkinesia in HD, tremor and bradykinesia in PD) but also the delay/arrest of the disease progression due to the neuroprotectant properties described for certain cannabinoids. However, most of the studies that have examined the therapeutic potential of these compounds in motor disorders have been conducted in animal models whereas the number of clinical trials is still limited. The importance of this intercellular signaling system demands further clinical investigation, as well as the development of novel compounds with more selectivity for the different proteins (CB_1 or CB_2 receptors, $TRPV_1$ receptor, endocannabinoid transporter, degradative enzymes) that constitute the cannabinoid system, this in an attempt to minimize the frequent side effects observed when classic cannabinoids are used in patients.

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