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Cannabinoids and Multiple Sclerosis

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Abstract This review discusses clinical and preclinical evidence that supports the use of cannabinoid receptor agonists for the management of multiple sclerosis. In addition, it considers preclinical findings that suggest that as well as ameliorating signs and symptoms of multiple sclerosis, cannabinoid CB_1 and/or CB_2 receptor activation may suppress some of the pathological changes that give rise to these signs and symptoms. Evidence that the endocannabinoid system plays a protective role in multiple sclerosis is also discussed as are potential pharmacological strategies for enhancing such protection in the clinic.

Keywords Δ^9-tetrahydrocannabinol · Dronabinol · Marinol® · Sativex® · Nabilone · Cesamet® · Multiple sclerosis · Spasticity · Pain · Cannabinoid receptors · Endocannabinoids · Anandamide · 2-arachidonoyl glycerol

Introduction

It is now generally accepted that there are at least two types of cannabinoid receptor, CB_1 and CB_2, both G-protein-coupled [1]. CB_1 receptors are found predominantly at nerve terminals where they mediate inhibition of neurotransmitter release. In contrast, CB_2 receptors are expressed mainly by immune cells, the functions of which include the modulation of cytokine release and of immune cell migration both within and outside the central nervous system. Evidence has also recently emerged that CB_2 receptors are expressed in the brain both by blood vessels [2] and by some neurons [3, 4]. However, the role of these central CB_2 receptors has yet to be established. Cannabinoid CB_1 and CB_2 receptors are targeted by agonists produced in mammalian tissues, this system of receptors and "endocannabinoids" together constituting the endocannabinoid system. The most investigated of the endocannabinoids are *N*-arachidonoylethanolamine (anandamide) and 2-arachidonoyl glycerol. As has been described elsewhere [5–8], these are synthesized on demand, removed from their sites of action by cellular uptake, and degraded by enzymes that include fatty acid amide hydrolase, monoacylglycerol lipase (for 2-arachidonoyl glycerol), cyclooxygenase-2, lipoxygenases, and cytochrome P450. Anandamide and/or 2-arachidonoyl glycerol most probably have both neuromodulatory and immunomodulatory roles that include inhibition of ongoing transmitter release through retrograde signaling [9] and regulation of cytokine release and of immune cell migration. Other ligands that may be endocannabinoids include 2-arachidonylglyceryl ether (noladin ether), *O*-arachidonoylethanolamine (virodhamine), *N*-dihomo-γ-linolenoylethanolamine, *N*-docosatetraenoylethanolamine, oleamide and *N*-arachidonoyl dopamine [8].

Turning now to cannabinoid receptor ligands that are not endogenous, their pharmacological properties have been reviewed recently elsewhere [1, 7, 8]. The "first generation" of these ligands consisted of agonists that interact more or less equally well with CB_1 and CB_2 receptors. Prominent examples of these CB_1/CB_2 agonists are the main psychoactive constituent of cannabis, Δ^9-tetrahydrocannabinol (Δ^9-THC), and the synthetic compounds nabilone, 11-hydroxy-Δ^8-THC-dimethylheptyl (HU-210), CP55940, and *R*-(+)-WIN55212. CB_1- and CB_2-selective ligands, both agonists and antagonists, have also been developed. Agonists include the CB_1-selective ligands, arachidonyl-2′-chloroethylamide (ACEA), arachidonylcyclopropylamide

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(ACPA) and *R*-(+)-methanandamide, and the CB_2-selective ligands, JWH-133, JWH-015, HU-308, and AM1241. As to antagonists, the most widely used of these are the CB_1-selective SR141716A, AM251, and AM281, and the CB_2-selective SR144528 and AM630. These all behave as inverse agonists, one indication that CB_1 and CB_2 receptors can exist in a constitutively active state [10]. As well as having an orthosteric site that is targeted by some of the agonists and antagonists just described, the CB_1 receptor also possesses an allosteric site [11]. The discovery of this site opens up the possibility of developing allosteric ligands for the CB_1 receptor that can enhance or inhibit its activation by exogenously administered or endogenously released direct agonists.

CB_1- and CB_2-selective agonists and antagonists serve as important pharmacological tools for establishing and distinguishing between the physiological and pathological roles of CB_1 and CB_2 receptors. It should be borne in mind, however, that some of these ligands also have non-CB_1, non-CB_2 targets, that anandamide include the vanilloid TRPV1 receptor [7]. As to additional pharmacological tools that are available for investigating the physiological and pathological roles of endocannabinoids, these include inhibitors of the cellular uptake of anandamide and of its metabolism by fatty acid amide hydrolase [8]. Other important strategies for exploring the roles of the endocannabinoid system rely on the availability of mice from which CB_1 and/or CB_2 cannabinoid receptors or fatty acid amide hydrolase have been genetically deleted. Animals that are monoacylglycerol lipase-deficient have yet to be developed. The feasibility of one other potential strategy, to develop animals unable to express an endocannabinoid transporter, is currently uncertain as there is as yet no conclusive evidence for the existence of such a transporter for anandamide and/or for other endocannabinoids [6, 12, 13].

This review begins by discussing the clinical and preclinical evidence that supports the use of cannabinoid receptor agonists for the management of multiple sclerosis. It then goes on to consider preclinical data, which suggest that cannabinoid CB_1 and/or CB_2 receptor activation may not only ameliorate signs and symptoms of multiple sclerosis, but also oppose the underlying causes and progression of this disease. Also discussed are preclinical findings suggesting that the endocannabinoid system plays a protective role in multiple sclerosis and pharmacological strategies by which such protection might be enhanced in the clinic.

Cannabinoid Receptor Agonists and the Management of Multiple Sclerosis

There is evidence from clinical trials that cannabinoid receptor agonists can ameliorate some of the characteristic signs and symptoms of multiple sclerosis (Table 1). Some of these studies have been performed with Δ^9-THC (dronabinol; Marinol®) or nabilone (Cesamet®), a synthetic analogue of Δ^9-THC. These drugs, which were administered orally in capsules, have been licensed for many years as medicines for the suppression of nausea and vomiting provoked by cancer chemotherapy (both drugs) or to stimulate appetite particularly in AIDS patients who are experiencing excessive loss of body weight (Δ^9-THC). Clinical trials have also been conducted with preparations of Δ^9-THC other than Marinol®, with Δ^9-THC hemisuccinate (administered in a rectal suppository), with inhaled cannabis and with cannabis extracts administered either in capsules (e.g., Cannador) or by a pump-action oromucosal spray (e.g., Sativex®) (Table 1). The main cannabinoid constituents of both Cannador and Sativex® are Δ^9-THC and the non-psychoactive plant cannabinoid, cannabidiol [33], the Δ^9-THC/CBD ratios being 2:1 for Cannador and approximately 1:1 for Sativex® (Table 1).

As detailed in Table 1, there have been several clinical trials that have yielded data indicating that cannabis, cannabis extracts, Δ^9-THC, nabilone, and/or Δ^9-THC hemisuccinate can ameliorate perceived spasticity, spasms and pain, and improve quality of sleep. In some studies but not others, significant cannabinoid-induced improvements have also been detected in objective measures of spasticity and mobility as well as in certain other signs of multiple sclerosis; for example, tremor and urinary dysfunction. Failure to detect cannabinoid-induced reductions in spasticity in some studies may well be a reflection of the poor sensitivity exhibited by available objective measures; for example, the Ashworth scoring system. In one study at least [17], such failure may also have stemmed from the use of oral doses of Δ^9-THC or cannabis extract that were too low even to produce any detectable subjective amelioration of spasticity.

The findings obtained in clinical trials are in line with anecdotal data obtained by sending out questionnaires to multiple sclerosis patients self-medicating with cannabis. The first such survey contains responses from 57 male and 55 female patients [34]. Over 90% of these patients who were experiencing the following symptoms reported improvement after taking cannabis: spasticity at sleep onset, pain in muscles, spasticity when waking at night, pain in the legs at night, tremor of arms/head, and depression. Of those patients experiencing other symptoms, the percentage reporting improvement in response to cannabis were

- 81 to 90% for anxiety, spasticity when waking in the morning or when walking, and tingling in face/arms/legs/trunk;
- 71 to 75% for numbness of chest/stomach, face pain, weight loss, and leg weakness;

- 61 to 66% for tiredness, urinary urgency, double vision, and sexual dysfunction;
- 51 to 59% for ability to walk, urinary hesitancy, vision dimness, defecation urgency, balance; urinary incontinence and slurred speech;
- 44% for fecal incontinence;
- 32% for memory loss;
- 30% for constipation.

Claims from multiple sclerosis patients that self-medication with cannabis ameliorates symptoms are also found in a number of more recent anecdotal reports [35–39].

With regard to the safety of pure cannabinoids or cannabis extracts for the management of serious disorders such as multiple sclerosis, there are no major concerns. Thus, the overall conclusion that can be drawn from the results obtained in the clinical trials listed in Table 1 are that such medication rarely produces unacceptable/troublesome adverse events or intolerable side effects in patients with multiple sclerosis. Some side effects of course were observed in these studies. These included

- dizziness/vertigo/lightheadedness [14, 17, 20, 22, 25, 26, 31, 40];
- dry mouth [14, 17, 18, 20, 23, 25, 26, 31, 40];
- mild drowsiness/somnolence/tiredness/sedation [17, 18, 22, 23, 25, 26, 28, 40];
- disorientation, feeling of drunkenness, "high sensation", mental clouding, and/or altered time perception [14, 22, 23, 27];
- impairment of memory or ability to concentrate [14, 26, 31, 40];
- musculoskeletal effects (myalgia, muscle weakness, tremor, balance impairment or lack of coordination) [14, 25, 26, 31, 40];
- nausea/feeling sick and blurred vision [18, 26, 40];
- constipation or diarrhea [20, 22, 26, 40];
- slight increase in heart rate [26];
- confusion, crying, dysphoria, disorientation, paranoia, and hallucinations [23, 26];
- mild withdrawal signs after sudden cessation of cannabinoid treatment [40].

As to which of these side effects occurred most frequently, an indication of this can be obtained by considering the data acquired by Zajicek et al. [20], Wade et al. [22], Svendsen et al. [25], and Rog et al. [26] in their clinical trials with Δ^9-THC or cannabis extracts. These data show the most common side effect to have been dizziness/lightheadedness, which was experienced by 33 to 59% of cannabinoid-treated patients and by 16 to 18% of patients receiving placebo. Corresponding values for some of the other more frequently reported side effects of Δ^9-THC or a cannabis extract are 12 to 26% and 0 to 7%, respectively, for dry mouth, 4 to 42% and 0 to 25%, respectively, for tiredness or fatigue, 9 to 25% and 0 to 20%, respectively, for muscle weakness, 25% and 4%, respectively, for myalgia (muscle pain), and 17% and 8%, respectively, for palpitations [20, 22, 25, 26]. There is evidence, at least for cannabis extracts delivered as an oromucosal spray [22, 23] and for orally administered dronabinol [25], that patients can reduce the incidence or severity of side effects and yet still achieve benefit by downward self-titration of the dose that is taken. There is also evidence that although multiple sclerosis patients do not seem to develop tolerance to the sought-after effects of pure cannabinoid or cannabis extract [18, 22, 23, 26, 31], the incidence of adverse events does decline as the treatment continues [25]. Evidence that tolerance develops more readily to some of the unwanted effects of cannabinoids than to certain sought-after therapeutic effects has also been obtained in experiments with rats [41].

The clinical and anecdotal evidence that cannabinoid receptor agonists can ameliorate motor dysfunction in multiple sclerosis is supported by data obtained from experiments with animal models of this disorder. These are models in which demyelination is induced by injection of mice with Theiler's murine encephalomyelitis virus (TMEV) or inoculation with mixtures containing material such as CNS tissue or myelin basic protein that gives rise to chronic relapsing experimental allergic encephalomyelitis (CREAE) in mice or to experimental autoimmune encephalomyelitis (EAE) in mice, rats, or guinea pigs. Results from these animal experiments indicate that the CB_1/CB_2 receptor agonists Δ^8-THC, Δ^9-THC, *R*-(+)-WIN55212, anandamide, and 2-arachidonoyl glycerol, the CB_1-selective agonists ACEA and methanandamide, and the CB_2-selective agonists JWH-015 and JWH-133 [1] ameliorate the signs of motor dysfunction exhibited by TMEV-infected, CREAE, or EAE animals. Such amelioration has been observed when one or another of these established cannabinoid receptor agonists was administered singly or repeatedly after inoculation for EAE or CREAE, or repeatedly before inoculation for EAE or at the time of and/or after TMEV infection (Table 2). Some of these preclinical studies have employed single measures of motor dysfunction: tremor, spasticity, impaired rotarod performance, and/or indicators of altered spontaneous activity. Others have made use of rating scales that provide a measure of the clinical severity of EAE or of TMEV infection. These are rating scales in which, for example, for rats or mice, 0 indicates no sign of motor dysfunction, 1 indicates mild gait abnormalities, tail flaccidity, or hind limb weakness, 2 or 3 indicates flaccid tail and generalized atonia/ataxia/waddling gait/severe gait abnormalities, 3 or 4 indicates paraparesis (e.g., hindlimb paralysis) or incontinence, 4 or 5 indicates paraparesis and incontinence, 5 or 6 indicates moribundity, and 5, 6, or 7 indicates death [42–46].

Table 1 Clinical trials in which the effects of cannabis, a cannabis extract, or a pure cannabinoid on signs and symptoms of multiple sclerosis have been investigated

Design[§]	Drug treatment	Outcome measures and results			Reference
Positive results		Spasticity	Pain	Other	
Double-blind, placebo-controlled, crossover study (n=8)	7.5 mg Δ^9-THC p.o. (ineffective at 2.5 or 5 mg; 10 mg was intolerable for some patients)	Objective test: no improvement; subjective improvement	–	–	[14]
Double-blind, placebo-controlled study (n=9)	5 or 10 mg Δ^9-THC p.o.	Objective test: improvement	–	Feel better able to walk (n=3)	[15]
Open-label study (n=1)	Inhaled cannabis	Objective test: improvement	–	Objective improvement in mobility, ataxia and hand and finger action tremor	[16]
Randomized, double-blind, placebo-controlled, twofold crossover study (n=16)	2.5 or 5 mg Δ^9-THC p.o. twice daily in Marinol® or cannabis extract	Ashworth scoring system and VAS: no significant improvement	–	–	[17]
Randomized, double-blind, placebo-controlled, crossover study (n=37)	Cannabis extract p.o. (7.5 to 27.5 mg Δ^9-THC per day)	Ashworth scoring system: significant improvement over total trial	–	Significant improvement in Rivermead Mobility Index, spasm frequency, and tremor over total trial	[18]
Open-label study (n=2)	10 or 15 mg Δ^9-THC p.o. or rectal administration of 2.5 or 5 mg Δ^9-THC hemisuccinate	Ashworth scoring system: improvement	Slight pain relief in one patient	Walking ability improved; no effect on micturition frequency	[19]
Randomized, double-blind, placebo-controlled, parallel-group multicenter study (n=206 to 213 per group)	Marinol® or Cannador p.o. twice daily (maximum daily dose of Δ^9-THC was 25 mg)	Ashworth scoring system: no significant improvement; subjective improvement	Pain rating scale: significant analgesia	No significant improvement in Rivermead Mobility Index or in urinary dysfunction; subjective amelioration of spasms; sleep quality and walk time improved[§]	[20]
Randomized, double-blind, placebo-controlled, single-patient crossover study (n=20) (14 patients with MS)	Sativex®, high-Δ^9-THC, low-CBD cannabis extract or low-Δ^9-THC, high-CBD cannabis extract[†]	VAS: improvement in some patients with all extracts	VAS: no significant efficacy for Sativex®; significant efficacy for the other two cannabis extracts	Subjective spasm amelioration by Sativex® and by high-Δ^9-THC extract (VAS); sleep quality improved by Sativex® (VAS)	[21]
Randomized, double-blind, placebo-controlled, parallel-group study (n=80 per group)	Sativex®[†]	Ashworth scoring system: no significant improvement; subjective improvement (VAS)	VAS: no significant analgesia	No subjective amelioration of spasms, tremor or bladder dysfunction (VAS); sleep quality improved (VAS)	[22]
Open-label study (15 patients were evaluated)	Sativex® or high-Δ^9-THC, low-CBD cannabis extract[†]	Subjective improvement (high-Δ^9-THC, low-CBD cannabis extract only)	VAS: significant analgesia (both cannabis extracts)	Significant improvement in sleep quality (high-Δ^9-THC, low-CBD cannabis extract only) and in urinary urgency and day-time frequency, in incontinence, and in nocturia (both cannabis extracts)	[23]
Open-label study (n=1)	1 mg nabilone p.o. twice daily	–	VAS: complete pain relief	–	[24]

Table 1 (continued)

Design[§]	Drug treatment	Outcome measures and results			Reference
Positive results		Spasticity	Pain	Other	
Randomized, double-blind, placebo-controlled, crossover study (n=24)	5 mg Marinol® p.o. twice daily	–	11-point numerical pain rating scale: significant analgesia	–	[25]
Randomized, double-blind, placebo-controlled, parallel-group study (n=34 and 32 per group; 32 patients per group completed study)	Sativex®[††]	–	11-point numerical pain rating scale: significant analgesia	Sleep quality improved (11-point numerical rating scale)	[26]
Single-blind, placebo-controlled study (n=8)	5 to 15 mg Δ^9-THC p.o.	–	–	Subjective improvement in tremor & sense of well-being (n=7); performance in handwriting test improved (n=2); long lasting decrease in head and neck tremor (n=1); little change in mild hand ataxia (n=1)	[27]
Double-blind, placebo-controlled, crossover study (n=1)	1 mg nabilone p.o. every second day	–	–	Subjective improvement in painful muscle spasms, mood and well-being; reduced frequency of nocturia	[28]
Double-blind, randomized, placebo-controlled, study (n=10)	Inhaled cannabis	–	–	Objective impairment but subjective improvement in posture and balance	[29]
Placebo-controlled study	Inhaled cannabis	–	–	Improvement in pendular nystagmus (amplitude but not frequency) and in visual acuity	[30]

The main cannabinoid constituents of both Cannador and Sativex® are Δ^9-THC andCBD, the Δ^9-THC/CBD ratios being 2:1 for Cannador and 1.08:1 for Sativex®.

MS = multiple sclerosis; VAS = visual analogue scale

[†] Administered by oromucosal spray; the maximum permitted 24-hourly dose of each cannabinoid was ca. 120 mg.

[††] Administered by oromucosal spray; the maximum permitted 24-hourly dose was 129.6 mg for Δ^9-THC and 120 mg for CBD.

[§] In a 12-month follow-up study performed with 80% of these patients, the Ashworth scoring system did detect a small but significant amelioration of spasticity in response to Marinol®, although not in response to Cannador [31]. Results from a more recent substudy in which subjects completed incontinence diaries showed that "urge incontinence episodes" were reduced significantly more by Marinol® and Cannador than by placebo [32].

Results from experiments with TMEV-infected or EAE animals suggest that as well as ameliorating unwanted signs of multiple sclerosis, repeated in vivo administration of a cannabinoid receptor agonist may oppose the progression of this disease. Thus, as indicated in Table 3, there are reports that such a drug treatment can reduce signs of EAE inflammation in the spinal cord, reduce demyelination, microglial activation, the T-cell population, and the production of proinflammatory cytokines in the spinal cord after TMEV infection and affect leukocyte trafficking by reducing two leukocyte–endothelial interactions (leukocyte rolling and adhesion) that are thought to contribute to the progression of EAE (and of multiple sclerosis) [45, 56]. It is noteworthy, however, that when administered repeatedly, neither Δ^9-THC (Marinol®) nor cannabis extracts have been found to influence serum cytokine levels in multiple sclerosis patients [57, 58].

It should be noted that there are some important differences between the animal models of multiple sclerosis that have been mentioned in this section. Thus, for example, EAE mice exhibit signs of neurodegeneration and constitute a chronic model of multiple sclerosis, whereas EAE rats exhibit only signs of inflammation and are thought to provide an acute model of this disease [59, 60].

Table 2 Preclinical studies in which motor function has been found to be ameliorated by cannabinoid receptor agonists administered before† or after inoculation for EAE, after inoculation for CREAE or after infection with TMEV

Experimental model	Species	Treatment*			
		Cannabinoid receptor agonist	Effective dose regimen	Outcome measure	Reference
EAE	Rat	Δ^9-THC	5, 10 or 25 mg/kg/day p.o.† 5 mg/kg/day p.o.	8-point rating scale	[42]
EAE	Guinea-pig	Δ^9-THC	5 mg/kg/day i.p.	6-point rating scale	[42]
EAE	Rat	Δ^8-THC	40 mg/kg/day p.o.	7-point rating scale	[43]
EAE	Rat	*R*-(+)-WIN55212	2 mg/kg/day i.p. or 4, then 4.5, 5, 5.5, 6, 6.5 and 7 mg/kg/day i.p. over 9 days	6-point rating scale	[44]
EAE	Mouse	*R*-(+)-WIN55212	10 mg/kg i.p. every 4 days	6-point rating scale	[45]
TMEV-IDD	Mouse	*R*-(+)-WIN55212	20 mg/kg/day i.p. for 6 days§	6-point rating scale	[46]
TMEV-IDD	Mouse	*R*-(+)-WIN55212 ACEA JWH-015	2.5 then 3.75 then 5 mg/kg/day i.p. 1.25 then 1.9 then 2.5 mg/kg/day i.p. 0.6 then 0.9 then 1.2 mg/kg/day i.p.	Rotarod performance	[47]
TMEV-IDD	Mouse	Methanandamide	2.5 then 3.75 mg/kg/day i.p.	Rotarod performance and spontaneous activity	[48]
CREAE	Mouse	Δ^9-THC	1 or 10 mg/kg i.v.¶	Tremor and spasticity	[49, 50]
CREAE	Mouse	*R*-(+)-WIN55212 Methanandamide	1 or 5 mg/kg i.p.¶ 5 mg/kg i.v.¶	Tremor and/or spasticity	[49, 51]
CREAE	Mouse	JWH-133	1.5 mg/kg i.v.¶	Spasticity	[49]
CREAE	Mouse	CP55940 *R*-(+)-WIN55212 RWJ400065	1 mg/kg i.p.¶ 5 mg/kg i.p.¶ 10 mg/kg i.v.¶	Spasticity	[52]
CREAE	Mouse	Anandamide 2-Arachidonoyl glycerol	10 mg/kg i.v.¶ 1 or 10 mg/kg i.v.¶	Spasticity	[53]

CREAE = chronic relapsing experimental allergic encephalomyelitis; EAE = experimental autoimmune encephalomyelitis; TMEV-IDD = Theiler's murine encephalomyelitis virus-induced demyelinating disease

*Unless stated otherwise, treatments were given at the time of and/or following EAE/CREAE inoculation or after infection with TMEV.

† Administered before inoculation for EAE.

§ Administered at the time of infection with TMEV and/or after TMEV infection.

¶ Single injection after inoculation for CREAE.

Cannabinoid Receptor Activation and Multiple Sclerosis

Most cannabinoids that have been found to show efficacy against the signs and symptoms of multiple sclerosis in patients or to ameliorate motor dysfunction or reduce signs of inflammation or demyelination in TMEV-infected, CREAE, or EAE animals ("Cannabinoid Receptor Agonists and the Management of Multiple Sclerosis") have in common the ability to activate cannabinoid receptors. More conclusive preclinical evidence that CB_1 and/or CB_2 receptor activation can indeed reduce signs and symptoms of multiple sclerosis or oppose its progression comes from reports that

- *R*-(+)-WIN55212-induced amelioration of tremor and spasticity in CREAE/EAE mice (Table 2) can be attenuated by the CB_1-selective antagonist SR141716A administered together with the CB_2-selective antagonist, SR144528, albeit at doses that exacerbate tremor and spasticity in these lesioned animals in the absence of *R*-(+)-WIN55212 [49, 51];
- amelioration by *R*-(+)-WIN55212 in EAE mice of tail limpness, hind limb weakness, hind limb paralysis and moribundity (Table 2) and its attenuation in EAE mice of leukocyte rolling and adhesion (Table 3) is opposed by SR144528, although interestingly, not by SR141716A [45];
- neither (1) the ability of *R*-(+)-WIN55212 to ameliorate spasticity in CREAE mice or to decrease motor dysfunction and other clinical disease signs in TMEV-infected mice (Tables 2 and 3) nor (2) its ability to activate cannabinoid CB_1 and CB_2 receptors [61] is shared by the *S*-(-)-isomer of this aminoalkylindole [46, 49];
- $CB_1^{-/-}$ CREAE mice exhibit earlier onset of spasticity, more immobility and residual paresis, and greater neuronal/axonal loss, demyelination, and mortality than wild-type CREAE mice [62, 63];

Table 3 Preclinical evidence that cannabinoid receptor agonists and inhibitors of the cellular uptake of anandamide oppose the progression of multiple sclerosis when administered before† or after inoculation for EAE or after infection with TMEV

Experimental model	Species	Treatment*		Effect	Reference
		Cannabinoid receptor agonist	Effective dose regimen		
EAE	Rat	Δ^9-THC	5, 10 or 25 mg/kg/day p.o.† 5 mg/kg/day p.o.	Reduced inflammmation in spinal cord#	[42]
EAE	Guinea pig	Δ^9-THC	5 mg/kg/day i.p.	Reduced inflammmation in spinal cord	[42]
EAE	Rat	*R*-(+)-WIN55212	4, then 4.5, 5, 5.5, 6, 6.5 and 7 mg/kg/day i.p. over 9 days	Reduced inflammatory cell infiltration of the spinal cord	[44]
EAE	Mouse	*R*-(+)-WIN55212	10 mg/kg i.p. every 4 days	Attenuation of EAE-induced leukocyte rolling and adhesion in cerebral venous microvessels	[45]
TMEV-IDD	Mouse	*R*-(+)-WIN55212	20 mg/kg/day i.p. for 6 days§	Reduced secretion of IFN-γ by T lymphocytes and of expression in spinal cord of mRNA for proinflammatory and antiviral mediators	[46]
TMEV-IDD	Mouse	*R*-(+)-WIN55212	2.5 then 3.75 then 5 mg/kg/day i.p.	Reduced demyelination, microglial activation and number of $CD4^+$ infiltrated T cells within spinal cord	[47]
		ACEA	1.25 then 1.9 then 2.5 mg/kg/day i.p.		
		JWH-015	0.6 then 0.9 then 1.2 mg/kg/day i.p.		
		Uptake inhibitor			
EAE	Mouse	Arvanil	0.5 mg/kg i.p.§§	Reductions in T-cell proliferation and in the production of IFN-γ and IL-10 by T cells	[54]
TMEV-IDD	Mouse	OMDM-1	7 mg/kg/day i.p.	Reduced macrophage/microglial activation in spinal cord	[55]
		OMDM-2	7 mg/kg/day i.p.		
TMEV-IDD	Mouse	UCM707	5 mg/kg/day i.p.	Spinal microglial cells switched from reactive to resting morphology	[48]

EAE = experimental autoimmune encephalomyelitis; IFN-γ = interferon-γ; IL-10 = interleukin-10; TMEV-IDD = Theiler's murine encephalomyelitis virus-induced demyelinating disease

*Unless stated otherwise, treatments were given at the time of and/or following EAE inoculation or after infection with TMEV.

In their EAE experiments (Table 2), Wirguin et al. [43] did not detect any Δ^8-THC-induced changes in inflammation in rat spinal cord or brain.

† Administered before inoculation for EAE.

§ Administered at the time of infection with TMEV and/or after TMEV infection.

§§ Arvanil was administered 3 days before and 3 days after inoculation for EAE.

– signs of apoptosis and of axonal damage associated with IFN-γ-induced demyelination are more marked in cultured brain cells from $CB_1^{-/-}$ mouse fetuses than from wild-type animals [64];

Further evidence that CB_1 and CB_2 receptors can each mediate amelioration of some signs of multiple sclerosis, at least in animal models, comes from the findings, first, that signs of motor impairment exhibited by CREAE mice or by TMEV-infected mice can be reduced both by the CB_1-selective agonists, ACEA and methanandamide, and by the CB_2-selective agonists, JWH-133 and JWH-015 (Table 2), and second, that both ACEA and JWH-015 can reduce demyelination, microglial activation, and the T-cell population in TMEV-infected mouse spinal cord (Table 3). There is also evidence that *R*-(+)-WIN55212 can act, at least in part, through CB_2 receptors to induce apoptosis of encephalitogenic T cells [44], an action that may contribute to its antiinflammatory effect in EAE rats (Table 3). It is noteworthy, however, that Pryce and Baker [52] have recently obtained evidence that it is activation of CB_1 rather than of CB_2 receptors that accounts for the ability of cannabinoids to ameliorate spasticity in CREAE mice. More specifically, they found that the antispastic effects of the CB_1/CB_2 receptor agonists, *R*-(+)-WIN55212 and CP55940, and of the CB_2-selective agonist, RWJ400065, were detectable in wild-type (Table 2), but not CB_1-deficient CREAE mice. Their results suggest that as CB_2-selective agonists all have an ability to activate CB_1 receptors when administered at doses above those required

to activate CB_2 receptors, it is CB_1 receptors through which such compounds act when they ameliorate spasticity in wild-type CREAE mice.

Whereas amelioration of effects of TMEV infection, EAE, or CREAE that are mediated by CB_2 receptors may result from the ability of these receptors to modulate immune cell migration and to inhibit proinflammatory cytokine release and enhance antiinflammatory cytokine release from immune cells [7, 65], amelioration mediated by the CB_1 receptor in these animal models may result at least in part from its ability to attenuate excessive neurotransmitter release when activated and so, for example, protect against excitotoxicity [66]. The findings of Arévalo-Martín et al. [47] described above ("Cannabinoid Receptor Activation and Multiple Sclerosis") suggest that in TMEV-infected mice at least, some amelioration may also result from CB_1 receptor-mediated changes in immune function, a possibility that is supported by the presence of CB_1 receptors in immune cells [65]. Clearly, further research is needed to identify in greater detail the parts that CB_1 and CB_2 receptors each play in cannabinoid-induced modulation of both the signs and the underlying causes of encephalomyelitis in animal models of multiple sclerosis.

The Endocannabinoid System and Multiple Sclerosis

The finding that adult or fetal $CB_1^{-/-}$ mice are more susceptible to neuronal damage than wild-type animals ([62–64], see "Cannabinoid Receptor Agonists and the Management of Multiple Sclerosis"); may be an indication, first, that the endocannabinoid system upregulates in multiple sclerosis, and second that this upregulation has a protective role. Additional support for these hypotheses comes from findings that

- the concentration of anandamide (but not of 2-arachidonoyl glycerol) has been reported to be 3.7 fold higher in lesioned brain tissue taken post mortem from patients with chronic active multiple sclerosis than in autopsied brain tissue obtained from healthy controls and to be 1.9 fold higher in lesioned brain tissue taken from patients with chronic silent multiple sclerosis than in control brain tissue [67];
- at times when they are exhibiting signs of spasticity, CREAE mice have elevated concentrations in their brains and spinal cords of the endocannabinoids, anandamide, and 2-arachidonoyl glycerol [53];

Table 4 Preclinical studies in which motor function has been found to be ameliorated by inhibitors of the cellular uptake of anandamide administered after inoculation for CREAE or EAE or after infection with TMEV

Experimental model	Species	Treatment		Outcome measure	Reference
		Uptake inhibitor	Effective dose regimen		
CREAE	Mouse	AM404*	2.5 and 10 mg/kg i.v.[¶]	Spasticity	[53, 68]
		VDM11	10 mg/kg i.v.[¶]		
		UCM707[§]	0.5 or 5 mg/kg i.v.[¶]		
CREAE	Mouse	Arvanil	0.1 mg/kg i.v.[¶]	Spasticity	[51]
CREAE	Mouse	OMDM-1	5 mg/kg i.v.[¶]	Spasticity	[69]
		OMDM-2	5 mg/kg i.v.[¶]		
CREAE	Mouse	O-2093	0.05 and 1 mg/kg i.v.[¶]	Spasticity	[70]
		O-3246	1 mg/kg i.v.[¶]		
		O-3262	1 mg/kg i.v.[¶]		
EAE	Rat	AM404[#]	5 mg/kg/day i.p.	Spontaneous activity	[59]
		OMDM-2	5 mg/kg/day i.p.		
		Arvanil	1 mg/kg/day i.p.		
EAE	Mouse	Arvanil	0.5 mg/kg i.p.[§§]	8-point rating scale	[54]
TMEV-IDD	Mouse	OMDM-1	7 mg/kg/day i.p.	Spontaneous activity and rotarod performance	[55]
		OMDM-2	7 mg/kg/day i.p.	Spontaneous activity but not rotarod performance	
TMEV-IDD	Mouse	UCM707	5 mg/kg/day i.p.	Spontaneous activity and rotarod performance	[48]

CREAE = chronic relapsing experimental allergic encephalomyelitis; EAE = experimental autoimmune encephalomyelitis; TMEV-IDD = Theiler's murine encephalomyelitis virus-induced demyelinating disease

*Spasticity was also ameliorated by a single injection of the fatty acid amide hydrolase inhibitor, AM374 (1 and 10 mg/kg i.v.).

[¶] Single injection.

[§] UCM707 (5 mg/kg/day i.p.) administered to rats after inoculation for EAE did not ameliorate motor function when this was assessed using a 5-point rating scale [68].

[#] VDM11 (5 mg/kg/day i.p.) administered after inoculation for EAE did not affect spontaneous activity.

[§§] Arvanil was administered 3 days before and 3 days after inoculation for EAE.

- exogenously administered anandamide and 2-arachidonoyl glycerol ameliorate spasticity in CREAE mice (Table 2);
- single or repeated administration of inhibitors of the cellular uptake of anandamide or of anandamide metabolism by fatty acid amide hydrolase reduces signs of motor dysfunction in EAE rats and in TMEV-infected and CREAE mice (Table 4) and signs of the progression in mice of TMEV-induced demyelinating disease and of EAE (Table 3);
- CREAE mice with mild spasticity become more spastic when injected with SR141716A and SR144528 [49];
- repeated administration of SR141716A increases CREAE mouse mortality [63].

The results that have been obtained to date with inhibitors of anandamide uptake or metabolism in experiments with TMEV-infected, CREAE, or EAE rodents and that are summarized in Tables 3 and 4 should be interpreted with particular caution. Thus, as the pharmacological characterization of these inhibitors is far from complete, it remains possible that some or all of these compounds also target other components of the endocannabinoid system when administered at a dose that inhibits cellular uptake. Indeed, there is already evidence from in vitro experiments to suggest that most of the uptake inhibitors that are mentioned in Tables 3 and 4 interact with CB_1, CB_2, and/or TRPV1 receptors at least as potently as they inhibit the cellular uptake of anandamide (Table 5). Evidence already exists that the anandamide uptake inhibitor, AM404, decreases motor dysfunction in EAE rats by direct TRPV1 receptor activation [59]. In addition, it may act as an indirect TRPV1 receptor agonist by inhibiting transporter-mediated removal of anandamide from neurons to cause this endocannabinoid to accumulate at intracellular TRPV1 receptors in a manner that results in an increased anandamide-induced activation of these receptors [59]. In contrast, although the anandamide uptake inhibitor, arvanil, can interact with both TRPV1 and CB_1 receptors (Table 5), there is evidence that it reduces spasticity in CREAE and EAE mice through a mechanism that does not rely on the activation of either of these receptor types [51]. It follows that it will be important to investigate the effects of more selective inhibitors of anandamide uptake in TMEV-infected, CREAE, or EAE rodents and to extend these experiments to selective inhibitors of the anandamide-metabolizing enzyme, fatty acid amide hydrolase, as the effects of such inhibitors in animal models of multiple sclerosis have been little investigated. To further address the hypothesis that endocannabinoids are released in a protective manner in multiple sclerosis, it would also be of interest to investigate the consequences of genetically deleting fatty acid amide hydrolase, monoacylglycerol lipase, or the putative anandamide transporter from TMEV-infected, CREAE, or EAE mice or indeed of inhibiting monoacylglycerol lipase in an animal model of multiple sclerosis.

There is some evidence that the antispasticity effect induced in CREAE mice by the irreversible fatty acid amide hydrolase inhibitor, AM374 (footnote to Table 4), is CB_1/CB_2 receptor-mediated. Thus, just as combined pretreatment with SR141716A and SR144528 has been found to oppose reductions in spasticity induced in CREAE mice by *R*-(+)-WIN55212 ("Cannabinoid Receptor Activation and Multiple Sclerosis"), so too such pretreatment has been reported to attenuate the antispasticity effect of AM374 in these animals [53]. This evidence is weakened by the finding that SR141716A and SR144528 enhance spasticity in CREAE mice when administered together in the absence of other drugs [49]. Even so, it remains possible that

Table 5 Some reported in vitro pharmacological properties of inhibitors of anandamide cellular uptake or of fatty acid amide hydrolase (FAAH), the effects of which have been investigated in animal models of multiple sclerosis

Inhibitor	Uptake inhibition K_i or IC_{50} (μM)	FAAH inhibition K_i or IC_{50} (μM)	CB_1 K_i or IC_{50} (μM)	CB_2 K_i or IC_{50} (μM)	TRPV1 K_i or EC_{50} (μM)	Reference
AM374†	–	0.013, 0.05	0.52	–	–	[71, 72]
AM404	1 to 11	0.5 to 5.9, 22, >30	>1.0*; 1.76*	13*	0.026	[73–78]
VDM11	6.1 to 11.2	1.2 to 3.7, >50	>5* to 10*	>5* to 10*	¶	[76, 78, 79]
Arvanil	3.6	32	1.8*, 2.6*	>15*	0.28*	[80, 81]
UCM707	0.8, 25, 41	30, >100	4.7*	0.067*	>5*	[77, 78]
OMDM-1	2.4*, 2.6, >20	>50*, >100	12.1*	>10	>10	[78, 82]
OMDM-2	3.0*, 3.2, 17	>50*, 54, >100	5.1*	>10	10	[78, 82]
O–2093	17.3	>50	1.29*	2.38*	§	[70]
O–3246	1.4	>50	2.69*	2.18*		
O–3262	2.8	>50	2.02*	1.31*		

*K_i

† Irreversible inhibitor of FAAH [71].

¶VDM11 was almost devoid of TRPV1 agonist activity at 30 μM [76].

§*O*-2093, *O*-3246, and *O*-3262 exhibited only slight TRPV1 agonist activity at 10 μM [70].

AM374 ameliorates spasticity in CREAE mice by causing endocannabinoid accumulation at cannabinoid receptors.

The enhancement of spasticity induced in CREAE mice by SR141716A and SR144528 may be a further indication of an ongoing "protective" release of endocannabinoids onto cannabinoid receptors. However, this is not the only possible explanation. Thus, as these agents are both inverse agonists [10], it could be that they were acting in an endocannabinoid-independent manner by reducing the extent to which cannabinoid receptors were coupling spontaneously to their effector mechanisms. The magnitude of such inverse agonism is influenced by the degree of ongoing spontaneous coupling and this is expected to increase in parallel with any increase in receptor expression level or signaling efficiency [10]. It is noteworthy, therefore, that Berrendero et al. [83] have found CB_1 receptor coupling efficiency to increase in certain brain areas of EAE rats: the superficial layer of the cerebral cortex and the lateral and medial caudate putamen. However, they also detected *decreases* in CB_1 receptor density or CB_1 mRNA levels in these brain areas of the EAE animals. Decreases in CB_1 receptor density, although not in CB_1 mRNA levels, have also been detected in some brain regions of CREAE mice [60]. These changes, which were restricted to some of the areas of the brain that control motor function, were observed either in the acute, chronic, and remission phases of CREAE (globus pallidus) or else just in the acute and chronic phases (cerebellum, lateral caudate putamen). Also detected, albeit only in the cerebellum during the chronic phase of CREAE, were signs of a decrease in CB_1 receptor coupling efficiency. In contrast to CB_1 receptor expression levels, CB_2 receptor expression levels may increase in multiple sclerosis. Thus, signs of such an increase have been detected post mortem in microglial cells/macrophages of the spinal cords of multiple sclerosis patients [84]. Moreover, Maresz et al. [85] have reported that CB_2 but not CB_1 mRNA increases markedly in activated microglial cells and peripheral macrophages harvested from the central nervous system of EAE mice. They also obtained evidence that these increases were triggered by proinflammatory cytokines. No increases in CB_2 mRNA were seen in resting microglial cells or in encephalitogenic T cells of EAE animals.

CREAE mice experience relapsing–remitting paralytic episodes, and, although increases in endocannabinoid levels have been reported to occur in the brains and spinal cords of such animals when they are exhibiting signs of spasticity, no such increases have been detected in nonspastic CREAE remission animals [53]. There have also been reports that anandamide and/or 2-arachidonoyl glycerol levels remain unchanged in the brains of EAE mice [86] that were lesioned in a manner that does not induce remission/relapsing episodes [60] and in the spinal cords of TMEV-infected or EAE mice [55, 85] and, indeed, that levels of both these endocannabinoids decrease in certain brain areas of EAE rats [59]. These rat brain areas were found to be the midbrain for anandamide and 2-arachidonoyl glycerol, the cerebral cortex, hippocampus, caudate putamen and brain stem for anandamide, and the limbic forebrain and diencephalon for 2-arachidonoyl glycerol. Interestingly, there is evidence that the failure to detect any elevation in brain levels of 2-arachidonoyl glycerol in some experiments with EAE mice may be an indication that IFN-γ is being released in the brains of these animals by invading primed T cells in a manner that prevents any detectable 2-arachidonoyl glycerol production by microglia and invading macrophages [86]. Such a mechanism may well also operate in rats, Thus, although the inhibitor of the cellular uptake of anandamide, UCM707, can improve motor function when administered once to CREAE mice or repeatedly to TMEV-infected mice, it has been found to lack detectable activity against motor dysfunction when given repeatedly to EAE rats (Table 4). It should of course also be borne in mind that endocannabinoids are synthesized on demand rather than stored and that the central nervous system contains efficient mechanisms for rapidly removing anandamide and 2-arachidonoyl glycerol from their sites of action after their release ("Introduction"). Consequently, increased endocannabinoid release may well not always be accompanied by any detectable change in the tissue levels of anandamide or 2-arachidonoyl glycerol unless endocannabinoid cellular uptake or enzymatic hydrolysis is compromised either pharmacologically by the administration of an inhibitor, or pathologically. Indeed, although anandamide and 2-arachidonoyl glycerol levels were found by Mestre et al. [55] to be not elevated in the spinal cords of TMEV-infected mice, increases in anandamide but not 2-arachidonoyl glycerol levels were detected in the spinal cords of these animals in response to OMDM-1 and OMDM-2 when one or another of these uptake inhibitors was administered at a dose (7 mg/kg i.p.) that also ameliorated signs of TMEV infection (Tables 3 and 4).

Neuropathic Pain

Because neuropathic pain is often a major symptom of multiple sclerosis, it is important to note that there is evidence that the emergence of this symptom is associated with a protective upregulation of the endocannabinoid system. La Rana et al. [87] found that signs of neuropathic pain exhibited by rats with unilateral chronic constriction injury (CCI) induced by loose ligation of the sciatic nerve can be ameliorated by single or repeated administration of either AM404 or UCM707, both of which inhibit the cellular uptake of anandamide. Costa et al. [88] have shown that repeated (but not single) administration of either

AM404 or its structural analogue, VDM11, can induce antinociception in the same rat model. Similar results with AM404 have been obtained by Palazzo et al. [89] in rat experiments in which it was also discovered that CCI elevates anandamide but not 2-arachidonoyl glycerol levels in the dorsal raphe region of the brain stem. That CCI elevates anandamide but not 2-arachidonoyl glycerol in the rat dorsal raphe has been confirmed by Petrosino et al. [90] who also found CCI to elevate both anandamide and 2-arachidonoyl glycerol in rat spinal cord, periaqueductal gray, and rostral ventral medial medulla. Chang et al. [91] found that signs of neuropathic pain induced in rats by tight spinal nerve ligation can be ameliorated by the reversible fatty acid amide hydrolase inhibitor, OL135, when this is injected once at a dose that also elevates rat brain levels of anandamide. In contrast, Jayamanne et al. [92] found that the irreversible fatty acid amide hydrolase inhibitor, URB597, does not ameliorate signs of neuropathic pain induced in rats by tight partial sciatic nerve ligation when it is administered once at a dose that does relieve signs of chronic inflammatory pain. Also, against the hypothesis that neuropathic pain can be relieved by fatty acid amide hydrolase inhibition is the finding that signs of neuropathic pain induced by sciatic nerve ligation are no less in mice from which fatty acid amide hydrolase has been genetically deleted than in wild-type mice [93]. The extent to which signs of neuropathic pain relief that have been reported to be induced in rats by AM404 and OL135 are mediated by CB_1 or CB_2 receptors is currently unclear. La Rana et al. [87] found AM404 to be antagonized by the CB_1-selective antagonist SR141716A, but not by the CB_2-selective antagonist SR144528. However, Costa et al. [88] found AM404 to be antagonized to the same extent by both these antagonists, and also by the TRPV1 antagonist capsazepine. Palazzo et al. [89] have also reported AM404-induced antinociception in rats with CCI to be antagonized by SR141716A. As to OL135, this was found by Chang et al. [91] to be antagonized by SR144528, but not by SR141716A.

There is evidence that neuropathic pain is associated not only with an increase in endocannabinoid release onto cannabinoid CB_1 and/or CB_2 receptors, but also with an increased expression of these receptors [94–97]. Thus, expression levels of cannabinoid receptors have been reported to increase in rat models of neuropathic pain in dorsal root ganglion neurons (CB_1) and in the thalamus (CB_1) and spinal cord (CB_1 and CB_2). For CB_1 receptors, this increase presumably takes place on neurons not only in the dorsal root ganglion [97], but also in the thalamus and spinal cord [94, 95], whereas for CB_2 receptors it most probably occurs on activated microglia that have migrated into the spinal cord [96]. It will be important to establish whether such upregulation of CB_1 and/or CB_2 receptors is associated with neuropathic pain in man and, if it is, whether this upregulation is limited largely or entirely to sites at which cannabinoid receptors mediate symptom relief. It will be important to establish whether such upregulation of CB_1 and/or CB_2 receptors is associated with neuropathic pain in man and, if it is, whether this upregulation is limited largely or entirely to sites at which cannabinoid receptors mediate symptom relief. Thus, upregulation of this kind would be expected to improve the benefit-to-risk ratio of a cannabinoid receptor agonist by increasing the potency with which it produces analgesia without affecting the potency with which it produces unwanted effects. It is also expected to produce a selective augmentation of the maximal degree of analgesia that can be produced particularly by a partial agonist such as Δ^9-THC and so favor the use of a cannabinoid receptor partial agonist over a full agonist for the management of neuropathic pain. In line with this hypothesis is a recent report that the CB_1-selective agonist, ACEA, exhibited enhanced efficacy as an antinociceptive agent when administered directly into the inflamed hind paws of rats after peripheral inflammation had apparently caused primary afferent neurons to express an increased number of CB_1 receptors [98].

Summary and Future Directions

In conclusion, there is evidence both from clinical trials and from experiments with animal models of multiple sclerosis that signs and symptoms of this disease can be ameliorated by cannabinoid receptor agonists at doses that do not provoke unacceptably severe adverse events. Evidence has also emerged from animal experiments that CB_1 and CB_2 receptor agonists target some of the pathological events responsible for the clinical signs that characterize TMEV-infection, CREAE, and EAE. These signs may well include motor dysfunction, as it is possible that cannabinoids ameliorate this in one or more of these experimental models at least in part by reducing inflammation within the central nervous system that is giving rise to conduction block through the production of edema. However, it is still unclear whether cannabinoid receptor agonists oppose the progression of multiple sclerosis in patients with this disease or whether they simply lessen the severity of particular signs and symptoms. There is also still some confusion in the literature as to the relative importance of CB_1 and CB_2 receptor activation in the attenuation by exogenously administered or endogenously released cannabinoids both of signs of CREAE, EAE, and TMEV infection in animals and of the underlying pathology. Consequently, there is a need for further research which might, for example, extend investigations already carried out with $CB_1^{-/-}$ CREAE mice to experiments with TMEV-infected, CREAE, or EAE mice

from which CB_2 or both CB_1 and CB_2 receptors have been genetically deleted. Experiments with such animals would of course, in addition, help to address the question of whether cannabinoids ameliorate encephalomyelitis or TMEV infection to any significant degree by interacting with targets other than CB_1 or CB_2 receptors.

There is also some evidence that endocannabinoid release increases in TMEV-infected, CREAE, or EAE animals in a manner that ameliorates the characteristic signs of motor dysfunction exhibited by these animals and probably also the pathological changes that give rise to this motor dysfunction. Much of this evidence comes from experiments that have demonstrated that such amelioration can be induced by drugs that have in common the ability to delay removal of anandamide from its sites of action by inhibiting its cellular uptake. Further research is now required to establish

- the relative extent to which anandamide and 2-arachidonoyl glycerol are released in a protective manner in animal models of multiple sclerosis as this in turn would give some indication as to whether this disease might best be managed by drugs that inhibit anandamide uptake and/or metabolism, by drugs that inhibit 2-arachidonoyl glycerol uptake and/or metabolism, or by a combination of these drugs;
- the relative extent to which each of the growing number of putative endocannabinoids other than anandamide and 2-arachidonoyl glycerol protects against multiple sclerosis or its signs and symptoms;
- whether, when developed, drugs that target the CB_1 allosteric site ("Introduction") as enhancers ameliorate motor dysfunction and/or the underlying pathological changes in TMEV-infected, CREAE, or EAE animals;
- whether multiple sclerosis patients release endocannabinoids in a protective manner such that inhibitors of endocannabinoid uptake or metabolism, or CB_1 allosteric enhancers, would oppose the progression of this disease and/or ameliorate its signs and symptoms.

Given the evidence that *R*-(+)-WIN55212 reduces inflammation in TMEV-infected mice (Table 3), a recent report that this cannabinoid induces both COX-2 protein upregulation in cultures of TMEV-infected murine brain endothelial cells and PGE_2 release from these cells and that it does this in a manner that is both CB_1 and CB_2 receptor-independent [99] also merits further investigation.

In some investigations, no increases in anandamide or 2-arachidonoyl glycerol levels have been detected in the brains of EAE animals. Although this may simply be a reflection of the efficiency with which these endocannabinoids are metabolized after their release, it could also be an indication that endocannabinoid release is never or only sometimes triggered by multiple sclerosis. Such a situation would of course strengthen the case for managing this disorder in the clinic with directly acting cannabinoid receptor agonists rather than with agents that act less directly by augmenting the levels or actions of released endocannabinoids.

Finally, since the completion of this review, further preclinical evidence has emerged supporting the hypotheses first, that activation of CB_1 receptors on central neurons will ameliorate clinical signs of multiple sclerosis such as spasticity, second, that activation of CB_2 receptors expressed by T cells within the central nervous system will decrease inflammation in multiple sclerosis and possibly also slow progression of the disease, and third, that CB_2 receptor activation reduces leukocyte trafficking into inflamed tissue [100, 101].

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