# The endocannabinoid system in targeting inflammatory neurodegenerative diseases

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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).

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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-$\alpha$ 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

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**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.
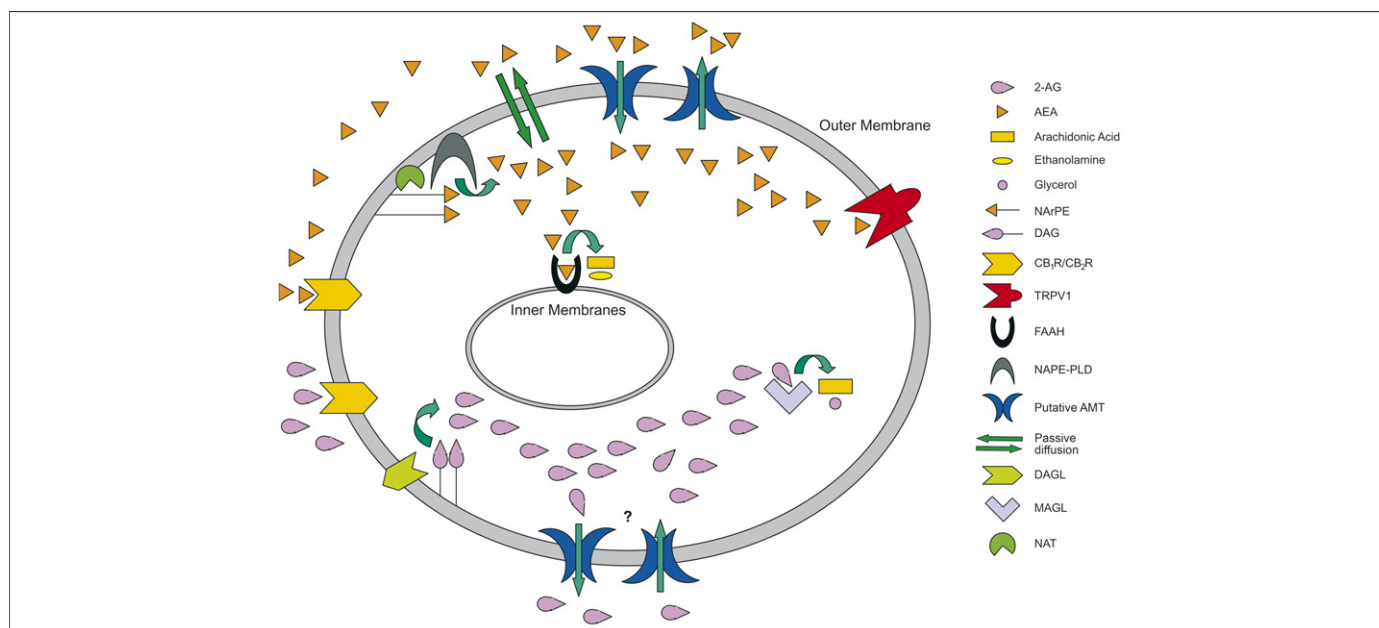
- **Retina**
  Control of scotopic vision.

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'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-$\alpha$; (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

**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.
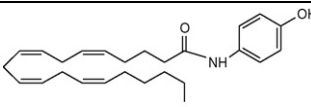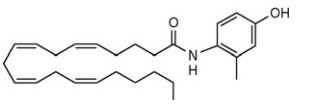
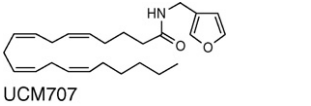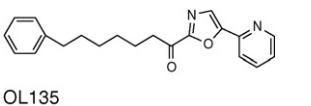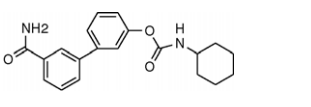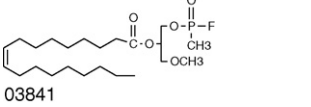### Amyotrophic lateral sclerosis
Pharmacological agonists of CB receptors and increased levels of endocannabinoids, obtained through genetic ablation of fatty acid amide hydrolase (FAAH) [38], exert robust anti-inflammatory and neuroprotective effects in hSOD1G93A mice, delaying disease progression [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 inflam-

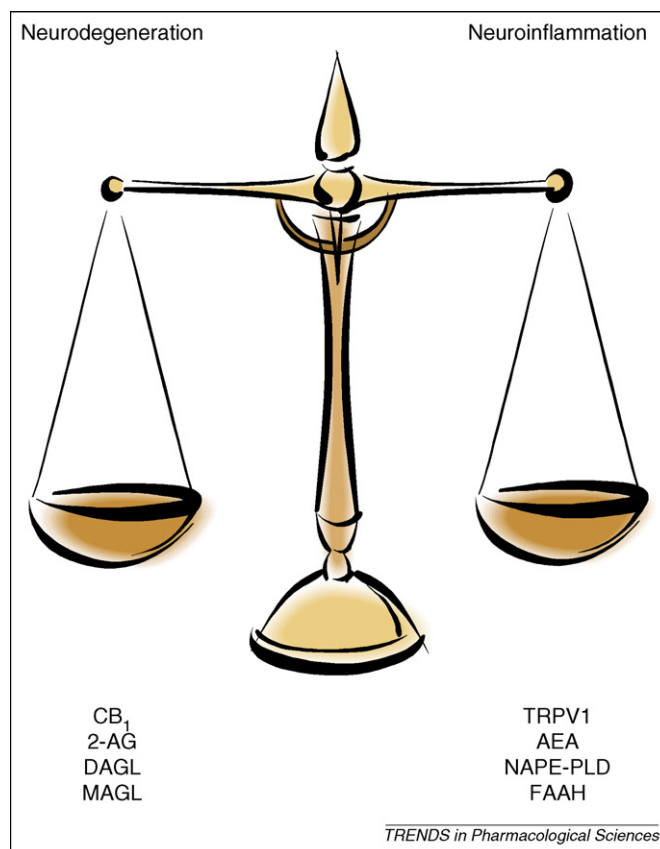matory neurological diseases in animal models [36,49] (Table 1).

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].

## 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.

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