Review TRENDS in Pharmacological Sciences Vol.28 No.4

The endocannabinoid system in targeting inflammatory neurodegenerative diseases

Diego Centonze[1,2], Alessandro Finazzi-Agrò[3], Giorgio Bernardi[1,2] and Mauro Maccarrone[2,4]

[1] Neurological Clinics, Department of Neurosciences, University of Rome Tor Vergata, Rome 00133, Italy
[2] European Center for Brain Research (CERC) and Santa Lucia Foundation, Rome 00196, Italy
[3] Department of Experimental Medicine and Biochemical Sciences, University of Rome Tor Vergata, Rome 00133, Italy
[4] Department of Biomedical Sciences, University of Teramo, Teramo 64100, Italy

The classical divide between degenerative and inflammatory disorders of the CNS is vanishing as accumulating evidence shows that inflammatory processes are important in the pathophysiology of primarily degenerative disorders, and neurodegeneration complicates primarily inflammatory diseases of the brain and spinal cord. Here, we review the contribution of degenerative and inflammatory processes to CNS disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis and HIV-associated dementia. An early combination of neuroprotective and anti-inflammatory approaches to these disorders seems particularly desirable because isolated treatment of one pathological process might worsen another. We also discuss the apparently unique opportunity to modify neurodegeneration and neuroinflammation simultaneously by pharmacological manipulation of the endocannabinoid system in the CNS and in peripheral immune cells. Current knowledge of this system and its involvement in the above CNS disorders are also reviewed.

Inflammation is linked to neurodegeneration

A strong link between inflammation and neurodegeneration has recently emerged with evidence indicating that the two processes coexist from the very early stages of both classical neurodegenerative disorders and classical inflammatory diseases of the CNS. Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ALS) are among the best examples of neurodegenerative disorders associated with intense inflammation, whereas multiple sclerosis (MS) and HIV-associated dementia are inflammatory disorders that lead to diffuse neuronal damage (see Glossary). Indeed, recognition of the inflammatory reaction accompanying neurodegeneration and the neurodegeneration accompanying inflammation is not new. For example, activation of microglia and of astrocytes, which are part of the innate immune system in the CNS, has been identified as a cardinal feature of AD pathology in the brain. Similarly, neuronal injury has been known to be involved in MS since the first description of the disease by Charcot [1]. Such findings did not attract much attention in the past, however, because reactive gliosis was considered to be only an unspecific, scar-like response to neuronal death during

Glossary

Alzheimer's disease (AD): a chronic degenerative disorder of the CNS that afflicts more than 4 000 000 people in the USA alone; AD accounts also for the most common form of dementia in the elderly. Neuritic plaques, fibrillary tangles, neuronal loss, damaged synaptic connections and reactive gliosis are the pathological hallmarks of AD. Neuritic plaques comprise mainly deposits of β-amyloid peptide in the extracellular space, and are formed owing to abnormal processing of the amyloid precursor protein. The β-amyloid peptide deposits are thought to exert toxic effects in nearby neurons, leading to hyperphosphorylation of tau and to intraneuronal aggregates of this protein.
Amyotrophic lateral sclerosis (ALS): a fatal neurodegenerative disorder that selectively damages upper and lower motor neurons of the spinal cord, brainstem and motor cortex. The clinical manifestations of this disorder include progressive weakness, spasticity and muscular atrophy. Most cases of ALS are sporadic, but about 10% are familial. Mutations of the gene encoding Cu^{2+}–Zn^{2+} superoxide dismutase (*SOD1*) account for ~20% of the genetically determined forms of ALS. Mutations of other genes, such as those encoding alsin, the vesicle-associated membrane protein VAPB and senataxin (a protein involved in RNA processing) have also been identified as causes of familial forms of ALS.
HIV-associated dementia: in the USA, HIV-associated dementia is among the most common causes of dementia in young adults. An estimated 10% of HIV-infected individuals develop significant cognitive and behavioral dysfunctions despite adequate antiretroviral therapy, and neuropathological alterations of the CNS can be identified at autopsy in over 90% of HIV-positive individuals. Perivascular macrophages and microglia are the principal infected cells in the brain. Immune activation of these cells results in the secretion of several bioactive molecules, such as cytokines, chemokines and neurotoxins.
Innate immunity: a native, non-specific immunity that is present at birth in all species. It is also known as hereditary (genetic) or constitutive immunity and represents the first line of defense for non-immunized subjects. Innate immunity depends on phagocytes, natural killer cells, lysozyme, interferon and the complement complex, and also engages brain astroglia and microglia.
Multiple sclerosis (MS): the most common cause of neurological disability in young adults. Recurrent inflammation of the white matter of the CNS is the primary hallmark of the disease, at least in the early phases. Environmental factors in genetically susceptible individuals are thought to trigger immune-mediated aggression of central myelin in MS. The resulting inflammatory demyelination causes various clinical signs and symptoms, depending on the specific myelinated tract that is interrupted during each attack of the disease.
Parkinson's disease (PD): a chronic, progressive neurodegenerative disorder. The main pathological feature of PD is the degeneration of dopamine-containing neurons of the substantia nigra, leading to severe dopamine denervation of the striatum. The irreversible loss of the dopamine-mediated control of striatal function results in the typical motor symptoms observed in PD (i.e. bradykinesia, tremor and rigidity).

Corresponding author: Maccarrone, M. (mmaccarrone@unite.it).
Available online 12 March 2007.

 0165-6147/$ – see front matter doi:10.1016/j.tips.2007.02.004

degenerative damage, and neuronal loss was thought to be a late consequence of axon demyelination in MS.

Recent discoveries have now imposed reconsideration of the perceived relationship between inflammation and neurodegeneration, by making it clear that one is not simply a culmination of the other. Indeed, inflammation and neurodegeneration seem to occur in parallel rather than in series, and thus have a mutual influence on many neurological diseases. Common molecular pathways that bring these two processes together have been described [2,3], in addition to the capacity of activated immune cells to damage neurons in the absence of any antigen specificity [4,5] and the ability of damaged neurons to trigger local immune responses [6].

On the basis of these considerations, an early combination of neuroprotective and anti-inflammatory strategies seems a rational approach to CNS diseases, irrespective of the nature of the primary insult. Such an approach is particularly desirable because isolated treatment of one pathological process might even worsen another. In treatment of MS with interferon-β, for example, the inflammatory component of the disease is controlled, whereas neuronal survival can be negatively affected, by this immunomodulatory agent [7].

In this review, we discuss the contribution of inflammation to neurodegenerative diseases and, conversely, that of degeneration to neuroinflammatory disorders. In addition, we review the current knowledge of the endocannabinoid system and its involvement in these CNS disorders.

Inflammation in neurodegenerative disorders

The identification of brain astroglia and microglia as important components of innate immunity has been crucial to understanding the role of inflammation in neurodegenerative disorders. Reactive gliosis, in fact, is well-known to accompany both acute and chronic neuronal damage, but its importance as part of a harmful inflammatory response had been neglected until recently.

Activated astroglia can mediate both protective and toxic effects during neurodegenerative diseases [8]. By contrast, microglia have an active role in the defensive attack against viruses and bacteria, but intense microglial activation, such as that produced to clear up apoptotic cells or neuron debris during neurodegenerative disorders, can be detrimental to the survival of neighboring cells. Cytokines, bioactive peptides produced through complement activation, and other soluble factors mediate the toxic effects of microglia-produced inflammatory milieu on neurons [3,9]. Indeed, several inflammatory mediators are increased in the brain tissues or cerebrospinal fluid of individuals affected with AD, PD and ALS [3,9].

Alzheimer's disease

Since the discovery of MHC class II antigens in the microglia surrounding amyloid plaques and dystrophic neuritis, several inflammatory processes have been described in the brains of individuals affected with AD [10]. These processes include complement and glial cell activation, acute phase protein synthesis, and chemokine expression [3,9]. The role of inflammatory neurodegeneration in AD is supported by studies demonstrating associations between the disease and polymorphisms in genes encoding some cytokines or acute phase proteins [11], and by data showing the protective action against the disease exerted by anti-inflammatory agents [12].

Parkinson's disease

More recently, the importance of neuroinflammation in PD pathophysiology has also emerged. Indeed, activated microglia have been described in close proximity to degenerating dopamine neurons in individuals with PD, and activation of microglia into the substantia nigra has been shown to cause selective destruction of dopamine neurons [13]. Furthermore, experimental parkinsonism, induced by neurotoxins specific to dopamine neurons, is coupled with the activation of nigral and striatal microglia and with the production of proinflammatory molecules. In addition, in models of PD, inhibition of microglia is neuroprotective [3]. As in AD, polymorphisms of some cytokines have been identified as risk factors for PD, whereas epidemiological studies have reported that chronic users of anti-inflammatory drugs have a decreased risk for PD [14].

By contrast, recent data have shown that prior delivery of a peripheral, pro-inflammatory stimulus induces neuroprotection in a rodent model of PD. This protective effect is paralleled by a concomitant reduction in the associated microglial response and moderate, transient increases in cytokine levels at the sites of neurodegeneration [15]. Therefore, modulation of the neuroprotective effect of peripheral inflammation might be exploited for improving the treatment of PD.

Amyotrophic lateral sclerosis

A strong inflammatory process has been described in the brains of individuals with ALS, and a correlation between the intensity of inflammation and progression of the disease has also been observed [16]. Studies on postmortem spinal cord tissues have found that the number of activated microglia is greater in individuals with ALS than in controls [17]. Further evidence of microglial involvement in ALS pathology has come from studies showing pro-survival effects of the pharmacological suppression of microglial activation on rat spinal cord neurons exposed to cerebrospinal fluid from individuals with ALS [18]. Lastly, anti-inflammatory agents prolong the survival of transgenic mice expressing human SOD1 with a G93A mutation (hSOD1G93A), an animal model of familial ALS [19], and a clinical trial exploring the effect of a immunomodulatory agent in individuals with ALS is currently ongoing [20].

Neurodegeneration in inflammatory disorders

Neurons are unusual targets of inflammatory diseases. Normally, in fact, they do not express MHC molecules, which is an essential requirement for cell susceptibility to immune attacks [1]. Nevertheless, the neuronal compartment of the CNS is frequently injured during inflammatory responses against glial cells, as is the case in MS, a chronic inflammatory disease of oligodendrocytes and myelin sheaths, and in HIV-associated dementia, a disorder secondary to HIV infection of microglia. Inflammatory autoimmune responses can

also counteract neurodegeneration, however, indicating that the relationship between the two processes is far more complex [21].

Multiple sclerosis

Accumulating evidence indicates that neurodegeneration might occur not only as a late consequence of axon demyelination in MS, but also as a very early event in the course of the disease. Accordingly, neuronal damage and axonal loss are common and abundant in MS, affecting both overt inflammatory lesions and normal-appearing white matter [22]. In addition, neuronal loss in gray matter also contributes to brain damage in MS, as indicated by the extensive cortical and subcortical deposition of iron, caudate atrophy and neuronal apoptosis observed in MS brains.

Several mechanisms have been proposed to explain the massive involvement of neurons in MS, including axon transection by cytotoxic T cells and damage by soluble products released by resident and invading inflammatory cells. These products include axon-specific antibodies, complement, nitric oxide, oxygen radicals, proteases and eicosanoids [23]. Acquired neuronal channelopathies, altered activity of Na^+–Ca^{2+} exchangers, glutamate-mediated excitotoxicity, intraneuronal Ca^{2+} accumulation and inhibition of mitochondrial respiratory chain are other crucial factors that contribute to neuronal damage during the course of MS and experimental MS [23,24].

HIV-associated disease

HIV-associated dementia is characterized by severe brain atrophy and neuronal apoptosis in the absence of the direct localization of HIV in neurons. The mechanisms by which a primary infection of glial cells damages neurons are not fully understood, but the release of neurotoxic cytokines and chemokines from activated astrocytes and microglia seems to be important. The presence of chemokine receptors on neurons indicates that they might have a role in neuronal damage by favoring glutamate-mediated excitotoxicity and Ca^{2+} entry through voltage-dependent Ca^{2+} channels. In addition, microglia and macrophages activated by HIV seem to damage neurons through the release of neurotoxins such as arachidonic acid, glutamate, tumor necrosis factor-α and interleukin-1 [25].

Anti-inflammatory and neuroprotective effects of endocannabinoids

A unique opportunity to improve inflammation and neurodegeneration simultaneously might be offered by pharmacological agents that can modulate the activity of cannabinoid (CB) receptors (Box 1). Indeed, both CB receptor subtypes, CB_1 and CB_2, are abundantly expressed in neurons and in central and peripheral immune cells, and regulate degeneration and inflammation in diseases of the CNS. Despite the potential benefits of drugs acting on CB receptors, however, their clinical use is hampered mainly because of their psychotropic effects [26].

Progressive characterization of the biochemical machinery that regulates the synthesis, transport and degradation of the endogenous ligands of CB receptors – namely, endocannabinoids – has prompted extensive investigations into the therapeutic effects of agents targeting the 'endocannabinoid system' (ECS) in several pathological conditions, including CNS degenerative and inflammatory disorders [27–29]. In this context, we recall that the release of endocannabinoids during neuronal injury might constitute a protective response [30]. Indeed, exogenous and endogenous cannabinoids have been shown to exert neuroprotection in various *in vitro* and *in vivo* models of neuronal injury [31,32].

This neuroprotective activity occurs through different mechanisms, including (i) prevention of excitotoxicity by CB_1-receptor-mediated inhibition of glutamate-mediated transmission via the closing of N- and P/Q-type Ca^{2+} channels; (ii) reduction of Ca^{2+} influx at both the pre- and postsynaptic level, followed by inhibition of subsequent noxious cascades; (iii) antioxidant activity, mainly owing to the phenol group of various resorcinol-type cannabinoids; (iv) suppression of the production of tumor necrosis factor-α; (v) activation of the phosphatidylinositol 3-kinase and protein kinase B pathway; (vi) induction of phosphorylation of extracellular regulated kinases; and (vii) induction of the expression of transcription factors and neurotrophins.

A central point of contention has been, and still remains, the receptor dependency of (endo)cannabinoid neuroprotection, because several not fully defined CB receptors are certainly part of the ECS, and at present we have only a vague idea about the possible interaction of the (endo)cannabinoids with these targets [31,32]. Several elements of the ECS have been characterized so far

Box 1. Signal transduction and biological actions of endocannabinoids in the CNS

Signal transduction

- **Signaling through CB_1 and CB_2 receptors**
  Inhibition of adenylyl cyclase, activation of mitogen-activated protein kinase, inhibition of voltage-gated L, N and P/Q-type Ca^{2+} channels, activation of K^+ channels, activation of focal adhesion kinase, activation of cytosolic phospholipase A_2, activation (CB_1R) or inhibition (CB_2R) of nitric oxide synthase.
- **Signaling through non-CB_1 and non-CB_2 receptors**
  Modulation of L-type Ca^{2+} channels, opening of intracellular Ca^{2+} stores, inhibition of AMT and FAAH, antioxidative effects.
- **Signaling through TRPV1 receptors**
  Activation of non-selective ion channels, activation of protein kinases, opening of intracellular Ca^{2+} stores, dissipation of mitochondrial membrane potential (mitochondrial uncoupling), cytochrome *c* release from mitochondria, activation of caspases.

Biological actions

- **Cortex, cerebellum and spinal cord**
  Blockade of *N*-methyl-D-aspartate (NMDA) receptors, control of tremor and spasticity.
- **Basal ganglia, striatum and globus pallidus**
  Control of psychomotor disorders, interference with dopamine transmission, inhibition of GABA-mediated transmission, induction of long-term depression, potentiation of GABA-mediated catalepsy.
- **Thalamus, hypothalamus and hippocampus**
  Control of pain initiation, control of wake–sleep cycles, control of thermogenesis, control of appetite and food intake, impairment of working memory, impairment of memory consolidation, inhibition of long-term potentiation, inhibition of glutamate-mediated transmission.
- **Retina**
  Control of scotopic vision.

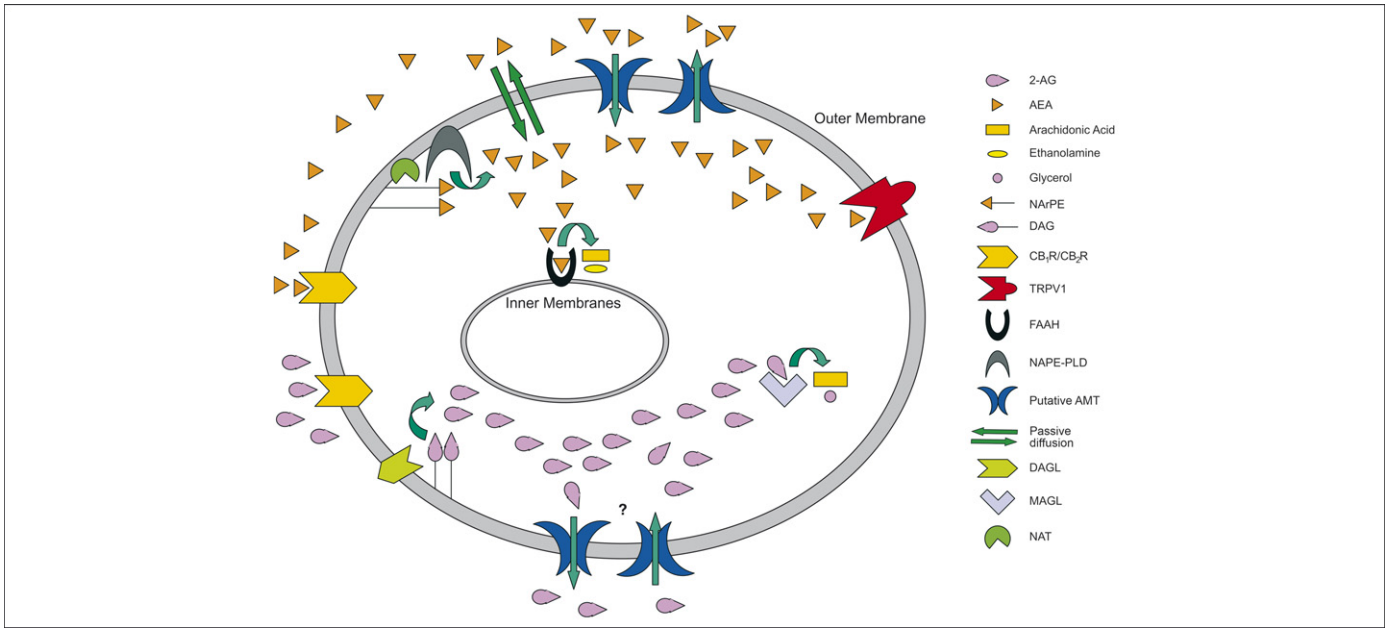


Figure 1. The endocannabinoid system. Endocannabinoids are lipid mediators, isolated from brain and peripheral tissues, that include amides and esters of long-chain polyunsaturated fatty acids. Two arachidonic acid derivatives, AEA and 2-AG, are the most biologically active endocannabinoids described so far. Unlike classical neurotransmitters and neuropeptides, AEA is not stored in intracellular compartments but is produced 'on demand' by receptor-stimulated cleavage of lipid precursors. There is now general consensus that AEA is generated by a transacylase-phosphodiesterase-mediated synthesis, starting from membrane phosphoglycerides and phosphatidylethanolamine. The AEA precursor *N*-arachidonoylphosphatidylethanolamine (NArPE) is thought to originate from transfer of arachidonic acid from the *sn*-1 position of 1,2-*sn*-diarachidonoylphosphatidylcholine to phosphatidylethanolamine, catalyzed by a Ca^{2+}-dependent *N*-acyltransferase or *trans*-acylase (NAT). NArPE is then cleaved by NAPE-PLD, a recently characterized phospholipase D, which releases AEA and phosphatidic acid. By contrast, the biological activity of AEA at CB receptors is terminated by its removal from the extracellular space, which occurs through cellular uptake by a purported high-affinity transporter (AMT). As yet, the existence of a true AMT is a matter of debate, and it is possible that AEA might cross the plasma membrane by passive diffusion. Nevertheless, once taken up by cells, AEA is a substrate for the hydrolase FAAH, which breaks the amide bond and releases arachidonic acid and ethanolamine. Like AEA, 2-AG is not stored in intracellular compartments, but it is produced on demand by receptor-stimulated cleavage of lipid precursors. A biosynthetic pathway provides for 2-AG formation through the hydrolysis of phospholipids by a specific DAGL. Subsequently, 2-AG is subjected to rapid transport across the plasma membrane, possibly through a purported 2-AG membrane transporter that might be the same as AMT. Once accumulated in the cell, 2-AG can be degraded by FAAH or, more efficiently, by a specific MAGL. Both AEA and 2-AG bind to and activate CB_1 and CB_2 receptors; however, AEA is a weaker agonist than 2-AG at CB_1 and is only a partial agonist at CB_2. As a result, 2-AG has been proposed as the true endogenous ligand for both subtypes of CB receptor. TRPV1 is another key molecular target of AEA, but importantly not of 2-AG; thus, AEA is also considered to be a true 'endovanilloid'. The main signal transduction pathways triggered by AEA and 2-AG through CB_1 or CB_2 receptors, and by AEA through TRPV1, are summarized in Box 1, along with the biological actions of these endocannabinoids in the CNS. All elements of the ECS are located in the plasma membrane apart from FAAH, which is bound to intracellular membranes, and MAGL, which is cytosolic.

(Figure 1). The list of characterized ECS elements often includes the type-1 vanilloid receptor (TRPV1), although this receptor does not strictly belong to the ECS. In fact, TRPV1 has emerged as a key target of the amide *N*-arachidonoylethanolamine (AEA; also known as 'anandamide') – the most prominent member of the endocannabinoids – to such an extent that AEA is also considered to be a true 'endovanilloid' [33]. Below, we summarize the involvement of ECS components in neurodegenerative disorders.

Alzheimer's disease

Stimulation of CB_1, CB_2, and non-CB_1 or non-CB_2 receptors (such as that obtained with cannabidiol) prevents microglial activation and microglia-mediated neurotoxicity and neurodegeneration in experimental models of AD [34]. Similar effects can be achieved by increasing endogenous levels of endocannabinoids through inhibition of the cellular uptake of AEA (by compounds that we term 'AMT inhibitors') [35] (Table 1).

Parkinson's disease

Degeneration of dopamine neurons during experimental PD can be reduced by agonists of CB_1, CB_2, and non-CB_1 or non-CB_2 receptors – an effect that involves modulating the interactions between glial cells and neurons [36]. CB_1 receptors, however, also exert detrimental effects on dopamine cell survival by potentiating the toxic effects of the TRPV1 agonist capsaicin [37]. It is thus conceivable that endocannabinoids such as AEA, which activates both TRPV1 and CB_1 receptors [33], might contribute to PD pathophysiology by favoring apoptosis of dopamine neurons.

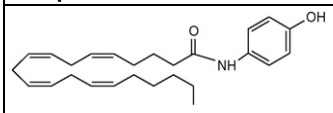
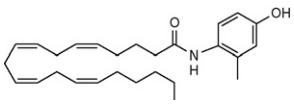
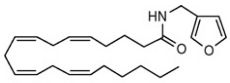
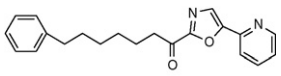
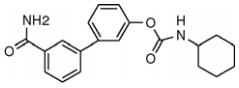
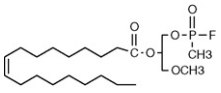
Amyotrophic lateral sclerosis

Pharmacological agonists of CB receptors and increased levels of endocannabinoids, obtained through genetic ablation of fatty acid amide hydrolase (FAAH) [38], exert robust anti-inflammatory and neuroprotective effects in hSOD1G93A mice, delaying disease progression [39,40]. The neuroprotective effects observed in hSOD1G93A mice after pharmacological and genetic augmentation of endocannabinoids levels seem to be selectively dependent on stimulation of the CB_2 receptor; by contrast, activation of the CB_1 receptor has a negative influence on motor neuron survival [40].

Multiple sclerosis

The use of cannabis-based medicine for the treatment of MS has a long history, and has been recently reviewed [41].

Table 1. Chemical structures of inhibitors of endocannabinoid metabolism with therapeutic potential in disorders of the CNS

Compound	Effect on ECS	Therapeutic activity
 AM404	AMT inhibitor[a,b]	Improvement of hyperkinesia and sensorimotor orientation in animal models of PD; amelioration of spasticity in animal models of MS
	AMT inhibitor[a]	Improvement of hyperkinesia and sensorimotor orientation in animal models of PD; amelioration of spasticity in animal models of MS
 UCM707	AMT inhibitor[a]	Improvement of hyperkinesia and sensorimotor orientation in animal models of PD; amelioration of spasticity in animal models of MS
 OL135	FAAH inhibitor	Decrease in pain sensation in hyperalgesia
	FAAH inhibitor	Decrease of anxiety; decrease in pain sensation in hyperalgesia
 03841	DAGL inhibitor	Unknown

[a]Throughout this review, the phrase 'AMT inhibitor' is used to designate a compound that blocks the cellular uptake of anandamide.
[b]AM404 can also act as a TRPV1 agonist or cyclooxygenase-1 or cyclooxygenase-2 inhibitor.

In models of experimental MS, stimulation of CB_1 and of CB_2 receptors has been shown to be beneficial against the inflammatory process [42,43], lending support to early findings showing that individuals with MS experience a reduction in the frequency of relapses when smoking marijuana [44]. Anti-inflammatory effects have also been reported in experimental MS in response to pharmacological AMT inhibitors, which can increase levels of AEA [45,46]. Interestingly, stimulation of CB_1 receptors also ensures neuroprotection in mice with experimental MS [47].

HIV-associated dementia

Cannabinoids have been proposed to exert beneficial effects in HIV-associated dementia, owing to their ability to modulate microglia activation [48]; however, the absence of reliable animal models of this disorder prevents direct exploration of this possibility.

Therapeutic potential of ECS-targeting drugs in CNS disorders

Although still at its infancy, exploration of the therapeutic effects of drugs targeting endocannabinoid metabolism is increasingly encouraging with evidence showing altered levels of endocannabinoids in several pathological conditions of the CNS [26–29]. There are now several examples of the successful use of ECS-directed drugs to alleviate the clinical symptoms of degenerative and inflammatory neurological diseases in animal models [36,49] (Table 1).

Promising results have been reported with AMT inhibitors such as AM404 (see Chemical names), VDM11 and UCM707. Indeed, treatment with AM404 reduces the neurochemical defects associated with neurodegenerative damage, but this action seems to be due to the synthesis of new AEA after the direct activation of TRPV1 by AM404, rather than to AMT inhibition. Furthermore, AM404 also inhibits purified cyclooxygenase-1 and cyclooxygenase-2 – and thus prostaglandin synthesis – in activated macrophages [50]. Therefore, AM404 might also directly curb the inflammatory component of neurological disorders. Administration of AM404 or VDM11 has been shown to reduce significantly the frequency of spontaneous glutamate-mediated activity recorded from striatal neurons in an experimental model of PD, thereby exerting anti-excitotoxic effects. Notably, another AMT inhibitor, UCM707, significantly protects mice against the excitotoxin kainic acid. Similarly, systemic administration of AM404 improves akinesia and sensorimotor orientation – two anti-parkinsonian effects. Indeed, it has been found that administration of AM404 and VDM11 in mice suffering from chronic relapsing experimental allergic encephalomyelitis, a model of MS, markedly ameliorates spasticity.

Several studies have provided strong evidence that FAAH, owing to its broad distribution, might represent

an attractive therapeutic target for the treatment of neurological diseases (Table 1). For example, inhibition of FAAH by URB597 can augment endogenous brain levels of AEA and produce anxiolytic, analgesic and anti-nociceptive actions. These effects are mediated by CB_1 receptor stimulation, suggesting that URB597 might exert not only symptomatic but also neuroprotective effects in CNS disorders. Similarly, AM374, another FAAH inhibitor, has been shown to exert potent neuroprotective effects *in vivo* and *in vitro* by enhancing CB_1-dependent activation of mitogen-activated protein kinase [51]. The advantage of these compounds is that they do not seem to induce the side-effects common to typical agonists of CB receptors, such as hypomotility, catalepsy or hypothermia, because they do not interact directly with CB receptors. Furthermore, another selective and powerful FAAH inhibitor that has been used for the treatment of pathological states is OL135 – a reversible α-keto heterocyclic inhibitor that enhances AEA-induced analgesia *in vivo*.

A recent study has demonstrated that the high level of interferon-γ in the CNS in mice affected with experimental autoimmune encephalomyelitis (EAE), a model of MS, disrupts endocannabinoid-mediated neuroprotection, although functional CB receptors are maintained [52]. Therefore, this study provides additional support for the concept that the balance between inflammation and neurodegeneration has considerable bearing on the endocannabinoid tone, and thus favors the use of FAAH inhibitors to treat the inflammatory and neurodegenerative damage associated with pathological conditions such as MS.

By contrast, the recent discovery of potent and specific inhibitors of diacylglycerol lipase (DAGL), such as O3841 [53] (Table 1), suggests that it could become possible to dissect the contribution of 2-arachidonoylglycerol (2-AG) and that of AEA to neurological disorders. In addition, there is active search for selective inhibitors of monoacylglycerol lipase (MAGL): the availability of such inhibitors might be crucial to design therapeutic strategies based on the preferential recruitment of one endocannabinoid over the other depending on the clinical context. Indeed, AEA and 2-AG have distinct pharmacological profiles on CB_1, CB_2 and TRPV1 receptors; thus, it can be anticipated that differential modulation of endocannabinoid levels might be indicated in distinct diseases. In general terms, the higher affinity of 2-AG versus AEA for CB_1 receptors [54], coupled with the fact that AEA but not 2-AG binds to TRPV1 [33], suggests that agents that can modulate 2-AG levels within the CNS could be more suitable for treating diseases in which the neurodegenerative aspect prevails over inflammation. Stimulation of CB_1 receptors, in fact, reduces transmitter release at excitatory synapses and exerts clear anti-excitotoxic effects [30]. By contrast, stimulation of TRPV1 favors inflammation [27]; as a result, modulation of AEA-dependent activation of vanilloid receptors is likely to be more effective for treatment of the inflammatory component of neurodegenerative diseases [55]. These ideas can be formulated into a working model of the modulation of degeneration and inflammation in the CNS by the ECS (Figure 2).

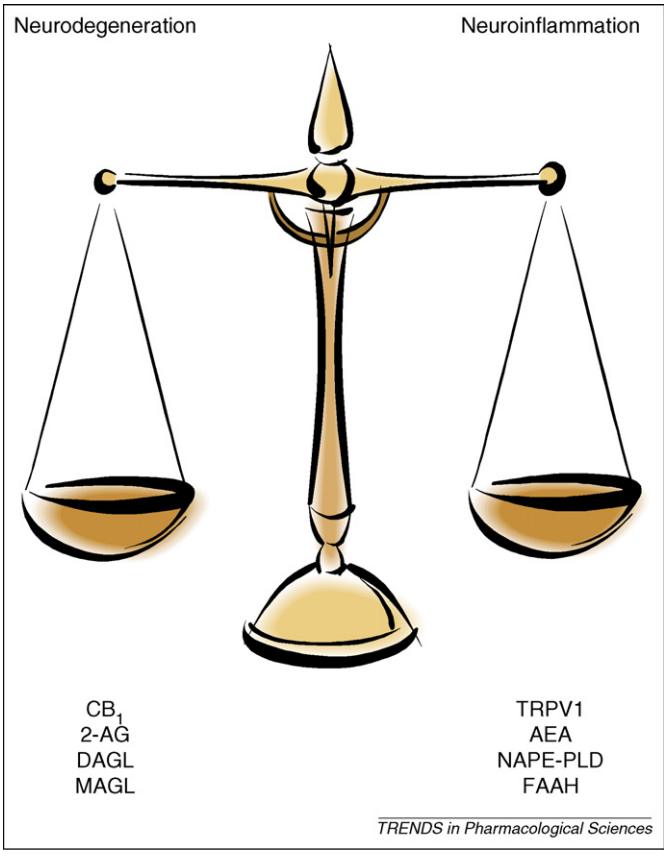


Figure 2. Working hypothesis of the modulation of degeneration and inflammation in the CNS by the ECS. On the one hand, AEA might control inflammatory processes by binding to TRPV1, such that drugs that are able to modulate the AEA metabolic enzymes NAPE-PLD and FAAH might be exploited to curb neuroinflammation. On the other hand, 2-AG could regulate neurotransmitter release through CB_1 receptors, thereby affecting neurodegeneration. Thus, drugs that modulate 2-AG metabolism through DAGL and MAGL might be exploited to curb diseases in which the neurodegenerative aspect prevails over inflammation.

In addition, 2-AG might further control the balance between neurodegeneration and neuroinflammation at the peripheral level, where CB_2 receptors are most abundant [54]. Unlike AEA, which is a weak partial agonist for CB_2 receptors, 2-AG fully activates these receptors [56] and regulates the release and function of cytokines [55]. As a result, drugs directed towards DAGL or MAGL might be better suited to modulate the contribution of peripheral cells to neurodegenerative disorders. In the same context, it should be kept in mind that essential activities of 2-AG that are independent of those of AEA are emerging in both the CNS [57] and the periphery [58]; therefore, an understanding of MAGL and DAGL regulation and the role of these lipases in maintaining the endocannabinoid tone *in vivo* is of utmost importance, as it has been for *N*-acylphosphatidylethanolamine (NAPE)-specific phospholipase D (NAPE-PLD) [59] and FAAH [60,61] with respect to AEA. Notably, it seems that our hypothesis that a balance between CB receptors and TRPV1 modulates the dual nature of neurological diseases finds a nice parallel in pathological pain sensation, where it has been demonstrated that TRPV1 functions to oppose CB-receptor-dependent effects [62].

Chemical names

AM374: palmitylsulfonyl fluoride
AM404: *N*-(4-hydroxyphenyl)-arachidonamide
O3841: octadec-9-enoic acid 1-methoxymethyl-2-(fluoro-methyl-phosphinoyloxy)-ethyl ester
OL135: 1-oxo-1[5-(2-pyridyl)-2-yl]-7-phenylheptane
UCM707: *N*-(3-furylmethyl)-arachidonamide
URB597: cyclohexylcarbamic acid 3′-carbamoyl-biphenyl-3-yl-ester
VDM11: *N*-(4-hydroxy-2-methylphenyl)-arachidonamide

Concluding remarks and future perspectives

The role of the ECS in regulating brain activity during physiological and pathological conditions is emerging. For example, control of the cellular activity of AEA seems to be largely dependent on its hydrolysis by FAAH, rather than on its synthesis by Ca^{2+}-dependent *N*-acyltransferase or NAPE-PLD. Overall, it seems that modulating endocannabinoid metabolism, rather than agonizing or antagonizing CB and non-CB receptors, might be the way to understand better the pathophysiological implications of these bioactive lipids and to exploit them for therapeutic purposes [27–29]. If not therapeutic agents *per se*, inhibitors of NAPE-PLD, FAAH, AMT, DAGL or MAGL could be used together with AEA or 2-AG analogs to lower the doses or to shorten the treatment necessary *in vivo* to observe an effect, and thus to minimize the possible psychotropic side-effects of endocannabinoids when they are used as pharmaceutical agents. Along this line, novel compounds such as cannabidiol, a major non-psychotropic constituent of cannabis that does not bind to CB_1 or CB_2 receptors, have promising anti-convulsive, anti-anxiety and anti-psychotic properties [54]. The mechanism of action of cannabidiol relies on AMT and FAAH inhibition, and on antioxidative properties; as a result, this natural drug might be a lead compound for the development of therapeutics against the inflammatory component of neurodegenerative disorders.

Lastly, it seems necessary to recall that, first, several endogenous endocannabinoids or endocannabinoid-like compounds, whose functions are not understood, are present in our body, and their biological activity might be affected in unexpected ways by drugs that modulate known or unknown proteins of the ECS; and second, metabolic enzymes have been recently identified that hydrolyze AEA analogs such as *N*-palmitoylethanolamine [63] and that catalyze novel biosynthesis [64] or hydrolysis [65] pathways of AEA, and it remains to be elucidated how these pathways might contribute to the overall tone and biological activity of endocannabinoids.

Acknowledgements

We thank all colleagues who have contributed over the years to our studies of the endocannabinoid system in the CNS, and Natalia Battista and Andrea Paradisi for the artwork. This work was supported by funding from the Ministero dell'Istruzione, dell'Università e della Ricerca (FIRB 2006) to D.C. and M.M., the Ministero della Salute (grants 2006) to D.C., the Agenzia Spaziale Italiana (DCMC and MoMa projects) to A.F-A. and M.M., and the Fondazione TERCAS (Research Programs 2004 and 2005) to M.M.

References

1 Zipp, F. and Aktas, O. (2006) The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases. *Trends Neurosci.* 29, 518–527
2 Aktas, O. *et al.* (2005) Neuronal damage in autoimmune neuroinflammation mediated by the death ligand TRAIL. *Neuron* 46, 421–432
3 Block, M.L. and Hong, J.S. (2005) Microglia and inflammation-mediated neurodegeneration: multiple triggers with a common mechanism. *Prog. Neurobiol.* 76, 77–98
4 Allan, S.M. *et al.* (2005) Interleukin-1 and neuronal injury. *Nat. Rev. Immunol.* 5, 629–640
5 Linker, R.A. *et al.* (2005) EAE and β-2 microglobulin-deficient mice: axonal damage is not dependent on MHC-I restricted immune responses. *Neurobiol. Dis.* 19, 218–228
6 Babcock, A.A. *et al.* (2003) Chemokine expression by glial cells directs leukocytes to sites of axonal injury in the CNS. *J. Neurosci.* 23, 7922–7930
7 Chaudhuri, A. (2005) Interferon β, progressive MS, and brain atrophy. *Lancet Neurol.* 4, 208–209
8 Maragakis, N.J. and Rothstein, J.D. (2006) Mechanisms of disease: astrocytes in neurodegenerative disease. *Nat. Clin. Pract. Neurol.* 2, 679–689
9 Bonifati, D.M. and Kishore, U. (2007) Role of complement in neurodegeneration and neuroinflammation. *Mol. Immunol.* 44, 999–1010
10 McGeer, P.L. *et al.* (1988) Reactive microglia are positive for HLA-DR in the substantia nigra of Parkinson's and Alzheimer's disease brains. *Neurology* 38, 1285–1291
11 Marchetti, B. and Abbracchio, M.P. (2005) To be or not to be (inflamed) – is that the question in anti-inflammatory drug therapy of neurodegenerative disorders? *Trends Pharmacol. Sci.* 26, 517–525
12 Zandi, P.P. *et al.* (2002) Reduced incidence of AD with NSAID but not H2 receptor antagonists: the Cache County Study. *Neurology* 59, 880–886
13 Gao, H.M. *et al.* (2003) Novel anti-inflammatory therapy for Parkinson's disease. *Trends Pharmacol. Sci.* 24, 395–401
14 Bonuccelli, U. and Del Dotto, P. (2006) New pharmacologic horizons in the treatment of Parkinson disease. *Neurology* 67 (Suppl. 2), S30–S38
15 Armentero, M.T. *et al.* (2006) Peripheral inflammation and neuroprotection: systemic pretreatment with complete Freund's adjuvant reduces 6-hydroxydopamine toxicity in a rodent model of Parkinson's disease. *Neurobiol. Dis.* 24, 492–505
16 Alexianu, M.E. *et al.* (2001) Immune reactivity in a mouse model of familial ALS correlates with disease progression. *Neurology* 57, 1282–1289
17 Henkel, J.S. *et al.* (2004) Presence of dendritic cells, MCP-1, and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. *Ann. Neurol.* 55, 221–235
18 Tikka, T.M. *et al.* (2002) Minocycline prevents neurotoxicity induced by cerebrospinal fluid from patients with motor neurone disease. *Brain* 125, 722–731
19 Pompl, P.N. *et al.* (2003) A therapeutic role for cyclooxygenase-2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. *FASEB J.* 17, 725–727
20 Gordon, P.H. *et al.* (2006) Randomized controlled phase II trial of glatiramer acetate in ALS. *Neurology* 66, 1117–1119
21 Schwartz, M. and Kipnis, J. (2005) Protective autoimmunity andneuroprotection in inflammatory and noninflammatory neurodegenerative diseases. *J. Neurol. Sci.* 233, 163–166
22 Filippi, M. and Rocca, M.A. (2005) MRI evidence for multiple sclerosis as a diffuse disease of the central nervous system. *J. Neurol.* 252, S16–S24
23 Hauser, S.L. and Oksenberg, J.R. (2006) The neurobiology of multiple sclerosis: genes, inflammation, and neurodegeneration. *Neuron* 52, 61–76
24 Waxman, S.G. (2006) Axonal conduction and injury in multiple sclerosis: the role of sodium channels. *Nat. Rev. Neurosci.* 7, 932–941
25 Ozdener, H. (2005) Molecular mechanisms of HIV-1 associated neurodegeneration. *J. Biosci.* 30, 391–405
26 Fowler, C.J. (2005) Pharmacological properties and therapeutic possibilities for drugs acting upon endocannabinoid receptors. *Curr. Drug Targets CNS Neurol. Disord.* 4, 685–696
27 Di Marzo, V. *et al.* (2004) The endocannabinoid system and its therapeutic exploitation. *Nat. Rev. Drug Discov.* 3, 771–784
28 Ortega-Gutiérrez, S. (2005) Therapeutic perspectives of inhibitors of endocannabinoid degradation. *Curr. Drug Targets CNS Neurol. Disord.* 4, 697–708

29 Maccarrone, M. (2006) Fatty acid amide hydrolase: a potential target for next generation therapeutics. *Curr. Pharm. Des.* 12, 759–772
30 Marsicano, G. *et al.* (2003) CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science* 302, 84–88
31 Sarne, Y. and Mechoulam, R. (2005) Cannabinoids: between neuroprotection and neurotoxicity. *Curr. Drug Targets CNS Neurol. Disord.* 4, 677–684
32 Van der Stelt, M. and Di Marzo, V. (2005) Cannabinoid receptors and their role in neuroprotection. *Neuromol. Med* 7, 37–50
33 Di Marzo, V. *et al.* (2001) Anandamide: some like it hot. *Trends Pharmacol. Sci.* 22, 346–349
34 Ramirez, B.G. *et al.* (2005) Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J. Neurosci.* 25, 1904–1913
35 Van der Stelt, M. *et al.* (2006) Endocannabinoids and β-amyloid-induced neurotoxicity *in vivo*: effect of pharmacological elevation of endocannabinoid levels. *Cell. Mol. Life Sci.* 63, 1410–1424
36 Lastres-Becker, I. *et al.* (2005) Cannabinoids provide neuroprotection against 6-hydroxydopamine toxicity *in vivo* and *in vitro*: relevance to Parkinson's disease. *Neurobiol. Dis.* 19, 96–107
37 Kim, S.R. *et al.* (2005) Transient receptor potential vanilloid subtype 1 mediates cell death of mesencephalic dopaminergic neurons *in vivo* and *in vitro*. *J. Neurosci.* 25, 662–671
38 McKinney, M.K. and Cravatt, B.F. (2005) Structure and function of fatty acid amide hydrolase. *Annu. Rev. Biochem.* 74, 411–432
39 Raman, C. *et al.* (2004) Amyotrophic lateral sclerosis: delayed disease progression in mice by treatment with a cannabinoid. *Amyotroph. Lateral Scler. Other Motor Neuron Disord.* 5, 33–39
40 Bilsland, L.G. *et al.* (2006) Increasing cannabinoid levels by pharmacological and genetic manipulation delay disease progression in SOD1 mice. *FASEB J.* 20, 1003–1005
41 Huntley, A. (2006) A review of the evidence for efficacy of complementary and alternative medicines in MS. *Int. MS J.* 13, 5–12
42 Arevalo-Martin, A. *et al.* (2003) Therapeutic action of cannabinoids in a murine model of multiple sclerosis. *J. Neurosci.* 23, 2511–2516
43 Eljaschewitsch, E. *et al.* (2006) The endocannabinoid anandamide protects neurons during CNS inflammation by induction of MKP-1 in microglial cells. *Neuron* 49, 67–79
44 Consroe, P. *et al.* (1997) The perceived effects of smoked cannabis on patients with multiple sclerosis. *Eur. Neurol.* 38, 44–48
45 Mestre, L. *et al.* (2005) Pharmacological modulation of the endocannabinoid system in a viral model of multiple sclerosis. *J. Neurochem.* 92, 1327–1339
46 Ortega-Gutierrez, S. *et al.* (2005) Activation of the endocannabinoid system as therapeutic approach in a murine model of multiple sclerosis. *FASEB J.* 19, 1338–1340
47 Pryce, G. *et al.* (2003) Cannabinoids inhibit neurodegeneration in models of multiple sclerosis. *Brain* 126, 2191–2202
48 Ehrhart, J. *et al.* (2005) Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. *J. Neuroinflammation* 2, 29–32
49 Bari, M. *et al.* (2006) New insights into endocannabinoid degradation and its therapeutic potential. *Mini Rev. Med. Chem.* 6, 109–120
50 Hogestatt, E.D. *et al.* (2005) Conversion of acetaminophen to the bioactive *N*-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system. *J. Biol. Chem.* 280, 31405–31412
51 Karanian, D.A. *et al.* (2005) Dual modulation of endocannabinoid transport and fatty acid amide hydrolase protects against excitotoxicity. *J. Neurosci.* 25, 7813–7820
52 Witting, A. *et al.* (2006) Experimental autoimmune encephalomyelitis disrupts endocannabinoid-mediated neuroprotection. *Proc. Natl. Acad. Sci. U. S. A.* 103, 6362–6367
53 Bisogno, T. *et al.* (2006) Development of the first potent and specific inhibitors of endocannabinoid biosynthesis. *Biochim. Biophys. Acta* 176, 205–212
54 Pertwee, R.G. (2005) Pharmacological actions of cannabinoids. *Handb. Exp. Pharmacol.* 168, 1–51
55 Klein, T.W. (2005) Cannabinoid-based drugs as anti-inflammatory therapeutics. *Nat. Rev. Immunol.* 5, 400–411
56 Kishimoto, S. *et al.* (2003) 2-Arachidonoylglycerol induces the migration of HL-60 cells differentiated into macrophage-like cells and human peripheral blood monocytes through the cannabinoid CB2 receptor-dependent mechanism. *J. Biol. Chem.* 278, 24469–24475
57 Melis, M. *et al.* (2004) Prefrontal cortex stimulation induces 2-arachidonoyl-glycerol-mediated suppression of excitation in dopamine neurons. *J. Neurosci.* 24, 10707–10715
58 Oka, S. *et al.* (2005) Evidence for the involvement of the cannabinoid CB2 receptor and its endogenous ligand 2-arachidonoylglycerol in 12-*O*-tetradecanoylphorbol-13-acetate-induced acute inflammation in mouse ear. *J. Biol. Chem.* 280, 18488–18497
59 Okamoto, Y. *et al.* (2004) Molecular characterization of a phospholipase D generating anandamide and its congeners. *J. Biol. Chem.* 279, 5298–5305
60 Kathuria, S. *et al.* (2003) Modulation of anxiety through blockade of anandamide hydrolysis. *Nat. Med.* 9, 76–80
61 Tarzia, G. *et al.* (2006) Synthesis and structure–activity relationships of FAAH inhibitors: cyclohexylcarbamic acid biphenyl esters with chemical modulation at the proximal phenyl ring. *ChemMedChem* 1, 130–139
62 Singh Tahim, A. *et al.* (2005) Inflammatory mediators convert anandamide into a potent activator of the vanilloid type 1 transient receptor potential receptor in nociceptive primary sensory neurons. *Neuroscience* 136, 539–548
63 Tsuboi, K. *et al.* (2005) Molecular characterization of *N*-acylethanolamine-hydrolyzing acid amidase, a novel member of the choloylglycine hydrolase family with structural and functional similarity to acid ceramidase. *J. Biol. Chem.* 280, 11082–11092
64 Liu, J. *et al.* (2006) A biosynthetic pathway for anandamide. *Proc. Natl. Acad. Sci. U. S. A.* 103, 13345–13350
65 Wei, B.Q. *et al.* (2006) A second fatty acid amide hydrolase with variable distribution among placental mammals. *J. Biol. Chem.* 281, 36569–36578