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 lead to the medicinal application of cannabis. Indeed, reports of medicinal efficacy of cannabis can been 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, Δ9-tetrahydrocannabinol (Δ9-THC) occurred long before the serendipitous identification of a G-protein coupled receptor at which Δ9-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; Δ8-THC, Δ8-tetrahydrocannabinol; AD, Alzheimer’s disease; AEA, anandamide/arachidonoylethanolamide; 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 hydrolase; GABA, γ-aminobutyric 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, Δ9-tetrahydrocannabinol (Δ9-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 cannabinimetic 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
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., 2008); 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-Miklaszewksa 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 (Byberg 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 ‘endocannabinoindoids’ have been identified; arachidonoyl ethanolamide (anandamide, AEA) (Devane et al., 1992), 2-arachidonoyl glycerol (2-AG) (Mechoulam et al., 1995), O-arachidonoyl ethanolamine (virothamine) (Porter et al., 2002), N-arachidonoyldopamine (NADA) (Huang et al., 2002) and 2-arachidonoyl glycerol 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; Sugita et al., 2000). However, reports of specific mechanisms regulating the synthesis (Stella and Piomelli, 2001) and metabolism (Tsutboi 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; Sudharhar et al., 2009) and more controversially in vitro at CB2 (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; Sugita 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 manner, 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 (García-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 (Egerova et al., 1998; Gulyás 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 (Vandevoorde and
Fowler, 2005; Alexander and Cravatt, 2006; Kaczocha et al., 2006), but not all (Ortiz 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 hypothalamic 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. Intrastriatal 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 (Mangiarnini et al., 1996). Unlike lesion models, the progression of disease in these animals occurs in the absence of overt cell death (Mangiarnini 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 UMC404 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 CB2 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 Δ2-THC. However, the same group had previously reported an exacerbation of malonate lesion with either Δ2-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 quino-linic 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 CB2-null 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 (Spier 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 Δ⁹-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 characterised 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 CB1 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 Δ⁹-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., 1998).
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., 2006). 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; Compton 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). CB2-expressing oligodendrocytes, their precursors, and macrophages, have been identified clustered around active lesions, and CB2-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 tended 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 (Striam 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 CB2 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 CB2 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 Δ⁸-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 Δ⁸-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 Δ⁸-THC, found no measurable improvement in motor function or spasticity itself, although Δ⁸-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 Δ⁸-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-1β 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), CB2 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 Δ⁹-THC in multiple sclerosis to the activation of both CB2 receptors expressed by T cells and CB2 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 (Wolfgang 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 (Hampton 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 (Yangou 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; Buijink 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 (Buijink 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; Bilsland 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; Bilsland 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 CB₂ mRNA and protein compared with wildtype animals (Zhao et al., 2008). Up-regulation of CB₂ may underpin the findings of a recent study which demonstrated heightened control of both inhibitory and excitatory transmission by striatal CB₂ 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 Δ⁹-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 Δ⁹-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.

Bilsland 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 (Bilsland 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 improved survival in motor neuron survival, supporting the theory that endocannabinoids are neuroprotective in ALS (Bilsland 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 (Bilsland et al., 2006). It will be important to define the role of CB₁ and the relationship between CB₁ and CB₂, in modifying disease progression in a single standard background.

Microglia from ALS mice possess increased cytotoxic potential (Woydt 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. Δ⁹-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 Δ⁹-THC in ALS patients, symptomatic benefits were seen in insomnia, appetite and spasticity (Gelinas et al., 2002). Other endocannabinoid compounds may have a similar tolerability profile without the psychotropic side effects found with Δ²-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 (Holtmann and Herkenham, 2000; Herrmann et al., 2002) and exhibit complex signalling interactions (Glass and Felder, 1997; Meschler and Howlett, 2001; Kearn et al., 2005). Endocannabinoids and FAAH inhibitors influence the firing activity of dopaminergic neurons through PPARα (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 (Giulfirda 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 CB₂ 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., 2000). 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 CB1 expression are induced by the inflammatory environment. Activation of CB1 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 oralomucosal spray delivering Δ9-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 intrinsically 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 pathologi-
cal 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 degra-
dation enzymes also holds promise for providing more
temporally and regionally appropriate enhancement of can-
nabinoid activity. Cannabinoid agonists in human trials to
date have been well tolerated and safe, but clearly psychoac-
tivity following CB1 activation is often an unacceptable con-
sequence, 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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