

# Cannabinoids and Neuroprotection in Basal Ganglia Disorders

Onintza Sagredo · Moisés García-Arencibia ·  
Eva de Lago · Simone Finetti · Alessandra Decio ·  
Javier Fernández-Ruiz

Received: 19 December 2006 / Accepted: 19 March 2007 / Published online: 23 June 2007  
© Humana Press Inc. 2007

**Abstract** Cannabinoids have been proposed as clinically promising neuroprotective molecules, as they are capable to reduce excitotoxicity, calcium influx, and oxidative injury. They are also able to decrease inflammation by acting on glial processes that regulate neuronal survival and to restore blood supply to injured area by reducing the vasoconstriction produced by several endothelium-derived factors. Through one or more of these processes, cannabinoids may provide neuroprotection in different neurodegenerative disorders including Parkinson's disease and Huntington's chorea, two chronic diseases that are originated as a consequence of the degeneration of specific nuclei of basal ganglia, resulting in a deterioration of the control of movement. Both diseases have been still scarcely explored at the clinical level for a possible application of cannabinoids to delay the progressive degeneration of the basal ganglia. However, the preclinical evidence seems to be solid and promising. There are two key mechanisms involved in the neuroprotection by cannabinoids in experimental models of these two disorders: first, a cannabinoid receptor-independent mechanism aimed at producing a decrease in the oxidative injury and second, an induction/upregulation of cannabinoid CB<sub>2</sub> receptors, mainly in reactive microglia, that is capable to regulate the influence

of these glial cells on neuronal homeostasis. Considering the relevance of these preclinical data and the lack of efficient neuroprotective strategies in both disorders, we urge the development of further studies that allow that the promising expectatives generated for these molecules progress from the present preclinical evidence till a real clinical application.

**Keywords** Cannabinoids · Cannabinoid signaling system · CB<sub>1</sub> receptors · CB<sub>2</sub> receptors · Basal ganglia · Neurodegeneration · Neuroprotection · Parkinson's disease · Huntington's disease

## The Cannabinoid System and the Basal Ganglia

The cannabinoid system is a novel intercellular signaling system that plays modulatory roles in different neurobiological functions, including the control of the basal ganglia function (1). Thus, a series of anatomical, biochemical, electrophysiological, and pharmacological studies have strongly demonstrated (a) that the basal ganglia contain high levels of endogenous cannabinoids, as well as of the receptors for these ligands, including the cannabinoid CB<sub>1</sub> and CB<sub>2</sub> receptor types and also the related transient receptor potential vanilloid 1 (TRPV<sub>1</sub>) receptor (2,3); (b) that the activation or the blockade of these receptors produces important changes in the control of movement (2,4), (c) that these motor effects are caused by the modulatory action exerted by the cannabinoid system on various classic neurotransmitters, such as  $\gamma$ -aminobutyric acid, dopamine, or glutamate, that operate at the basal ganglia (2,5); and (d) that different elements of the cannabinoid signaling system, in particular the CB<sub>1</sub> and CB<sub>2</sub> receptors, experience important changes in various basal ganglia disorders (2,6). This solid evidence relating

O. Sagredo · M. García-Arencibia · E. de Lago · S. Finetti ·  
A. Decio · J. Fernández-Ruiz (✉)  
Departamento de Bioquímica y Biología Molecular,  
Facultad de Medicina III, Universidad Complutense,  
Ciudad Universitaria s/n,  
28040 Madrid, Spain  
e-mail: jjfr@med.ucm.es

O. Sagredo · M. García-Arencibia · E. de Lago ·  
J. Fernández-Ruiz  
CIBER de Enfermedades Neurodegenerativas (CIBERNED),  
Instituto de Salud Carlos III,  
28029-Madrid, Spain

endocannabinoids and their receptors to the function of the basal ganglia, both in the healthy and the pathological brain, provides support to the possibility that cannabinoid-based medicines, with selectivity for different targets of the cannabinoid signaling system (enzymes, receptors, inactivation system), might have therapeutic potential in basal ganglia disorders, in particular Parkinson's disease (PD) and Huntington's disease (HD) (2, 6). These are the two disorders related to the control of movement that have concentrated more interest in terms of a potential therapeutic application of cannabinoids. This therapy might cover the alleviation of specific motor symptoms, such as the antihyperkinetic effects demonstrated by direct or indirect agonists of cannabinoid receptors in HD (7, 8) or the improvement of the motor inhibition caused by CB<sub>1</sub> receptor antagonists in PD (9, 10). However, this type of effects on symptom relief will be addressed here only marginally (see Table 1 for a comparative summary of symptom relieving effects of cannabinoids in different neurological disorders), because the objective of this article is to concentrate on the capability of cannabinoids to delay the progression of both diseases (6, 11, 12). This is particularly important in view of the poor clinical outcome with substances that might exert a neuroprotective action in HD, PD, and also in other neurodegenerative disorders affecting other brain functions (13). In this sense, over the last decade, a considerable volume of preclinical work has accumulated solid evidence to assume that the cannabinoid system plays a role in the protection against acute or chronic brain damage (6, 11, 12; see Table 1 for a comparative summary of neuroprotective effects of cannabinoids in different neurological disorders), which would be a part of an important dual function exerted by cannabinoids and their receptors on the control of the cell decision of death/survival (14). The pharmacological exploitation of this

function would represent a clinically promising goal for next years with a likely application for the treatment of neurodegenerative disorders and brain tumors (15). The present article will review the basic knowledge on the neuroprotective potential of cannabinoids in these two neurodegenerative disorders, HD and PD, trying to establish the future lines of research for the clinical application of this potential in patients affected by this type of basal ganglia disorders.

### Activation of the Cannabinoid System in Response to Neuronal Damage

There is solid evidence indicating that the cannabinoid system, in concordance with its suggested role in the protection of the brain, becomes activated in response to different stimuli that may damage nerve cells. This has been documented in different experimental paradigms of neurodegeneration, although with variable results, depending on the age, animal species, type and severity of injury, and mechanism(s) activated for cell death (reviewed in 6, 11, 16, 17). For instance, a repeated observation is that neuronal damage of different etiology is frequently accompanied by an increase in the production of endocannabinoids in the brain (11, 16). This has been observed in response to excitotoxic conditions in the rat (18, 19) and the mouse (20). In both cases, the levels of anandamide (arachidonylethanolamide [AEA]) increased, but this did not occur with the levels of 2-arachidonoylglycerol (2-AG) (18–20). Similar results were reported by Gubellini et al. (21) in a rat model of PD. However, other authors found that 2-AG is the endocannabinoid that is massively produced in the mouse brain after closed head injury (22). This endogenous response has been also described in humans where elevated

**Table 1** Summary of therapeutic benefits of the management of the cannabinoid system in neurodegenerative disorders

Neurological disorder	Symptom relief	Disease progression
Huntington's disease	TRPV1 agonists reduce hyperkinesia CB1 agonists produce only modest effects	CB2 agonists reduce inflammatory events Cannabidiol and $\Delta^9$ -THC reduce oxidative stress
Parkinson's disease	CB1 antagonists reduce bradykinesia CB1 agonists reduce tremor	Antioxidant cannabinoids are neuroprotective
Tourette's syndrome Dystonia	Plant-derived cannabinoids and analogs reduce tics Classic and non-classic cannabinoid agonists have antidystonic effects	
Dyskinesia	CB1 agonists or antagonists delay L-dopa-induced dyskinesia	
Amyotrophic lateral sclerosis	Unknown	$\Delta^9$ -THC delays neurological decline CB2 agonists are also effective
Alzheimer's disease	CB1 antagonists reduce memory deficits CB1 agonists increase appetite	CB1 and CB2 agonists reduce $\beta$ -amyloid toxicity
Multiple sclerosis	Direct or indirect cannabinoid agonists reduce spasticity	CB1 agonists reduce spinal inflammation and degeneration



levels of AEA and other fatty acid amides have been recorded around the site of damage in a microdialysis study performed on a single stroke patient (23). Similar findings were obtained in studies with inhibitors of the endocannabinoid uptake. For instance, the compound UCM707 increased protection against kainate-induced seizures in mice, where AEA levels were reported to be elevated (20). By contrast, other authors (24) found a protection after exogenous administration of AEA in a neonatal model of secondary excitotoxicity, but they did not record any increases in AEA or 2-AG levels and, concomitantly, they did not find any effect of another uptake inhibitor, VDM11, on lesion volume.

As happens with endocannabinoid ligands, their receptors are also induced/upregulated in brain cells in response to injury and/or inflammation (6, 19, 25, 26). Thus, CB<sub>1</sub> receptors were upregulated in response to experimental stroke generated in adult animals (25) or in response to excitotoxic stimuli in neonatal rats (19). As regards to CB<sub>2</sub> receptors, a receptor type that is relatively scarce in the brain in healthy conditions (see below), recent reports have shown induction of this receptor type in Alzheimer's disease and HD (6, 15, 26). This occurs mainly in reactive microglia that surround senile plaques in human Alzheimer's disease brain samples (26) or that migrate at lesioned sites in rats with striatal atrophy generated by mitochondrial complex II inhibition, an experimental model of HD (6, 15). As will be detailed below, these observations suggest that CB<sub>2</sub> receptors might play a role in reducing cytotoxic influence exerted by reactive microglia on neurons.

### **Mechanisms Involved in Neuroprotection by Cannabinoids**

The molecular mechanisms underlying the neuroprotectant properties of cannabinoids are quite diverse and, frequently, complementary. They include some events not mediated by cannabinoid receptors, such as the blockade of *N*-methyl-D-aspartate (NMDA) receptors or the reduction of oxidative injury exerted by some specific groups of cannabinoids with particular chemical characteristics (6). By contrast, other neuroprotective actions of cannabinoids are definitively mediated by either CB<sub>1</sub> (6) or CB<sub>2</sub> receptors (15) and even through the activation of TRPV<sub>1</sub> receptors (27). These receptor-mediated events would be involved in the inhibition of glutamate release, reduction of calcium influx, improvement of blood supply to the injured brain, and/or decrease of local inflammatory events exerted by cannabinoids (for review, see 6, 11, 12].

*Events Mediated Preferentially by CB<sub>1</sub> Receptors* Cannabinoids are certainly antigitamatergic substances, as they

are able to reduce excitotoxicity (6, 11, 12, 17). This has been demonstrated both *in vitro* (28, 29) and *in vivo* (30). This antigitamatergic effect seems to be dual. On one side, most of cannabinoid agonists reduce glutamate release, an effect likely exerted by the activation of CB<sub>1</sub> receptors located presynaptically on glutamatergic terminals, as it is sensitive to the blockade of these receptors with rimonabant, a selective CB<sub>1</sub> receptor antagonist (see 6, 17, 31 for reviews). In fact, rimonabant by itself increased lesion volume in a rat model of HD (32), although there are some studies reporting no effects (24) or, even, improvement (33). On the other side, some specific cannabinoids, such as dexamabinol (34) and also anandamide, although only in special circumstances (35), are also able to directly act on NMDA glutamatergic receptors. This is an action not mediated by CB<sub>1</sub> receptors, but it may be an alternative way to reduce, at the postsynaptic level, the influence of high glutamate concentrations typical of excitotoxic situations. Concomitantly to their antigitamatergic effects, cannabinoid agonists, acting again through the activation of CB<sub>1</sub> receptors, may also close voltage-sensitive calcium channels (36) that are activated in response to the depolarization associated with NMDA receptor overactivation. This results in a reduction of the overall intracellular calcium levels avoiding the negative influence of numerous destructive pathways that are overactivated by an excessive elevation of intracellular levels of this ion (6, 37). Finally, cannabinoid agonists may also reduce neuronal degeneration by improving blood supply to the injured brain, an effect particularly interesting in the case of ischemic episodes. This vasodilation effect would be exerted by inhibiting the production of several endothelium-derived mediators, such as endothelin-1 or nitric oxide (38–40), that are associated with the vasoconstriction typical of ischemic episodes. This would be certainly a CB<sub>1</sub> receptor-mediated event (38–40), although the involvement of other additional novel types of cannabinoid receptors that have been recently proposed for cardiovascular effects of cannabinoids cannot be ruled out (41).

*Events Mediated Preferentially by CB<sub>2</sub> Receptors* Cannabinoid agonists also behave as anti-inflammatory molecules able to reduce local inflammatory events that frequently accompany both acute and chronic neurodegenerative disorders and that have been related to the activation of glial cells at the lesioned sites (i.e., recruitment of reactive microglia and astrogliosis). Cannabinoids possibly act through the control of specific functions exerted by glial cells that are aimed at regulating the neuronal homeostasis (see 6 for review). For instance, cannabinoids would reduce the release of cytotoxic factors, such as inflammatory cytokines (tumor necrosis factor- $\alpha$  [TNF- $\alpha$ ], interleukin-1 $\beta$  [IL-1 $\beta$ ]), reactive oxygen species, or nitric oxide, by



reactive microglia (15,42,43), an effect preferentially mediated by the activation of CB<sub>2</sub> receptors. In addition, they might also increase the production of pro-survival molecules, such as several neurotrophic factors (transforming growth factor- $\beta$ ) or anti-inflammatory cytokines (IL-10, IL-1ra) (44,45), or improve the trophic support exerted by astrocytes on neurons (46), an effect possibly mediated by the activation of CB<sub>1</sub> receptors, although a role for CB<sub>2</sub> receptors cannot be excluded (15).

*Events Mediated by Cannabinoid Receptor-Independent Mechanisms* Certain cannabinoids provide neuroprotection through an effect that restores the normal balance between oxidative events and antioxidant endogenous mechanisms that is frequently disrupted (by an excessive production of reactive oxygen species, by a deficiency in antioxidant endogenous mechanisms, or by both causes) during neurodegenerative conditions (47). This capability is available for compounds such as the plant-derived cannabinoids cannabidiol (CBD),  $\Delta^9$ -tetrahydrocannabinol ( $\Delta^9$ -THC), and cannabinol or their analogs nabilone, levonantradol, and dexanabinol. It appears well demonstrated that this would be a cannabinoid receptor-independent effect (47–50) that is sustained by an action of these compounds as scavengers of reactive oxygen species allowed by the particular chemical structure of antioxidant cannabinoids, which contain phenolic groups (47). However, additional mechanisms involving a direct improvement of endogenous antioxidant enzymes have been also proposed, although this would require further demonstration (51,52).

### **Cannabinoids and Neuroprotection in Huntington's Disease**

HD is an inherited neurodegenerative disorder caused by a mutation in the gene encoding the protein huntingtin, which consists of a CAG triplet repeat expansion translated into an abnormal polyglutamine tract in the amino-terminal portion of this protein (for review see 53). The disease presents in midlife and is ultimately fatal. The major neuropathological consequence of this mutation is the preferential and progressive degeneration of striatal neurons that project to the globus pallidus and the substantia nigra [these neurons contain CB<sub>1</sub> receptors (54)]. This results in a biphasic pattern of motor abnormalities that evolves from an early hyperkinetic phase (choreic movements) to a late akinetic and more disabling phase (see 53 for review). The disease also presents cognitive dysfunction and psychiatric symptoms at advanced phases. Although it has been demonstrated that HD is a disease of genetic origin and the mutated gene has been already identified, mechanisms underlying

striatal degeneration are still unknown, and consequently, the therapeutic outcome for HD patients is still too poor. In this context, cannabinoid agonists might provide therapeutic benefits in this disorder (2,6,15,55), and they can reasonably do it in two complementary aspects: (a) acting as antihyperkinetic substances and (b) serving as neuroprotective molecules.

Cannabinoid agonists can develop an antihyperkinetic effect through acutely recovering the neurochemical deficits typical of first grades of this disorder (2,7,8,55). This potential, however, seems to be restricted to certain cannabinoids that combine the capability to enhance the cannabinoid signaling and also to activate TRPV<sub>1</sub> receptors (2,8). As mentioned above, we will not address this capability here to concentrate in the second therapeutic benefit that cannabinoids can provide in HD, that is, their capability to delay the progress of striatal degeneration (4,6,12,55). This capability has been tested only in animal models of this disease (32,56,57), and although the matter is still far to be completely elucidated, some results have provided promising expectatives for a clinical application in patients. The rationale for this type of studies is based on the idea that the loss and/or dysfunction of CB<sub>1</sub> receptors, which have been reported that occur in the basal ganglia in HD patients and animal models (7,56,58–60), is a very early event that takes place before the appearance of major neuropathological signs and when the cell death does not exist or is minimal. This hypothesis has been also suggested by other authors (17,57), who considering the data obtained in HD and also in other pathologies, proposed that the malfunctioning of the cannabinoid system (i.e., AEA or 2-AG synthesis is inhibited, CB<sub>1</sub> receptors are inactive or their expression is reduced) might be a signal to trigger an unbalance in glutamate homeostasis and initiate excitotoxicity. Although this hypothesis remains to be demonstrated, there are some data supporting its validity. Thus, analyses done in human brains at very early grades of HD pathogenesis or studies conducted in different models of transgenic mice that express mutated forms of huntingtin, which develop neuronal malfunctioning rather than neuronal degeneration, proved the early occurrence of downregulatory responses of CB<sub>1</sub> receptors (58–60). In addition, rats with striatal atrophy generated by the administration of mitochondrial toxins exhibited profound changes in G-protein activation by CB<sub>1</sub> receptor agonists, several days before overt striatal degeneration and appearance of severe motor symptoms, and in absence of significant modifications in binding sites and messenger RNA levels for this receptor (56). Therefore, one may assume that defects in CB<sub>1</sub> receptor signaling could render neurons more vulnerable to the degenerative process associated with HD, a finding that would be in favor of the hypothesis that early functional changes in CB<sub>1</sub>



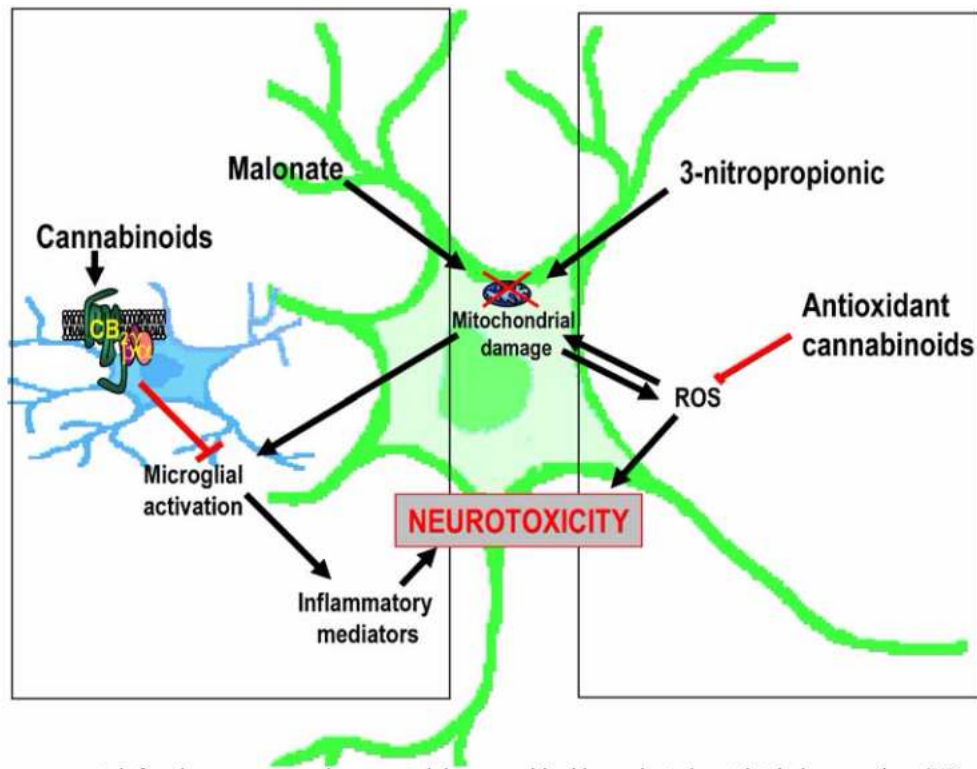
receptors might be involved in the pathogenesis of HD. If this were the case, one may also consider that these defects might play an instrumental role, so that the early stimulation of these receptors might reduce/delay the progression of striatal degeneration. On the other hand and in contrast to the progressive decrease experienced by CB<sub>1</sub> receptors during the course of this disease, CB<sub>2</sub> receptors, whose presence in the healthy striatum is relatively modest, are however markedly upregulated when striatal degeneration progresses (see 15 for review). In this context, it would be expected that compounds targeting selectively this receptor type may be also effective to attenuate striatal degeneration in this disorder.

Therefore, there is consistent molecular evidence to support the hypothesis that cannabinoids, acting through CB<sub>1</sub> or CB<sub>2</sub> receptors, may delay the progression of striatal damage in HD, although the number of studies that have addressed this issue may be still too short. There are two recent *in vitro* studies conducted by two different groups (61,62), who screened a library of 1,040 compounds for their capability to protect cultured PC12 cells from death caused by an expanded polyglutamine form of huntingtin exon 1. Whereas Aiken et al. (61) reported that various cannabinoids showed reproducible protection in this assay, Wang et al. (62) did not find any positive result using the same methodological approach and the same cannabinoid compounds. The issue has been also recently evaluated *in vivo*, using different animal models that replicate different aspects of the cytotoxic events that cooperatively contribute to HD pathogenesis [i.e., mitochondrial dysfunction, excitotoxicity, inflammation, and oxidative stress (53)]. Thus, Pintor et al. (57) reported that the activation of CB<sub>1</sub> receptors reduced the striatal damage generated in rats by the administration of the excitotoxin quinolinic acid. This might indicate that CB<sub>1</sub> receptor agonists may behave as neuroprotectant against the excitotoxic death that occurs in HD. Cannabinoids, however, may also be effective against other types of neurotoxic events that also operate in HD. For instance, we have found that  $\Delta^9$ -THC protects striatal neurons against the *in vivo* toxicity of 3-nitropropionic acid (56), a mitochondrial toxin that replicates the complex II deficiency characteristic of HD patients (63). Striatal injury in this animal model progresses by mechanisms that mainly involve non-apoptotic pathways, as neuronal death in this model is caspase 3 independent and produced via the Ca<sup>++</sup>-regulated protein calpain (64,65). This neuroprotective effect of  $\Delta^9$ -THC might be exerted through different mechanisms, as this plant-derived cannabinoid may activate CB<sub>1</sub> or CB<sub>2</sub> receptors, as well as it can act through cannabinoid receptor-independent mechanisms (i.e., inhibition of cyclooxygenase-2, antioxidant effect). Recently, we have seen that the neuroprotective effect of  $\Delta^9$ -THC in rats lesioned with 3-nitropropionic acid (56) was also produced,

to a similar extent, by CBD but not by selective agonists of CB<sub>1</sub>, CB<sub>2</sub>, or TRPV<sub>1</sub> receptors (52). CBD has a particular pharmacological profile in comparison with  $\Delta^9$ -THC, as CBD does not bind CB<sub>1</sub> or CB<sub>2</sub> receptors but exhibits an antioxidant potential equivalent to that of  $\Delta^9$ -THC. This suggests that both cannabinoids would protect striatal neurons from 3-nitropropionic-induced non-apoptotic death through an antioxidant action mediated by cannabinoid receptor-independent mechanisms (52,56), possibly by acting as scavengers of free radicals, as has been outlined in Fig. 1.

In contrast with the data published in rats lesioned with quinolinic acid (57) or 3-nitropropionic acid (52,56) that propose a role for CB<sub>1</sub> receptors or for cannabinoid receptor-independent mechanisms, respectively, there is another rat model of HD where the neuroprotective effects of cannabinoids have been reported to be mainly mediated by CB<sub>2</sub> receptors (see 15 for review). This is the model of striatal injury 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 [it activates NMDA receptors and caspase-3 (66)]. Using this model, we found neuroprotection when rats were administered with a selective CB<sub>2</sub> receptor agonist, but not with a selective CB<sub>1</sub> receptor agonist or with an antioxidant cannabinoid with no affinity for both receptor types as CBD (15). The involvement of CB<sub>2</sub> receptors in this effect was also confirmed by additional experiments with SR144528, a selective CB<sub>2</sub> receptor antagonist (15). An important issue derived from these pharmacological observations is that CB<sub>2</sub> receptors are induced/upregulated in the lesioned striatum in response to neuronal damage. As this receptor type is usually absent or scarcely concentrated in the brain in healthy conditions, this phenomenon should be interpreted as a part of an endogenous response against the neuronal death caused by the inhibition of the mitochondrial function with malonate (see above and 6 for review). On the other hand, immunohistochemical studies have confirmed that induction/upregulation of CB<sub>2</sub> receptors occurs in glial cells, mainly in reactive microglia that are recruited and migrate to the striatum in response to the lesion (15). In reactive microglia, CB<sub>2</sub> receptors might play a role in the control of the influence that these cells exert on neurons, for instance, reducing the production of cytotoxic factors, such as nitric oxide, reactive oxygen species, and in particular, pro-inflammatory cytokines (see Fig. 1 for an outline), which are released by microglial cells and that deteriorate neuronal homeostasis (15,42,43). Two specific observations support this hypothesis. First, it has been largely demonstrated that activation of glial cells (astrocytes, oligodendroglia, or microglia) occurs in HD (67,68) as in other neurodegenerative pathologies. Second, we have





**Fig. 1** Mechanisms suggested for the neuroprotection exerted by cannabinoids against the striatal degeneration (HD models) caused by mitochondrial complex II inhibition with malonate (neuronal death is apoptotic) or 3-nitropropionic acid (neuronal death is non-apoptotic)

preliminary and still unpublished evidence indicating that the activation of CB<sub>2</sub> receptors reduces the increase in the production of TNF- $\alpha$ , a pro-inflammatory mediator, generated by the intrastriatal administration of malonate in rats (unpublished results).

Therefore, there are three key mechanisms that enable cannabinoids to provide neuroprotection in HD: (a) their capability to normalize glutamate homeostasis, an effect mediated by CB<sub>1</sub> receptors, which would allow to reduce excitotoxic events that occur in this pathology; (b) the antioxidant potential of certain cannabinoids, which would be exerted through cannabinoid receptor-independent mechanisms and which would allow to reduce the oxidative injury that also takes place in HD; and (c) their activity at the CB<sub>2</sub> receptors to control the microglial influence on neuronal survival, thus reducing the local inflammatory events that are associated with the striatal degeneration. The availability of animal models of HD that reproduce independently these phenomena has allowed to identify the type of cannabinoid mechanism that is more effective against each of these cytotoxic processes. However, as mentioned above, these cytotoxic processes occur in a cooperative manner during the pathogenesis of HD in humans (53). This is an important insight to be considered at the time to decide about the best type of cannabinoid compound that might be subjected to clinical evaluation as a novel neuroprotective agent for HD patients. The use of

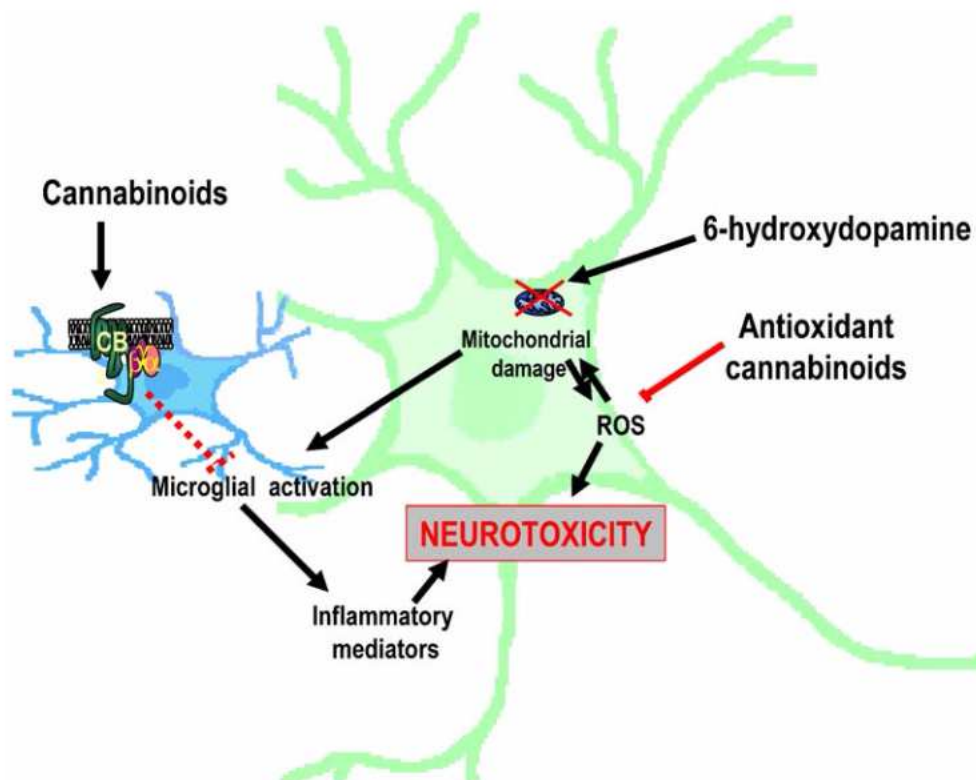
nonselective or hybrid compounds with indistinct activity at these different targets might be perhaps the best solution.

### Cannabinoids and Neuroprotection in Parkinson's Disease

The major clinical neuropathology in PD includes bradykinesia (slowness of movement), rigidity and tremor caused by the progressive degeneration of dopaminergic neurons of the substantia nigra pars compacta that leads to a severe dopaminergic denervation of the striatum (see a recent review in 69). Although the etiology of PD is presently unknown, major pathogenic processes, which trigger the progressive loss of nigral dopaminergic neurons, are oxidative stress, mitochondrial dysfunction, and inflammatory stimuli (70–72). Dopaminergic-replacement therapy with L-dopa represents a useful remedy to release rigidity and bradykinesia in PD patients (73), at least in the early and middle phases of this disease. However, PD patients, as what happens with HD, do not have an efficient therapy to arrest/delay the progression of nigral degeneration. Cannabinoid agonists have been proposed as potentially useful neuroprotective substances in PD, although the issue has been explored only very recently. In part, this is a consequence of the hypokinetic profile of most of canna-

binoid agonists that represents a disadvantage for this disease. Thus, despite their neuroprotectant activity in long-term treatments, cannabinoid agonists can acutely enhance rather than reduce motor disability, as a few clinical data have already revealed (2, 74, 75). However, in recent preclinical studies carried out with  $\Delta^9$ -THC, we have obtained solid evidence that this plant-derived cannabinoid may reverse the impairment of dopaminergic transmission in the basal ganglia of rats with hemiparkinsonism caused by the unilateral application of 6-hydroxydopamine (76). These effects did not occur in the contralateral structures, thus indicating that the effect of  $\Delta^9$ -THC was produced by reducing dopaminergic cell death in the lesioned side rather than producing upregulatory effects in surviving neurons (76). The analysis of tyrosine hydroxylase, the rate-limiting enzyme in dopamine synthesis, in the substantia nigra of these animals corroborated this finding (76). It is important to point out that equivalent neuroprotective effects were exerted by  $\Delta^9$ -THC and also by CBD (76). As mentioned above, CBD has negligible affinity for CB<sub>1</sub> and CB<sub>2</sub> receptors in contrast with the activity described for  $\Delta^9$ -THC on these two receptor types. This suggests that the neuroprotective effects exerted by both plant-derived cannabinoids against 6-hydroxydopamine toxicity, are cannabinoid receptor independent and imply the antioxidant properties of both cannabinoids (76), in concordance with data obtained using the same two compounds but in other

in vitro or in vivo models of neurodegeneration (49, 52). This has been confirmed in recent in vivo studies conducted in our laboratory (51), in which we evaluated the potential of different cannabinoid-based molecules with selectivity for different elements of the cannabinoid system (CB<sub>1</sub> receptor, CB<sub>2</sub> receptor, endocannabinoid transporter). Our results again supported that only compounds with antioxidant properties, such as the anandamide analogue AM404, were able to reduce the toxicity of 6-hydroxydopamine for nigral neurons (51). By contrast, selective agonists for the CB<sub>1</sub> or the CB<sub>2</sub> receptor failed to protect these neurons, whereas the same happened with inhibitors of the endocannabinoid transporter (51). This last observation was important to determine that the neuroprotective potential of AM404 in this disease is caused by its antioxidant properties derived from the presence of a phenolic group in its chemical structure (51). AM404 also behaves as inhibitor of the endocannabinoid transporter, but other inhibitors devoid of antioxidant potential failed to reproduce the neuroprotective effects of AM404 (51). Therefore, one may assume that the antioxidant and cannabinoid receptor-independent properties of certain cannabinoids are the key element to determine the neuroprotective potential of these compounds in PD, as has been outlined in Fig. 2. This fact is of special relevance in a degenerative disorder in which oxidative stress is a major hallmark in the pathogenesis (69).



**Fig. 2** Mechanisms suggested for the neuroprotection exerted by cannabinoids against the nigral degeneration (PD model) caused by 6-hydroxydopamine



Despite the above finding and as happens in HD, it is possible that some additional mechanisms might also contribute to the neuroprotective effects of cannabinoids in PD. One of these mechanisms might be related to the influence of glial cells on neuronal survival. In this sense, although the cause of dopaminergic cell death in PD is still unknown, it has been postulated that alterations in glial cell function (i.e., microglial activation) might play an important role in the initiation and/or early progression of the neurodegenerative process (77). This might be crucial in a region like the substantia nigra, which is particularly enriched in microglia and other glial cells (78). In fact, several glial-derived cytotoxic factors, such as TNF- $\alpha$ , IL-1 $\beta$ , nitric oxide, and others, have been reported to be elevated in the substantia nigra and the caudate–putamen of PD patients (79). With this idea in mind, we conducted some *in vitro* studies addressed to evaluate the importance of glial metabolism in the effects of cannabinoid agonists on the toxicity of 6-hydroxydopamine for neurons. We found a marked increase in neuronal survival when cells were incubated with conditioned media generated by exposing glial cells to the nonselective cannabinoid agonist HU-210, which contrasts with the poor improvement of neuronal survival caused by the direct exposure of neuronal cells to HU-210 (76). This cannabinoid, HU-210, is also antioxidant, but in our study (76), it acted through increasing the trophic support exerted by glial cells on neurons. Because of the role suggested for CB<sub>2</sub> receptors in glial-mediated effects of cannabinoids (43), it is possible that this receptor is involved, as an additional target, in a part of the neuroprotective effects observed for cannabinoids in PD, as happens in HD (6). However, our *in vivo* data only reflect a very modest contribution of CB<sub>2</sub> receptors to the neuroprotective effect of cannabinoids observed in hemiparkinsonian rats (51).

Therefore, the different studies conducted so far to elucidate the molecular and cellular substrates involved in the neuroprotection by cannabinoids in PD strongly indicate that the key feature here is the antioxidant potential of certain cannabinoids (51, 76). This would be a cannabinoid receptor-independent mechanism, although there is some evidence that suggest that CB<sub>2</sub> receptors may also play a role, possibly very modest, in the control of glial influence on neuronal homeostasis (42, 43). Together with the development of further preclinical studies aimed at identifying the true potential of CB<sub>2</sub> receptors in PD, we also urge the development of clinical trials that should validate the usefulness of these two capabilities of cannabinoids in PD patients.

### Concluding Remarks and Futures Perspectives

Among a variety of pharmacological effects, cannabinoids have been proposed as potentially useful and clinically

promising neuroprotective molecules. Along this article, we have reviewed the cellular and molecular mechanisms that might be involved in the neuroprotective effects of cannabinoids described in HD and PD, paying emphasis in their potential (a) to reduce excitotoxicity exerted either by inhibiting glutamate release or, in some specific cases, by blocking glutamatergic receptors, (b) to block NMDA receptor-induced calcium influx exerted directly, as a consequence of the antagonism of these receptors, or indirectly, through the inhibition of selective channels for this ion, (c) to decrease oxidative injury by acting as scavengers of reactive oxygen species, a property independent of cannabinoid receptor and restricted to specific classic cannabinoids, and (d) to reduce inflammation by acting predominantly through the activation of CB<sub>2</sub> receptors on the glial processes that regulate neuronal survival. Through one or more of these processes, cannabinoids may be used to delay the progression of neurodegeneration in PD, HD, and also in other neurodegenerative diseases. However, most of the studies that have examined the therapeutic potential of cannabinoids in these disorders have been conducted in animal or cellular models, whereas the number of clinical trials is still very limited. Therefore, it should be expected that the number of studies examining this potential increases in coming years, as soon as the promising expectatives generated for these molecules progress from the present preclinical evidence to a true clinical application.

**Acknowledgments** The experimental work carried out by our group and that has been mentioned in this review article, has been supported during the last years by the MEC (grants SAF2003-08269 and SAF2006-11333), MSC (CIBERNED, CB06/05/0089) and CAM (S-SAL-0261/2006). The authors are indebted to all colleagues who contributed in this experimental work.

### References

1. Marsicano G, Lutz B (2006) Neuromodulatory functions of the endocannabinoid system *J Endocrinol Invest* 29:27–46
2. Fernández-Ruiz J, González S (2005) Cannabinoid control of motor function at the basal ganglia. *Handb Exp Pharmacol* 168:479–507
3. van der Stelt M, Di Marzo V (2003) The endocannabinoid system in the basal ganglia and in the mesolimbic reward system: implications for neurological and psychiatric disorders. *Eur J Pharmacol* 480:133–150
4. Romero J, Lastres-Becker I, de Miguel R, Berrendero F, Ramos JA, Fernández-Ruiz JJ (2002) The endogenous cannabinoid system and the basal ganglia: biochemical, pharmacological and therapeutic aspects. *Pharmacol Ther* 95:137–152
5. Giuffrida A, Piomelli D (2000) The endocannabinoid system: a physiological perspective on its role in psychomotor control. *Chem Phys Lipids* 108:151–158
6. Fernández-Ruiz J, González S, Romero J, Ramos JA (2005) Cannabinoids in neurodegeneration and neuroprotection. In:



- Mechoulam R (ed) *Cannabinoids as therapeutics* (MDT). Birkhäuser, Switzerland, pp79–109
7. Lastres-Becker I, Hansen HH, Berrendero F, de Miguel R, Pérez-Rosado A, Manzanares J, Ramos JA, Fernández-Ruiz J (2002) Loss of cannabinoid CB<sub>1</sub> receptors and alleviation of motor hyperactivity and neurochemical deficits by endocannabinoid uptake inhibition in a rat model of Huntington's disease. *Synapse* 44:23–35
  8. Lastres-Becker I, de Miguel R, De Petrocellis L, Makriyannis A, Di Marzo V, Fernández-Ruiz J (2003) Compounds acting at the endocannabinoid and/or endovanilloid systems reduce hyperkinesia in a rat model of Huntington's disease. *J Neurochem* 84:1097–1109
  9. Fernández-Espejo E, Caraballo I, Rodríguez de Fonseca F, El Banoua F, Ferrer B, Flores JA, Galán-Rodríguez B (2005) Cannabinoid CB<sub>1</sub> antagonists possess antiparkinsonian efficacy only in rats with very severe nigral lesion in experimental parkinsonism. *Neurobiol Dis* 18:591–601
  10. González S, Scorticati C, García-Arencibia M, de Miguel R, Ramos JA, Fernández-Ruiz J (2006) Effects of rimonabant, a selective cannabinoid CB<sub>1</sub> receptor antagonist, in a rat model of Parkinson's disease. *Brain Res* 1073–1074:209–219
  11. Mechoulam R, Panikashvili A, Shohami E (2002) Cannabinoids and brain injury: therapeutic implications. *Trends Mol Med* 8:58–61
  12. Grundy RI (2002) The therapeutic potential of the cannabinoids in neuroprotection. *Expert Opin Investig Drugs* 11:1–10
  13. Akwa Y, Allain H, Bentue-Ferrer D, Berr C, Bordet R, Geerts H, Nicoullon A, Onteniente B, Vercelletto M (2005) Neuroprotection and neurodegenerative diseases: from biology to clinical practice. *Alzheimer Dis Assoc Disord* 19:226–239
  14. Guzmán M, Sánchez C, Galve-Roperh I (2001) Control of the cell survival/death decision by cannabinoids. *J Mol Med* 78:613–625
  15. Fernández-Ruiz J, Romero J, Velasco G, Tolón RM, Ramos JA, Guzmán M (2007) Cannabinoid CB<sub>2</sub> receptor: a new target for the control of neural cell survival? *Trends Pharmacol Sci* 28:39–45
  16. Hansen HS, Moesgaard B, Petersen G, Hansen HH (2002) Putative neuroprotective actions of *N*-acyl-ethanolamines. *Pharmacol Ther* 95:119–126
  17. van der Stelt M, Veldhuis WB, Maccarrone M, Bar PR, Nicolay K, Veldink GA, Di Marzo V, Vliegenthart JF (2002) Acute neuronal injury, excitotoxicity, and the endocannabinoid system. *Mol Neurobiol* 26:317–346
  18. Hansen HS, Moesgaard B, Hansen HH, Schousboe A, Petersen G (1999) Formation of *N*-acyl-phosphatidylethanolamine and *N*-acylethanolamine (including anandamide) during glutamate-induced neurotoxicity. *Lipids* 34:S327–S330
  19. Hansen HH, Schmid PC, Bittigau P, Lastres-Becker I, Berrendero F, Manzanares J, Ikonomidou C, Schmid HH, Fernández-Ruiz JJ, Hansen HS (2001) Anandamide, but not 2-arachidonoylglycerol, accumulates during in vivo neurodegeneration. *J Neurochem* 78:1415–1427
  20. Marsicano G, Goodenough S, Monory K, Hermann H, Eder M, Cannich A, Azad SC, Cascio MG, Gutierrez SO, van der Stelt M, Lopez-Rodríguez ML, Casanova E, Schutz G, Zieglansberger W, Di Marzo V, Behl C, Lutz B (2003) CB<sub>1</sub> cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 302:84–88
  21. Gubellini P, Picconi B, Bari M, Battista N, Calabresi P, Centonze D, Bernardi G, Finazzi-Agrò A, Maccarrone M (2002) Experimental parkinsonism alters endocannabinoid degradation: implications for striatal glutamatergic transmission. *J Neurosci* 22:6900–6907
  22. Panikashvili D, Simeonidou C, Ben-Shabat S, Hanus L, Breuer A, Mechoulam R, Shohami E (2001) An endogenous cannabinoid (2-AG) is neuroprotective after brain injury. *Nature* 413:527–531
  23. Schabitz WR, Giuffrida A, Berger C, Aschoff A, Schwaninger M, Schwab S, Piomelli D (2002) Release of fatty acid amides in a patient with hemispheric stroke: a microdialysis study. *Stroke* 33:2112–2124
  24. van der Stelt M, Veldhuis WB, van Haften GW, Fezza F, Bisogno T, Bär PR, Veldink GA, Vliegenthart JF, Di Marzo V, Nicolay K (2001) Exogenous anandamide protects rat brain against acute neuronal injury in vivo. *J Neurosci* 21:8765–8771
  25. Jin KL, Mao XO, Goldsmith PC, Greenberg DA (2000) CB<sub>1</sub> cannabinoid receptor induction in experimental stroke. *Ann Neurol* 48:257–261
  26. Benito C, Nuñez E, Tolon RM, Carrier EJ, Rabano A, Hillard CJ, Romero J (2003) Cannabinoid CB<sub>2</sub> receptors and fatty acid amide hydrolase are selectively overexpressed in neuritic plaque-associated glia in Alzheimer's disease brains. *J Neurosci* 23:11136–11141
  27. Veldhuis WB, van der Stelt M, Wadman MW, van Zadelhoff G, Maccarrone M, Fezza F, Veldink GA, Vliegenthart JF, Bar PR, Nicolay K, Di Marzo V (2003) Neuroprotection by the endogenous cannabinoid anandamide and arvanil against in vivo excitotoxicity in the rat: role of vanilloid receptors and lipoxygenases. *J Neurosci* 23:4127–4133
  28. Shen M, Thayer SA (1998) Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. *Mol Pharmacol* 54:459–462
  29. Abood ME, Rizvi G, Sallapudi N, McAllister SD (2001) Activation of the CB<sub>1</sub> cannabinoid receptor protects cultured mouse spinal neurons against excitotoxicity. *Neurosci Lett* 309:197–201
  30. Nagayama T, Sinor AD, Simon RP, Chen J, Graham SH, Jin KL, Greenberg DA (1999) Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. *J Neurosci* 19:2987–2995
  31. Schlicker E, Kathmann M (2001) Modulation of transmitter release via presynaptic cannabinoid receptors. *Trends Pharmacol Sci* 22:565–572
  32. Lastres-Becker I, Bizat N, Boyer F, Hantraye P, Brouillet E, Fernández-Ruiz J (2003) Effects of cannabinoids in the rat model of Huntington's disease generated by an intrastriatal injection of malonate. *Neuroreport* 14:813–816
  33. Hansen HH, Azcoitia I, Pons S, Romero J, Garcia-Segura LM, Ramos JA, Hansen HS, Fernández-Ruiz J (2002) Blockade of cannabinoid CB<sub>1</sub> receptor function protects against in vivo disseminating brain damage following NMDA-induced excitotoxicity. *J Neurochem* 82:154–158
  34. Shohami E, Mechoulam R (2000) A non-psychoactive cannabinoid with neuroprotective properties. *Drug Dev Res* 50:211–215
  35. Hampson AJ, Bornheim LM, Scanziani M, Yost CS, Gray AT, Hansen BM, Leonoudakis DJ, Bickler PE (1998) Dual effects of anandamide on NMDA receptor-mediated responses and neurotransmission. *J Neurochem* 70:671–676
  36. Demuth DG, Molleman A (2006) Cannabinoid signalling. *Life Sci* 78:549–563
  37. Fowler CJ (2003) Plant-derived, synthetic and endogenous cannabinoids as neuroprotective agents. Non-psychoactive cannabinoids, 'entourage' compounds and inhibitors of *N*-acyl ethanolamine breakdown as therapeutic strategies to avoid psychotropic effects. *Brain Res Rev* 41:26–43
  38. Wagner JA, Varga K, Kunos G (1998) Cardiovascular actions of cannabinoids and their generation during shock. *J Mol Med* 76:824–836
  39. Randall MD, Harris D, Kendall DA, Ralevic V (2002) Cardiovascular effects of cannabinoids. *Pharmacol Ther* 95:191–202
  40. Chen Y, McCarron RM, Ohara Y, Bemby J, Azzam N, Lenz FA, Shohami E, Mechoulam R, Spatz M (2000) Human brain capillary endothelium: 2-arachidonoylglycerol (endocannabinoid) interacts with endothelin-1. *Circ Res* 87:323–327
  41. Begg M, Pacher P, Batkai S, Osei-Hyiaman D, Offertaler L, Mo FM, Liu J, Kunos G (2005) Evidence for novel cannabinoid receptors. *Pharmacol Ther* 106:133–145
  42. Stella N (2004) Cannabinoid signaling in glial cells. *Glia* 48:267–277



43. Walter L, Stella N (2004) Cannabinoids and neuroinflammation. *Br J Pharmacol* 141:775–785
44. Smith SR, Terminelli C, Denhardt G (2000) Effects of cannabinoid receptor agonist and antagonist ligands on production of inflammatory cytokines and anti-inflammatory interleukin-10 in endotoxemic mice. *J Pharmacol Exp Ther* 293:136–150
45. Molina-Holgado F, Pintaux E, Moore JD, Molina-Holgado E, Guaza C, Gibson RM, Rothwell NJ (2003) Endogenous interleukin-1 receptor antagonist mediates anti-inflammatory and neuroprotective actions of cannabinoids in neurons and glia. *J Neurosci* 23:6470–6474
46. Guzman M, Sanchez C (1999) Effects of cannabinoids on energy metabolism. *Life Sci* 65:657–664
47. Marsicano G, Moosmann B, Hermann H, Lutz B, Behl C (2002) Neuroprotective properties of cannabinoids against oxidative stress: role of the cannabinoid receptor CB<sub>1</sub>. *J Neurochem* 80:448–456
48. Eshhar N, Striem S, Kohen R, Tirosh O, Biegon A (1995) Neuroprotective and antioxidant activities of HU-211, a novel NMDA receptor antagonist. *Eur J Pharmacol* 283:19–29
49. Hampson AJ, Grimaldi M, Axelrod J, Wink D (1998) Cannabidiol and (-)- $\Delta^9$ -tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci U S A* 95:8268–8273
50. Chen Y, Buck J (2000) Cannabinoids protect cells from oxidative cell death: a receptor-independent mechanism. *J Pharmacol Exp Ther* 293:807–812
51. García-Arencibia M, González S, de Lago E, Ramos JA, Mechoulam R, Fernández-Ruiz J (2007) Evaluation of the neuroprotective effect of cannabinoids in a rat model of Parkinson's disease: importance of antioxidant and cannabinoid receptor-independent properties. *Brain Res* 1134:162–170
52. Sagredo O, Ramos JA, Decio A, Mechoulam R, Fernández-Ruiz J (2007) Cannabidiol reduced the striatal atrophy caused 3-nitropropionic acid in vivo by mechanisms independent of the activation of cannabinoid receptors. *Eur J Neurosci* (in press)
53. Borrell-Pages M, Zala D, Humbert S, Saudou F (2006) Huntington's disease: from huntingtin function and dysfunction to therapeutic strategies. *Cell Mol Life Sci* 63:2642–2660
54. Herkenham M, Lynn AB, Little MD, Melvin LS, Johnson MR, de Costa DR, Rice KC (1991) Characterization and localization of cannabinoid receptors in rat brain: a quantitative in vitro autoradiographic study. *J Neurosci* 11:563–583
55. Lastres-Becker I, De Miguél R, Fernández-Ruiz J (2003) The endocannabinoid system and Huntington's disease. *Curr Drug Target CNS Neurol Disord* 2:335–347
56. Lastres-Becker I, Bizat N, Boyer F, Hantraye P, Fernández-Ruiz JJ, Brouillet E (2004) Potential involvement of cannabinoid receptors in 3-nitropropionic acid toxicity in vivo: implication for Huntington's disease. *Neuroreport* 15:2375–2379
57. Pintor A, Tebano MT, Martire A, Grieco R, Galluzzo M, Scattoni ML, Pezzola A, Coccorello R, Felici F, Cuomo V, Piomelli D, Calamandrei G, Popoli P (2006) The cannabinoid receptor agonist WIN 55,212-2 attenuates the effects induced by quinolinic acid in the rat striatum. *Neuropharmacology* 51:1004–1012
58. Glass M, Dragunow M, Faull RLM (2000) The pattern of neurodegeneration in Huntington's disease: a comparative study of cannabinoid, dopamine, adenosine and GABA-A receptor alterations in the human basal ganglia in Huntington's disease. *Neuroscience* 97:505–519
59. Lastres-Becker I, Berrendero F, Lucas JJ, Martín E, Yamamoto A, Ramos JA, Fernández-Ruiz J (2002) Loss of mRNA levels, binding and activation of GTP-binding proteins for cannabinoid CB<sub>1</sub> receptors in the basal ganglia of a transgenic model of Huntington's disease. *Brain Res* 929:236–242
60. Denovan-Wright EM, Robertson HA (2000) Cannabinoid receptor messenger RNA levels decrease in subset neurons of the lateral striatum, cortex and hippocampus of transgenic Huntington's disease mice. *Neuroscience* 98:705–713
61. Aiken CT, Tobin AJ, Schweitzer ES (2004) A cell-based screen for drugs to treat Huntington's disease. *Neurobiol Dis* 16:546–555
62. Wang W, Duan W, Igarashi S, Morita H, Nakamura M, Ross CA (2005) Compounds blocking mutant huntingtin toxicity identified using a Huntington's disease neuronal cell model. *Neurobiol Dis* 20:500–508
63. Gu M, Gash MT, Mann VM, Javoy-Agid F, Cooper JM, Schapira AH (1996) Mitochondrial defect in Huntington's disease caudate nucleus. *Ann Neurol* 39:385–389
64. Bizat N, Hermel JM, Humbert S, Jacquard C, Creminon C, Escartin C, Saudou F, Krajewski S, Hantraye P, Brouillet E (2003) In vivo calpain/caspase cross-talk during 3-nitropropionic acid-induced striatal degeneration: implication of a calpain-mediated cleavage of active caspase-3. *J Biol Chem* 278:43245–43253
65. Galas MC, Bizat N, Cuvclier L, Bantubungi K, Brouillet E, Schiffmann SN, Blum D (2004) Death of cortical and striatal neurons induced by mitochondrial defect involves differential molecular mechanisms. *Neurobiol Dis* 15:152–159
66. Toulmond S, Tang K, Bureau Y, Ashdown H, Degen S, O'Donnell R, Tam J, Han Y, Colucci J, Giroux A, Zhu Y, Boucher M, Pikounis B, Xanthoudakis S, Roy S, Rigby M, Zamboni R, Robertson GS, Ng GY, Nicholson DW, Fluckiger JP (2004) Neuroprotective effects of M826, a reversible caspase-3 inhibitor, in the rat malonate model of Huntington's disease. *Br J Pharmacol* 141:689–697
67. Sapp E, Kegel KB, Aronin N, Hashikawa T, Uchiyama Y, Tohyama K, Bhide PG, Vonsattel JP, DiFiglia M (2001) Early and progressive accumulation of reactive microglia in the Huntington disease brain. *J Neuropathol Exp Neurol* 60:161–172
68. Rajkowska G, Selemon LD, Goldman-Rakic PS (1998) Neuronal and glial somal size in the prefrontal cortex: a postmortem morphometric study of schizophrenia and Huntington disease. *Arch Gen Psychiatry* 55:215–224
69. Blandini F, Nappi G, Tassorelli C, Martignoni E (2000) Functional changes in the basal ganglia circuitry in Parkinson's disease. *Prog Neurobiol* 62:63–88
70. McGeer PL, Yasojima K, McGeer EG (2001) Inflammation in Parkinson's disease. *Adv Neurol* 86:83–89
71. Sherer TB, Betarbet R, Greenamyre JT (2001) Pathogenesis of Parkinson's disease. *Curr Opin Investig Drugs* 2:657–662
72. Sethi KD (2002) Clinical aspects of Parkinson disease. *Curr Opin Neurol* 15:457–460
73. Carlsson A (2002) Treatment of Parkinson's with L-DOPA. The early discovery phase, and a comment on current problems. *J Neural Transm* 109:777–787
74. Consroe P (1998) Brain cannabinoid systems as targets for the therapy of neurological disorders. *Neurobiol Dis* 5:534–551
75. Müller-Vahl KR, Kolbe H, Schneider U, Emrich HM (1999). Cannabis in movement disorders. *Forsch Komplementmed* 6:23–27
76. Lastres-Becker I, Molina-Holgado F, Ramos JA, Mechoulam R, Fernández-Ruiz J (2005) Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity in vivo and in vitro: relevance to Parkinson's disease. *Neurobiol Dis* 19:96–107
77. Gao HM, Jiang J, Wilson B, Zhang W, Hong JS, Liu B (2002) Microglial activation-mediated delayed and progressive degeneration of rat nigral dopaminergic neurons: relevance to Parkinson's disease. *J Neurochem* 81:1285–1297
78. Kim WG, Mohny RP, Wilson B, Joehn GH, Liu B, Hong JS (2000) Regional difference in susceptibility to lipopolysaccharide-induced neurotoxicity in the rat brain: role of microglia. *J Neurosci* 20:6309–6316
79. Nagatsu T, Mogi M, Ichinose H, Togari A (2000) Changes in cytokines and neurotrophins in Parkinson's disease. *J Neural Transm* 60:277–290