Drugs of the Future 2007, 32(4): 341-351

CCC: 0377-8282/2007
DOI: 10.1358/dof.2007.032.04.1083482

Review Article

The endocannabinoid system for the development of new drugs for spasticity

Vincenzo Di Marzo
Endocannabinoid Research Group, Institute of Biomolecular Chemistry, Consiglio Nazionale delle Ricerche, Via Campi Flegrei 34, Pozzuoli, Naples, Italy. Correspondence: vdimarzo@icmib.na.cnr.it

CONTENTS

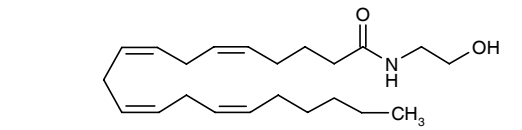
Abstract

Recent clinical trials have highlighted the possibility that agents that activate cannabinoid CB_1 receptors, or extracts of *Cannabis sativa*, might have beneficial effects on symptoms of multiple sclerosis (MS). In particular, spasticity and neuropathic pain, two major debilitating consequences of this serious neurodegenerative disorder, appear to be improved following treatment with these pharmaceutical preparations. Furthermore, agents capable of activating the two subtypes of cannabinoid receptors, either directly or indirectly, exhibit palliative effects, and in some cases delay disease progression, in experimental models of MS. In one such model, chronic-relapsing experimental allergic encephalomyelitis (CREAE) in mice, compounds inhibiting the processes leading to inactivation of endogenous cannabinoid receptor ligands exhibit strong antispastic effects in the chronic phase of the disorder. The role of the endocannabinoid system (*i.e.*, cannabinoid CB_1 and CB_2 receptors and their endogenous ligands, the endocannabinoids) in MS is discussed in this article, together with the possible future use of endocannabinoid-based drugs for spasticity associated with MS and other neurological conditions.

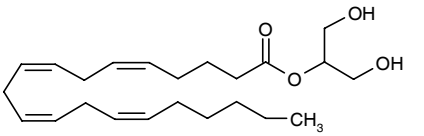
Introduction

By definition, endocannabinoids are endogenous mediators that act by binding to and activating the cannabinoid receptors CB_1 and CB_2, the two molecular targets for the *Cannabis sativa* psychoactive principle Δ^9-tetrahydrocannabinol (THC) (1). *N*-Arachidonoylethanolamine (AEA, anandamide) and 2-arachidonoylglycerol (2-AG) (Fig. 1) are the two most extensively studied endocannabinoids. It is now well established that these compounds act as neuromodulators in the nervous system, while they act as autocrine or paracrine regulators of the homeostasis of other chemical mediators in peripheral tissues. Of the two endocannabinoids, 2-AG is the most abundant in most tissues, and is also effective and selective at both CB_1 and CB_2 receptors *versus* other molecular targets. AEA, on the other hand, is not very effective at the CB_2 receptor and appears to act on other targets, of which the transient receptor potential vanilloid type 1 (TRPV1) channel, expressed in both sensory and central neurons, is probably the best characterized (2).

Both AEA and 2-AG are biosynthesized “on demand” and released from cells immediately after their production



Anandamide (AEA)



2-Arachidonoylglycerol (2-AG)

Fig. 1. Chemical structures of anandamide and 2-arachidonoylglycerol, the two most extensively studied endocannabinoids.

(3). These events are triggered by the enhancement of intracellular Ca^{2+} concentrations that follows cell depolarization, the mobilization of intracellular Ca^{2+} stores subsequent to stimulation of $G_{q/11}$ protein-coupled receptors, or both (4-10). However, the levels of the two endocannabinoids are in many cases modulated in different, and sometimes even opposing, ways via the modulation of the activity and/or expression of biosynthetic or degrading enzymes, or both (3). AEA is produced from the processing of *N*-arachidonoylphosphatidylethanolamine (NArPE), which in turn is obtained from the enzymatic transfer of arachidonic acid esterified on the *sn*-1 position of phospholipids to the nitrogen atom of phosphatidylethanolamine, via an as yet unidentified *N*-acyltransferase. The Ca^{2+}-sensitive enzyme catalyzing the one-step conversion of NArPE and other *N*-acylphosphatidylethanolamines (NAPEs) to AEA and other *N*-acylethanolamines is the same and is termed NAPE-selective phospholipase D (NAPE-PLD). However, alternative pathways have been identified recently that can transform NArPE into AEA.

In contrast, 2-arachidonate-containing diacylglycerols are the most frequent biosynthetic precursors of 2-AG (3). They are produced from the hydrolysis of phosphoinositol bis-phosphate, catalyzed by the PIP_2-selective phospholipase C (PLC), or in some cases from the hydrolysis of phosphatidic acid, catalyzed by a phosphohydrolase. DAGs are then converted to 2-AG by the action of two Ca^{2+}-sensitive plasma membrane *sn*-1-selective DAG lipases, DAGLα and DAGLβ. DAGLα is more abundant in the adult brain and DAGLβ in the developing brain. Both enzymes are co-localized with CB_1 receptors in neuronal axons of the perinatal nervous system and "move" to postsynaptic neurons in the adult brain.

AEA is inactivated through intracellular enzymatic hydrolysis to arachidonic acid and ethanolamine. The enzyme catalyzing this reaction was termed "fatty acid amide hydrolase" (FAAH), as it recognizes other long-chain fatty acid amides as substrates, including *N*-acylethanolamines, primary amides, *N*-acylamino acids and *N*-acyltaurines. FAAH also efficiently catalyzes the hydrolysis of long-chain fatty acid esters, including 2-AG. Whether or not FAAH degrades 2-AG constitutively is still controversial. Selective FAAH inhibitors produce a significant elevation of 2-AG tissue levels under certain conditions of administration and in certain tissues. However, monoacylglycerol lipase (MAGL) enzymatic activities also inactivate 2-AG. An MAGL was recently cloned from the rat and evidence for its role in 2-AG degradation in isolated cells was provided by the use of "silencing RNA" techniques. The cloned MAGL seems to account for only 50% of the total 2-AG-hydrolyzing activity in soluble fractions of rat brain, and pharmacological evidence for the existence of other MAGL isoforms has been provided. The cloned MAGL recognizes both *sn*-1- and -2-acylglycerols as substrates, with almost any unsaturated long-chain fatty acid esterified to the glycerol backbone, but is inactive with fatty acid amides. It is distributed in the CNS in the same brain regions as CB_1 receptors and is a presynaptic enzyme, in agreement with the necessity of inactivating 2-AG acting as a retrograde signal. However, our current knowledge of the role of MAGL is still limited due to the lack of a "knockout" mouse for this enzyme and of selective inhibitors (3).

AEA and 2-AG are transported across the membrane in order to interact with cannabinoid receptors (released following *de novo* biosynthesis) or intracellular hydrolyzing enzymes (reuptake following action at receptors) (3). It is still being debated whether or not endocannabinoid uptake by and release from cells occur via a membrane transporter. Intracellular degradation of AEA by FAAH was suggested to be the only drive behind its cellular uptake via simple passive diffusion. However, strong indirect evidence exists for specific proteins facilitating the membrane transport of both AEA and 2-AG according to the gradient of concentrations across the plasma membrane. Experiments carried out using cells from FAAH knockout mice, confocal microscopy to assess the spatial and functional separation between AEA uptake and hydrolysis, and synthetic compounds capable of distinguishing between FAAH and proteins responsible for the cell membrane binding/cellular uptake of AEA, support the existence of one or more specific proteins for AEA transport. Nevertheless, no such protein has been cloned to date.

"On demand" or "long-term" regulation of endocannabinoid levels

Regulation of the tissue concentrations of endocannabinoids and the nature of the signal that they convey are strongly determined by their chemical nature. A typical example is provided by the role proposed for these mediators in synaptic plasticity. In fact, endocannabinoids are biosynthesized and released from postsynaptic neurons to activate presynaptic cannabinoid CB_1 receptors, which, in most cases, reduce the release of both excitatory (*e.g.,* glutamate) and inhibitory (*e.g.,* GABA) neurotransmitters via inhibition of voltage-activated calcium channels, thereby modulating the strength of neuronal synapses in both the short and long term (11). This "retrograde" signaling has been suggested to participate in several physiological functions of the brain, such as the control of food intake and habit-forming and mnemonic processes, but also in pathological situations, such as the control of excitotoxicity. Since the phospholipid biosynthetic precursors for endocannabinoids appear to be ubiquitous in membranes, it is the relative pattern of expression of Ca^{2+}-sensitive endocannabinoid-biosynthesizing enzymes and cannabinoid receptors that determines the specificity of endocannabinoid action. The localization of the degrading enzymes, however, determines its duration. In support of this mode of action, at least for 2-AG, recent studies showed that the enzyme catalyzing the last step in the biosynthesis of this compound, DAGLα (12), is expressed in postsynaptic dendritic "spines" establishing synapses with CB_1-expressing axon terminals (13, 14). Furthermore, MAGL is located in

presynaptic neurons, thus allowing for the immediate inactivation of the endocannabinoid signal (15-17). Elegant experiments established that endocannabinoid retrograde action can distinguish, as targets, between neighboring CB_1-expressing glutamate- and GABA-releasing neurons (18, 19).

Intracellular Ca^{2+} concentrations are frequently elevated in nervous tissue under pathological conditions, where they activate a cascade of biochemical events leading to cell damage, further entry of Ca^{2+} and cell death. Therefore, it is very likely that the "on-demand" biosynthesis and release of endocannabinoids are a hallmark of neuropathological conditions. In this case, local activation of cannabinoid receptors can counteract not only the cause of Ca^{2+} elevation, such as, for example, excessive excitatory signaling in the CNS (20, 21), but also its subsequent effects, such as neuroinflammation and gliosis, for example by downregulating the activity of microglial and glial cells (22) (see below). In the case of chronic or degenerative conditions, sustained elevation of endocannabinoid levels can be achieved by long-term modification of the expression of genes encoding endocannabinoid metabolic enzymes, for example by: 1) overexpressing the above-mentioned DAGLα (such as following β-amyloid-induced neurotoxicity [23]); or 2) by downregulating FAAH (which catalyzes AEA and 2-AG hydrolysis), the cellular reuptake of endocannabinoids, or both (such as in animal models of Parkinson's disease or in HIV-induced neurotoxicity [24-27]). Stimulation of purinergic $P2X_7$ receptors was shown to lead to sustained 2-AG formation in microglia via simultaneous elevation of DAGLα and decrease in MAGL activity (4), and interferon gamma-induced DAGLα downregulation was recently suggested to disrupt this process and cause neuronal damage during experimental allergic encephalomyelitis (EAE) in mice (28), an animal model of multiple sclerosis (MS).

The endocannabinoid system controls excitotoxicity in acute neurological conditions

Certain acute pathological conditions of the CNS offer ideal examples of the "on-demand" regulation of endocannabinoid biosynthesis (29). In the hippocampus, glutamate-induced neuronal depolarization and/or $G_{q/11}$-mediated intracellular Ca^{2+} mobilization cause excitotoxicity, along with transient elevations in the levels of endocannabinoids, which can then be released and act retrogradely to inhibit glutamate release from principal neurons (19), thus dampening neuronal excitability (9, 20, 30, 31) and inducing the expression of early neuroprotective genes (9, 20, 32). These phenomena, which have been studied *in vitro* in hippocampal cultures (33, 34), might occur during epileptic seizures or during other neurological conditions, such as ischemia (21). The endocannabinoid involved might depend on the type of noxious stimulus used and the brain area under examination, with: 1) both AEA and 2-AG being involved in kainate-induced excitotoxicity in the hippocampus (9); 2) only 2-AG being elevated during ischemia-induced excitotoxicity of dopaminergic neurons in the ventral tegmental area both *in vitro* and *in vivo* (21); and 3) only AEA and its acylethanolamide congeners being elevated in whole brain during transient or permanent middle cerebral artery occlusion (35, 36) or intracerebral NMDA injection (37) in rats. In the latter case, elevated levels of the biosynthetic precursors of AEA and other acylethanolamides were also observed, and receptors other than CB_1 were suggested to participate in the neuroprotective action of these compounds.

Excitotoxic brain lesions initially result in primary destruction of brain parenchyma, which attracts macrophages and microglia. These inflammatory cells release toxic cytokines and free radicals, resulting in secondary neuronal damage, which also occurs during several neurodegenerative conditions, including MS. Using an experimental model of microglia-mediated neuroinflammation in the brain, the endocannabinoid system was found to be highly activated during CNS inflammation. In living brain tissue, a 2.8-fold increase in AEA concentrations after induction of excitotoxic damage by NMDA was potentiated up to 13.2-fold after invasion of BV-2 microglial cells. AEA was shown to protect neurons from inflammatory damage by CB_1 and CB_2 receptor-mediated rapid induction of mitogen-activated protein kinase phosphatase-1 (MKP-1) in microglial cells, associated with histone H3 phosphorylation of the *mkp1* gene sequence. As a result, AEA-induced rapid MKP-1 expression switched off MAPK signal transduction in activated microglial cells. The authors suggested that "the release of AEA in injured CNS tissue might represent a new mechanism of neuroimmune communication during CNS injury, which controls and limits immune response after primary CNS damage" (38).

The endocannabinoid system is neuroprotective in animal models of MS

Multiple sclerosis (MS) is a relatively common neurodegenerative disorder, the exact etiology of which is still not entirely understood, but which has strong autoimmune and neuroinflammatory components. Both components lead to nerve demyelinization and subsequent malfunctioning of neuronal firing and excitotoxicity, which eventually cause motor disturbances such as tremors, paralysis and limb spasticity, as well as neuropathic pain. Furthermore, since overexcitation of nerves from electrical activity within an inflammatory environment can cause neurodegeneration (39), factors that dampen excessive neuronal activity are also neuroprotective. As outlined above, endocannabinoids possess both neurotransmitter-inhibitory and antiinflammatory actions, and hence appear to be ideal endogenous candidates to afford neuroprotection from "inside" (40).

Although MS is clearly a neurodegenerative disorder, it is characterized by alternating acute phases of the most typical overt symptoms, particularly paralysis. This typical pattern of symptoms can be simulated in mice with the

model known as chronic-relapsing experimental allergic encephalomyelitis (CREAE), which is different from the various EAE models used to mimic mostly the inflammatory aspects of human MS (see below). In this model, a particular strain of mice, the ABH Biozzi mouse, is injected subcutaneously in the flank with murine spinal cord homogenate in complete Freund's adjuvant (containing 60 µg *Mycobacterium tuberculosis* H37Ra and *Mycobacterium butyricum* [4:1] per injection). After an "incubation phase", attacks of paralysis alternate with remission periods. In the late, chronic phase of the disorder, spasticity is observed during remission, normally after 3-4 episodes of paralysis. Using this model, it has been possible to demonstrate that activation of cannabinoid CB_1, but not CB_2, receptors reduces spasticity during the acute phase of this late symptom (41, 42). More importantly, CB_1 receptor "knockout" mice with CREAE exhibit significantly greater spasticity and susceptibility to neurodegeneration than wild-type mice with this disorder, as do mice treated with CB_1 receptor antagonists (41-43). There is an increased incidence (> 5%) of CREAE-induced mortality in CB_1-deficient mice and also in heterozygotes which have reduced CB_1 expression. More strikingly, CB_1-deficient mice exhibited significantly more immobility and residual paresis and axonal pathology than wild-type mice following recovery after the first paralytic episode. These animals relapse and accumulate more deficits, which rapidly reach an unacceptable severity limit, including the development of permanent hindlimb paralysis. Consistent with the enhanced neurodegeneration after a single attack, spasticity developed earlier in CB_1-null mice with CREAE (43). These findings are strongly suggestive of a possible enhanced endocannabinoid tone during CREAE aimed at counteracting the neurological consequences of this disorder. Indeed, in mice with CREAE, both AEA and 2-AG levels in the spinal cord are highest during the spasticity phase and return to basal levels when hindlimbs are no longer spastic (44). The function of this short-term form of endocannabinoid spasticity is clearly to reduce this symptom, since systemically administered endocannabinoids and inhibitors of endocannabinoid cellular reuptake and enzymatic hydrolysis significantly ameliorate spasticity in CREAE mice (41, 44-47), in some cases also acting via non-CB_1, non-CB_2, non-TRPV1 receptors (48).

Dynamic changes in the endocannabinoid system also occur in the brain of CREAE mice, where, however, only minor increases in endocannabinoid levels are observed and only during spasticity, but not relapse (44). Instead, a moderate decrease in the density of CB_1 receptors in the striatum was observed during the acute phase of CREAE. This reduction disappeared during the remission phase, but was again observed in the chronic phase to a more marked extent. The same pattern for CB_1 receptor density was observed in the cerebellum, and, in this case, was accompanied by a progressive decrease in the ability of these receptors to activate GTP-binding proteins, a phenomenon that becomes more dramatic in the chronic phase. The decrease in the density of CB_1 receptors in the acute phase was also found in the globus pallidus, but in this case, the reduction was maintained during the subsequent phases. No changes were observed in CB_1 receptor mRNA levels in any of the different regions examined. Finally, in contrast with the observations in motor structures, the status of CB_1 receptors remained unaltered in cognition-related regions, such as the cerebral cortex and hippocampus, during the different phases of CREAE (49). Thus, brain CB_1 receptors are affected by the development of CREAE with down-regulated responses that are limited to motor-related regions and generally more marked during the chronic phase, when spasticity appears during remission.

A different scenario is found in the brains of non-CREAE animal models of MS, where changes in endocannabinoid and cannabinoid receptor levels have been demonstrated. The acute neuroinflammatory component of MS can be best studied using rats or mice with EAE induced by autoimmune activation (*e.g.,* with complete Freund's adjuvant containing myelin basic protein [MBP] and *Mycobacterium* or proteolipid protein peptide) or by Theiler's murine encephalomyelitis virus (TMED) infection. In these models, the levels of endocannabinoids in the brain were found to be either reduced (50) or unchanged (28) in the chronic phase of the disorder when paralysis appears, whereas, unlike the CREAE mouse model, no change was found in the spinal cord (51, 52). A decrease in endocannabinoid levels and CB_1 receptor expression in the striatum was suggested to underlie the motor impairment typical of EAE rats (50). In fact, reduction of endocannabinoid-mediated long-term depression of the indirect pathway of motor control from the striatum to the external layer of the globus pallidus was recently suggested to be associated with motor impairment (53). In EAE mice, however, the absence of changes in endocannabinoid levels reported in another study was explained by the fact that high brain levels of interferon gamma associated with EAE inhibit purinergic $P2X_7$-induced stimulation of 2-AG biosynthesis in microglial cells (28). In EAE mice, plant-derived and synthetic cannabinoids had previously been shown to reduce both the symptoms and the progression of EAE by interfering with the release of inflammatory cytokines (54), microglia/macrophage activation (55) and leukocyte/endothelial cell interactions (56), thus suggesting the involvement of both CB_1 and CB_2 receptors in the potential protection afforded by endocannabinoids against neuroinflammation. In fact, CB_2 receptor stimulation was recently shown to contribute to endocannabinoid inhibition of EAE progression and interferon gamma-induced inflammatory responses (51, 57, 58), and the expression of this receptor was found to be significantly upregulated in activated microglial cells of the spinal cord of EAE mice (52). Interestingly, CB_2 receptors were also suggested to participate in the antiexcitotoxic effects of the cannabinoid agonist HU-210 in mice with EAE induced by TMEV (59).

In summary, the endocannabinoid system appears to participate in EAE in at least three ways, *i.e.*, by being: 1)

activated, particularly in the spinal cord during spasticity (as demonstrated in studies with CREAE mice) in order to counteract excessive muscle contraction and perhaps neuronal excitotoxic damage, possibly by regulating excitatory neurotransmitter release at the presynaptic and prejunctional level; 2) defective at the brain level, as one of the possible underlying causes of motor impairment (as observed in EAE mice or rats); and 3) activated at the level of immunocompetent cells resident in the spinal cord and participating in the inflammatory component of MS in order to dampen inflammation (as observed in mice with EAE). This multifunctional protective role of endocannabinoids suggests that elevation of endocannabinoid levels by endocannabinoid uptake inhibitors might produce beneficial effects not only on the symptoms but also on disease progression, as has been observed in several EAE models (47, 51, 57). Activation of TRPV1 receptors appears to be involved in the protective effect of these compounds (50), and this might explain why the "hybrid" CB_1 agonist, endocannabinoid uptake inhibitor and TRPV1 agonist arvanil strongly reduces the progression of EAE in mice (60).

The endocannabinoid system in other disorders causing spasticity

Amyotrophic lateral sclerosis (ALS) and spinal cord injury (SCI) are examples of irreversible chronic disorders affecting peripheral nervous tissues more directly than the brain, and often resulting in spasticity and pain. In these disorders, stimulation of either CB_1 or CB_2 receptors can provide therapeutic effects. In the case of ALS, which is caused in humans by degeneration of either upper or lower motor neurons, all studies performed so far have been carried out in the superoxide dismutase (*SOD1*) mutant mouse model, which, although quite different from the human condition, exhibits several of the features of this untreatable disorder causing degeneration and death of motor neurons. Two reports have shown long-term elevation of endocannabinoid levels in the spinal cord (61) and both spinal cord and brain (62) with increasing age in *SOD1* G93 mutant mice. In the latter study, it was also shown that congenital FAAH knockout results in prolonged survival in these mice. Importantly, CB_1 receptor knockout also increased survival, thus indicating that the endocannabinoids do not act via this receptor to protect from this disorder. Accordingly, recent studies have shown that stimulation of CB_2 receptors, even when initiated at symptom onset, can prolong survival of *SOD1* G93 mutant mice (63, 64), and that mRNA, receptor binding and function of CB_2, but not CB_1, receptors are dramatically and selectively upregulated in the spinal cords of these mice in a temporal pattern paralleling disease progression (64).

In the case of SCI and spinal nerve lesions, several animal models have been used to investigate the effect of cannabinoids. A recent study evaluated the efficacy of the nonselective CB receptor agonist Win-55,212-2 on tactile hypersensitivity in rats following a brief compression to the thoracic spinal cord. The withdrawal thresholds of the hind paws following this type of SCI were significantly decreased, indicating tactile hypersensitivity (allodynia). Systemic injection of Win-55,212-2 increased withdrawal thresholds in a dose-dependent manner. Pretreatment with the CB_1 receptor subtype-selective antagonist AM-251 completely abolished the antinociceptive effect of Win-55,212-2, whereas pretreatment with the CB_2 receptor subtype-selective antagonist AM-630 did not alter the antinociceptive effect of Win-55,212-2 (65). Importantly, it was also shown that while opioids lose their capability to suppress C-fiber-induced spinal neuron activation in the injured L(5) but not in the intact L(4) ganglion after spinal nerve ligation between L(4) and L(5) ganglia, a CB_1 receptor agonist maintained its efficacy on both spinal ganglia under these conditions (66). These data indicate that CB_1-selective agonists might be novel templates for the development of therapeutic drugs for clinical pain induced by spinal cord and nerve injuries. On the other hand, in other studies, the sensitization of mechanically activated wide dynamic range dorsal horn neurons and the subsequent allodynia induced by spinal nerve ligation were inhibited by CB_2 agonists (67-69). Accordingly, spinal nerve ligation is accompanied by upregulation of both CB_1 (70) and CB_2 receptors, possibly due to microglial cell activation (71), and endocannabinoid levels (70). This latter event might represent an adaptive response aimed at reducing mechanical sensitivity, since inhibition of endocannabinoid hydrolysis attenuated evoked responses of spinal neurons in rats with spinal nerve ligation (72).

Clinical trials

Recent findings indicate that the endocannabinoid system undergoes tissue-specific plastic changes and might participate in the control of symptoms and disease progression in subjects with MS. CNS tissue from inflammatory lesions of patients with active or silent MS and normal control patients was analyzed in a recent study, showing a 3.7-fold higher concentration of AEA in inflammatory lesions in patients with active MS and a 1.9-fold higher concentration in lesions of patients with silent MS in comparison to healthy controls (38). Cannabinoid CB_2 immunoreactivity was found to be increased in activated microglial cells/macrophages of the spinal cord of patients with MS and ALS (73). In brain plaques, CB_1 receptors appear to be upregulated in cortical neurons, oligodendrocytes, oligodendrocyte precursors, macrophages and infiltrated T-lymphocytes, whereas CB_2 receptors are overexpressed in T-lymphocytes, astrocytes and perivascular and reactive microglia, and FAAH is overexpressed in neurons and hypertrophic astrocytes (74). From a therapeutic point of view, after several decades of merely anecdotal reports (see [75] for a comprehensive review), the results of a multicenter, randomized, placebo-controlled study in over 600 patients with MS were published in 2003 (76). Although no benefit on spasticity was found with a low oral dose of THC (5

mg/day) when this symptom was assessed using the Ashworth scale, over 60% of the treated patients exhibited objective improvements in mobility and reported subjective improvements in pain, with an overall very low incidence of adverse events. Furthermore, the 12-month follow-up of this study indicated beneficial effects for THC beyond those observed in the first part of the study (77).

Independent clinical trials extended the efficacy against spasticity to *Cannabis* extracts administered either as capsules or as an oromucosal spray (GW Pharmaceuticals' Sativex®). These extracts contain THC together with nonpsychotropic plant cannabinoids, the most abundant of which is cannabidiol (CBD), which is endowed with a wide range of pharmacological properties *in vitro* and *in vivo* in animals (78). In one case, 57 MS patients with persistent spasticity were enrolled in a prospective, randomized, double-blind, placebo-controlled, crossover study of *Cannabis* extract capsules standardized to contain 2.5 mg THC and 0.9 mg CBD each. Significantly lower spasm frequency and increased mobility, with tolerable side effects, were observed in the 37 treated patients who completed the study (79). In another study, 160 MS patients were administered oromucosal sprays of matched placebo or a whole-plant extract containing equal amounts of THC and cannabidiol at a dose of 2.5-120 mg/day of each. The primary outcome measure was a visual analogue scale (VAS) score for each patient's most troublesome symptom. Spasticity scores were significantly reduced by the extract (Sativex®) in comparison with placebo ($p = 0.001$) and there were no significant adverse effects on cognition or mood, and intoxication was generally mild (80). In another study, 24 patients with MS, SCI, brachial plexus damage and limb amputation due to neurofibromatosis were treated with Sativex® or matched placebo by sublingual spray at doses determined by titration within the range of 2.5-120 mg/24 h. Measures used were patient-recorded symptoms, well-being and intoxication scores on a daily basis using VAS. At the end of each 2-week period, an observer rated the severity and frequency of symptoms on numerical rating scales, administered standard measures of disability (Barthel Index), mood and cognition, and recorded adverse events. The authors reported significant pain relief associated with Sativex® compared to placebo. Impaired bladder control, muscle spasms and spasticity were also improved by the extract in some patients with these symptoms. Three patients had transient hypotension and intoxication with rapid initial dosing of the THC-containing extract (81).

With regard to the safety profile of *Cannabis* extracts, the results of a large study were recently published. A total of 137 MS patients with symptoms not controlled satisfactorily using standard drugs and treated with Sativex® entered an open-label trial following a 10-week, placebo-controlled study. Patients were assessed every 8 weeks using VAS and diary scores of main symptoms, and were followed for an average of 434 days. A total of 58 patients (42.3%) withdrew due to lack of efficacy, adverse events, withdrawn consent, loss to follow-up and other reasons. Patients reported 292 adverse events, of which 251 (86%) were mild to moderate, including oral pain, dizziness, diarrhea, nausea and oromucosal disorder. Three patients had five 'serious adverse events': two cases of seizures, one fall, one case of aspiration pneumonia and one case of gastroenteritis. Four patients had first-ever seizures. The improvements recorded and dose taken in the acute study remained stable. Planned, sudden interruption of treatment for 2 weeks in 25 patients (of 62 approached) did not cause a consistent withdrawal syndrome, although 11 (46%) patients reported at least one occurrence of tiredness, interrupted sleep, hot and cold flushes, mood alteration, reduced appetite, emotional lability, intoxication or vivid dreams. Twenty-two (88%) patients re-started the treatment. The authors concluded that "long-term use of oromucosal Sativex® maintains its effect in those patients who perceive initial benefit", and that "the precise nature and rate of risks with long-term use, especially epilepsy, will require larger and longer term studies" (82).

More recent studies extended the potential efficacy of *Cannabis*-based medicines to MS-related pain and urinary incontinence, as well as to spasticity caused by other disorders. In an open-label pilot study in 21 MS patients taking Sativex® for 8 weeks followed by THC only (2.5 mg/spray) for a further 8 weeks, a reduction in lower urinary tract symptoms was also reported (83). A single-center, 5-week (1-week run-in, 4-week treatment), randomized, double-blind, placebo-controlled, parallel-group trial was recently carried out in 66 patients with MS and central pain states (59 dysesthetic, 7 painful spasms) administered Sativex® oromucosal spray as adjunctive analgesic treatment. Each spray delivered 2.7 mg of THC and 2.5 of CBD, and patients could gradually self-titrate to a maximum of 48 sprays in 24 h. The extract was generally well tolerated, although more patients on treatment reported dizziness, dry mouth, and somnolence compared to placebo. Cognitive side effects were limited to long-term memory storage. Most importantly, the treatment was effective in reducing pain and sleep disturbances (84). A meta-analysis of several published clinical trials recently concluded that cannabinoids, including the cannabidiol/THC buccal spray, are effective in treating neuropathic pain in MS, although the authors emphasized that their review was based on a small number of trials and patients, and that pain related to MS was assumed to be similar to neuropathic pain (85).

Another double-blind, placebo-controlled, crossover study evaluated the safety and efficacy of low-dose treatment with the synthetic cannabinoid receptor agonist nabilone (1 mg/day) on spasticity-related pain in patients with upper motor neuron syndrome (UMNS). Eleven of 13 patients completed the study. An 11-point box scale showed a significant decrease in pain on nabilone ($p < 0.05$), while spasticity, motor function and activities of daily living did not change. Five patients reported side effects: one case of moderate transient weakness of the lower limbs (nabilone phase, dropout), three cases of mild drowsiness (two nabilone, one placebo) and one

case of mild dysphagia (placebo). One patient was excluded from the study due to an acute relapse of MS (nabilone phase, dropout). The authors concluded that nabilone 1 mg/day is a safe and easily applicable option in the care of patients with chronic UMNS and otherwise uncontrollable spasticity-related pain (86).

Finally, a double-blind study was performed several years ago comparing 5 mg THC p.o., 50 mg codeine p.o. and placebo in a patient with spasticity and pain due to SCI. The three treatments were applied 18 times each in a randomized and balanced order. THC and codeine both had an analgesic effect in comparison with placebo. Only THC showed a significant beneficial effect on spasticity. At the dose of THC used, no altered consciousness occurred (87). Unfortunately, this pilot study was never followed by larger clinical trials in patients with SCI.

New antispastic drugs from the endocannabinoid system?

As reviewed in this article, endocannabinoids appear to be ideal candidates for neuroprotection in several possible insults to central and peripheral nervous tissues, including those eventually resulting in spasticity. By activating cannabinoid CB_1 receptors and, in the case of AEA, by activating/desensitizing vanilloid TRPV1 receptors, these compounds can reduce the excitotoxicity involved in all neurodegenerative disorders causing limb spasticity, and by activating CB_2 receptors they can downregulate the immunological neuroinflammatory components of these disorders that may render the neuronal damage caused by excitotoxicity irreversible, and are, in the case of MS, one of the causes of the disorder. Accordingly, the levels of endocannabinoids and/or their molecular targets undergo plastic changes during disorders causing spasticity, and in the case of the CREAE model of MS, are elevated during spasticity itself. In fact, these changes either represent adaptive mechanisms aimed at counteracting the symptoms of the disorder, as in the case of spasticity and pain, or contribute to disease progression, particularly when they result in a decrease in endocannabinoid tone. In either case, *Cannabis* preparations or synthetic molecules that prolong the half-life of the endocannabinoids or elevate otherwise impaired endocannabinoid tone, have shown efficacy in the treatment of several symptoms of neurodegenerative disorders, including spasticity, in both preclinical and clinical studies. In particular, inhibitors of endocannabinoid degradation (Fig. 2), such as blockers of endocannabinoid cellular reuptake and enzymatic hydrolysis (46, 88-93), inhibit spasticity in the CREAE model of MS (44-47), or restore locomotion and interfere with the inflammatory

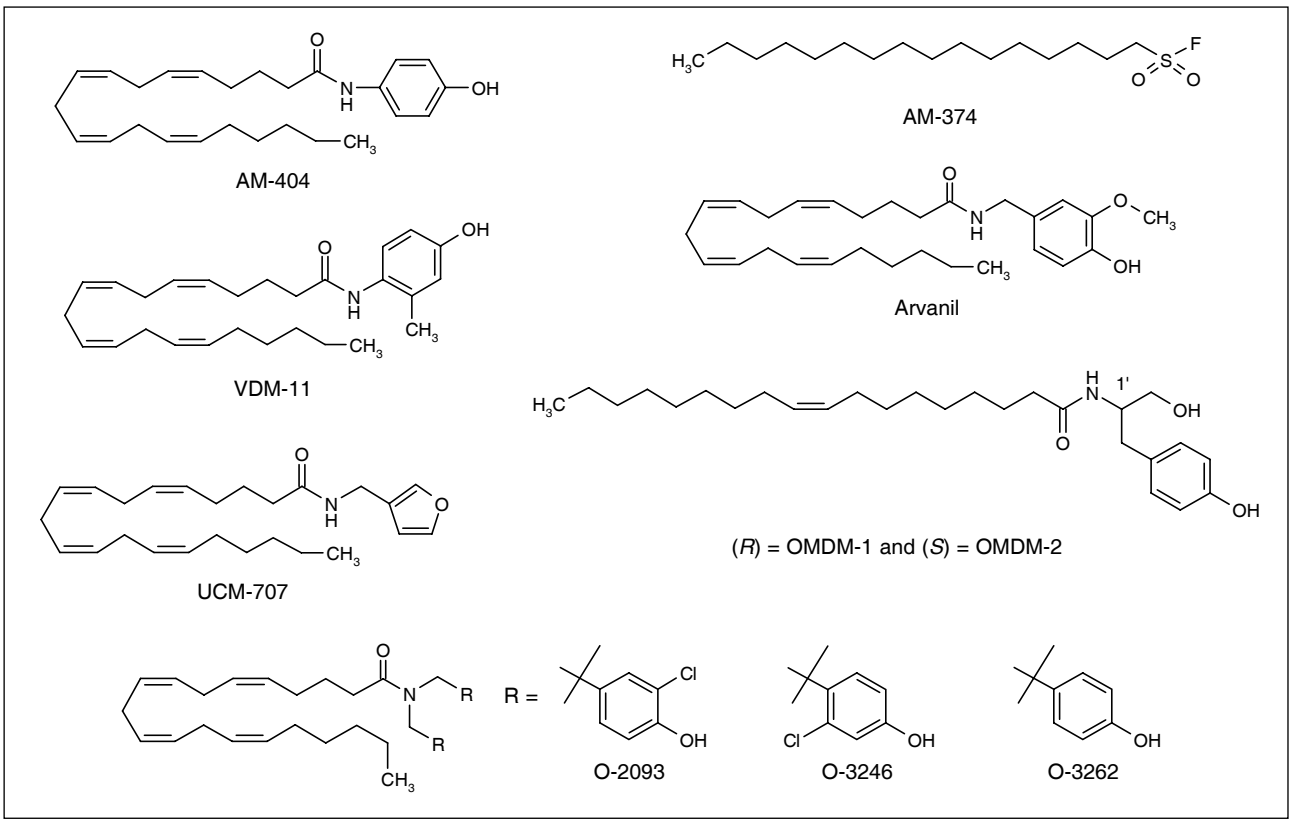


Fig. 2. Chemical structures of inhibitors of endocannabinoid inactivation (by cellular reuptake via the putative endocannabinoid membrane transporter or enzymatic hydrolysis by fatty acid amide hydrolase [FAAH]) that have been found to date to be effective against spasticity in the chronic-relapsing experimental allergic encephalomyelitis (CREAE) model of multiple sclerosis (45, 46, 88, 89).

components of EAE (50, 51, 57). The utility of these "indirect" agonists is underlined by the fact that they might also act by exploiting the activity at non-CB_1, non-CB_2 receptors of the compounds whose half-life they prolong (48, 50, 60). At the same time, in view of the "on-demand "character of endocannabinoid biosynthesis, these inhibitors will produce their effects only in those tissues where endocannabinoids are being produced and degraded, thus acting in a potentially very selective manner and showing few side effects. On the other hand, preparations containing THC (or nabilone) have proven effective at reducing spasticity, particularly when the neuroprotective and antispastic effect of THC is accompanied by the strong antiinflammatory actions of cannabidiol, which also seems to "buffer" its unwanted psychoactive effects (94). Future studies should extend to the clinic the utility of inhibitors of endocannabinoid inactivation against spasticity observed in animal models, and also determine whether selective and nonpsychotropic CB_2 agonists reduce spasticity by retarding the progression of disorders like MS and ALS. Finally, further experiments in animal models will need to clarify the exact neurochemical mechanism(s) underlying CB_1 receptor-mediated inhibition of spasticity (42), in order to develop new therapeutics capable of ameliorating this symptom while being devoid of the collateral effects of psychoactive cannabinoids.

References

1. Pertwee, R.G. *Pharmacological actions of cannabinoids*. In: Cannabinoids. Handbook of Experimental Pharmacology, Vol. 168. Pertwee, R.G. (Ed.). Springer, New York, 2005, 1-51.

2. Ross, R.A. *Anandamide and vanilloid TRPV1 receptors*. Br J Pharmacol 2003, 140: 790-801.

3. Di Marzo, V., Petrosino, S. *Endocannabinoids and the regulation of their levels in health and disease*. Curr Opin Lipidol 2007, 18: 129-40.

4. Witting, A., Walter, L., Wacker, J., Moller, T., Stella, N. *P2X7 receptors control 2-arachidonoylglycerol production by microglial cells*. Proc Natl Acad Sci USA 2004, 101: 3214-9.

5. van der Stelt, M., Trevisani, M., Vellani, V. et al. *Anandamide acts as an intracellular messenger amplifying Ca2+ influx via TRPV1 channels*. EMBO J 2005, 24: 3026-37.

6. Jung, K.M., Mangieri, R., Stapleton, C.M. et al. *Stimulation of endocannabinoid formation in brain slice cultures through activation of group I metabotropic glutamate receptors*. Mol Pharmacol 2005, 68: 1196-202.

7. Hashimotodani, Y., Ohno-Shosaku, T., Tsubokawa, H. et al. *Phospholipase C beta serves as a coincidence detector through its Ca2+ dependency for triggering retrograde endocannabinoid signal*. Neuron 2005, 45: 257-68.

8. Maejima, T., Oka, S., Hashimotodani, Y. et al. *Synaptically driven endocannabinoid release requires Ca2+-assisted metabotropic glutamate receptor subtype 1 to phospholipase Cbeta4 signaling cascade in the cerebellum*. J Neurosci 2005, 25: 6826-35.

9. Wettschureck, N., van der Stelt, M., Tsubokawa, H. et al. *Forebrain-specific inactivation of Gq/G11 family G proteins results in age-dependent epilepsy and impaired endocannabinoid formation*. Mol Cell Biol 2006, 26: 5888-94.

10. Edwards, D.A., Kim, J., Alger, B.E. *Multiple mechanisms of endocannabinoid response initiation in hippocampus*. J Neurophysiol 2006, 95: 67-75.

11. Chevaleyre, V., Takahashi, K.A., Castillo, P.E. *Endocannabinoid-mediated synaptic plasticity in the CNS*. Annu Rev Neurosci 2006, 29: 37-76.

12. Bisogno, T., Howell, F., Williams, G. et al. *Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain*. J Cell Biol 2003, 163: 463-8.

13. Yoshida, T., Fukaya, M., Uchigashima, M. et al. *Localization of diacylglycerol lipase-alpha around postsynaptic spine suggests close proximity between production site of an endocannabinoid, 2-arachidonoyl-glycerol, and presynaptic cannabinoid CB1 receptor*. J Neurosci 2006, 26: 4740-51.

14. Katona, I., Urban, G.M., Wallace, M. et al. *Molecular composition of the endocannabinoid system at glutamatergic synapses*. J Neurosci 2006, 26: 5628-37.

15. Gulyas, A.I., Cravatt, B.F., Bracey, M.H., Dinh, T.P., Piomelli, D., Boscia, F., Freund, T.F. *Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala*. Eur J Neurosci 2004, 20: 441-58.

16. Szabo, B., Urbanski, M.J., Bisogno, T., Di Marzo, V., Mendiguren, A., Baer, W.U., Freiman, I. *Depolarization-induced retrograde synaptic inhibition in the mouse cerebellar cortex is mediated by 2-arachidonoylglycerol*. J Physiol 2006, 577: 263-80.

17. Hashimotodani, Y., Ohno-Shosaku, T., Kano, M. *Presynaptic monoacylglycerol lipase activity determines basal endocannabinoid tone and terminates retrograde endocannabinoid signaling in the hippocampus*. J Neurosci 2007, 27: 1211-9.

18. Hentges, S.T., Low, M.J., Williams, J.T. *Differential regulation of synaptic inputs by constitutively released endocannabinoids and exogenous cannabinoids*. J Neurosci 2005, 25: 9746-51.

19. Monory, K., Massa, F., Egertova, M. et al. *The endocannabinoid system controls key epileptogenic circuits in the hippocampus*. Neuron 2006, 51: 455-66.

20. Marsicano, G., Goodenough, S., Monory, K. et al. *CB1 cannabinoid receptors and on-demand defense against excitotoxicity*. Science 2003, 302: 84-8.

21. Melis, M., Pillola, G., Bisogno, T. et al. *Protective activation of the endocannabinoid system during ischemia in dopamine neurons*. Neurobiol Dis 2006, 24: 15-27.

22. Walter, L., Stella, N. *Cannabinoids and neuroinflammation*. Br J Pharmacol 2004, 141: 775-85.

23. van der Stelt, M., Mazzola, C., Esposito, G. et al. *Endocannabinoids and beta-amyloid-induced neurotoxicity in vivo: Effect of pharmacological elevation of endocannabinoid levels*. Cell Mol Life Sci 2006, 63: 1410-24.

24. Esposito, G., Ligresti, A., Izzo, A.A. et al. *The endocannabinoid system protects rat glioma cells against HIV-1 Tat protein-induced cytotoxicity. Mechanism and regulation*. J Biol Chem 2002, 277: 50348-54.

25. Gubellini, P., Picconi, B., Bari, M. et al. *Experimental parkinsonism alters endocannabinoid degradation: Implications for striatal glutamatergic transmission*. J Neurosci 2002, 22: 6900-7.

26. Maccarrone, M., Gubellini, P., Bari, M. et al. *Levodopa treatment reverses endocannabinoid system abnormalities in experimental parkinsonism*. J Neurochem 2003, 85: 1018-25.

27. Maccarrone, M., Piccirilli, S., Battista, N. et al. *Enhanced anandamide degradation is associated with neuronal apoptosis induced by the HIV-1 coat glycoprotein gp120 in the rat neocortex*. J Neurochem 2004, 89: 1293-300.

28. Witting, A., Chen, L., Cudaback, E. et al. *Experimental autoimmune encephalomyelitis disrupts endocannabinoid-mediated neuroprotection*. Proc Natl Acad Sci USA 2006, 103: 6362-7.

29. van der Stelt, M., Di Marzo, V. *Cannabinoid receptors and their role in neuroprotection*. Neuromol Med 2005, 7: 37-50.

30. Wallace, M.J., Martin, B.R., DeLorenzo, R.J. *Evidence for a physiological role of endocannabinoids in the modulation of seizure threshold and severity*. Eur J Pharmacol 2002, 452: 295-301.

31. Wallace, M.J., Blair, R.E., Falenski, K.W., Martin, B.R., DeLorenzo, R.J. *The endogenous cannabinoid system regulates seizure frequency and duration in a model of temporal lobe epilepsy*. J Pharmacol Exp Ther 2003, 307: 129-37.

32. van der Stelt, M., Veldhuis, W.B., van Haaften, G.W. et al. *Exogenous anandamide protects rat brain against acute neuronal injury in vivo*. J Neurosci 2001, 21: 8765-71.

33. Blair, R.E., Deshpande, L.S., Sombati, S., Falenski, K.W., Martin, B.R., DeLorenzo, R.J. *Activation of the cannabinoid type-1 receptor mediates the anticonvulsant properties of cannabinoids in the hippocampal neuronal culture models of acquired epilepsy and status epilepticus*. J Pharmacol Exp Ther 2006, 317: 1072-8.

34. Deshpande, L.S., Blair, R.E., Ziobro, J.M., Sombati, S., Martin, B.R., Delorenzo, R.J. *Endocannabinoids block status epilepticus in cultured hippocampal neurons*. Eur J Pharmacol 2007, 558: 52-9.

35. Berger, C., Schmid, P.C., Schabitz, W.R., Wolf, M., Schwab, S., Schmid, H.H. *Massive accumulation of N-acylethanolamines after stroke. Cell signalling in acute cerebral ischemia?* J Neurochem 2004, 88: 1159-67.

36. Muthian, S., Rademacher, D.J., Roelke, C.T., Gross, G.J., Hillard, C.J. *Anandamide content is increased and CB1 cannabinoid receptor blockade is protective during transient, focal cerebral ischemia*. Neuroscience 2004, 129: 743-50.

37. Hansen, H.H., Schmid, P.C., Bittigau, P. et al. *Anandamide, but not 2-arachidonoylglycerol, accumulates during in vivo neurodegeneration*. J Neurochem 2001, 78: 1415-27.

38. Eljaschewitsch, E., Witting, A., Mawrin, C. et al. *The endocannabinoid anandamide protects neurons during CNS inflammation by induction of MKP-1 in microglial cells*. Neuron 2006, 49: 67-79.

39. Kapoor, R., Davies, M., Blaker, P.A., Hall, S.M., Smith, K.J *Blockers of sodium and calcium entry protect axons from nitric oxide-mediated degeneration*. Ann Neurol 2003, 53: 174-80.

40. Di Marzo, V., Bifulco, M., De Petrocellis, L. *Endocannabinoids and multiple sclerosis: A blessing from the 'inner bliss'?* Trends Pharmacol Sci 2000, 21: 195-7.

41. Baker, D., Pryce, G., Croxford, J.L., Brown, P., Pertwee, R.G., Huffman, J.W., Layward, L. *Cannabinoids control spasticity and tremor in a multiple sclerosis model*. Nature 2000, 404: 84-7.

42. Pryce, G., Baker, D. *Control of spasticity in a multiple sclerosis model is mediated by CB(1), not CB(2), cannabinoid receptors*. Br J Pharmacol 2007, 150: 519-25.

43. Pryce, G., Ahmed, Z., Hankey, D.J. et al. *Cannabinoids inhibit neurodegeneration in models of multiple sclerosis*. Brain 2003, 126: 2191-202.

44. Baker, D., Pryce, G., Croxford, J.L. et al. *Endocannabinoids control spasticity in a multiple sclerosis model*. FASEB J 2001, 15: 300-2.

45. de Lago, E., Ligresti, A., Ortar, G. et al. *In vivo pharmacological actions of two novel inhibitors of anandamide cellular uptake*. Eur J Pharmacol 2004, 484: 249-57.

46. Ligresti, A., Cascio, M.G., Pryce, G. et al. *New potent and selective inhibitors of anandamide reuptake with antispastic activity in a mouse model of multiple sclerosis*. Br J Pharmacol 2006, 147: 83-91.

47. de Lago, E., Fernandez-Ruiz, J., Ortega-Gutierrez, S. et al. *UCM707, an inhibitor of the anandamide uptake, behaves as a symptom control agent in models of Huntington's disease and multiple sclerosis, but fails to delay/arrest the progression of different motor-related disorders*. Eur Neuropsychopharmacol 2006, 16: 7-18.

48. Brooks, J.W., Pryce, G., Bisogno, T. et al. *Arvanil-induced inhibition of spasticity and persistent pain: Evidence for therapeutic sites of action different from the vanilloid VR1 receptor and cannabinoid CB(1)/CB(2) receptors*. Eur J Pharmacol 2002, 439: 83-92.

49. Cabranes, A., Pryce, G., Baker, D., Fernandez-Ruiz, J. *Changes in CB1 receptors in motor-related brain structures of chronic relapsing experimental allergic encephalomyelitis mice*. Brain Res 2006, 1107: 199-205.

50. Cabranes, A., Venderova, K., de Lago, E. et al. *Decreased endocannabinoid levels in the brain and beneficial effects of agents activating cannabinoid and/or vanilloid receptors in a rat model of multiple sclerosis*. Neurobiol Dis 2005, 20: 207-17.

51. Mestre, L., Correa, F., Arevalo-Martin, A. et al. *Pharmacological modulation of the endocannabinoid system in a viral model of multiple sclerosis*. J Neurochem 2005, 92: 1327-39.

52. Maresz, K., Carrier, E.J., Ponomarev, E.D., Hillard, C.J., Dittel, B.N. *Modulation of the cannabinoid CB2 receptor in microglial cells in response to inflammatory stimuli*. J Neurochem 2005, 95: 437-45.

53. Kreitzer, A.C., Malenka, R.C. *Endocannabinoid-mediated rescue of striatal LTD and motor deficits in Parkinson's disease models*. Nature 2007, 445: 643-47.

54. Croxford, J.L., Miller, S.D. *Immunoregulation of a viral model of multiple sclerosis using the synthetic cannabinoid R+WIN55,212*. J Clin Invest 2003, 111: 1231-40.

55. Arevalo-Martin, A., Vela, J.M., Molina-Holgado, E., Borrell, J., Guaza, C. *Therapeutic action of cannabinoids in a murine model of multiple sclerosis*. J Neurosci 2003, 23: 2511-6.

56. Ni, X., Geller, E.B., Eppihimer, M.J., Eisenstein, T.K., Adler, M.W., Tuma, R.F. *Win 55212-2, a cannabinoid receptor agonist,*

attenuates leukocyte/endothelial interactions in an experimental autoimmune encephalomyelitis model. Mult Scler 2004, 10: 158-64.

57. Ortega-Gutierrez, S., Molina-Holgado, E., Arevalo-Martin, A. et al. *Activation of the endocannabinoid system as therapeutic approach in a murine model of multiple sclerosis*. FASEB J 2005, 19: 1338-40.

58. Sanchez, A.J., Gonzalez-Perez, P., Galve-Roperh, I., Garcia-Merino, *A. R-(+)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl)-pyrrolo-[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphtalenylmethanone (WIN-2) ameliorates experimental autoimmune encephalomyelitis and induces encephalitogenic T cell apoptosis: Partial involvement of the CB(2) receptor*. Biochem Pharmacol 2006, 72: 1697-706.

59. Docagne, F., Muneton, V., Clemente, D. et al. *Excitotoxicity in a chronic model of multiple sclerosis: Neuroprotective effects of cannabinoids through CB1 and CB2 receptor activation*. Mol Cell Neurosci 2007, Epub ahead of print.

60. Malfitano, A.M., Matarese, G., Pisanti, S. et al. *Arvanil inhibits T lymphocyte activation and ameliorates autoimmune encephalomyelitis*. J Neuroimmunol 2006, 171: 110-9.

61. Witting, A., Weydt, P., Hong, S., Kliot, M., Moller, T., Stella, N. *Endocannabinoids accumulate in spinal cord of SOD1 G93A transgenic mice*. J Neurochem 2004, 89: 1555-7.

62. Bilsland, L.G., Dick, J.R., Pryce, G., Petrosino, S., Di Marzo, V., Baker, D., Greensmith, L. *Increasing cannabinoid levels by pharmacological and genetic manipulation delay disease progression in SOD1 mice*. FASEB J 2006, 20: 1003-5.

63. Kim, K., Moore, D.H., Makriyannis, A., Abood, M.E. *AM1241, a cannabinoid CB2 receptor selective compound, delays disease progression in a mouse model of amyotrophic lateral sclerosis*. Eur J Pharmacol 2006, 542: 100-5.

64. Shoemaker, J.L., Seely, K.A., Reed, R.L., Crow, J.P., Prather, P.L. *The CB2 cannabinoid agonist AM-1241 prolongs survival in a transgenic mouse model of amyotrophic lateral sclerosis when initiated at symptom onset*. J Neurochem 2007, Epub ahead of print.

65. Hama, A., Sagen, J. *Antinociceptive effect of cannabinoid agonist WIN 55,212-2 in rats with a spinal cord injury*. Exp Neurol 2006, 4: 454-7.

66. Kawasaki, Y., Kohno, T., Ji, R.R. *Different effects of opioid and cannabinoid receptor agonists on C-fiber-induced extracellular signal-regulated kinase activation in dorsal horn neurons in normal and spinal nerve-ligated rats*. J Pharmacol Exp Ther 2006, 316: 601-7.

67. Ibrahim, M.M., Deng, H., Zvonok, A. *Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: Pain inhibition by receptors not present in the CNS*. Proc Natl Acad Sci USA 2003, 100: 10529-33.

68. Elmes, S.J., Jhaveri, M.D., Smart, D., Kendall, D.A., Chapman, V. *Cannabinoid CB2 receptor activation inhibits mechanically evoked responses of wide dynamic range dorsal horn neurons in naive rats and in rat models of inflammatory and neuropathic pain*. Eur J Neurosci 2004, 20: 2311-20.

69. Scott, D.A., Wright, C.E., Angus, J.A. *Evidence that CB-1 and CB-2 cannabinoid receptors mediate antinociception in neuropathic pain in the rat*. Pain 2004, 109: 124-31.

70. Mitrirattanakul, S., Ramakul, N., Guerriero, A.V. *Site-specific increases in peripheral cannabinoid receptors and their endogenous ligands in a model of neuropathic pain*. Pain 2006, 126: 102-14.

71. Zhang, J., Hoffert, C., Vu, H.K., Groblewski, T., Ahmad, S., O'Donnell, D. *Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not inflammatory chronic pain models*. Eur J Neurosci 2003, 17: 2750-4.

72. Jhaveri, M.D., Richardson, D., Kendall, D.A., Barrett, D.A., Chapman, V. *Analgesic effects of fatty acid amide hydrolase inhibition in a rat model of neuropathic pain*. J Neurosci 2006, 26: 13318-27.

73. Yiangou, Y., Facer, P., Durrenberger, P. *COX-2, CB2 and P2X7-immunoreactivities are increased in activated microglial cells/macrophages of multiple sclerosis and amyotrophic lateral sclerosis spinal cord*. BMC Neurol 2006, 6: 12.

74. Benito, C., Romero, J.P., Tolon, R.M. et al. *Cannabinoid CB1 and CB2 receptors and fatty acid amide hydrolase are specific markers of plaque cell subtypes in human multiple sclerosis*. J Neurosci 2007, 27: 2396-402.

75. Robson, P. *Human studies of cannabinoids and medicinal cannabis*. In: Cannabinoids. Handbook of Experimental Pharmacology, Vol. 168. Pertwee, R.G. (Ed). Springer, New York, 2005, 719-56.

76. Zajicek, J., Fox, P., Sanders, H., Wright, D., Vickery, J., Nunn, A., Thompson, A. *UK MS Research Group. Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): Multicentre randomised placebo-controlled trial*. Lancet 2003, 362: 1517-26.

77. Zajicek, J.P., Sanders, H.P., Wright, D.E. et al. *Cannabinoids in multiple sclerosis (CAMS) study: Safety and efficacy data for 12 months follow up*. J Neurol Neurosurg Psychiatry 2005, 76: 1664-9.

78. Pertwee, R.G. *The pharmacology and therapeutic potential of cannabidiol*. In: Cannabinoids, Vol. 216. Di Marzo, V. (Ed.). Kluwer Academic-Plenum Publishers, New York, 2004, 32-83.

79. Vaney, C., Heinzel-Gutenbrunner, M., Jobin, P. et al. *Efficacy, safety and tolerability of an orally administered cannabis extract in the treatment of spasticity in patients with multiple sclerosis: A randomized, double-blind, placebo-controlled, crossover study*. Mult Scler 2004, 10: 417-24.

80. Wade, D.T., Makela, P., Robson, P., House, H., Bateman, C. *Do cannabis-based medicinal extracts have general or specific effects on symptoms in multiple sclerosis? A double-blind, randomized, placebo-controlled study on 160 patients*. Mult Scler 2004, 10: 434-41.

81. Wade, D.T., Robson, P., House, H., Makela, P., Aram, J. *A preliminary controlled study to determine whether whole-plant cannabis extracts can improve intractable neurogenic symptoms*. Clin Rehabil 2003, 17: 21-9.

82. Wade, D.T., Makela, P.M., House, H., Bateman, C., Robson, P. *Long-term use of a cannabis-based medicine in the treatment of spasticity and other symptoms in multiple sclerosis*. Mult Scler 2006, 12: 639-45.

83. Brady, C.M., DasGupta, R., Dalton, C., Wiseman, O.J., Berkley, K.J., Fowler, C.J. *An open-label pilot study of cannabis-based extracts for bladder dysfunction in advanced multiple sclerosis*. Mult Scler 2004, 10: 425-33.

84. Rog, D.J., Nurmikko, T.J., Friede, T., Young, C.A. *Randomized, controlled trial of cannabis-based medicine in central pain in multiple sclerosis*. Neurology 2005, 65: 812-9.

85. Iskedjian, M., Bereza, B., Gordon, A., Piwko, C., Einarson, T.R. *Meta-analysis of cannabis based treatments for neuropathic and multiple sclerosis-related pain*. Curr Med Res Opin 2007, 23: 17-24.

86. Wissel, J., Haydn, T., Muller, J., Brenneis, C., Berger, T., Poewe, W., Schelosky, L.D. *Low dose treatment with the synthetic cannabinoid nabilone significantly reduces spasticity-related pain: A double-blind placebo-controlled cross-over trial*. J Neurol 2006, 253: 1337-41.

87. Maurer, M., Henn, V., Dittrich, A., Hofmann, A. *Delta-9-tetrahydrocannabinol shows antispastic and analgesic effects in a single case double-blind trial*. Eur Arch Psychiatry Clin Neurosci 1990, 240: 1-4.

88. Beltramo, M., Stella, N., Calignano, A., Lin, S.Y., Makriyannis, A., Piomelli, D. *Functional role of high-affinity anandamide transport, as revealed by selective inhibition*. Science 1997, 277: 1094-7.

89. Gifford, A.N., Bruneus, M., Lin, S., Goutopoulos, A., Makriyannis, A., Volkow, N.D., Gatley, S.J. *Potentiation of the action of anandamide on hippocampal slices by the fatty acid amide hydrolase inhibitor, palmitylsulphonyl fluoride (AM 374)*. Eur J Pharmacol 1999, 383: 9-14.

90. De Petrocellis, L., Bisogno, T., Davis, J.B., Pertwee, R.G., Di Marzo, V. *Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: Inhibitors of anandamide uptake with negligible capsaicin-like activity*. FEBS Lett 2000, 483: 52-6.

91. Lopez-Rodriguez, M.L., Viso, A., Ortega-Gutierrez, S., Lastres-Becker, I., Gonzalez, S., Fernandez-Ruiz, J., Ramos, J.A. *Design, synthesis and biological evaluation of novel arachidonic acid derivatives as highly potent and selective endocannabinoid transporter inhibitors*. J Med Chem 2001, 44: 4505-8.

92. Ortar, G., Ligresti, A., De Petrocellis, L., Morera, E., Di Marzo, V. *Novel selective and metabolically stable inhibitors of anandamide cellular uptake*. Biochem Pharmacol 2003, 65: 1473-81.

93. Di Marzo, V., Ligresti, A., Morera, E., Nalli, M., Ortar, G. *The anandamide membrane transporter. Structure-activity relationships of anandamide and oleoylethanolamine analogs with phenyl rings in the polar head group region*. Bioorg Med Chem 2004, 12: 5161-9.

94. Russo, E., Guy, G.W. *A tale of two cannabinoids: The therapeutic rationale for combining tetrahydrocannabinol and cannabidiol*. Med Hypotheses 2006, 66: 234-46.