

THEMED ISSUE: CANNABINOIDS

REVIEW

The endocannabinoid system as a target for the treatment of neurodegenerative disease

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The *Cannabis sativa* plant has been exploited for medicinal, agricultural and spiritual purposes in diverse cultures over thousands of years. Cannabis has been used recreationally for its psychotropic properties, while effects such as stimulation of appetite, analgesia and anti-emesis have led to the medicinal application of cannabis. Indeed, reports of medicinal efficacy of cannabis can be traced back as far as 2700 BC, and even at that time reports also suggested a neuroprotective effect of the cultivar. The discovery of the psychoactive component of cannabis resin, Δ^9 -tetrahydrocannabinol (Δ^9 -THC) occurred long before the serendipitous identification of a G-protein coupled receptor at which Δ^9 -THC is active in the brain. The subsequent finding of endogenous cannabinoid compounds, the synthesis of which is directed by neuronal excitability and which in turn served to regulate that excitability, further widened the range of potential drug targets through which the endocannabinoid system can be manipulated. As a result of this, alterations in the endocannabinoid system have been extensively investigated in a range of neurodegenerative disorders. In this review we examine the evidence implicating the endocannabinoid system in the cause, symptomatology or treatment of neurodegenerative disease. We examine data from human patients and compare and contrast this with evidence from animal models of these diseases. On the basis of this evidence we discuss the likely efficacy of endocannabinoid-based therapies in each disease context.

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Abbreviations: 2-AG, 2-arachidonoylglycerol; 3-NP, 3-nitropropionic acid; 6-OHDA, 6-hydroxy dopamine; Δ^9 -THC, Δ^9 -tetrahydrocannabinol; AD, Alzheimer's disease; AEA, anandamide/arachidonylethanolamide; ALS, amyotrophic lateral sclerosis; AMT, anandamide membrane transporter; BAP, β -amyloid peptide; CB₁, cannabinoid receptor 1; CB₂, cannabinoid receptor 2; CNS, central nervous system; CREAE, chronic relapsing EAE; CSF, cerebrospinal fluid; DAGL, diacylglycerol lipase; EAAT2, glutamate transporter/excitatory amino acid transporter 2; EAE, experimental autoimmune encephalomyelitis; FAAH, fatty acid amide hydrolyase; GABA, γ -amino butyric acid; HD, Huntington's disease; hSOD1, human superoxide dismutase 1; LID, levodopa-induced dyskinesia; MAGL, monoacylglycerol lipase; MS, multiple sclerosis; NAPE-PLD, N-acyl phosphatidylethanolamine phospholipase D; PEA, palmitoylethanolamide; PD, Parkinson's disease; PPAR, peroxisome proliferator-activated receptor; TMEV, Theiler's murine encephalomyelitis virus; TNF, tumour necrosis factor; TRPV1, transient receptor potential (vanilloid) receptor 1; WT, wildtype

The psychoactive component of cannabis resin, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), was first isolated in 1964 (Gaoni and Mechoulam, 1964), and at least 70 other structurally related 'phytocannabinoid' compounds have since been

isolated (Elsohly and Slade, 2005). The development of synthetic cannabimimetic drugs has aided in the pharmacological characterization of an endogenous system which responds to cannabis. However, it was the serendipitous identification of a G-protein coupled cannabinoid receptor at which cannabinoid compounds are active in the brain (Matsuda *et al.*, 1990), which heralded an explosion in endocannabinoid research. In this review, we examine the reports of changes to endocannabinoid levels in human patients and compare and contrast this with evidence from animal models. We also

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consider the reported changes to other elements of the endocannabinoid system, with a view to understanding their relationship to endocannabinoid levels and disease symptomatology. Based upon these examinations, and upon the reported effects of manipulation of the cannabinoid system in each disease context, we discuss the likely efficacy of endocannabinoid-based therapies.

The endocannabinoid system

The endocannabinoid system comprises the cannabinoid receptors CB₁, CB₂ and possibly others; the fatty acid derivatives which act as their endogenous ligands; and the proteins responsible for the synthesis, reuptake and degradation of these endogenous ligands.

Cannabinoid receptors

Various studies have mapped the localization of cannabinoid receptors in tissues and at a subcellular level, and these have been critical to our understanding of the effects of cannabinoids in disease. CB₁ receptors are expressed in both the central nervous system and periphery. They are the most abundant GPCR in the brain, with high expression levels in the basal ganglia nuclei and moderately high expression in the hippocampus, cerebellum and neocortex (Herkenham *et al.*, 1990; Glass *et al.*, 1997). At the subcellular level, CB₁ has been localized to pre-synaptic terminals, and is found at significantly higher levels on GABAergic than glutamatergic neurons in various brain regions (Katona *et al.*, 1999; Katona *et al.*, 2001; Puighermanal *et al.*, 2009).

CB₁ is expressed at much lower levels peripherally than in the brain. Nonetheless, CB₁ expression has been detected in a variety of circulating immune cells (Bouaboula *et al.*, 1993), and in resident microglia in rat brain (Sinha *et al.*, 1998). Activation of microglial CB₁ has been shown to inhibit the release of nitric oxide, suggesting that CB₁ may be anti-inflammatory (Waksman *et al.*, 1999). There is evidence that changes in CB₁ expression may occur with activation in immune cell lines; however, reports are conflicting as to whether CB₁ is up- or down-regulated in these activated cells (reviewed in Klein, 2005). Astrocytic expression of CB₁ has been demonstrated in cultured cells (Bouaboula *et al.*, 1995; Sheng *et al.*, 2005), and after injury (Garcia-Ovejero *et al.*, 2009); however, there has been some controversy over whether normal astrocytes *in situ* express CB₁. Several studies have found no overlap between astrocytic and CB₁ immunostaining (McDonald and Mascagni, 2001; Marchalant *et al.*, 2007; Marchalant *et al.*, 2008), therefore there was a call for more definitive studies to confirm that the CB₁-positive perivascular glia reported by several other groups were indeed astrocytes (Moldrich and Wenger, 2000; Rodriguez *et al.*, 2001; Salio *et al.*, 2002). A recent investigation has gone some way towards achieving this, by demonstrating that the response to cannabinoids by astrocytes in hippocampal slices was blocked by a CB₁-specific antagonist (Navarrete and Araque, 2008).

The myriad peripheral and immune activities of cannabinoid compounds, despite the low levels of CB₁ expression in cognate immune organs (reviewed in Klein *et al.*, 2003), prompted the search for a peripheral receptor for cannabinoids, CB₂ (Munro *et al.*, 1993). CB₂ shows only 44% overall identity to CB₁ (Munro *et al.*, 1993). CB₂ is found in particular abundance in peripheral organs with immune function, including macrophages, spleen, tonsils, thymus and leucocytes, as well as the lung and testes (Munro *et al.*, 1993; Galiegue *et al.*, 1995; Brown *et al.*, 2002). Early studies suggested that CB₂ was absent from the brain (Griffin *et al.*, 1999; Brown *et al.*, 2002). However, a number of studies have now shown CB₂ expression in diseased brain cells, including astrocytomas (Sanchez *et al.*, 2001; Ellert-Miklaszewska *et al.*, 2007), microglia and astrocytes in Alzheimer's disease (Benito *et al.*, 2003; Esposito *et al.*, 2007b), and T cells, microglia and astrocytes in multiple sclerosis (Benito *et al.*, 2007).

These studies and others provide strong evidence that CB₂ is up-regulated in response to inflammatory cues or immune cell activation. It should be noted, however, that a specific marker to distinguish macrophages and microglia remains to be identified, making it difficult to determine whether CB₂ up-regulation is attributable to expression by activated resident microglia or by peripheral macrophages invading the brain. More recently, CB₂ expression has also been reported to occur in normal brain; in neural progenitors (Palazuelos *et al.*, 2006); select neuronal subsets in the brainstem (Van Sickle *et al.*, 2005), cerebellar granule layer (Skaper *et al.*, 1996), and dorsal root of the spinal cord (Beltramo *et al.*, 2006); and in microglial populations (Klegeris *et al.*, 2003; Nunez *et al.*, 2004; Ashton *et al.*, 2006), but not normal astrocytes (Ashton *et al.*, 2006; Benito *et al.*, 2007; Racz *et al.*, 2008; Garcia-Ovejero *et al.*, 2009). Several reports originating from one research group, of extensive CB₂ immunoreactivity in various neuronal subtypes throughout the brain, have been somewhat controversial and warrant further investigation (Gong *et al.*, 2006; Onaivi *et al.*, 2006).

The possible existence of other cannabinoid-responsive receptors has been brought to light by the inability of specific CB₁ or CB₂ antagonists to abolish the effects or binding of certain cannabinoids, and by the persistence of cannabinoid effects in CB₁ and/or CB₂ knockout animals. One such receptor is the abnormal-cannabidiol receptor, which is activated by abnormal cannabidiol, AEA, 2-AG and WIN55,212-2, and antagonized by cannabidiol, the cannabidiol analogue O-1918 and the endogenous compound N-arachidonoyl L-serine (Jarai *et al.*, 1999; Begg *et al.*, 2003; McHugh *et al.*, 2008; Kreutz *et al.*, 2009). The orphan receptor designated GPR55 has also received considerable attention as a possible cannabinoid receptor. Endo-, phyto- and synthetic cannabinoids have been shown to bind and activate GPR55 (Ryberg *et al.*, 2007; Lauckner *et al.*, 2008), although subsequent studies dispute a number of these findings (Henstridge *et al.*, 2009; Kapur *et al.*, 2009). Indeed, those studies reported that an array of cannabinoids failed to activate GPR55, with the exception of AM251 and SR141716A, which are CB₁ antagonists, and lysophosphatidylinositol, which is not a cannabinoid (Oka *et al.*, 2007; Henstridge *et al.*, 2009; Kapur *et al.*, 2009). Two of the studies suggested that CP55,940, a cannabinoid receptor agonist, acts as an antagonist or partial agonist

at GPR55 (Henstridge *et al.*, 2009; Kapur *et al.*, 2009). The adoption of either the abnormal-cannabidiol receptor or GPR55 into the cannabinoid receptor family remains an issue of debate. However, a possible role for non-CB₁/CB₂ receptors in the effects of cannabinoids in neurodegenerative disorders should not be overlooked.

Endocannabinoids

Endocannabinoids were first discovered in brain, but are also present in the periphery in both humans and animals. They are produced by cultured neurons (Di Marzo *et al.*, 1994), microglia (Walter *et al.*, 2003) and astrocytes (Walter *et al.*, 2002), as well as by isolated macrophages (Wagner *et al.*, 1997; Di Marzo *et al.*, 1999). To date, five endogenous cannabinoids or 'endocannabinoids' have been identified; arachidonoyl ethanolamide (anandamide, AEA) (Devane *et al.*, 1992), 2-arachidonoyl glycerol (2-AG) (Mechoulam *et al.*, 1995), O-arachidonoyl ethanolamine (virodhamine) (Porter *et al.*, 2002), N-arachidonoyldopamine (NADA) (Huang *et al.*, 2002) and 2-arachidonoyl glyceryl ether (noladin ether) (Hanus *et al.*, 2001). While publications subsequent to Hanus *et al.* also report noladin ether to occur in brain, several other groups have failed to corroborate this finding (Oka *et al.*, 2003; Richardson *et al.*, 2007), leaving doubt as to whether it is a *bona fide* endocannabinoid.

In addition to these compounds, the AEA analogue palmitoylethanolamide (PEA) is receiving increasing interest as a potential endocannabinoid. PEA shows no affinity for CB₁, and reports are conflicting as to whether it activates CB₂ (Facci *et al.*, 1995; Ryberg *et al.*, 2007) or not (Showalter *et al.*, 1996; Lambert *et al.*, 1999; Sugiura *et al.*, 2000). However, reports of specific mechanisms regulating the synthesis (Stella and Piomelli, 2001) and metabolism (Tsuboi *et al.*, 2005) of PEA support the notion that this endogenous fatty acid may act as more than just an 'entourage' molecule for the effects of 'true' endocannabinoids (for review, see Mackie and Stella, 2006). There has also been significant interest in oleamide as an endocannabinoid. It has shown efficacy both *in vivo* at the abnormal-cannabidiol receptor (Hoi and Hiley, 2006; Sudhar *et al.*, 2009) and more controversially *in vitro* at CB₁ (for commentary see Fowler, 2004; Leggett *et al.*, 2004). The investigation of oleamide as an endocannabinoid may be complicated by its use in, and organic solvent-mediated leaching from, disposable laboratory plastic ware (McDonald *et al.*, 2008).

As some of these potential endocannabinoids remain under debate, and because the majority of studies of endocannabinoids levels in neurodegenerative disease have quantified only AEA and 2-AG, these two endocannabinoids will thus form the focus of our review.

AEA has been found both in the brain and periphery (Felder *et al.*, 1996). AEA is an agonist for CB₁ (Devane *et al.*, 1992; Mackie *et al.*, 1993; Showalter *et al.*, 1996), CB₂ (Felder *et al.*, 1995; Showalter *et al.*, 1996), and the vanilloid TRPV1 receptor (Zygmunt *et al.*, 1999). In the brain, AEA levels are high in the hippocampus, thalamus, striatum and brainstem and lower, but still detectable, in the cerebral cortex and cerebellum (Felder *et al.*, 1996; Bisogno *et al.*, 1999). This pattern of

distribution shows poor correlation with that described for CB₁ (Herkenham *et al.*, 1990; Bisogno *et al.*, 1999). AEA is also found, albeit at far lower levels than in brain, in human blood (serum and plasma) and CSF (Felder *et al.*, 1996).

Like AEA, 2-AG is found in both the brain and periphery, although in the brain it is found at concentrations approximately 150 times that of AEA (Bisogno *et al.*, 1999). 2-AG is found at high levels in the brainstem, hippocampus, striatum and medulla in rats, showing a correlation with AEA but not CB₁ localization (Bisogno *et al.*, 1999). 2-AG is an agonist at CB₁ and also CB₂, where its potency is greater than that of AEA; while this has been taken to suggest that 2-AG may be the endogenous ligand for CB₂, this finding could equally be due to greater stability of 2-AG than AEA (Mechoulam *et al.*, 1995; Gonsiorek *et al.*, 2000; Sugiura *et al.*, 2000).

Endocannabinoid synthesis and inactivation

Various synthetic and degradative enzymes have been identified which dynamically regulate the levels of endogenous cannabinoids under normal and diseased conditions, and which may be key targets for therapeutics. Both AEA and 2-AG are produced by cleavage of plasma membrane phospholipids. They are synthesized 'on demand' in an activity-dependent fashion, whereby calcium acts as a biosensor of membrane depolarization to induce synthesis as required (Di Marzo *et al.*, 1994; Stella *et al.*, 1997). AEA is synthesized from its arachidonic acid and phosphatidylethanolamine precursors by the sequential actions of two intracellular enzymes; an N-acyltransferase and a phospholipase (NAPE-PLD) (Di Marzo *et al.*, 1994; Cadas *et al.*, 1997). 2-AG is formed by the hydrolysis of membrane-derived diacylglycerol by sn1 diacylglycerol lipase (DAGL) found in the membranes of neuronal dendritic spines (Bisogno *et al.*, 2003; Katona *et al.*, 2006). DAGL expression may also be induced in reactive astrocytes (Garcia-Ovejero *et al.*, 2009). How these highly lipophilic endocannabinoids are released from the membrane into synaptic and extra-synaptic spaces remains poorly understood.

Inactivation of endocannabinoids occurs rapidly *in vivo* (Di Marzo *et al.*, 1994; Maccarrone *et al.*, 1998). The fatty acid amide hydrolase (FAAH) enzyme is found intracellularly on the membranes of organelles in mainly post-synaptic neurons, showing complementary expression to CB₁ (Eger *et al.*, 1998; Gulyas *et al.*, 2004). Reactive astrocytes show increased expression of FAAH (Benito *et al.*, 2005; Benito *et al.*, 2007; Nunez *et al.*, 2008). FAAH is responsible for the degradation of AEA predominantly, although 2-AG can also act as a substrate (Di Marzo *et al.*, 1998; Goparaju *et al.*, 1998). The inactivation of 2-AG occurs preferentially through hydrolysis by the pre-synaptically localized monoacylglycerol lipase (MAGL) enzyme (Dinh *et al.*, 2002). The membrane transporter for AEA (AMT) is yet to be cloned and its existence is a topic of hot debate. On one side of the controversy, the saturable, temperature-dependent and specific nature of AEA uptake argues against passive diffusion through the membrane (Di Marzo *et al.*, 1994). In addition, specific inhibitors of uptake but not hydrolysis bolster the claims for the AMT's existence. Many (Vandevorode and

Fowler, 2005; Alexander and Cravatt, 2006; Kaczocha *et al.*, 2006), but not all (Ortar *et al.*, 2003; Fegley *et al.*, 2004), of these uptake inhibitors have subsequently been shown to inhibit FAAH, which would reduce the concentration gradient for diffusion. Recently, intracellular transporters were identified which facilitate the delivery of AEA to FAAH through the hydrophilic cytosol (Kaczocha *et al.*, 2009). This finding may help reconcile the data supporting both passive membrane diffusion and carrier-mediated kinetics for AEA uptake; however, while this model abrogates the need for an AMT, it does not yet rule out the possibility of its existence.

Endocannabinoid function

Our growing understanding of the roles of the endogenous cannabinoids has suggested two main pathways by which cannabinoids may impact upon neurodegenerative processes; neuromodulation and immunomodulation. The neuromodulatory action of endocannabinoids has been well characterized in several elegant studies, and CB₁ and CB₂ signal transduction have been detailed in various reviews (Felder *et al.*, 1995; McAllister and Glass, 2002; Dalton *et al.*, 2009; Scotter *et al.*, 2009). Briefly, it has been shown that endocannabinoids synthesized by depolarized post-synaptic dendrites, particularly 2-AG (Kim and Alger, 2004), can act as retrograde ligands at CB₁ located at pre-synaptic terminals to inhibit the release of excitatory or inhibitory neurotransmitter from the pre-synaptic neuron (Maejima *et al.*, 2001; Wilson and Nicoll, 2001).

Aside from this crucial regulatory role in the activity of neurons, endocannabinoids also play a key role in peripheral and brain immune function. As mentioned, CB₂ is expressed on various circulating and resident immune cells, particularly when these cells are activated, and its agonism is typically associated with a dampening of their pro-inflammatory activities. This includes the inhibition of release of inflammatory mediators, including nitric oxide, interleukin-2 and TNF- α , inhibition of the activation of the cell-mediated immune processes, and inhibition of proliferation and chemotaxis (Ehrhart *et al.*, 2005; Coopman *et al.*, 2007; Maresz *et al.*, 2007; Romero-Sandoval *et al.*, 2009; and reviewed in Walter and Stella, 2004a).

Endocannabinoids in neurodegenerative disease

There has been anecdotal and preliminary scientific evidence of cannabis affording symptomatic relief in diverse neurodegenerative disorders. These include multiple sclerosis, Huntington's, Parkinson's and Alzheimer's diseases, and amyotrophic lateral sclerosis. This evidence implied that hypofunction or dysregulation of the endocannabinoid system may be responsible for some of the symptomatology of these diseases. Given also the abundance of CB₁ in areas associated with movement and executive thought, interest soon developed in measuring endocannabinoids levels in patients with degenerative movement disorders.

Huntington's disease

Huntington's disease (HD) is a hereditary disorder with an incidence of approximately 1 in 15 000 and is characterized by a triad of symptoms; disturbances in movement, mood and cognition (for reviews, see Rosenblatt and Leroy, 2000; and Ross and Margolis, 2001). In 1993 the *huntingtin* gene, mutation of which is responsible for the disease, was mapped, and its misfolding was identified as a requisite pathological event in the degeneration of neurons in the striatum, cortex and other diffuse brain regions affected by HD (The Huntington's Disease Collaborative Research Group, 1993). However, despite this finding and much subsequent research, both the wildtype function of the huntingtin protein and the pathophysiological pathways activated by the mutant protein remain ill-defined.

Endocannabinoid changes system in human HD

One of the first detectable signs of cellular dysfunction in human HD brains is the loss of CB₁ from GABAergic efferent terminals and somata. In HD patients with early symptoms but without gross neuropathology (Grade zero (Vonsattel *et al.*, 1985)), there is a significant decrease in CB₁ density in the internal and external globus pallidus and substantia nigra (Glass *et al.*, 2000). While CB₁ loss in the external segment of the globus pallidus is likely due to the degeneration of the terminals of the GABA/enkephalin efferents lost first in HD (Reiner *et al.*, 1988), in the internal segment significant CB₁ loss is seen prior to changes in co-localized receptors or GABA/substance P neuronal pathology (Glass *et al.*, 2000; Allen *et al.*, 2009). Contrary to findings with CB₁, CB₂ has recently been demonstrated to be up-regulated in post-mortem HD striatum (Palazuelos *et al.*, 2009), consistent with marked gliosis in this region.

Recently, it was reported that lymphocyte preparations from HD patients contained levels of AEA that were sixfold greater than those of control patient lymphocytes (Battista *et al.*, 2007). This was attributed to an inhibition of function of FAAH in AEA metabolism. While the relationship between the peripheral and central endocannabinoid systems remains unclear, in the same study a corresponding reduction in FAAH activity was also detected in the cerebral cortex of post-mortem HD brains.

Endocannabinoid changes in animal models of HD

Various animal models have been developed which reproduce the selective degeneration of medium spiny neurons of the striatum in HD. Intra-striatal administration of the mitochondrial toxin 3-nitropropionic acid (3-NP) to rats produces an interneuron-sparing striatal lesion and HD-like motor abnormalities (Beal *et al.*, 1993). An examination of endocannabinoids levels in this model revealed decreases in AEA and 2-AG in the striatum, but not the cerebral cortex, and increases in the region corresponding to the substantia nigra (Lastres-Becker *et al.*, 2001b). These changes are difficult to interpret however, given the acute ablation of medium spiny

neurons which likely produce these endocannabinoids (van der Stelt *et al.*, 2003). Transgenic mouse models of HD, carrying the pathologically expanded huntingtin gene with 115 (R6/1) or 150 (R6/2) repeats, also recapitulate the motor phenotype of HD (Mangiarini *et al.*, 1996). Unlike lesion models, the progression of disease in these animals occurs in the absence of overt cell death (Mangiarini *et al.*, 1996), which may enable the analysis of endocannabinoid changes associated with early cellular dysfunction. Loss of CB₁ is recapitulated by various transgenic rodent models, despite their lack of overt cell death (Denovan-Wright and Robertson, 2000; Lastres-Becker *et al.*, 2002a; McCaw *et al.*, 2004), suggesting that CB₁ loss may represent an early marker of, or exacerbating factor in, neuronal dysfunction.

Interestingly, these CB₁ changes occur considerably earlier than alterations in endocannabinoid levels in R6/2 mice; with receptor changes occurring pre-symptomatically while endocannabinoid alterations (as described below) were concurrent with distinct motor symptomatology (Denovan-Wright and Robertson, 2000; McCaw *et al.*, 2004; Bisogno *et al.*, 2008). This indicates that changes to cannabinoid receptor levels may be a part of or induced by HD pathology, rather than occurring secondarily to changes in endocannabinoid levels.

Profiles of endocannabinoid levels in pre-symptomatic R6/1 mice (12 weeks gestational age) showed decreased AEA in the hippocampus and increased 2-AG in the cerebral cortex. Interestingly no changes were detected for either endocannabinoid in the striatum (Dowie *et al.*, 2009a), the brain region in which dysfunction occurs early in both this model and human sufferers (Vonsattel *et al.*, 1985; Mangiarini *et al.*, 1996). Endocannabinoid quantification in pre-symptomatic R6/2 mice (4.5 weeks gestational age) suggested a decrease, rather than an increase, in 2-AG levels in the cerebral cortex, while AEA levels were unchanged in all areas examined (Bisogno *et al.*, 2008). Analysis of motor symptomatic R6/2 mice (10 weeks gestational age) in the same study showed 2-AG levels to be decreased in the cerebral cortex, striatum, and also trending towards a decrease in the hippocampus. AEA levels were also decreased in the striatum and hippocampus, but increased in the cerebral cortex (Bisogno *et al.*, 2008). This latter finding, of increased AEA in the cerebral cortex, could be the result of inhibition of FAAH, as was reported for human HD cerebral cortex (Battista *et al.*, 2007).

Recently, CB₂ alterations have also been detected in the human and transgenic animal HD brain. Palazuelos *et al.* (2009) detected increased CB₂ protein in the striatum in both human HD patients and R6/2 mice compared with controls. In mouse models the increase in CB₂ occurred concurrently with increased immunoreactivity for the microglial (and macrophage) markers CD68 and CD11b, with which CB₂ was shown to co-localize (Palazuelos *et al.*, 2009). Surprisingly, the greatest increase in CB₂ immunoreactivity in R6/2 mice (~3.5-fold) was seen at a pre-symptomatic time-point, suggesting that increases in CB₂-positive microglia occurred early in the disease. This is in accordance with human HD studies which have found that striatal microglia are activated in preclinical and low grade patients (Sapp *et al.*, 2001; Tai *et al.*, 2007). The intrinsic activation of CB₂ in HD may reduce pro-inflammatory microglial cascades to some extent; however,

this mechanism is clearly insufficient to prevent the inevitable progression of neuronal death.

Cannabinoid agents as therapeutics in HD

The question of whether CB₁ activation may be therapeutic in HD has been explored in various rodent lesion models; however, reports are conflicting as to whether CB₁ agonism is neuroprotective, exacerbatory, or is more useful in the treatment of HD symptoms. It has been found that administration of the endocannabinoid uptake inhibitors UM404 or UCM707 attenuated both the neurotransmitter deficits and increase in ambulation ('hyperkinesia') associated with 3-NP lesion (Lastres-Becker *et al.*, 2002b; de Lago *et al.*, 2006). However, further investigation suggested that the target responsible for much of the anti-hyperkinetic effect may have been the vanilloid TRPV1 receptor, with CB₁ playing only a minor role (Lastres-Becker *et al.*, 2003b).

A neuroprotective role for CB₁ in HD has also been tested. Lastres-Becker *et al.* (2004) found that the number of rats which developed a significant lesion due to 3-NP administration was reduced in a cohort treated daily with Δ^9 -THC. However, the same group had previously reported an exacerbation of malonate lesion with either Δ^9 -THC or SR141716A treatment, despite their opposing actions at CB₁ (Lastres-Becker *et al.*, 2003a), and later the endocannabinoid uptake inhibitor UCM707 also failed to reduce lesion volume in the malonate model (de Lago *et al.*, 2006). In contrast, in quinolinic acid-lesioned rats, pre- and then co-administration of WIN55,212-2 with the excitotoxin reduced the induction of glutamate release and reduced the lesion volume (Pintor *et al.*, 2006). Similarly, following lesion with kainic acid, wildtype but not CB₁-knockout mice showed an injury-induced recruitment of brain-derived neurotrophic factor which decreased neuronal damage and gliosis (Marsicano *et al.*, 2003; Khaspekov *et al.*, 2004). The ability of the neuromodulatory cannabinoid system to attenuate excitotoxic damage, as is inflicted by the aforementioned toxins, is widely accepted. However, the contribution of excitotoxicity to the pathophysiology of HD is unknown, and as such care must be taken in extrapolating the efficacy of cannabinoids in lesion models to transgenic or human HD.

In the R6/1 transgenic mouse model of HD, exposure to enriched environments caused an up-regulation of CB₁ and also delayed the onset of symptoms (van Dellen *et al.*, 2000; Glass *et al.*, 2004). Environmental enrichment was also found to almost completely rescue the deficit in brain-derived neurotrophic factor associated with mutant huntingtin expression (Spires *et al.*, 2004). However, despite the efficacy of CB₁ signalling in attenuating molecular and behavioural HD, the early and dramatic loss of these receptors may preclude their use as therapeutics (Dowie *et al.*, 2009b).

In contrast, CB₂ on microglia in the R6/2 striatum has been shown to increase prior to symptom-onset (Palazuelos *et al.*, 2009). Because CB₂ knockout leads to enhanced gliosis and an exacerbation of motor symptoms, the reported CB₂ up-regulation may be a protective compensatory mechanism (Palazuelos *et al.*, 2009). The selective CB₂ agonist HU308 has been shown to reduce neuronal loss, potentially through

suppression of glial activation, in the quinolinic acid, 3-NP and malonate lesion models (Sagredo *et al.*, 2007; Palazuelos *et al.*, 2009; Sagredo *et al.*, 2009); however, neuroprotection via CB₂ manipulation is yet to be confirmed in transgenic HD models. Indeed the CB₁/CB₂ agonist HU210 failed to modify disease in the R6/1 transgenic model of HD (Dowie *et al.*, 2009b). Interestingly, cannabidiol has been found to almost completely reverse the neuronal changes induced by 3-NP administration to rats, suggesting that anti-oxidant effects, abnormal-cannabinoid receptor antagonism, or as yet unidentified cannabinoid receptors may be protective in this model of HD (Sagredo *et al.*, 2007). A 6-week clinical trial of cannabidiol in humans failed to ameliorate symptoms (Consroe *et al.*, 1991), but further studies need to be performed to evaluate the effectiveness of cannabinoid treatment in patients with HD.

Alzheimer's disease

The disruptive effects of Δ^9 -THC on memory are well documented and have recently been more fully characterized at the molecular level (Puighermanal *et al.*, 2009). Alzheimer's disease (AD), a disease with major impact on memory systems, has therefore been investigated for evidence of dysfunction of the endocannabinoid system resulting from, or contributing to, disease pathophysiology. AD is the most common neurodegenerative disorder, with a prevalence of approximately 10% in humans over 80 years old (Ferri *et al.*, 2005). There is both genetic and idiopathic aetiology for the disease, which is characterized by gross atrophy of cholinergic neurons projecting to the cerebral cortex and hippocampus, and also of glutamatergic neurons of those regions (Whitehouse *et al.*, 1982; Greenamyre *et al.*, 1985; Wenk, 2003). Neurodegeneration appears to follow the extracellular deposition of β -amyloid protein in 'plaques' and/or the formation of intracellular 'tangles' of hyperphosphorylated tau protein (see Minati *et al.* 2009 for a recent review).

There is much debate regarding which, if either, of these proteins is central to the neurodegenerative process (reviewed in Mudher and Lovestone, 2002). While anti- β -amyloid vaccination of humans (Hock *et al.*, 2003; Nicoll *et al.*, 2003; Gilman *et al.*, 2005) and transgenic animals (Schenk *et al.*, 1999; Dodart *et al.*, 2002) has led to various degrees of cognitive or histological improvement, a Phase III trial currently underway may further implicate or absolve β -amyloid plaques in the impairment of cognitive function. Already there is much compelling evidence for β -amyloid cascade/neuroinflammation hypothesis, which proposes that misfolded β -amyloid is both injurious to neurons and also invokes a microglial response which, while perhaps evolved to phagocytically clear plaques, is itself neurotoxic (Haga *et al.*, 1989; Itagaki *et al.*, 1989; Hardy and Higgins, 1992; Streit *et al.*, 2005; Hickman *et al.*, 2008). The finding that CB₂ is expressed on the microglia clustered around β -amyloid plaques therefore suggests that endocannabinoids may have the ability to modulate the effector cells of AD (Benito *et al.*, 2003).

Endocannabinoid system changes in human AD

Studies have found CB₁ expression on neurons to be reduced (Westlake *et al.*, 1994; Ramirez *et al.*, 2005) or unchanged (Benito *et al.*, 2003) in human AD brain. Remaining CB₁ protein was shown to be excessively nitrated and to have decreased efficacy of G-protein coupling (Ramirez *et al.*, 2005). In contrast, CB₂ expression is dramatically up-regulated, particularly in the microglial cells surrounding β -amyloid plaques in human AD brain (Benito *et al.*, 2003; Ramirez *et al.*, 2005).

Endocannabinoid levels *per se* have not been assayed in post-mortem human AD brain, however, enhanced enzymatic activity of both DAGL and MAGL, the synthetic and catabolic enzymes for 2-AG, respectively, has been detected in the hippocampus in human AD (Farooqui *et al.*, 1988). In addition, FAAH protein expression and activity are reported to be elevated in activated astrocytes adjacent to the plaques (Benito *et al.*, 2003). These data have been suggested to imply that local production and turnover of 2-AG may also be elevated in the disease. Interestingly, quantification of AEA and 2-AG from plasma of Alzheimer's disease patients did not detect any significant differences from age-matched controls (Koppel *et al.*, 2009).

Endocannabinoid system changes in animal models of AD

Animal models of AD provide support to the hypothesis that 2-AG levels may be elevated in the hippocampus in human AD patients. Rats injected unilaterally in the frontal cortex with fragments of β -amyloid peptide (BAP rats) develop AD-like molecular pathology with significant neuronal and neuritic degeneration in the distantly located hippocampus, and impaired learning and memory (Kowall *et al.*, 1991; van der Stelt *et al.*, 2006). In agreement with the increased activity of DAGL and MAGL in human AD (six- to eightfold) (Farooqui *et al.*, 1988), mRNA expression of these enzymes was elevated by 1.2- and 1.6-fold, respectively, in BAP rats (van der Stelt *et al.*, 2006). 2-AG levels were rapidly and dramatically elevated in the ipsilateral and, to a lesser degree, the contralateral hippocampus in these animals. Increases in AEA levels were of a greater magnitude in the contralateral than ipsilateral hippocampus (van der Stelt *et al.*, 2006). *In vitro*, C6 glioma cells exposed to β -amyloid produced fourfold less AEA and 1.5-fold more 2-AG than control cells, suggesting that elevated 2-AG levels at least are a conserved feature of these AD models (Esposito *et al.*, 2007a). Consistent with human studies, CB₁ has been found to be reduced in BAP rats and C6 glioma cells challenged with β -amyloid (Esposito *et al.*, 2007a).

Cannabinoid agents as therapeutics in AD

Synthetic Δ^9 -THC (dronabinol) has been shown to alleviate behavioural disturbances and weight loss, and night-time agitation symptoms in human studies of Alzheimer's and severe dementia respectively (Volicer *et al.*, 1997; Walther *et al.*,

2006). As yet however, cannabinoid neuroprotection studies have only been conducted in animals. In BAP rats, the AMT inhibitor VDM11 was able to attenuate hippocampal neuron damage through the elevation of AEA levels alone, while in BAP mice neuronal rescue was associated with elevations in both AEA and 2-AG levels (van der Stelt *et al.*, 2006). Pharmacological dissection suggests that these endocannabinoids may mediate neuroprotection through activation of CB₁, and inhibit the inflammatory microglial response through activation of CB₂ (Ramirez *et al.*, 2005). CB₂ agonists have been shown to inhibit TNF- α and nitric oxide production by microglia/macrophages, as well as stimulating their phagocytosis of β -amyloid peptide (Ehrhart *et al.*, 2005; Tolon *et al.*, 2009). In a study by Esposito *et al.* a CB₂ antagonist was able to attenuate markers of astrogliosis (Esposito *et al.*, 2007a). Interestingly, the same group showed similar anti-inflammatory effects in another *in vivo* AD model following administration of cannabidiol, which does not bind to CB₂ (Esposito *et al.*, 2007b).

The unifying hypothesis encompassing most of these studies is that pathologic changes in endocannabinoid levels and CB₂ expression are induced by the inflammatory environment which occurs in AD. Activation of CB₂ by up-regulated endocannabinoids goes some way towards halting microglial activation; however, this innate compensation is insufficient to prevent the subsequent inflammatory damage to neurons, which may also suffer from a loss of protection due to the down-regulation of CB₁. On the basis of the pre-clinical efficacy already demonstrated, cannabinoid stimulators may have therapeutic benefit by augmenting the brain's innate response.

Multiple sclerosis

Multiple sclerosis (MS) is an inflammatory CNS disease which in contrast to the other neurodegenerative disorders reviewed here, presents most frequently in early adulthood (Liguori *et al.*, 2000). The CNS infiltration of autoreactive T cells, with specificity for myelin or other CNS proteins, is followed by their clonal expansion. Other immune mediators are recruited, including B cells and microglia, and together with T cells differentiated into 'cytotoxic' effectors, cause demyelination of neurons of the brain and spinal cord (Friese and Fugger, 2005). This is particularly catastrophic for motor and sensory function, and symptoms of MS include spasticity, hyperreflexia, pain and sensory disturbance (Noseworthy *et al.*, 2000). Focal inflammatory lesions or 'plaques' in the white matter are characterized by clusters of immune cells causing axonal demyelination and destruction, death of oligodendrocytes and bystander neurons, and a sclerotic astroglial 'scar' (Frohman *et al.*, 2006). Aetiology of the disease is enigmatic; however, correlations have been found between MS and birth/residence in non-equatorial countries, viral infection and mutations in several genes with immune function (Marrie, 2004; Compston and Coles, 2008). The 1-year prevalence for MS is approximately 0.9 per 1000 (Hirtz *et al.*, 2007). The majority of MS cases (~85%) are initially of relapsing-remitting nature, where occasional 'attacks' are followed by periods of remission where no progression of disease

occurs and indeed damage sustained during the attack may be partially or completely repaired (Lublin and Reingold, 1996). The other subtypes of MS; primary or secondary progressive (subsequent to relapsing-remitting disease), and progressive relapsing, are characterized by progressive decline in motor function with infrequent or no remission respectively (Lublin and Reingold, 1996).

Endocannabinoid system changes in human MS

Both CB₁- and CB₂-expressing cells are reported to collect around plaques in human MS (Benito *et al.*, 2007). CB₁-expressing oligodendrocytes, their precursors, and macrophages, have been identified clustered around active lesions, and CB₁-positive perivascular T cells are also evident in MS. CB₂ expression has been localized to plaque-associated microglia and macrophages, nearby astrocytes, and perivascular T cells. FAAH was detected in plaque-associated astrocytes (Benito *et al.*, 2007). In the same study neuronal CB₁ expression was noted to appear more intense in areas of demyelination, although the authors suggested increased CB₁ epitope availability rather than *bona fide* up-regulation.

Interestingly, an up-regulation of CB₁ and CB₂ levels has also been detected in blood sampled from primary progressive MS patients, suggesting that peripheral immune regulation of the endocannabinoid system paralleled that occurring in the human brain (Jean-Gilles *et al.*, 2009).

Endocannabinoid levels in human MS patients have been investigated in several studies. The first of these reported a significant increase in AEA, but not 2-AG levels, in the CSF of relapsing-remitting MS patients experiencing current relapse, with a strong correlation between AEA levels and the number of inflammatory lesions visible on imaging (Centonze *et al.*, 2007). Another recent study also found elevated AEA levels compared with controls in MS patients across the clinical spectrum, this time in the plasma, again suggesting that the peripheral endocannabinoid system may be subject to similar processes to those occurring centrally (Jean-Gilles *et al.*, 2009). In contrast, Di Filippo *et al.* (2008) reported a deficit in all endocannabinoids tested (AEA, 2-AG, PEA and OEA) in the CSF of MS patients compared with controls. Interestingly, despite this underlying hypofunctionality, the authors suggest that the inducible nature of the endocannabinoid system was retained. Compared with patients in remission, endocannabinoid levels were higher (though still below those of controls) in the subset of patients currently in relapse, and higher again in patients with active lesions (Di Filippo *et al.*, 2008). This is in keeping with an earlier report which demonstrated that AEA was released from both silent human MS lesions, and to a greater degree from active lesions (Eljaschewitsch *et al.*, 2006). All three of these studies suggest a relationship between disease state and the regulation of endocannabinoid levels, and demonstrate induction of the endocannabinoid system by inflammatory cues or neuronal activity. FAAH, which is expressed by macrophages (Di Marzo *et al.*, 1999; Di Marzo *et al.* 1998), platelets (Maccarrone *et al.*, 2000) and mast cells (Maccarrone *et al.*, 2000) in blood, showed decreased expression in secondary progressive MS and trended towards a decrease in relapsing-remitting and

primary progressive MS patient blood (Jean-Gilles *et al.*, 2009). Decreased expression of the inactivating enzyme FAAH may underpin the elevations in AEA detected in human blood and CSF described earlier.

Endocannabinoid changes in animal models of MS

In the experimental autoimmune encephalomyelitis (EAE) model, reductions in the order of 25–65% in AEA and 2-AG have been detected in motor (striatum, midbrain) and other brain areas (brainstem, hippocampus and cerebral cortex); and in the midbrain, diencephalon and limbic forebrain respectively (Cabranes *et al.*, 2005). Another group reported no change in brain levels of either endocannabinoid in the same model (Witting *et al.*, 2006). EAE is induced in rats by inoculation with immunogenic myelin components, causing significant inflammation and infiltration of mononuclear cells into the spinal cord, while infiltrated lesions in the brain, as seen in human MS, are rare (Berrendero *et al.*, 2001; Cabranes *et al.*, 2005). It has been suggested that these differences from the pattern of human MS, and the acute nature of EAE, make it a poor model of MS, in which the majority of patients show chronic relapse/remission (Sriram and Steiner, 2005). Indeed, this model may not appropriately recapitulate the acute attack phase either, as the reported reductions in endocannabinoid levels in EAE rats are in conflict with the relapse-induced elevations in endocannabinoids seen in human MS patients.

In the chronic relapsing experimental allergic encephalomyelitis (CREAE) model, induced by sensitizing specific strains of mice, with foreign CNS myelin, AEA and 2-AG were increased in the brain (~19% and ~70% respectively) and spinal cord (~200% and ~70% respectively) of spastic mice compared with non-spastic and control mice (Baker *et al.*, 2001). CREAE mice, like EAE rats, also show a lack of infiltrating lesions of the brain, despite abundant lesions in the spinal cord (Baker *et al.*, 1990). However, findings from the CREAE model are more in keeping with the trends in human multiple sclerosis patients, and together these studies suggest that 'on-demand' production of endocannabinoids may occur in response to relapse/lesion.

In the EAE model the changes in endocannabinoid levels and receptor expression conflict somewhat with those described in the human brain and periphery. A reduction in CB₁ expression in the cerebral cortex and striatum has been detected in EAE rats (Berrendero *et al.*, 2001; Centonze *et al.*, 2007). Interestingly, given the decrease in endocannabinoid levels described above for EAE models, an increase in both the AEA biosynthetic (NAPE-PLD) and inactivation (FAAH) enzymes has been identified in both EAE mouse striatum and in the lymphocytes of humans with current relapse of MS (Centonze *et al.*, 2007). Also of interest, CREAE mice, which more closely recapitulated the endocannabinoid changes seen in the acute phases of human MS than did EAE rats, showed a pattern of CB₁ reduction that was remarkably similar to that in EAE rats. Despite these conflicting patterns one may conclude that: i) that components of the endocannabinoid system are altered in multiple sclerosis and models thereof; and ii) that those components may be altered independently of one another.

Cannabinoid agents as therapeutics in MS

Sativex, an oromucosal spray delivering Δ^9 -THC and cannabidiol, is licensed for use in multiple sclerosis in several countries, following demonstrations of its efficacy as a treatment for symptoms of neuropathic pain and disturbed sleep (Rog *et al.*, 2005; Rog *et al.*, 2007; and reviewed in Wade *et al.*, 2006). It is generally well-tolerated even with long-term use. The large majority of adverse events reported in trials have been mild to moderate and the use of Sativex has not been correlated with any decline in cognitive measures in MS patients (Rog *et al.*, 2007; Aragona *et al.*, 2009; reviewed in Wade *et al.*, 2006; Smith, 2007). Sativex may be useful not only in the control of pain, but also spasticity, as several trials have found. A 2007 study of 189 patients found nearly twice the reduction in spasticity in patients taking Sativex compared with placebo over a 6-week period (Collin *et al.*, 2007). A more recent 6-week Sativex study failed to detect any clinical improvement, alterations to NAPE-PLD or FAAH enzyme activity, or receptor expression; however, significant findings may have been limited by the small scale of the study (20 patients) (Centonze *et al.*, 2009).

Similar findings have emerged from trials delivering synthetic or plant-derived Δ^9 -THC (dronabinol or nabilone respectively). Meta-analysis of three such trials found these cannabinoids to significantly reduce neuropathic and spasticity-induced pain (Karst *et al.*, 2003; Wade *et al.*, 2003; Svendsen *et al.*, 2004; Iskedjian *et al.*, 2007; and see Wissel *et al.*, 2006). Those studies, and a large 15-week clinical trial for oral Δ^9 -THC, found no measurable improvement in motor function or spasticity itself, although Δ^9 -THC-treated patients perceived improvement (Zajicek *et al.*, 2003). A 12-month follow-up to this large trial showed that perceived improvement now correlated with a measurable reduction in spasticity in patients treated with Δ^9 -THC, and to a lesser degree, those treated with cannabis extract (Zajicek *et al.*, 2005).

Cannabinoids and endocannabinoid modulation have also elicited a therapeutic response in the EAE, CREAE and Theiler's murine encephalomyelitis virus (TMEV) models of MS. In the EAE model, the endocannabinoid/endovanilloid agonist arvanil and the AMT inhibitors AM404 and OMDM2 reduced neurological decline, while VDM11 and UCM707, also AMT inhibitors, did not (Cabranes *et al.*, 2005; de Lago *et al.*, 2006). While AM404 and arvanil are agonists at the vanilloid TRPV1 receptor, the protective OMDM2 has virtually no efficacy at TRPV1 (Ortar *et al.*, 2003) and indeed the TRPV1-selective agonist capsaicin was unable to ameliorate the neurological deficit in these mice, indicating that protection more likely occurred via cannabinoid than vanilloid receptors (Cabranes *et al.*, 2005). In the CREAE model, limb spasticity was attenuated by each of the endocannabinoids AEA, 2-AG or PEA, or exogenous cannabinoids or cannabinoid modifiers WIN, AM404, AM374, VDM11, OMDM1, OMDM2 or UCM707 (Baker *et al.*, 2001; de Lago *et al.*, 2006; de Lago *et al.*, 2004). It appears that both CB₁ and CB₂ receptors may have a role in this improved symptomatic profile. While the CB₁-selective antagonist SR141716A more efficiently antagonized the improvement in spasticity and tremor, the CB₂-selective ligand JWH-133 was also able to reduce spasticity, at a dose which was sub-effective via CB₁ (Baker *et al.*, 2000).

In the much-studied TMEV mice, endocannabinoid augmentation has been shown to attenuate inflammatory events such as the differentiation of myelin-specific T cells, the production of pro-inflammatory mediators (TNF- α , nitric oxide, interleukin-1 β and interleukin-6) and the activation and infiltration of microglia, leading to a slowed progression of the experimental disease (Croxford and Miller, 2003; Ortega-Gutierrez *et al.*, 2005). As Ortega-Gutierrez *et al.* (2005) found these effects to be only partially blocked by a combination of selective CB₁ and CB₂ antagonists, additional, non-CB₁/CB₂ receptors may also be involved. A recent study suggests inhibition of the TASK1 channel by AEA may play an important role in the regulation of T cells in EAE (Bittner *et al.*, 2009). Several studies have also implicated the PPAR- γ receptor as contributing to the therapeutic profile of some cannabinoid agonists (Mestre *et al.*, 2009; Loria *et al.*, 2010).

Most of these studies have shown cannabinoids, via CB₂, to control the runaway inflammatory cascade which initiates neuronal damage in MS. However, as suggested by Benito *et al.* (2007), CB₁ may also help to limit excitotoxic damage to neurons, by suppressing both the neuronal release of glutamate and the neuronal depolarization response to glutamate. Maresz *et al.* (2007) attributed the neuroprotective properties of Δ^9 -THC in multiple sclerosis to the activation of both CB₂ receptors expressed by T cells and CB₁ receptors expressed by neurons (Maresz *et al.*, 2007).

In addition to potentially preventing inflammatory and excitotoxic damage in MS, cannabinoids may also have a role in promoting repair of the axonal myelin sheath. Several studies have indicated that cannabinoids, via CB₁ or CB₂ (or both), may regulate myelination in the developing brain (Arevalo-Martin *et al.*, 2007), the normal adult brain (Kittler *et al.*, 2000) and the inflamed brain in the TMEV model of MS (Arevalo-Martin *et al.*, 2003). The increased remyelination seen in the TMEV model may reflect the ability of cannabinoids to reduce inflammatory mediators which retard remyelination processes. Alternatively, the cannabinoids may have a *bona fide* stimulatory effect upon myelination, by enhancing the survival (Molina-Holgado *et al.*, 2002), migration and differentiation towards an oligodendrocyte fate (Arevalo-Martin *et al.*, 2007) of oligodendrocyte progenitor cells in the inflamed brain. If these exciting findings of cannabinoid-mediated attenuation of inflammation, stimulation of remyelination, and behavioural and symptomatic recovery translate from model systems to humans, cannabinoids may be promising therapeutics in MS.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is the third most common neurodegenerative cause of adult death, after Alzheimer's disease and Parkinson's disease (Nicholson *et al.*, 2000). ALS results in the degeneration of motor neurons in the cortex, brainstem and spinal cord (Brown, 1997; Nicholson *et al.*, 2000). Most causes of ALS are presently unknown and several mechanisms of insult to motor neurons have been suggested (Ludolph *et al.*, 2000; Robberecht, 2000; Cleveland and Rothstein, 2001). Two of the primary theories underlying motor neuron vulnerability are susceptibility to excitotoxicity and

oxidative damage, including neuroinflammation (Ludolph *et al.*, 2000; Robberecht, 2000).

Further insights into the aetiology of sporadic ALS, responsible for 90% of the diagnosed cases of the disease, have come from studies of regulation of glutamate activity. Plasma, CSF and post-mortem brain tissue from patients with ALS showed significant increases in glutamate levels (Plaitakis and Carosio, 1987; Rothstein *et al.*, 1990). Thus, glutamate may be the (or one of the) neurotoxic agent(s) reported in serum from ALS patients (Wolfgram and Myers, 1973). Rothstein *et al.* (Rothstein *et al.* 1992) demonstrated impaired glutamate uptake in vesicular preparations from post-mortem spinal cord and motor cortex of ALS patients. They subsequently confirmed a loss of function of EAAT2 expressed exclusively by glia, in the motor cortex and spinal cord of 60–70% of sporadic ALS patients (Rothstein *et al.*, 1995). Importantly, compounds that act on the endocannabinoid system have the potential to reduce excitotoxic and oxidative cell damage as well as neuroinflammation (Hampson *et al.*, 1998; Carter and Rosen, 2001; Walter and Stella, 2004b).

Endocannabinoid system changes in human ALS

To date there have been few studies on the endocannabinoid system in human ALS. Changes to the endocannabinoid system in ALS may reflect the neuroinflammatory component of disease pathogenesis. In the spinal cord of human ALS patients, areas of motor neuron damage were marked by an increased cohort of CB₂-positive microglia/macrophages (Yiangou *et al.*, 2006b).

Endocannabinoid changes in animal models of ALS

Mutations in Cu/Zn superoxide dismutase (hSOD1) are the primary cause of up to 20% of familial ALS cases (Rosen *et al.*, 1993). Over 100 different base-pair substitutions have been documented in human patients to date. It should be noted that familial ALS constitutes only 10% of ALS cases, nonetheless, to date hSOD1 mutations are one of the few aetiologies established for the disease. Until recently, no animal model accurately reflected the pathology of ALS. Now, transgenic mice expressing human SOD1 mutations have been generated (hSOD1G93A, hSOD1G85R, hSOD1G37R) (Gurney *et al.*, 1994; Wong *et al.*, 1995; Bruijn *et al.*, 1997). The three mutant mouse strains have slightly different pathologies; however, they exhibit pathologic and cytologic motor neuron degeneration similar to patients with familial as well as sporadic ALS. Transgenic mice over-expressing wild-type (WT) hSOD1 and mice lacking hSOD1 do not show these disease signs (Bruijn *et al.*, 1998). The hSOD1G93A mice are the strain predominantly used for preclinical testing of compounds for treating ALS; the disease in these animals follows a consistent onset, progression and outcome that closely mimics human ALS (Gurney *et al.*, 1996; Klivenyi *et al.*, 1999; Zhu *et al.*, 2002).

Recent studies have described the involvement of the endocannabinoid system in the progression of disease in ALS mice and the benefits associated with the administration of

cannabinoid agonists (Raman *et al.*, 2004; Witting *et al.*, 2004; Weydt *et al.*, 2005; Bilslund *et al.*, 2006; Kim *et al.*, 2006; Yiangou *et al.*, 2006a; Shoemaker *et al.*, 2007). AEA and 2-AG accumulate in the lumbar spinal cord of ALS mice during disease progression and are presumed to be part of an endogenous defence mechanism (Witting *et al.*, 2004; Bilslund *et al.*, 2006).

Unlike in human tissue, increased CB₂ immunoreactivity was not detected in G93ASOD1 mice; however, there was a pre-symptomatic decrease, followed by a symptomatic increase, in CB₁ mRNA and protein compared with wildtype animals (Zhao *et al.*, 2008). Up-regulation of CB₁ may underpin the findings of a recent study which demonstrated heightened control of both inhibitory and excitatory transmission by striatal CB₁ in symptomatic G93ASOD1 mice (Rossi *et al.*, 2009).

Cannabinoid agents as therapeutics in ALS

Support for a protective role of the endocannabinoid system comes from *in vivo* studies in ALS mice. Interestingly, treatment with Δ^9 -THC was effective if administered either before or after onset of signs in the ALS mouse model (Raman *et al.*, 2004). Administration at the onset of tremors delayed motor impairment by 6% and prolonged survival by 5% in Δ^9 -THC treated mice when compared with vehicle controls. This delay, while modest, is similar to that seen with ceftriaxone (Rothstein *et al.*, 2005), which is in a clinical trial for ALS.

Bilslund *et al.* (2006) found a significant delay in disease progression when another cannabinoid agonist WIN55,212-2 was administered to ALS mice beginning after symptom onset (at 90 days of age); however, survival was not extended. Importantly, functional motor unit survival was enhanced in the cannabinoid-treated animals at 120 days, a relatively late stage in disease; these functional studies were supported by evaluating motor neuron numbers in the treated animals (Bilslund *et al.*, 2006). FAAH knockout mice, which have increased AEA levels due to the lack of its hydrolytic enzyme, crossed with ALS mice also showed improvement in motor neuron survival, supporting the theory that endocannabinoids are neuroprotective in ALS (Bilslund *et al.*, 2006).

Deletion of CB₁ in ALS mice (in the ABH strain background), while not altering motor neuron survival, extended lifespan by 15 days, a 13% increase in survival (Bilslund *et al.*, 2006). It will be important to define the role of CB₂, and the relationship between CB₁ and CB₂, in modifying disease progression in a single standard background.

Microglia from ALS mice possess increased cytotoxic potential (Weydt *et al.*, 2004). CB₂ activation blocks β -amyloid-induced microglial activation (Ramirez *et al.*, 2005). Conversely, with other stimuli, CB₂ activation can increase microglial migration and proliferation (Walter *et al.*, 2003; Carrier *et al.*, 2004). One study using a selective CB₂ agonist, AM1241, in ALS mice showed slowing of disease progression when administered after disease onset (Kim *et al.*, 2006). A second study using a different dosing paradigm found that a dose of 3 mg·kg⁻¹ AM1241 produced a 56% increase in survival interval (an 11% increase in lifespan) (Shoemaker *et al.*, 2007). A recent study in human ALS patients demonstrated

increased CB₂ immunostaining in activated microglia from spinal cord (Yiangou *et al.*, 2006a). These results suggest that CB₂-mediated processes may modify disease progression in ALS.

An important consideration for treatment is that ALS is a chronic disease therefore long-term toxicity of treatment drugs becomes an important issue. Δ^9 -THC is well tolerated and already in clinical usage for nausea associated with cancer chemotherapy and appetite stimulation with the AIDS wasting syndrome. In a pilot study of the safety and tolerability of Δ^9 -THC in ALS patients, symptomatic benefits were seen in insomnia, appetite and spasticity (Gelinis *et al.*, 2002). Other endocannabinoid compounds may have a similar tolerability profile without the psychotropic side effects found with Δ^9 -THC. If they are effective in a pre-clinical model of ALS, they could be evaluated for its effectiveness in the human disease. Cannabinoids may prove to be novel therapeutic targets for the treatment of ALS.

Parkinson's disease

Parkinson's disease (PD) is characterized by muscle rigidity, tremor and a slowing of physical movement (bradykinesia). The primary symptoms are the results of decreased stimulation of the motor cortex by the basal ganglia, normally caused by the insufficient formation and action of dopamine, due to death of dopaminergic neurons of the substantia nigra. Secondary symptoms may include high level cognitive dysfunction and subtle language problems. The neuromodulatory effects of the endocannabinoid system are of particular importance to the dopaminergic system, which in turn exerts reciprocal regulation upon the endocannabinoid system. For example, CB₁ and D1/D2-like receptors are co-localized in striatal neurons (Hohmann and Herkenham, 2000; Hermann *et al.*, 2002) and exhibit complex signalling interactions (Glass and Felder, 1997; Meschler and Howlett, 2001; Kern *et al.*, 2005). Endocannabinoids and FAAH inhibitors influence the firing activity of dopaminergic neurons through PPAR α (Melis *et al.*, 2008) and TRPV1 receptors (Marinelli *et al.*, 2003). Cannabinoid CB₁-mediated effects on dopamine release are complex; AEA has been demonstrated to reduce dopamine release in striatal slice cultures (Cadogan *et al.*, 1997), and to increase dopamine release in the nucleus accumbens *in vivo* (Cheer *et al.*, 2004; Solinas *et al.*, 2006). Likewise, activation of dopamine D2 receptors has been demonstrated to increase AEA levels in the basal ganglia (Giuffrida *et al.*, 1999; Ferrer *et al.*, 2003).

Endocannabinoid changes in human PD

In Parkinsonian tissue the level of CB₁ mRNA has been shown to be decreased in the caudate nucleus, anterior dorsal putamen and external segment of the globus pallidus (Hurley *et al.*, 2003). In contrast to this, an increase in CB₁ binding in the caudate nucleus and the putamen has been observed by others (Lastres-Becker *et al.*, 2001a). These studies are complicated to interpret as all patients have undergone drug treatment, and the effects of drug treatment on the cannabinoid

system are not clear. To date endocannabinoids levels have only been investigated in one study. Pisani *et al.* showed AEA levels in the CSF of PD patients (either untreated or undergoing drug-washout) were more than twice that of controls (Pisani *et al.*, 2005).

Endocannabinoid changes in animal models of PD

Conflicting data exist around the alterations in both cannabinoid receptors and endocannabinoids in models of Parkinson's disease. Increased CB₁ binding and CB₁ mRNA levels have been reported in the brain of MPTP-treated primates (Lastres-Becker *et al.*, 2001a) and in rats with 6-OHDA lesions (Romero *et al.*, 2000), however, others have seen no alteration in receptor levels (Zeng *et al.*, 1999). Decreased (Giuffrida *et al.*, 1999) and increased AEA (Gubellini *et al.*, 2002) have both been observed following 6-OHDA lesion, while dopamine depletion has been shown to elevate 2-AG levels in rat globus pallidus following reserpine treatment (Di Marzo *et al.*, 2000). In MPTP non-human primate models, both AEA and 2AG have been demonstrated to be elevated (van der Stelt *et al.*, 2005). A recent study has suggested that in models of PD, indirect-pathway endocannabinoid-mediated long-term depression is absent but is rescued by a D2 receptor agonist or inhibitors of endocannabinoid degradation, consistent with the suggestion of decreased endocannabinoid tone in 6-OHDA-treated rats (Kreitzer and Malenka, 2007). Interestingly, administration of levodopa, a dopamine precursor that is the mainstay treatment for PD, does not elevate AEA in the basal ganglia of 6-OHDA-treated rats (Giuffrida *et al.*, 1999), but does lead to up-regulation of striatal CB₁ receptors (Zeng *et al.*, 1999) in the dopamine-depleted striatum. Collectively, these observations indicate that degeneration of nigro-striatal projections dramatically affects endocannabinoid transmission although the precise nature of these changes may depend on the model utilized and the timeframe of the observations.

Cannabinoid agents as therapeutics in PD

Studies on the potential therapeutic utility of cannabinoid agonists and antagonists in PD have also produced conflicting results. In several studies of MPTP-treated primates, rimonabant and other cannabinoid antagonists failed to alleviate the motor deficits of parkinsonism (Meschler and Howlett, 2001; Mesnage *et al.*, 2004; Cao *et al.*, 2007), although Cao *et al.* (2007) found increased responses to suboptimal doses of levodopa in the presence of the antagonist. However, in contrast to these studies, motor activity was improved with rimonabant following both MPTP treatment in a primate (van der Stelt *et al.*, 2005) and in a recent 6-OHDA rodent lesion study (Kelsey *et al.*, 2009). This recent study has also found improved responses to low (but not high) levodopa concentrations in the presence of sub-optimal concentrations of rimonabant (Kelsey *et al.*, 2009). Levodopa-induced dyskinesias (LID), a disabling motor complication resulting from long-term use of levodopa, are however, alleviated by activation of CB₁ (Ferrer *et al.*, 2003; Morgese *et al.*, 2009), and

recent data suggest that this is through CB₁-mediated alterations in dopamine and glutamate outputs (Morgese *et al.*, 2009). FAAH inhibitors failed to produce an anti-dyskinetic effect when administered alone in levodopa-treated 6-OHDA-lesioned rats (Morgese *et al.*, 2007), suggesting that AEA elevation is not sufficient to attenuate LID. Interpretation of this finding, however, is complicated by the finding that URB597 in combination with the TRPV1 antagonist capsazepine produced a significant anti-dyskinetic effect, suggesting that the beneficial actions of CB₁ stimulation on LID may be counteracted by TRPV1 agonism (Morgese *et al.*, 2007). Furthermore, Lee *et al.* (2006) showed that URB597 alone or stimulation of TRPV1 receptors by capsaicin can attenuate levodopa-induced hyperactivity in reserpine-treated rats. Whether these discrepancies are due to the different animal models and/or the type of behavioural measure used remains to be clarified.

As for the animal studies, drug trials in humans have produced conflicting results. In a randomized, double-blind, placebo-controlled, crossover trial ($n = 7$), the cannabinoid receptor agonist nabilone significantly reduced LID in PD (Sieradzan *et al.*, 2001). In contrast, in a seventeen patient double-blind, cross-over study, cannabis, while well tolerated, had no effect on LID (Carroll *et al.*, 2004).

Conclusion

An overarching paradigm in the diseases summarized in this review is that hypofunction or dysregulation of the endocannabinoid system may be responsible for some of the symptomatology of these diseases. In Huntington's disease, Alzheimer's disease, as well as in ALS, pathologic changes in endocannabinoid levels and CB₂ expression are induced by the inflammatory environment. Activation of CB₂ by up-regulated endocannabinoids goes some way towards halting microglial activation; however, this innate compensation is insufficient to prevent the subsequent inflammatory damage to neurons, which may also suffer from the loss of protection conferred by the down-regulated CB₁ in HD and AD. In multiple sclerosis, cannabinoids have shown promise in animal models. Furthermore, Sativex, an oromucosal spray delivering Δ^9 -THC and cannabidiol, is licensed for use in multiple sclerosis in several countries, following demonstrations of its efficacy as a treatment for symptoms of neuropathic pain and disturbed sleep. If the exciting findings of cannabinoid-mediated attenuation of inflammation, stimulation of remyelination, and behavioural and symptomatic recovery translate from model systems to humans, cannabinoids may be promising therapeutics in MS.

However, there are conflicting data emerging from animal models of all of the diseases, which highlight the need for better models. In addition, improved technologies for studying human disease as it progresses (rather than just *post-mortem*) will be critical for evaluating the therapeutic potential of any new treatments, including cannabinoids. While alterations in the endocannabinoid system in a range of diseases has led to speculation that the endocannabinoid system is intricately involved in the pathology of these disorders, the extensive cell loss and inflammatory environment that characterizes these diseases makes it difficult to ascertain

whether the alterations are simply the result of the pathological process. Whether integral to the disease, or a symptom of it, the studies described in this review highlight the potential role that endocannabinoids may play in either protecting cells from the disease process, or treating the symptoms of the disease. CB₁ activation has been shown to be effective in limiting cell death following excitotoxic lesions, while CB₂ is involved in dampening inflammatory immune cell response to disease. These two targets may therefore work together to provide both neuroprotection to acute injury and immune suppression during more chronic responses. Modulation of endocannabinoid levels through targeting synthesis or degradation enzymes also holds promise for providing more temporally and regionally appropriate enhancement of cannabinoid activity. Cannabinoid agonists in human trials to date have been well tolerated and safe, but clearly psychoactivity following CB₁ activation is often an unacceptable consequence, particularly for long-term drug treatment; it is hoped that modulation of endocannabinoid levels may provide a more suitable alternative.

Conflict of interest

The authors declare no conflict of interest.

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