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Review article

The endocannabinoid system and its therapeutic exploitation in multiple sclerosis: Clues for other neuroinflammatory diseases

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ABSTRACT

Multiple sclerosis is the most common inflammatory demyelinating disease of the central nervous system, caused by an autoimmune response against myelin that eventually leads to progressive neurodegeneration and disability. Although the knowledge on its underlying neurobiological mechanisms has considerably improved, there is a still unmet need for new treatment options, especially for the progressive forms of the disease. Both preclinical and clinical data suggest that cannabinoids, derived from the *Cannabis sativa* plant, may be used to control symptoms such as spasticity and chronic pain, whereas only preclinical data indicate that these compounds and their endogenous counterparts, i.e. the endocannabinoids, may also exert neuroprotective effects and slow down disease progression. Here, we review the preclinical and clinical studies that could explain the therapeutic action of cannabinoid-based medicines, as well as the medical potential of modulating endocannabinoid signaling in multiple sclerosis, with a link to other neuroinflammatory disorders that share common hallmarks and pathogenetic features.

Contents

Abbreviations: 2-AG, 2-arachidonoylglycerol; Aβ, β-amyloid; GABA, γ-amino butyric acid; AD, Alzheimer's diseases; ALS, amyotrophic lateral sclerosis; AEA, anandamide; EAE, autoimmune encephalomyelitis; BBB, blood brain barrier; CDB, cannabidiol; CB, cannabinoid receptor; CNS, central nervous system; COX, cyclooxygenase; DAGL, diacylglycerol lipase; CB, endocannabinoids; FAAH, fatty acid amide hydrolase; HD, Huntington's disease; IFN, interferon; IL, interleukin; MS, multiple sclerosis; MAGL, monoacylglycerol lipase; NAPE-PLD, *N*-acylphosphatidyl-ethanolamine-specific phospholipase D; PD, Parkinson's disease; PPAR, peroxisome proliferator-activated receptors; PP, primary progressive; PR, progressive relapsing; RR, relapsing-remitting; MRI, resonance imaging; SP, secondary progressive; SOD, superoxide dismutase; THC, tetrahydrocannabinol; Th, -helper; TRPV 1, transient receptor potential vanilloid 1; TNF, tumour necrosis factor.

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1. Introduction to neuroinflammation and multiple sclerosis

Although the central nervous system (CNS) has been long considered an immune-privileged site, mainly due to the presence of the blood brain barrier (BBB), immune activities occur and are sometimes ncesessary for neuronal function and host defence (Banks, 2015). Any insult of the brain is associated with acute inflammation, characterized by endothelial cell activation, tissue oedema and release of inflammatory mediators that provide to eliminate the insult and restore brain function. However, neuroinflammation is widely regarded as a chronic process that involves both sustained activation of resident glial cells (microglia and astroglia) and recurrent infiltration of peripheral leukocytes and soluble inflammatory mediators. Microglial cells, either derived from mesenchymal monocyte precursors of the mesoderm or from non-hematopoietic microglial precursors in the yolk sac, both entering the brain during embryonic and fetal stages, are the immune sentinels of the CNS. Microglial cells are extremely heterogeneous, because they can exist in many different forms and activation states, from neuro-protective to neuro-destructive (Nayak et al., 2014). Microglial cells are critical for the development and surveillance of the CNS, shaping the immune phenotype of the brain and critically modulating neuroinflammation (Mosser et al., 2017). Astrocytes are by far the most abundant cells in the CNS, where they regulate virtually every physiological process, from physically forming the BBB and giving nutritional support to sustaining neurotransmitters turnover, synaptic plasticity and immune functions. Indeed, immune activation of astrocytes in response to signals released by injured neurons or activated microglia leads to the so-called astrogliosis, a hallmark of neuroinflammation, which leads to glial scars that prevent axonal regeneration (Jensen et al., 2013). During neuroinflammation also immune cells of both innate (monocytes/macrophages and dendritic cells) and adaptive (T and B lymphocytes) immunity are persistently recruited to the brain, and release inflammatory cytokines and chemokines that exacerbate neuroinflammation (Schwartz and Baruch, 2014). As a matter of fact, neuroinflammation often may lead to neurodegeneration, axonal loss and synaptic dysfunction, and thus it is typically associated with several neurological disorders, of which multiple sclerosis (MS) is the prototypical example. MS is a progressive, chronic neurodegenerative disease, which affects approximately 2.3 million people worldwide (Browne et al., 2014). It is the most common neurological disorder in young adults, and is regarded as an autoimmune disease in which inflammation leads to demyelination of the axons in the CNS. Although the aetiology of MS is still unknown, it is almost unanimously believed that both genetic and environmental components play a central role in disease onset and development (Hafler et al., 2007; Huynh and Casaccia, 2013). The MS prevalence ratio of women to men (2.3–3.5:1) has increased markedly during the last decades and this rapid increase probably reflects a differential gender response to unidentified changes in environment or nutrition (Harbo et al., 2013). MS is characterized by a series of episodic acute attacks (referred to as relapses) and remissions, but it gradually leads to progressive neurodegeneration and deterioration of neurologic function without any further remission. Although the different clinical courses of MS (also called “types” or “phenotypes”) were defined in 1996, the International Advisory Commette on Clinical Trials of MS, based on advances in the understanding of the disease process in MS and MRI technology, classified MS into only four independent subtypes: (i) relapsing-remitting (RR), defined by unpredictable relapses with full recovery or with sequelae; (ii) primary progressive (PP), which progresses continuously from the onset without attacks; (iii) secondary progressive (SP), which follows initial RR and then progresses with decline without remissions; and (iv) progressive relapsing (PR), characterized by a steady decline onset with superimposed attacks (Fig. 1). The RR-MS is the most prevalent form and accounts for approximately 85% of all cases (Compston and Coles, 2008; Lublin et al., 2014). However, the Committee also recognized the clinically isolated syndrome (CIS) as a first episode of neurologic symptoms caused by inflammation and demyelination that must last at least 214 h and that is characteristic of MS even though does not yet meet the criteria for its diagnosis because patients with CIS might also not end up developing MS (Lublin et al., 2014). MS pathogenesis and pathophysiology have been extensively studied, especially in the experimental autoimmune encephalomyelitis (EAE) mouse model, and are thought to involve initially the disruption of the immune system and of central myelin-producing cells. In the course of MS, damage to the BBB and over-activation of brain microglia lead to a substantial infiltration of autoreactive lymphocytes, causing oligodendrocyte death and axonal damage and ultimately resulting in demyelination, synaptic alteration and neuronal loss (Compston and Coles, 2008; Dutta and Trapp, 2011; Calabrese et al., 2015; Mahad et al., 2015) (Fig. 2).

Though the immune-mediated neuroinflammation hypothesis has dominated MS research for over 50 years, recent evidence seems to point to a neurodegenerative and microglia-centered process, according to which MS is primarily a neurodegenerative disease that starts in the brain, and then develops because of inflammation (Kassmann et al., 2007; Lassmann et al., 2012). This has led to the current “inside-out” and “outside-in” models of MS immunopathogenesis, whereby in the first model the immune response that destroys myelin and leads to BBB breakdown is driven by a dysfunction of brain cells, whereas in the second model a dysfunction residing in the periphery leads to BBB damage, myelin disruption and axonal death (Tsunoda and Fujinami, 2002; Stys et al., 2012).

The immune-mediated attacks are mainly driven by cells of adaptive immunity, namely myelin-specific and self-reactive CD8 amd CD4 T-cells (T-helper 1 and T-helper 17), with a key contribution of B-cells that produce high levels of autoantibodies and that have been recently shown to contribute to neurodegeneration and cortical demyelination, especially for meningeal ectopic B cell follicles (Fraussen et al., 2016). During these attacks, the myelin sheath is damaged, thus impairing axonal conduction and the correct communication between different parts of the nervous system (Compston and Coles, 2008; Gandhi et al., 2010; Mahad et al., 2015). These processes are sustained by a subsequent recruitment of cells of the innate immunity from the periphery that further amplify the activation of pathogenic T-cells and the destruction of neurons and oligodendrocytes. In addition, there is a permanent activation of resident microglia and astrocytes that further potentiate the neuroinflammatory response by producing proinflammatory mediators (Gandhi et al., 2010; Chiurchiù, 2014) (Fig. 2). Oligodendrocytes are responsible for myelin production

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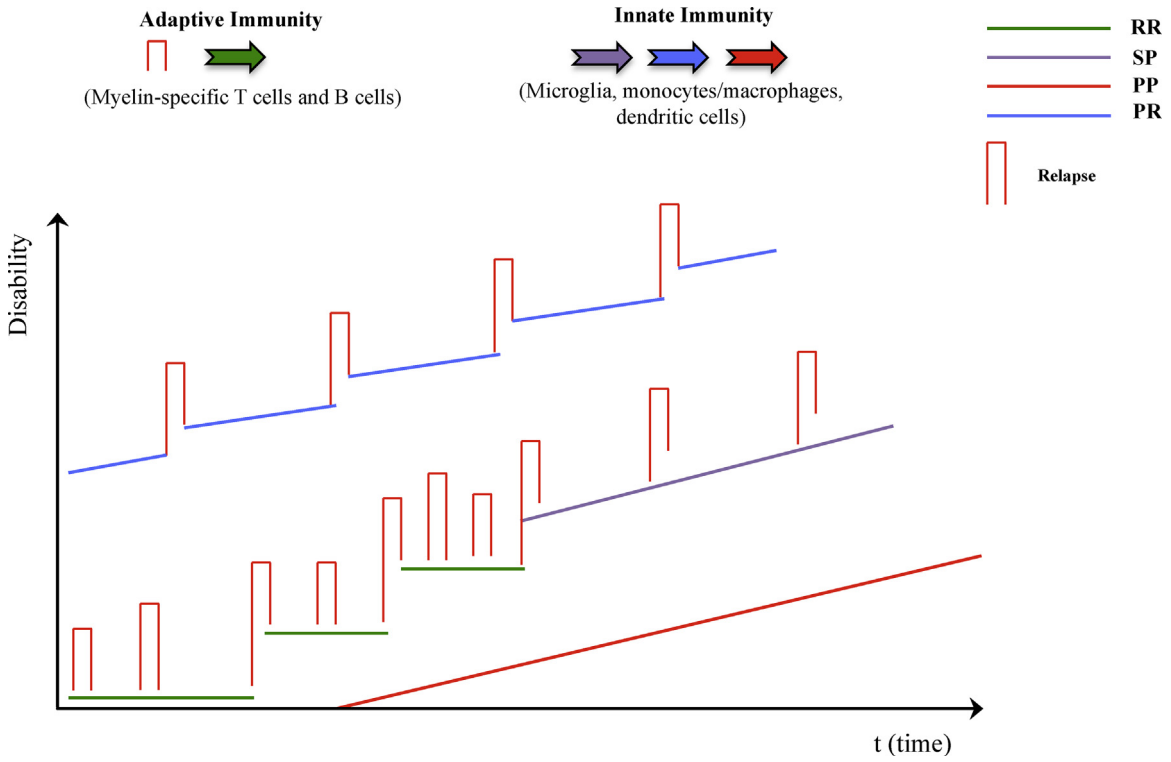

Fig. 1. The immunological basis of the different clinical forms of MS.

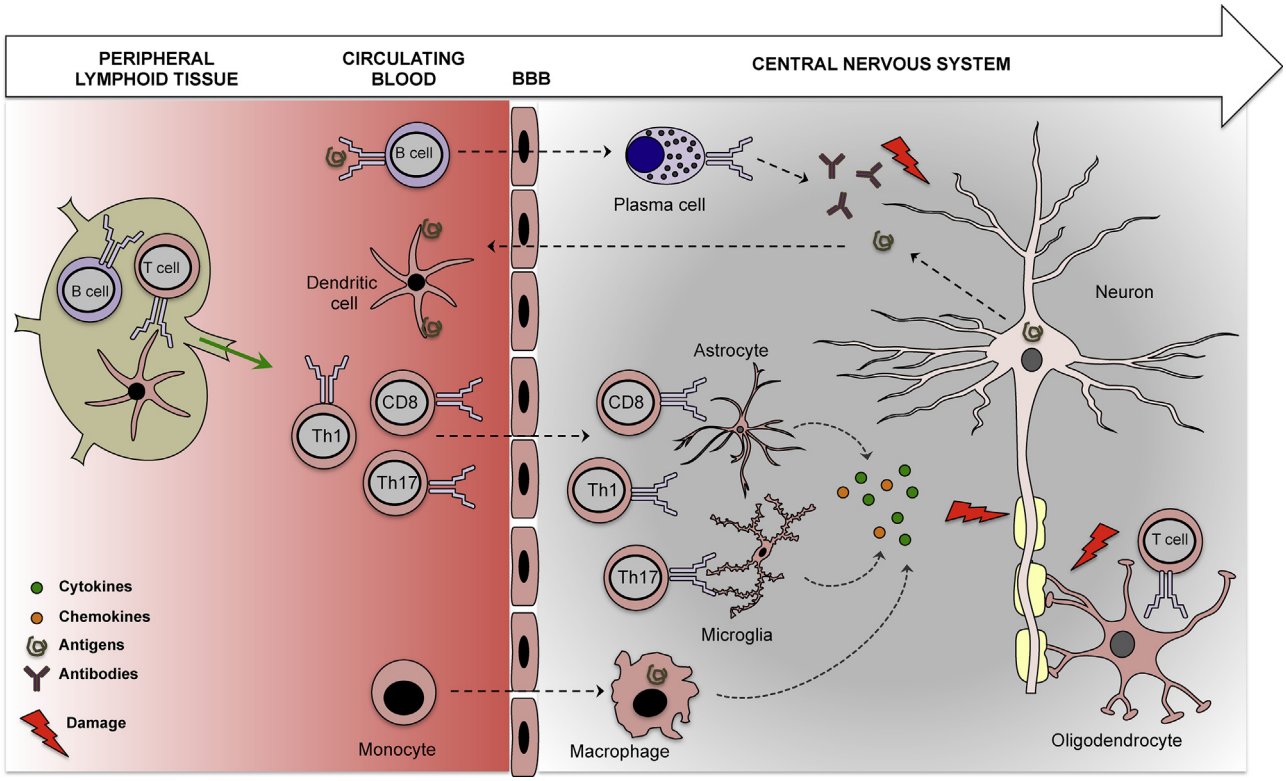

Fig. 2. Immunopathogenesis of MS. Injury to blood-brain barrier (BBB) and/or hyperactivation of brain microglial cells lead to a massive infiltration in the central nervous system of autoreactive T cells (CD8 and CD4 T-helper cells), B cells and antigen-presenting cells (APCs), including monocytes/macrophages. In the brain, T cells and monocytes/macrophages release cytokines and chemokines that damage the oligodendroglial cells, and hence the myelin sheath. Concomitantly, B cells (plasma cells) produce myelin-specific antibodies, which form membrane-attack complexes that further damage myelin sheath. These processes also lead to an over-activation of microglia and astrocytes, which further potentiate the inflammatory response.

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and generate new myelin in a process termed remyelination, especially in the early phases of the disease. Remyelination is one of the reasons why symptoms tend to decrease or disappear temporarily in RR-MS (Peferoen et al., 2014). However, oligodendrocytes are unable to completely rebuild the myelin sheath and repeated attacks lead to less and less effective remyelination, until scar-like plaques (scleroses) build up around the damaged axons with subsequent axonal loss (Cambron et al., 2012). These scars, usually more than 10 as visualized by magnetic resonance imaging (MRI), determine a wide range of symptoms, including physical disability, fatigue, and cognitive impairment (Polman et al., 2011). Approximately 10 years after disease onset, frequent relapses and remissions give pace to a progressive clinical aggravation. Such an evolution from relapsing-remitting to progressive forms of MS certainly involves the loss of oligodendroglia and neurons most likely due to several concurrent events, including (i) disruption of peripheral immune tolerance and a subsequent shift to innate immunity (plaques contain more monocyte/macrophages and dendritic cells and fewer T cells in chronic disease), (ii) reduced neurotrophic abilities of astrocytes on neurons, (iii) increased loss of oligodendrocyte precursor cells and (iv) toxic effects on axons (Gandhi et al., 2010; Reynolds et al., 2011) (Fig. 1).

At any rate, all these noxious effects appear to be causing the widespread, cortical and subcortical grey matter atrophy since the early stages of the disease as observed by MRI and analysis of post-mortem MS patient samples (Mandolesi et al., 2015; Musella et al., 2016). Neurodegeneration leads to permanent and increasing disability, with spasticity and pain as important hallmarks of the disease.

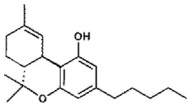
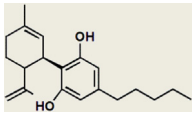
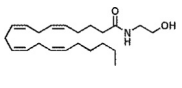
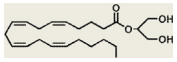
Current therapies for MS focus on the prevention of relapses in the early stage, mainly stopping the immune attacks with drugs that directly inhibit cell activation and the release of inflammatory mediators, or that prevent leukocyte recruitment into the CNS. However, almost no drugs that prevent or slow down the occurrence of neurodegeneration and disease progression are yet available, with the exception of the very recently approved humanized monoclonal antibody Ocrelizumab (Ocrevuz®) for primary progressive MS patients (Montalban et al., 2017). Approval of Nabiximol (Sativex®, a 1:1 mixture of Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD), both isolated from *Cannabis sativa*) to treat MS-induced spasticity was recently obtained in several countries (Zettl et al., 2016; Maccarrone et al., 2017). Since preclinical data show that cannabinoids also exert anti-inflammatory properties and neuroprotective effects (Chiurchiù et al., 2015a), it is hypothesized that these substances may limit progressive neurodegeneration in MS (Pryce et al., 2003; Jackson et al., 2005; Witting et al., 2006). Here, we will review the role of cannabinoids and their endogenous counterparts (i.e., the endocannabinoids, eCBs) in MS, their mode of action and preclinical and clinical significance, as well as how their pharmacological modulation could be useful to treat other neuroinflammatory diseases.

2. A glimpse to the eCB system

2.1. Cannabinoids and cannabinoid receptors

For over 5000 years, Cannabis (*Cannabis sativa*) has long been used for recreational and medicinal purposes for more than 5000 years. Cannabis extracts like marijuana contain more than 500 natural compounds, out of which over 110 belong to cannabinoids (Maccarrone et al., 2017). Although the first isolated plant-derived cannabinoids were cannabinol and cannabidiol (CBD), the identification of the major psychoactive constituent THC was achieved later on (Table 1) (Adams, 1942; Gaoni and Mechoulam, 1964), establishing the current field of cannabinoid research. Several cannabinoids have distinct biological activity, yet THC and CBD were particularly studied due to their broad spectrum of

Table 1
Major (endo)cannabinoids, and main metabolic enzymes of eCBs with a role in neuroinflammation.

Name (abbreviation)	Chemical structure
Δ^9-Tetrahydrocannabinol (THC)	
Cannabidiol (CBD)	
N-Arachidonoylethanolamine (Anandamide, AEA)	
2-Arachidonoylglycerol (2-AG)	
Biosynthetic enzyme of AEA	**Intracellular localization**
N-acylphosphatidyl ethanolamines (NAPE)-specific phospholipase D (NAPE-PLD)	Membrane-associated
Biosynthetic enzymes of 2-AG	**Intracellular localization**
Diacylglycerol lipase α (DAGLα)	Membrane-associated
Diacylglycerol lipase β (DAGLβ)	Membrane-associated
Degrading enzyme of AEA	**Intracellular localization**
Fatty acid amide hydrolase (FAAH)	Membrane-associated (mainly ER)
Degrading enzyme of 2-AG	**Intracellular localization**
Monoacylglycerol lipase (MAGL)	Membrane-associated and cytosolic

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action, including analgesic and anti-inflammatory effects. THC stereoselectively acts in the CNS (Mechoulam et al., 1988) by binding to a specific target identified as the type-1 cannabinoid receptor (CB_1) (Devane et al., 1988). Few years later a type-2 cannabinoid receptor (CB_2) was discovered in peripheral B- and T-lymphocytes (Munro et al., 1993). THC binds to both CB_1 and CB_2 with high affinity (Huffman, 2000; Mahadevan et al., 2000), whereas CBD, a non-psychoactive component, shows little affinity for both receptors (Mechoulam et al., 2007). Upon activation both receptors inhibit the formation of the second messenger cAMP, and modulate other signal transduction pathways such as extracellular regulated kinases, β-arrestin, and ion channels. There is a debate on the expression levels of CB_1 and CB_2 in different cell types and its subcellular localisation, but there is a general consensus that CB_1 is widely expressed within the CNS in cortical neurons and interneurons, astrocytes, oligodendrocytes and oligodendrocyte precursor cells, as well as in several leukocytes infiltrating the brain (Navarrete and Araque, 2010; Galve-Roperh et al., 2013). CB_1 regulates cognitive, memory and motor functions as well as analgesia and synaptic plasticity. On the contrary, the expression of CB_2 in cells of the CNS is more controversial, inasmuch as its presence for a long time was exclusively associated to microglia, but more recently it was also documented in brainstem neurons and astrocytes upon cellular activation by an insult or inflammation (Van Sickle et al., 2005; Onaivi et al., 2006; Atwood and Mackie, 2010; et al., 2015a, 2015b; et al., 2015a, 2015b). Furthermore, a substantial proportion of CB_1 in the brain is intracellular, being found in vesicles (Leterrier et al., 2004), mitochondria (Bénard et al., 2012) or lysosomes (Rozenfeld and Devi, 2008). Interestingly, the human brain has more CB_1 than any other G protein-coupled receptor.

2.2. Endogenous ligands of cannabinoid receptors and their metabolic routes

Unsurprisingly, in view of the lipophilic nature of THC, the endogenous ligands of CB receptors, i.e. the endocannabinoids (eCBs), are derivatives of the lipid arachidonic acid (AA). The most important eCBs are *N*-arachidonoylethanolamine (anandamide, AEA) and 2-arachidonoylglycerol (2-AG) (Table 1). These two endogenous ligands of CB_1 and CB_2 are the best-studied members of an ever-growing family of compounds (Maccarrone et al., 2014; Mechoulam et al., 2014) that include *N*-acylethanolamines, 2-monoacylglycerols, and now also peptidic eCBs (pepcans) (Bauer et al., 2012). This large family also includes the recently discovered ethanolamines of ω-3 (*n*-3) fatty acid, namely *N*-eicosapentaenoylethanolamine (EPEA) and *N*-docosahexaenoyl-ethanolamine (DHEA) (Artmann et al., 2008; Lucanic et al., 2011). EPEA and DHEA act indeed as CB_1/CB_2 agonists (Brown et al., 2010), but their function still needs to be clarified. There are additional compounds that do not bind to CB_1 and/or CB_2, but are processed by the same synthesizing and degrading enzymes as authentic eCBs; these so-called "eCB-like" substances include *N*-palmitoylethanolamine (PEA) and *N*-oleoylethanolamine (OEA) (Ueda et al., 2013).

Both AEA and 2-AG are often found together, but their individual levels vary between species, tissues, developmental stages and pathophysiological conditions. Within the CNS and amidst glial and neuronal cells, AEA and 2-AG act as retrograde messengers, i.e. they are released from somata and/or dendrites of neurons, and then act on afferent axon terminals or nearby astroglial processes to inhibit neurotransmitter release (Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). Depending on neuron type glutamatergic or γ-aminobutyric acid (GABAergic), activation of CB_1 may results in inhibition or activation of the neuronal circuit. Unlike other neurotransmitters, eCBs are not stored in vesicles, but are produced "on demand" (*i.e.*, when and where needed) upon different biological stimuli, to act paracrinally or autocrinally. At least five different pathways can generate AEA (Di Marzo et al., 1994; Leung et al., 2006; Liu et al., 2006), the most studied being the release of AEA from membrane precursors via *N*-acylphosphatidyl-ethanolamine (NAPE)-specific phospholipase D (NAPE-PLD) (Okamoto et al., 2004). Cleavage of AEA into AA and ethanolamine is mainly operated by fatty acid amide hydrolase (FAAH) (Cravatt et al., 1996). On the other hand, 2-AG is formed by the action of two diacylglycerol lipases, DAGLα and DAGLβ (Bisogno et al., 2003), and is primarily degraded into AA and glycerol by monoacylglycerol lipase (MAGL) (Dinh et al., 2002) and to a lesser extent by α/β-hydrolase domain (ABHD) 6 and ABHD12 (Blankman et al., 2007). Furthermore, AEA and 2-AG can also be metabolized by cyclooxygenase-2 (COX-2), several lipoxygenase isozymes and by cytochrome P450, generating oxidized compounds like prostaglandin-ethanolamides and glyceryl esters, hydroxy-anandamides and hydroxyeicosatetraenoyl-glycerols, respectively. All these substances can bind to CB_1 and CB_2, and are endowed with distinct, yet unclear, biological activities (van der Stelt et al., 2002; Rouzer and Marnett, 2011). All eCBs can also be inactivated through their uptake by a purported "endocannabinoid membrane transporter" (EMT), whose molecular identiy has yet to be identified but whose presence has been suggested by several reports as well as by its *in vitro* and *in vivo* pharmacological targeting (Chicca et al., 2017). Overall, the activity and function of eCBs is controlled by their endogenous tone, which depends on a finely regulated balance between biosynthetic and degradative pathways. At variance with this picture more recently it has been demonstrated that AEA can be stored in lipid droplets (adiposomes) and bound to intracellular transporters (Maccarrone et al., 2010). These additional players seem to be relevant, because neurophysiological actions of eCBs depend on the specific location of the proteins that synthesize, transport, bind and degrade them, altogether called "eCB system" (Maccarrone et al., 2014, 2015). The complexity and distinct distribution of eCB system components in pre- and post-synaptic neurons, as well as in microglia and astrocytes, is schematically depicted in Fig. 3.

2.3. Other receptors

In addition to CB_1 and CB_2, cannabinoids and eCBs can exert their effects engaging other non-CB receptors, including the transient receptor potential vanilloid 1 (TRPV1) channel, expressed mainly in peripheral tissues, including peripheral sensory neurons, epithelial and endothelial cells as well as immune cells (Xia et al., 2011). Other targets are peroxisome proliferator-activated receptors (PPAR) α and γ (Pistis and Melis, 2010) that belong to a family of nuclear receptors whose role is mainly to control lipid metabolism; and orphan G protein-coupled receptor GPR55 (Moriconi et al., 2010). The existence of such supplementary molecular targets support the view that an extension of the term "cannabinoid receptor" should really be encouraged. The multiplicity of cannabinoids and eCBs at interacting with many other non-CB receptors and the evidence that cannabinoid receptors are able to bind to other ligands other than cannabinoids and eCBs endorse such view.

3. Therapeutic exploitation of the endocannabinoid system in multiple sclerosis

3.1. Preclinical ex vivo and in vivo studies of eCB signaling in MS

To establish whether a drug is ready for clinical trials (the so-called move from bench to bedside) involves extensive preclinical studies that yield preliminary efficacy, pharmacokinetic, safety and toxicological information. The starting point of such studies is

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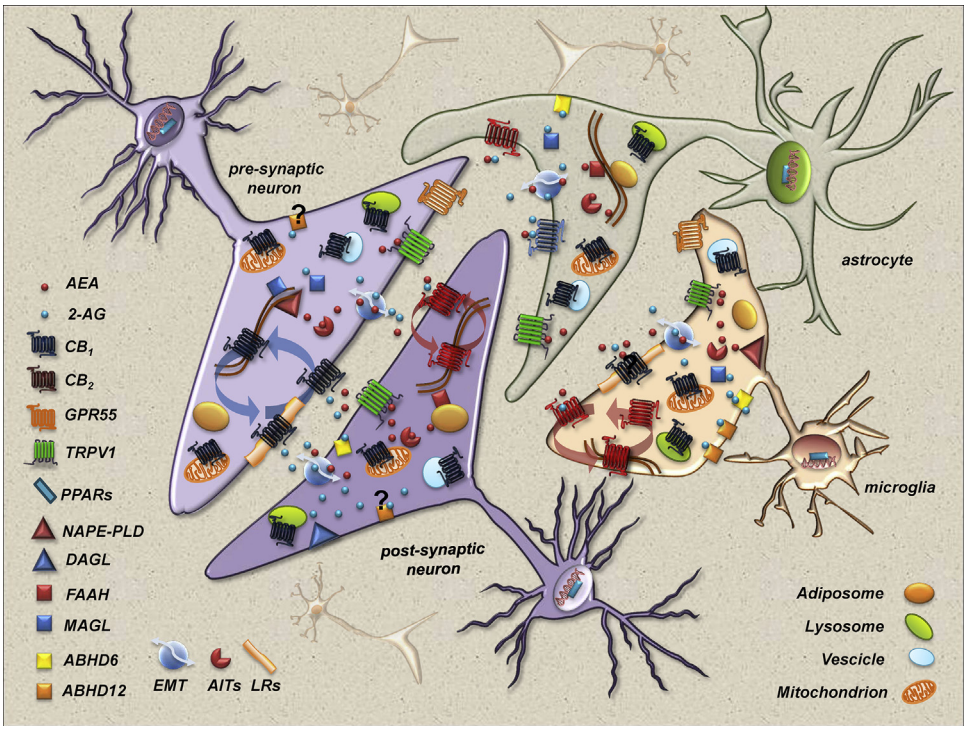

Fig. 3. A modern view of the eCB system in the CNS. eCB signaling is orchestrated by target receptors (CB_1, CB_2, GPR55, TRPV1 and PPARs), biosynthetic (NAPE-PLD and DAGL) and degradative (FAAH, MAGL, ABHD6 and ABHD12) enzymes, transmembrane transport mechanisms (like the putative EMT), intracellular trafficking by AITs like fatty acid binding proteins, heat shock protein 70, and FAAH-like AEA transporter, as well as by storage organelles (adiposomes or lipid droplets). Altogether, these proteins regulate the endogenous tone of eCBs, and hence their biological activity. For some of the elements of the eCB system a distinct distribution, both intracellularly and among pre- and post-synaptic neurons, microglia and astrocytes, has been documented. It should be noted that, unlike other eCB-binding receptors, CB_1 appears to be located in cholesterol-enriched membrane microdomains termed LRs, and that CB_2 is expressed in neurons mainly upon brain injury. Moreover, the role in neuroinflammatory diseases of eCB system elements shown here but not listed in Table 1 remains to be elucidated.
Abbreviations: ABHD6/12, α-β-hydrolase domain 6/12; AITs, AEA intracellular transporters; CB_1/CB_2, G-protein coupled type-1 and type-2 cannabinoid receptors; DAGL, diacylglycerol lipase α/β; eCBs, endocannabinoids; EMT, putative endocannabinoid transmembrane transporter; FAAH, fatty acid amide hydrolase; GPR55, G-protein coupled receptor 55; LRs, lipid rafts; MAGL, monoacylglycerol lipase; NAPE-PLD, N-acylphosphatidylethanolamine-specific phospholipase D; PPARs, peroxisome proliferator-activated nuclear receptors; TRPV1, transient receptor potential vanilloid 1 channels.

often the evidence of a significant alteration of activity or expression of a certain molecular target (often a protein) in disease models, or in affected patients, either *in vitro* (experiment and analysis performed outside a living organism) or *ex vivo* (experiment performed inside and analysis outside a living organism). In this line, accumulated *ex vivo* evidence showed a wide alteration of CB_1 and CB_2 expression in several experimental models of MS and in patients affected by different clinical forms of the disease (Benito et al., 2003, 2007; Cabranes et al., 2005; Loría et al., 2008; Jean-Gilles et al., 2009; Chiurchiù et al., 2013; Sánchez López et al., 2015; Maccarrone et al., 2017). This observation strongly suggests a functional role for cannabinoid receptors in MS pathology, and has stimulated preclinical studies directed at modulating their activity by *in vivo* administration of plant cannabinoids, synthetic agonists or antagonists (Table 2). The first such preclinical study demonstrated an amelioration of both tremor and spasticity in mice suffering from chronic relapsing EAE upon *in vivo* treatment with THC, methanandamide (a stable and non-hydrolizable AEA anolog), and synthetic CB_1 or CB_2 agonists (Baker et al., 2000). Selective antagonists of CB_1 or CB_2 inhibited these effects, indicating that the eCB system may participate in the control of MS. In addition to symptom management, cannabinoids also conferred neuroprotection via CB_1, slowing down the neurodegenerative process that eventually leads to chronic disability (Pryce et al., 2003). Furthermore, they caused immunosuppression-mediated amelioration of symptom progression and promoted remyelination also in a viral model of MS (Croxford and Miller, 2003; Arévalo-Martín et al., 2003). Such CB receptor-mediated neuroprotection and interference with MS progression were paralleled by downregulation of adhesion molecules and subsequent inhibition of T lymphocyte infiltration and microglial responses (Mestre et al., 2009; Zhang et al., 2009; Kozela et al., 2011), as well as by restoration of self-tolerance to myelin antigen (Arévalo-Martín et al., 2012). Selective activation of CB_2 in autoreactive T-cells was crucial to control inflammation in EAE (Maresz et al., 2007) by reducing highly pathogenic T-helper 17 differentiation and immune cell accumulation in the CNS, including activated macrophages/microglia (Kong et al., 2014). In line, selective activation of CB_2 on T lymphocytes isolated from MS patients inhibited cell proliferation and immune responses without inducing cell death (Malfitano et al., 2013). The involvement of CB receptors was confirmed not only by pharmacological modulation of their activity but also by their genetic deletion. Accordingly, mice whose CB_1 was genetically ablated poorly tolerated inflammatory and excitotoxic insults and developed substantial neurodegeneration (Pryce et al., 2003; Rossi et al., 2011a; Musella et al., 2014). Similarly, specific deletion of CB_2 on encephalitogenic T cells increased proliferation, reduced T-cell apoptosis, elevated production of proinflammatory cytokines and worsened the symptoms. This scenario suggested that activation of CB_2 on T-cells by eCBs was sufficient to control autoimmune inflammation, and possibly to slow down progression of the disease (Maresz et al., 2007). These studies indicate the involvement of both CB_1 and CB_2 in the therapeutic efficacy of the eCB system modulation. They also suggest a differential role played by these two receptor sub-types, whereby control of

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Table 2
Alterations of distinct elements of the eCB system, and their role in inflammation and neurodegeneration in MS.

ECS element	Model	Sample	Variation	Effects	Reference
AEA	Chronic EAE	Brain, spinal cord	↑	Early inhibition of spasticity	Baker et al. (2001)
	Lewis EAE rats	Brain	↓	Worsening of disease development and neurological impairment	Cabranes et al. (2005)
	RR-MS	Autopsied brain	↑	Microglia-induced neuroprotection	Eljaschewitsch et al. (2006)
	EAE and RR-MS patients	Brain, CSF, plasma, T cells	↑	Neuroprotection	Centonze et al. (2007a)
	RR-MS, SP-MS	CSF	↓	–	Di Filippo et al. (2008)
	RR-MS, PP-MP, SP-MS	Plasma	↑	Disease progression	Jean-Gilles et al. (2009)
	RR-MS	T cells, B cells, NK cells	↑	–	Sánchez López et al. (2015)
NAPE-PLD/ FAAH	EAE and RR-MS patients	Brain, CSF, plasma, T cells	↑ NAPE-PLD and ↓FAAH	Neuroprotection	Centonze et al. (2007a)
	SP-MS	Plasma	↓FAAH	Disease progression	Jean-Gilles et al. (2009)
	RR-MS	mDC and pDC	↓FAAH in mDC and ↑ in pDC	Lack of immunoregulation	Chiurchiù et al. (2013)
	RR-MS	T cells, B cells, NK cells	←→	–	Sánchez López et al. (2015)
2-AG	Chronic EAE	Brain, spinal cord	↑	Late inhibition of spasticity	Baker et al. (2001)
	Lewis EAE rats	Brain	↓	Worsening of disease development and neurological impairment	Cabranes et al. (2005)
	RR-MS patients	CSF	←→	–	Centonze et al. (2007a)
	RR-MS, SP-MS	CSF	↓	–	Di Filippo et al. (2008)
	TMEV-IDD	Spinal cord	↑	–	Loría et al. (2008)
	RR-MS	T cells, B cells, NK cells	Increased in NK cells	–	Sánchez López et al. (2015)
DAGL/ MAGL	EAE	–	–	Inhibition of MAGL ameliorates EAE progression	Hernández-Torres et al. (2014)
CB_1	Lewis EAE rats	Brain	↓	Worsening of disease development and neurological impairment	Cabranes et al. (2005)
	P-MS	Plasma	↑	Disease progression	Jean-Gilles et al. (2009)
	MS plaques	Neurons, oligodendrocytes, infiltrated T cells	↑	Disease progression	Benito et al. (2007)
	RR-MS	T cells, B cells, NK cells	↑in T cells	–	Sánchez López et al. (2015)
CB_2	TMEV-IDD	Spinal cord	↑	–	Loría et al. (2008)
	P-MS	Plasma	↑	Disease progression	Jean-Gilles et al. (2009)
	MS plaques	Infiltrated T cells, astrocytes, microglia	↑	Disease progression	Benito et al. (2007)
	RR-MS	mDC and pDC	↑in mDC and ←→in pDC	Lack of immunoregulation	Chiurchiù et al. (2013)
	RR-MS	T cells, B cells, NK cells	Increased in B cells	–	Sánchez López et al. (2015)

CSF, cerebrospinal fluid; EAE, experimental autoimmune encephalomyelitis; mDC, myeloid dendritic cells; RR, relapsing-remitting; P, progressive; pDC, plasmacytoid dendritic cells; PP, primary progressive; SP, secondary progressive; TMEV-IDD, Theiler's murine encephalomyelitis virus-induced demyelinating disease. ↑, increase; ↓, decrease; ←→, unchanged.

spasticity is mostly mediated by CB_1, while CB_2 seems to modulate overactive and exacerbated immune responses by (Pryce and Baker, 2007; Palazuelos et al., 2008).

Much alike CB_1 and CB_2, metabolic enzymes of eCBs have been found to be significantly altered in both MS patients (Baker et al., 2001; Cabranes et al., 2005; Benito et al., 2007; Centonze et al., 2007a,b; Di Filippo et al., 2008; Jean-Gilles et al., 2009; Chiurchiù et al., 2013; Sánchez López et al., 2015) and animal models of the disease (Baker et al., 2001; Pryce et al., 2003; Witting et al., 2006; Loría et al., 2008) (Table 2). These alterations of eCBs and of their metabolism are still somewhat controversial, most likely because of different experimental models used and variations in MS patients' recruitment and stratification. Despite these controversies, the anti-inflammatory and neuroprotective role of eCBs is widely documented in both *in vivo* and *ex vivo* experiments on cells obtained from either experimental models of MS or from authentic MS patients (Witting et al., 2006; Mestre et al., 2005; Ortega-Gutiérrez et al., 2005; Loría et al., 2010). Exogenous administration of eCBs improved control of spasticity (Baker et al., 2000, 2001) and protected neurons from inflammatory damage possibly by engaging CB receptors on microglial cells (Eljaschewitsch et al., 2006). Additionally, inhibition of endogenous AEA uptake and of its degradation by FAAH was shown to ameliorate motor symptoms (Baker et al., 2001; de Lago et al., 2004). This improvement was associated with reduced inflammatory responses in the spinal cord (Mestre et al., 2005) along with downregulation of macrophage and microglial function (Mestre et al., 2005; Ortega-Gutiérrez et al., 2005). In a virus-induced model of MS, AEA inhibited microglial activation by lowering interleukin (IL)-23 and IL-12 release (Correa et al., 2011), as well as IL-1β and IL-6 (Hernangómez et al., 2012). Inhibition of the last two pro-inflammatory cytokines was mediated by a mechanism involving CB_2 receptor-mediated recovery of the interaction between CD200 (expressed in neurons) and its receptor (expressed in macrophages/microglia). Such an interaction appears to be disrupted in MS, thus exposing neurons to macrophages/microglia-induced neurotoxicity and cytokine release (Hernangómez et al., 2014). Recently, we have demonstrated that the anti-inflammatory role of AEA in MS is cell type-specific, since it inhibits cytokine production only in myeloid dendritic cells (Chiurchiù et al., 2013). Instead, plasmacytoid dendritic cells were unresponsive to AEA due to a significant upregulation of FAAH compared to healthy subjects (Chiurchiù et al., 2013). Interestingly, chronic and long-term inhibition of FAAH (via genetic ablation) resulted in clinical remission and improved long-term outcome in EAE mice (Webb et al., 2008). Further, the acute phase of MS was ameliorated by increased levels of 2-AG that inhibited spasticity when given at 10 mg/kg (Baker et al., 2001) and caused a delayed onset in acute and chronic EAE

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models when administered intraperitoneally at 100 μg. The last effect was probably mediated by recruitment of anti-inflammatory macrophages (Lourbopoulos et al., 2011).

Based on these eCB-mediated neuroprotective actions, selective inhibitors of FAAH and MAGL, that degrade AEA and 2-AG respectively, were used *in vivo* to increase the tone and thus potentiate the effects of these eCBs. The blockade of AEA degradation by several potent and selective FAAH inhibitors was in fact able to control spasticity in Biozzi ABH mice (Pryce et al., 2013), another mouse model of MS that reproduces both relapsing-remitting course and secondary progressive neurological aspects (Amor et al., 2005). Likewise, MAGL inhibitors controlled spasticity (Pryce et al., 2013), preserved myelin integrity by suppressing oligodendrocyte excitotoxicity and microglial activation (Bernal-Chico et al., 2015), and slowed the clinical progression of EAE (Hernández-Torres et al., 2014; Brindisi et al., 2016).

Several preclinical studies have recently shown that eCB signalling controls synaptopathy, another hallmark of MS and neuroinflammation. Loss of homeostatic control of neurotransmission and subsequent synapse dysfunction is deeply affected by inflammation. Excitatory (glutamatergic) and inhibitory (GABAergic) synapses are privileged sites of action for eCBs and act in concert with proinflammatory cytokines (in particular IL-1β and tumour necrosis factor-α, TNF-α). Neuroinflammation unbalances synaptic transmission towards excitation, that ultimately results in excitotoxic neurodegeneration (Mandolesi et al., 2015), and activation of CB_1 dampens the enhancing effects of TNF-α on postsynaptic glutamate receptor expression and function (Rossi et al., 2011b), and reduces the frequency of spontaneous glutamate-mediated synaptic currents in striatal neurons, thus contrasting the effects of IL-1β on glutamate release from presynaptic nerve terminals (Musella et al., 2014). In this context, genetic deletion of CB_1 in glutamatergic or GABAergic neurons of EAE mice provided a conclusive demonstration that distinct subsets of these receptors are involved in both modulation of TNF-α-mediated postsynaptic exacerbation of glutamate currents, and IL-1β-dependent presynaptic increase of glutamate release induced in GABAergic projection neurons of the striatum (Musella et al., 2014). Moreover, IL-1β inhibits GABA synapses, and this effect further contributes to the excitotoxic damage of neurons during EAE and MS (Mandolesi et al., 2015). In keeping with its homeostatic role in neuroinflammatory disorders, CB_1 on GABAergic nerve terminals is selectively downregulated in the striatum of EAE mice, so that increased eCB levels in these animals only reduce excitatory transmission without affecting GABA signalling (Centonze et al., 2007a,b).

On the other hand, AEA can also bind to the transient receptor potential vanilloid type-1 (TRPV1) ion channel (Zygmunt et al., 1999), whose activation may negatively impact on neuronal survival. TRPV1 also modulates the synaptic effects of TNF-α and of IL-1β, hindering the synaptic deficits exerted by TNF-α in the peak phase of EAE and, in the chronic stages, enhancing IL-1β-induced GABAergic defects (Musumeci et al., 2011). These data suggest that the aversive or protective effects of TRPV1 on MS neurons are strictly dependent on the inflammatory milieu. Although it is still unclear which is the main cell type responding to central inflammation via eCB release, neural precursor cells appear good candidates, because they respond to inflammatory stimuli and to sensitized TRPV1 receptors (Stock et al., 2014), and limit inflammatory synaptic damage through AEA release (Butti et al., 2012). The mechanisms by which these elements of the eCB system modulate the presynaptic and postsynaptic perturbations induced by inflammation are depicted in Fig. 4.

Taken together, preclinical data strongly suggest that modulation of the eCB system is actively involved not only in the management of symptoms but also in neuroprotection and suppression of inflammation. These observations are generating considerable enthusiasm for bringing cannabinoid/eCB-based medicines into clinical practice for the treatment of both relapsing-remitting and progressive forms of MS. A further support to this perspective comes also from the experimental demonstration that plant-derived cannabinoids limit inflammation-induced neurodegeneration that drives progressive disability during EAE (Pryce et al., 2015). For example, in two different murine models of MS, Sativex slowed down MS progression by improving motor activity, reducing CNS infiltrates and microglial activity, together with promoting myelin repair (Feliú et al., 2015) and improving the neurological deficits and cell infiltrates within the CNS. This effect was attributed to THC activation of CB_1 receptors and not to CBD in the EAE model (Moreno-Martet et al., 2015), while in the viral model both THC and CBD were effective. However CBD, the cannabinoid that is devoid of psychoactive activity and potentially safe, has also been investigated as an effective alternative for alleviating neuroinflammation and neurodegeneration (Maccarrone et al., 2017). CBD ameliorates EAE clinical symptoms and reduces axonal damage and inflammation, as well as microglial activation, T-lymphocyte recruitment in the spinal cord (Kozela et al., 2011) and protection of oligodendrocyte progenitor cells from neuroinflammation-induced apoptosis (Mecha et al., 2012). These anti-inflammatory and neuroprotective actions of CBD are long-lasting (Mecha et al., 2013), counteract neuronal apoptosis (Giacoppo et al., 2015) and antagonistically interact with PEA (another non-psychotropic compound) in protecting against neurodegeneration and neuroinflammation (Rahimi et al., 2015). This is probably due to a cross-talk between the CBD-induced PI3K/Akt/mTOR pathway and upregulation of the PEA-binding PPARγ (Giacoppo et al., 2017). The most recognized effects of CBD are the immunoregulatory ones, namely (i) suppressing proinflammatory Th17 responses, (ii) promoting T cell exhaustion/tolerance, (iii) enhancing IFN-dependent anti-proliferative programs, (iv) hampering antigen presentation, and (v) inducing antioxidant milieu that resolves inflammation (Kozela et al., 2016).

3.2. eCB signaling from preclinical models of MS to clinical applications

A great amount of preclinical studies have convincingly demonstrated neuroprotective, neuromodulatory and immunomodulatory role of cannabinoids and eCBs, and their ability to alleviate symptoms and behavioural abnormalities in models of neurological diseases (Baker et al., 2001; Pryce et al., 2003; Centonze et al., 2007b; Chiurchiù, 2014; Chiurchiù et al., 2015a). Various cannabinoid preparations have also been examined in clinical studies to assess their efficacy in humans. Thus, distinct pharmaceutical preparations of cannabinoid agents have been or are being tested in numerous human diseases and, besides the aforementioned Sativex® and CBD, these include natural cannabinoid or phytocannabinoids such as Bedrocan® (19% THC, <1% CBD from *Cannabis sativa*), Bedrobinol® (12% THC, <1% CBD from *Cannabis sativa*), Bediol® (6% THC, 7,5% CBD from *Cannabis sativa*), Bedica® (14% THC, <1% CBD from *Cannabis indica*), Bedrolite® (19% THC, <1% CBD from *Cannabis sativa*), and Bedropuur® (20–24% THC, <1% CBD from *Cannabis indica*). In addition, also synthetic cannabinoids, such as Dronabinol (Marinol®, a synthetic THC), Nabilone (Cesamet®, a THC analog), and Levonantradol (a synthetic analog of Dronabinol) have been used in humans. These preparations can be taken *per os* mixed with food or made into tea, or by sublingual or topical administration, or they can be smoked or inhaled. However, these formulations are extremely heterogeneous in nature, making the results of the clinical studies incomparable, and contributing to inconclusive evidence of cannabinoid efficacy in most clinical studies.

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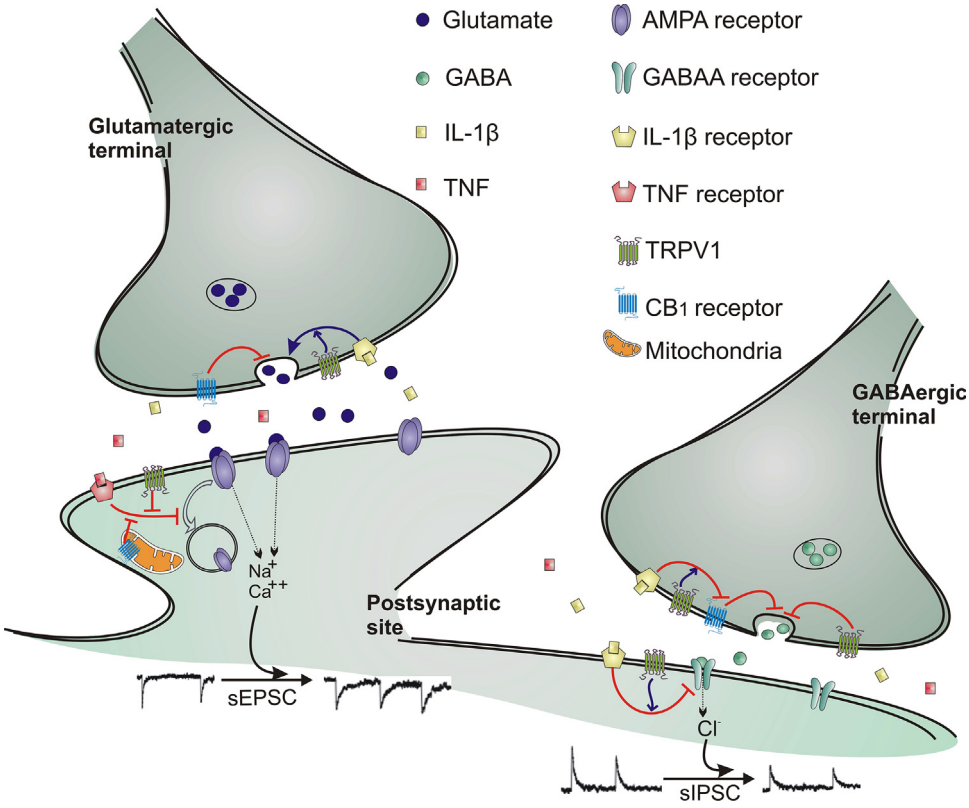

Fig. 4. Overall scheme of the presynaptic and postsynaptic perturbations mediated by proinflammatory cytokines and modulated by eCB system in EAE. IL-1β increases glutamate release at presynaptic terminals and TNF induces AMPA receptor upregulation, resulting in enhanced glutamate transmission. CB_1 contrasts the effects of IL-1β by reducing the frequency of spontaneous glutamate-mediated synaptic currents on presynaptic terminals, conversely TRPV1 is permissive for IL-1β synaptic effects on glutamate transmission. At the postsynaptic site, both CB_1 and TRPV1 restrain TNF-mediated potentiation on postsynaptic AMPA receptor. Moreover, IL-1β promotes the inhibition of CB_1 function on GABAergic synapses, thus mitigating the reduction of GABA release. Moreover, IL-1β reduces postsynaptic GABAA receptor function by promoting the decrease of GABA signaling. Finally, TRPV1 channels are permissive for IL-1β synaptic effects at both pre- and postsynaptic sites.

A recently published systematic review analyzed safety and efficacy results of randomized clinical trials that compared cannabinoids to placebo in three neurological disorders (MS, epilepsy and movement disorders) published in the preceding 50 years (1948–2013 interval) (Koppel et al., 2014; Gloss and Vickrey, 2014). According to the method of the American Academy of Neurology, quality evidence was rated as A (established with strong evidence as effective, ineffective or harmful, supported by 2 Class I studies), B (established with moderate evidence as probably effective, ineffective or harmful, supported by 1 Class I study), C (established with weak evidence as possibly effective, ineffective or harmful, supported by 1 Class II study), or U (insufficient, inadequate or conflicting data to make a determination). This rather stringent analysis approach showed that efficacy evidence of the published studies is generally weak, in clinical conditions other than MS-associated spasticity and neuropathic pain, and in the case of preparations other than nabiximols (Koppel et al., 2014; Maccarrone et al., 2017).

As reported in another recent and rigorous systematic review and meta-analysis considering ten diverse clinical conditions, cannabis-based medications were generally found to be effective for the majority of the explored clinical outcomes (Whiting et al., 2015). In the presented meta-analysis, only randomized trials comparing cannabinoid preparations with no treatment, placebo or standard care were considered, and non-randomized clinical studies were considered only if at least 25 patients were included (Whiting et al., 2015). Depression was the only clinical conditions in which placebo was reported to be more effective than nabiximols in three clinical studies, while cannabis-based medications were confirmed to be more effective than placebo on the primary outcomes of the selected studies in conditions like spasticity due to multiple sclerosis or paraplegia, neuropathic pain, cancer pain, but also anorexia secondary to HIV/AIDS, nausea and vomiting due to chemotherapy, sleep disorders, anxiety, psychosis and Tourette syndrome (Whiting et al., 2015).

Despite the limitations and poor quality of most clinical trials, the positive results of some rigorously conducted clinical studies and the acceptable side effects of most cannabinoid preparations (the most common adverse effects are dizziness, drowsiness and disorientation), led to the approval of Nabiximol as the only cannabis-based drug available in Canada, New Zealand and several European and Asian countries (but not yet in the U.S.A.) for the control of spasticity and neuropathic pain associated with MS.

There are few clinical trials assessing efficacy and pharmacological modulation of cannabinoids and eCBs as disease modifying treatments (Table 3). The CAMS study (Zajicek et al., 2003, 2005; Katona et al., 2005), failed to report any effect of cannabinoids on serum levels of proinflammatory cytokines and on T cell activation. The CUPID study (Zajicek et al., 2013) showed that Marinol®, (approved as an antiemetic and anorexic drug) has no overall effect on the progression of MS even in a long-term follow-up study (Ball et al., 2015). Furthermore, a two-fold cross over study measuring immune function in progressive MS patients revealed a pro-inflammatory role of Dronabinol (Killestein et al., 2003). Although MS progression and associated grey matter damage are both accelerated in human subjects carrying a genetic variant of CB_1 with reduced membrane expression (Ramil et al., 2010; Rossi et al., 2011a, 2013), clinical trials designed to investigate the neuroprotective effect of cannabinoid-based drugs did not show any impact on modulation of disease progression. In summary, the

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Table 3
Outcomes and results of cannabinoid-based clinical trials in MS.

Trial name (source)	Study design	Disease	No.	Formulation	Duration	Main/other clinical outcomes	Results
CAMS study Zajicek et al. (2003)	MC, PC, randomized	RR-MS, P-MS	630	Oral THC, THC/CBD, Placebo	15 weeks	Change in overall spasticity scores Admission for relapses	No significant improvement of spasticity Reduction of admissions for relapses in the treated groups
Killestein et al. (2003)	Two-fold crossover	P-MS	16	Oral THC/*Cannabis sativa* extract	4 weeks + 4 weeks	Immunomodulation	Increase of TNF-α in LPS-stimulated whole blood in the group treated with *Cannabis sativa* and increased IL-12p40 in patients with high adverse events score
CAMS study follow up Zajicek et al. (2005)	MC, PC, randomized	RR-MS, P-MS	502	Oral THC, THC/CBD, Placebo	12 months	Efficacy and long term safety in MS Admission for relapses	Small effect on muscle spasticity and disability, measured by EDSS and RMI No reduction of admission for relapses
Katona et al. (2005)	MC, PC, randomized	P-MS	100	Oral THC, THC/CBD, Placebo	13 weeks	Immunomodulation	No evidence for influence on serum levels of IFN-γ, IL-10, IL-12 and C-reactive protein
CUPID study Zajicek et al. (2013)	MC, DB, PC, randomised, parallel-group trial	P-MS	498	Dronabinol/Placebo	3 years	Efficacy of treatment in slowing MS progression New or enlarging T2 lesions or new T1 lesions Brain atrophy	No overall effect on the progression No difference No difference

Abbreviations: No., number of subjects; MC, multicentric; PC, placebo-controlled; DB, double-blind; EDSS, expanded disability status scale; P, progressive; RMI, rivermead mobility index; RR, relapsing-remitting.

results obtained from different clinical trials suggest that cannabinoids are able to control spasticity in MS patients, but do not confer neuroprotection in progressive MS. Curiously, notwithstanding the potent anti-inflammatory and neuroprotective effects of CBD and its low psychoactivity, all clinical trials for MS entail formulations with mixtures of THC and CBD but no formulation with only CBD. GW pharmaceuticals is seeking approval from the FDA to make Epidiolex (an oral formulation of 99% pure plant-derived CBD) commercially available for the treatment of several forms of epilepsies and epilepsy-associated rare syndromes; however, no clinical trials with this drug have been run or are being planned for MS.

As for drugs specifically targeting CB receptors, after the withdrawal of Rimonabant (a CB_1 inverse agonist) for its potentially serious side effects, alternative strategies would be either to develop novel CB_1 ligands that are peripherally restricted (*i.e.*, unable to cross the blood-brain barrier) and/or exhibit neutral antagonism, or to eventually bring CB_2 agonists into clinical practice. All these strategies are subject of intense research. Several clinical trials have been reported or are ongoing with MAGL and FAAH inhibitors (see for a recent review Chicca et al., 2015). For example, the irreversible MAGL inhibitor ABX-1431 has successfully completed phase 1a clinical trials and is now being evaluated for Tourette syndrome (phase 1b) and is scheduled for a phase 2 study in MS patients. The FAAH inhibitor PF-04457845 exhibited also a favourable safety profile in phase 1 and 2 clinical trials, but was ineffective in treating pain in osteoarthritis patients (Ahn et al., 2011; Li et al., 2012). The compound is currently being evaluated for the treatment of Tourette syndrome, fear responses and cannabis withdrawal. The development of FAAH inhibitors suffered a major setback with the phase 1 clinical trial of BIA 10-2474, which led to the death of one volunteer and mild-to-severe neurological symptoms in four others (Kerbrat et al., 2016). Recently, it was shown that BIA 10-2474 is a promiscuous drug that inhibits multiple lipases, including Neuropathy Target Esterase, that are not targeted by the highly selective and the clinically safe FAAH inhibitor PF-04457845 (van Esbroeck et al., 2017). BIA 10-2474, but not PF-04457845, produced substantial alterations in lipid networks in human cortical neurons, thereby suggesting that BIA 10-2474 has the potential to dysregulate metabolic pathways in the nervous system (van Esbroeck et al., 2017). It is, therefore, highly unlikely that inhibition of FAAH caused the adverse effects in the healthy volunteers. None of these drugs, nor any other selective inhibitor of the degradation of the other major eCB, 2-AG, has so far been used in clinical practice in relapsing or progressive forms of MS.

4. eCB signaling as a potential therapy for other neurodegenerative diseases with an inflammatory component

Despite the aetiology (where known) and the pathogenesis of other neuroinflammatory diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) are divergent and heterogeneous, it can be stated that neuroinflammation is a common hallmarks for all of them. Of note, all these disorders are characterized by a progressive loss of structure or function of neurons found at specific circuitries and brain areas. They all show subcellular histopathological similarities that include atypical protein deposits and cell death. In addition, the term neuroinflammation classically involves infiltration of peripheral immune cells within the CNS, but its current use has expanded to include those neurodegenerative diseases where, although leukocyte recruitment is not a critical feature, cellular and molecular signs of inflammation (i.e., expression of cytokines and other inflammatory mediators as well as glial activation) not only are present but are likely to contribute to disease pathogenesis and progression.

The role of the eCB system in these disorders has been extensively reviewed in recent papers (Chiurchiù et al., 2015a; Centonze et al., 2007b; Di Marzo et al., 2015; Fernández-Ruiz et al., 2015). Similarly to MS, perturbations of different components of the eCB system have been reported, being CB_1, CB_2 and eCB levels either increased or decreased in different brain areas (especially for CB_1), specific cell types or disease stages (Centonze et al., 2007a,b; Westlake et al., 1994; Benito et al., 2003; Lastres-Becker et al., 2003; Witting et al., 2004; Horne et al., 2013; Ramirez et al., 2005; van der Stelt et al., 2006; Yiangou et al., 2006; Battista et al., 2007; Zhao et al., 2008; Pisani et al., 2011; Jung et al., 2012; Bari et al., 2013; Altamura et al., 2015) (Table 4). For instance, stimulation of CB_1 leads to a generally reduced glutamate-induced excitotoxicity and improved neuronal viability (van der Stelt et al., 2001; Marsicano et al., 2003; Bilsland, 2006), whereas an increase in the expression of CB_2 is usually associated with activated microglia

Table 4
Alterations of distinct elements of the eCB system in neuroinflammatory diseases other than MS (pertinent references are in parenthesis).

ECS element	AD	PD	HD	ALS
AEA	↓AEA in brain (Jung et al., 2012)	↑AEA in basal ganglia, CSF and blood (Pisani et al., 2011; Di Filippo et al., 2008)	↓AEA in basal ganglia (Lastres-Becker et al., 2003) ↑AEA in blood (Battista et al., 2007)	↑AEA in spinal cord (Witting et al., 2004)
NAPE-PLD/ FAAH	↑FAAH in astrocytes and microglia (Benito et al., 2003)	–	↓NAPE-PLD and FAAH in striatum (Bari et al., 2013) ↓FAAH in blood (Battista et al., 2007)	–
2-AG	↑in brain (van der Stelt et al., 2006; Altamura et al., 2015)	↑in basal ganglia (Pisani et al., 2011)	↓in basal ganglia (Lastres-Becker et al., 2003)	↑in spinal cord (Witting et al., 2004)
DAGL/MAGL	↑DAGL in brain (van der Stelt et al., 2006; Altamura et al., 2015)	–	↓DAGL in striatum (Bari et al.[115]) ↓MAGL in cortex (Bari et al., 2013)	–
CB_1	↓in hippocampus and frontal cortex (Westlake et al., 1994; Ramirez et al., 2005) ←→in astrocytes and microglia (Benito et al., 2004)	↑in basal ganglia (Pisani et al., 2011)	↓in neuropeptide Y interneurons (Horne et al., 2013) ↓in GABAergic neurons (Centonze et al.[120])	↑in spinal cord and motor neurons (Witting et al., 2004; Zhao et al., 2008)
CB_2	↑in astrocytes and microglia (Benito et al., 2003)	–	–	in spinal cord and microglia (Witting et al., 2004; Yiangou et al., 2006)

AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; CSF, cerebrospinal fluid; HD, Huntington's disease; PD, Parkinson's disease. ↑, increase; ↓, decrease; ←→, unchanged.

and astrocytes (Westlake et al., 1994; Benito et al., 2003; Ramirez et al., 2005) and its activation induces anti-inflammatory and neuroprotective effects in AD (van der Stelt et al., 2006; Martín-Moreno et al., 2012), PD (Concannon et al., 2015), HD (Palazuelos et al., 2009) and ALS (Moreno-Martet et al., 2014). Moreover, the levels of eCBs, in particular of 2-AG, are mostly increased upon neuronal injury (Panikashvili et al., 2001), and overall they appear to exert neuroprotective effects. Accordingly, pharmacological and/or genetic inhibition of eCB degrading enzymes led to decreased neuroinflammation and neurodegeneration in various animal models of these neuroinflammatory diseases (Baker et al., 2001; Mestre et al., 2005; Ortega-Gutiérrez et al., 2005; Eljaschewitsch et al., 2006; de Lago et al., 2004; Webb et al., 2008; Pryce et al., 2013; Amor et al., 2005; Bernal-Chico et al., 2015; Hernández-Torres et al., 2014; Brindisi et al., 2016; Chen et al., 2012). Recent studies indicate that inhibition of biosynthesis or degradation of 2-AG may also exert anti-neuroinflammatory effects through a mechanism independent of CB_1 and CB_2. Preventing 2-AG degradation through selective MAGL inhibitors in mouse brain: i) abolished formation of pro-inflammatory prostaglandins that promote neuroinflammation (Nomura et al., 2011), ii) reduced TNF-α and IL-1β release, iii) suppressed microglial and astrocyte activation, and iv) promoted neuroprotection independently of CB receptors in different animal models of AD and PD (Chen et al., 2012; Nomura et al., 2011). Conversely inhibition of 2-AG biosynthesis through DAGL reduced lipopolysaccharide-stimulated pro-inflammatory cytokines (Ogasawara et al., 2016). Although these preclinical results seem promising, very few clinical trials with eCB-based drugs have been undertaken in patients affected by these neuroinflammatory diseases, exclusively entailing the use of Dronabinol or Nabiximol. The effects of the eCB system in these diseases are summarized in Fig. 5.

4.1. Alzheimer's disease

AD is the most common form of dementia in older adults. It usually starts slowly and irreversibly worsens over time, leading to a progressive decline in cognitive function and memory loss, caused by the formation of amyloid plaques and neurofibrillary tangles that cause neuronal death. Thus, neuritic plaques, fibrillary tangles, neuronal loss, damaged synaptic connections, and reactive gliosis are the histopathological hallmarks of AD. Amyloid plaques are made of aggregates of β-amyloid (Aβ), and other protein aggregates (like hyperphosphorylated Tau, ubiquitin, and presenilins 1 and 2). Aggregates of hyperphosphorylated Tau protein, instead, cause the formation of neurofibrillary tangles (Gosselet et al., 2013). Activated microglia and astrocytes are critical for neuritic plaques formation and for the onset of an inflammatory response during AD, likely contributing to the disease progression, but also to Aβ removal. Although a significant loss of CB_1 receptors has been reported in most brain regions involved in AD, possibly due to neuronal loss, CB_2 receptors are increased mainly in activated glial cells, and are often associated to an overexpression of FAAH in the same cells suggesting a key role for eCB signaling in the modulation of inflammation (Cassano et al., 2017). These findings led to a plethora of preclinical studies in several experimental models of AD, aimed at investigating the effects of specific cannabis extracts or selective CB_1/CB_2 agonists and inhibitors of eCB metabolic enzymes. THC inhibited the aggregation of Aβ induced by acetylcholine esterase (Eubanks et al., 2006), whereas CBD reduced the *in vivo* production of glial proinflammatory molecules in the hippocampus following Aβ-induced neuroinflammation (Esposito et al., 2007). In independent studies, CB_1 agonists prevented Aβ-induced microglial activation in AD patients (Ramirez et al., 2005) and amnesia in AD mice (Mazzola et al., 2003). Instead CB_2 activation ameliorated cognitive impairments by suppressing microglia inflammatory responses (Ramirez et al., 2012; Cassano et al., 2017). *In vivo* administration of the AMT inhibitor VDM11 or of the MAGL inhibitor JZL184, that increased the extracellular levels of endogenous AEA and 2-AG respectively, was beneficial against neurotoxicity by significantly reversing hippocampus damage, decreasing neuroinflammation and neurodegeneration and improving long-term synaptic plasticity and memory (van der Stelt et al., 2006; Chen et al., 2012). In addition, perturbation of MAGL activity has been investigated in animal models of AD, such as 5XFAD and PS1/APP$^+$ mice (Piro et al., 2012). Genetic and pharmacological inactivation of MAGL suppressed proinflammatory responses and amyloidosis in a PS1/APP$^+$ AD mice (Piro et al., 2012). Moreover, in 5XFAD AD mice, inhibition of

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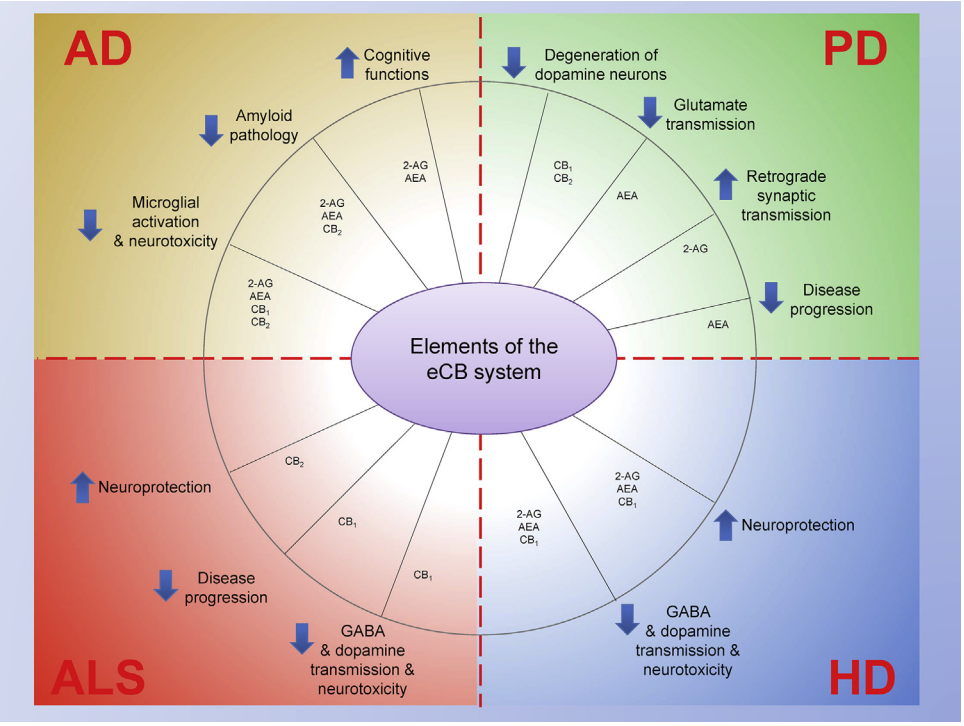

Fig. 5. Schematic representation of the main effects of distinct members of the eCB system in neuroinflammatory diseases other than MS. AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; eCB, endocannabinoid; HD, Huntington's disease; PD, Parkinson's disease.

MAGL reduces production and accumulation of β-amyloid (Aβ) and improves cognitive function (Chen et al., 2012)

Although the amount of literature on the role of eCB signaling in AD is impressive, a few case report studies and clinical trials are available for this disorder, and focus essentially on either Dronabinol or Nabilone. Although these studies showed a decreased severity of disturbed behavior and night-time agitation upon treatment (Volicer et al., 1997; Walther et al., 2006), they included a low number of patients and none of them evaluated cognitive or neurodegenerative markers. The first randomized, double-blind, placebo-controlled study conducted on a bigger cohort (50 patients), assessing also cognitive functions, reported that a low-dose of THC did not significantly reduce dementia-related neuropsychiatric symptoms after 21 days of treatment (van den Elsen et al., 2015). Overall, no actual evidence of cannabinoid effectiveness in the improvement of behavior and other parameters of dementia has been yet reported, and more controlled trials are needed to assess potential benefits on cognitive functions and neurodegeneration.

Interestingly, the very recent view that eCBs can also function as epigenetic modulators by inducing alterations in the transcriptional activity of key genes involved in various neurotransmitter systems (D'Addario et al., 2013), and the evidence that FAAH is epigenetically regulated in AD patients (D'Addario et al., 2012), has opened a novel avenue to the possible design of specific epigenetic drugs targeting elements of the eCB system.

4.2. Parkinson's disease

PD is the second commonest neurodegenerative disorder of the CNS. It is characterized by motor and non-motor symptoms, including tremor, bradykinesia, muscular stiffness or rigidity and loss of postural balance as well as constipation and depression. The underlying pathogenesis is determined by degeneration of dopamine-producing neurons of substantia nigra, leading to an impaired dopaminergic neurotransmission in the basal ganglia (Schapira and Tolosa, 2010). Several mechanisms seem to take part in the selective degeneration of dopaminergic neurons, including oxidative stress, mitochondrial dysfunction, excitotoxicity and also neuroinflammation. An important role of the immune system in the pathogenesis of PD is recent and it seems to involve either inflammation or autoimmunity, due to the identification of several autoantibodies directed towards PD-associated antigens (De Virgilio et al., 2016). Additionally, microglial activation at neurodegeneration sites follows nigral cell death in PD and is critical for initiation and/or progression of the neurodegenerative process through the production of a variety of inflammatory mediators, such as cytokines, glutamate and reactive oxygen species (De Virgilio et al., 2016). These observations led to the hypothesis that immune activation may be the cause, rather than a consequence, of the observed neuronal loss. Since CB_1 activity is increased in the basal ganglia and it affects the release of neurotransmitters and the activity of motoneurons, agonism of this receptor has proven to be a useful therapeutic strategy for PD. Indeed, CB_1 agonists exerted a protective effect on nigrostriatal dopamine neurons and on microglial activation in mouse models of PD (Price et al., 2009) and to attenuate the 6-hydroxydopamine-induced hypokinesia (Gonzalez et al., 2006). Furthermore, THC or CBD exerted neuroprotective effects by significantly preventing the loss of nigral dopaminergic neurons and by reducing the denervation of the ipsilateral striatum (Lastres-Becker et al., 2005). Furthermore, since eCBs become overactive and generally increase following dopaminergic neurons denervation, their anti-inflammatory effects might explain their protective role in PD, likely impacting on the activity of glial cells via CB_2 receptors on neurons. Nomura et al. used a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model for PD, where JZL184 provided neuroprotective effects, however, these effects remained unchanged by CB receptor antagonists and were recapitulated by COX inhibition (Nomura et al., 2011). This indicated that the neuroprotective effects were not mediated via the CB receptors, but were rather due to reductions in AA and proinflammatory prostaglandins. Mounsey

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et al. confirmed these findings (Mounsey Mustafa et al., 2015). Several recent reports have highlighted the critical role of CB_2 in preventing glial-derived neurotoxic mediator production, BBB leakage and infiltration of peripheral leukocytes. Moreover, CB_2 protects neurons from oxidative stress-induced dopaminergic neurodegeneration in several experimental models of PD (Chung et al., 2016; Javed et al., 2016), suggesting that selectively targeting this receptor could be indicated for novel clinical approaches. The first clinical studies conducted on PD reported reduction of levodopa-induced dyskinesia by Nabilone (Sieradzan et al., 2001), or by cannabis extract (Carroll et al., 2004). Yet, they failed to demonstrate efficacy in treating dyskinetic patients, probably due to methodological differences such as small cohort size and the difficult detection of small changes in dyskinesia. However, current clinical trials focused their attention to CBD instead of THC, due to its preliminary beneficial effects for the treatment of psychosis (Zuardi et al., 2009). On this basis, a recently concluded clinical trial aimed at exploring the effects of different doses of CBD on PD failed to document any significant difference in motor symptoms with possible neuroprotective effects (Chagas et al., 2014). A clinical trial assessing the effects of cannabis on PD-associated tremors has just been completed, and results should be available soon (ClinicalTrials.gov Identifier: NCT02028858). The evidence that MAGL-knockout animals show neuroprotection in a PD model (Nomura et al., 2011), and that FAAH inhibition decreases abnormal cortical glutamatergic drive (Gubellini et al., 2002) and reverses motor symptoms in PD experimental models (Celorrio et al., 2016), suggest that modulating the endogenous tone of eCBs could be of therapeutic value via multiple mechanisms of action.

4.3. Huntington's disease

HD is an autosomal dominant disorder that causes uncontrolled movements, emotional problems and cognitive loss due to a mutation in the huntingtin gene, determining an aberrant aggregation of huntingtin protein, ultimately leading to cytotoxic effects (Zuccato et al., 2010; Ross and Tabrizi, 2011). Early damage is mostly evident in the striatum, but as the disease progresses other areas of the brain are even more affected, like cerebral cortex, substantia nigra, hippocampus, Purkinje cells of the cerebellum and some parts of hypothalamus and thalamus. HD also causes an abnormal increase of astrocytes and microglia activation. Indeed, aberrant expression of huntingtin solely in microglia is sufficient to induce their priming and activation of a proinflammatory phenotype (Crotti and Glass, 2015). Also in HD an altered expression of several elements of the eCB system has been documented (Table 4). Furthermore, loss of CB_1 in neuropeptide Y interneurons seems to contribute to the impairment of basal ganglia functions linked to HD (Horne et al., 2013). Down regulation of CB_1 in GABAergic neurons is likely to be a compensatory mechanism in HD to counteract excitotoxic neuronal damage (Centonze et al., 2005), whereas CB_1 receptors located on glutamatergic terminals seem to be indispensable players in the neuroprotective activity of (endo)cannabinoids (Chiarlone et al., 2014). Loss of CB receptors has been recently corroborated by positron emission tomography in HD patients, suggesting that it could be among the potential biomarkers able to track HD pathology (Wilson et al., 2017). In addition, CB_2 receptors, especially located in microglial cells, are pivotal in attenuating microglial activation and preventing neurodegeneration (Palazuelos et al., 2009). In analogy with AD and PD, both similarly characterized by generalized motor and/or cognitive dysfunctions, cannabinoids were tested in pre-clinical models of HD, and also in the treatment of symptoms of HD patients. Again, in a mouse model of HD, CB_1/CB_2 agonists have been found to exert neuroprotection (Sagredo et al., 2012), and THC to attenuate deficits in motor coordination and protein aggregation (Blázquez et al., 2011). Furthermore, oral CBD has been used in the first clinical trial for HD patients, but its efficacy was not much different from placebo (Consroe et al., 1991). Lack of relieve of HD symptoms was also reported for Nabilone (Curtis et al., 2009). A recently concluded pilot study with Nabiximol proved its safety, tolerability and absence of severe adverse events, but no significant benefits on motor, cognitive, behavioral and functional scores (López-Sendón Moreno et al., 2016). If and how cannabinoids could be therapeutically used as drugs for the management of this disorder has to be further investigated. At any rate the presence of a neuroinflammatory component in HD should prompt the study of eCB signalling to potentially modulate microglial immune activation.

4.4. Amyotrophic lateral sclerosis

ALS is the most common degenerative disease of the motor neuron system of the spinal cord and brain stem, leading to a progressive atrophy of several voluntary muscles, thus impacting on key movements like chewing, swallowing, talking and even breathing, eventually causing death. Of note, 90–95% of all cases of ALS are sporadic but their aetiology is still unclear. On the contrary, a lot more is kown for familiar ALS (5–10%), whereby more than 20 genetic mutations have been associated with ALS. Mutations of Cu/Zn superoxide dismutase (SOD1) makes up to approximately 20% of all familial cases (Kiernan et al., 2011), but the pathogenesis of motoneuron degeneration remains unclear. Glutamate excitotoxicity, protein aggregation, mitochondrial dysfunction and oxidative stress, proteosomal dysfunction, axonal transport deficit, and cytoskeletal abnormalities seem all to be involved. Currently, ALS is recognized as a multifactorial disorder, whereby single genes or multiple determinants could cause it, including immunologic disturbances. In ALS misfolded protein aggregation and neuroinflammation also occur and are two hallmarks of this disease (Swinnen and Robberecht, 2014). Neuroinflammation is caused by an excessive microglial activation, in terms of the modulation of phagogytosis and inflammatory processes as well as regarding the cross-talk between neuronal and glial cells, that ultimately lead to motor neuron degeneration (Rodríguez and Mahy, 2016). Some of these pathogenic mechanisms are associated with SOD1 activity, but are also modulated by the eCB system. This could be the reason why increasing eCBs and their receptors activity in several ALS experimental models is neuroprotective (Table 4). Increased AEA and 2-AG levels during ALS-associated excitotoxic damage are likely to play a neuroprotective role, and thus manipulating the eCB system may be therapeutically exploited for the treatment of ALS. Although evidence on the effect of cannabinoids in ALS is still poor, these compounds are likely to exert powerful antioxidative, anti-inflammatory and neuroprotective effects and cause analgesia, muscle relaxation, prolonged neuronal survival and delayed disease onset and progression in experimental ALS models (Raman et al., 2004; Kim et al., 2006; Shoemaker et al., 2007; Rossi et al., 2010). Similar effects have been obtained using a Sativex®-like drug, namely an equimolecular combination of THC- and CBD-enriched botanical extracts (Moreno-Martet et al., 2014), calling for human clinical trials (Carter et al., 2010). The only completed trial conducted in ALS recruited 22 patients that were treated with orally administered Dronabinol for cramps. Although the trial failed to prove a subjective improvement of cramp intensity (Weber et al., 2010), an ongoing phase III trial (CANALS) is assessing safety and efficacy of Dronabinol on spasticity in motor neuron diseases including ALS, whereas a recently completed trial on 9 ALS patients assessed the pharmacokinetics and tolerability of THC and concluded that because of high interindividual variability in pharmacokinetics individual posology of THC is required for potential therapeutic use (Joerger et al., 2012). Thus, the possibility

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to treat ALS with cannabinoids needs to be further investigated and remains a major challenge for future clinical research. In view of recent evidence on the role of neuroinflammation and microglia involvement in ALS, along with increased CB_2 expression in activated microglia from spinal cord of human ALS patients (Yiangou et al., 2006), future investigations are warranted on strategies that selectively activate the latter receptor subtype.

5. Conclusions and future directions

Preclinical and clinical studies have highlighted the complexity of cannabinoids, eCBs, their receptors and metabolizing enzymes, which form an intricate network with multiple biological actions within the CNS and at the periphery. Remarkably, a wealth of preclinical and clinical data proved that cannabinoid-based medicines reduce spasticity symptoms in MS and also in other neurodegenerative diseases, probably through CB_1-dependent modulation of synaptic activity and neurotransmission. Yet, molecular mechanisms underlying dysregulation of neuronal circuits in MS are likely different from those of other neuroinflammatory diseases, so far leading to commercial approval of these medicines only for MS.

Furthermore, cannabinoid-based drugs are ineffective at halting neurodegeneration and disease progression for any of these neuroinflammatory disorders in patients. This lack of efficacy remains unclear, and could be related to: i) dosage and administration uncertainties, ii) illness progression stage of patients, and iii) inability to modulate CB_2-dependent anti-inflammatory and neuroprotective actions. These studies are also affected by the use of subjective scales as end-points, and by a strong placebo effect (Di Marzo et al., 2015). Therefore, it is deemed necessary to start large randomized placebo-controlled trials with objective end-points, in order to obtain conclusive results on the beneficial effects of cannabinoids and eCB-based drugs.

Additionally, patients are usually allowed to self-titrate the dose of the cannabinoid-based medicine to reduce the adverse side effects associated to CB_1 activation. Could it be that CB_1 is lost with disease progression? If so, cannabinoid-based therapy should start earlier. Or is perhaps the dose too low to stimulate CB_2? Selective CB_2 agonists would, therefore, be ideal drug candidates to be tested in both early and progressive phases of MS. Alternatively, enhancement of the endogenous levels of eCBs by inhibiting FAAH and/or MAGL appears an opportunity to be tested in MS patients, because it is expected to control spasticity symptoms, provide neuroprotection and exert anti-inflammatory actions. These options could represent an exciting translational frontier to obtain drugs to treat inflammatory-associated neurodegenerative diseases targeted towards different elements of the eCB system. Conceivably, cocktails of eCB-based drugs, or their combination with conventional therapies, might have more beneficial effects by acting synergistically. However, one possible obstacle to such a synergistic approach could be the heterogeneous plasticity of immune cells, especially those belonging to the innate compartment. Innate immune cells such as macrophages/microglia, on the one hand, can prevent overactive immune responses and autoimmunity by promoting a tolerogenic environment and by secreting neurotrophic growth factors. On the other hand, these cells can play an immunopathogenic role by inducing and/or exacerbating pro-inflammatory activities (Gandhi et al., 2010). For instance, similarly to peripheral macrophages, also microglia has different activation states that go from the classically-activated and pro-inflammatory to the alternatively-activated and resolving phenotype, and the fact that eCBs seem to be critical for inducing microglia to acquire the protective state (Mecha et al., 2015) suggests that eCB signalling could be explored to generate a reparative environment in neurodegenerative diseases (Mecha et al., 2016). Thus, as the role of resident or infiltrated innate immune cells becomes better defined, it will be possible to design therapies aimed at driving their plasticity in favour of an anti-inflammatory and neuroprotective activity.

Notably, distinct elements of the eCB system might be useful biomarkers for diagnostic purposes, and able to predict disease evolution in MS patients. For instance, AEA levels in cerebrospinal fluid are strikingly indicative of active phases of MS, even asymptomatic but nonetheless associated with acute blood brain barrier disruption and lymphocyte infiltration (Centonze et al., 2007a,b). However, standardized and optimized protocols for the determination of eCB levels in human biological fluids and biopsies are required to minimize variability in the determination of their content.

On a final note, MS and other neuroinflammatory diseases are multifactorial, where also environmental factors play an important role. In this context, due to the strong interplay between genes and the environment, an eCB-based "epigenetic therapy" could provide a new approach, with the aim of designing more specific epigenetic drugs devoid of genome-wide or off-target effects. In this context, physical therapy has gained room as a novel treatment able to rescue several functions in MS patients with disability, but also to mitigate the associated neuropathological alterations (Feinstein and Dalgas, 2014; Feinstein et al., 2015). The evidence that physical exercise modulates eCB signalling, for example by increasing eCB levels in the brain (Ferreira-Vieira et al., 2014; Galdino et al., 2014) and by sensitizing CB_1 at central synapses (De Chiara et al., 2010; Tantimonaco et al., 2014), suggests that its beneficial effects might be mediated in part by epigenetic modulation of eCB signalling (Dubreucq et al., 2013; Chaouloff et al., 2011). One way to give biochemical ground to these observations would be finding the genetically regulated CB_1 function in order to predict clinical response to cannabinoid treatment of spasticity (Coghe et al., 2015; Leocani et al., 2015), and to improve patient stratification as a tool for personalized rehabilitation of MS patients with disability.

Competing financial interests

The authors declare no competing financial interests.

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