Colloquium Series on Integrated Systems Physiology

The Endocannabinoid System in Local and Systemic Inflammation

Melanie E.M. Kelly • Christian Lehmann • Juan Zhou

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ABSTRACT

This book focuses on the role of the endocannabinoid system in local and systemic inflammation, with individual chapters written by experts in the field of cannabinoid research and medicine. The topics explore the actions of the endocannabinoid system on the immune system, including neuroinflammation in autoimmune disorders such as multiple sclerosis, and in neurodegenerative disorders such as Huntington's and Alzheimer's, as well as local and systemic inflammatory conditions affecting organs including the eye (uveitis and corneal inflammation), the bladder (interstitial cystitis), pancreas (diabetes), cardiovascular system (stroke), joints (arthritis), and sepsis. The objective of this book is to provide knowledge transfer on the use of cannabinoids in inflammatory disease by critically examining preclinical and clinical research on the immunomodulatory actions of the endocannabinoid system, with specific emphasis on the actions of cannabinoids in diseases where inflammation is a prominent component. By drawing these results together, we seek to provide further understanding of the complexities of endocannabinoid system modulation of immune function and identify potential uses and limitations for cannabinoid-based therapeutics.

KEY WORDS

Alzheimer's disease, amyloid β, arthritis, cannabinoids, cannabinoid receptors, cannabis, CNS injury, diabetic retinopathy, dronabinol, endocannabinoids, endocannabinoid system, endotoxin-induced uveitis, experimental autoimmune encephalomyelitis, experimental autoimmune uveoretinitis, Huntington's disease, immune system, immune cells, inflammation, marijuana, multiple sclerosis, Nabiximols, neuroinflammation, neurodegeneration, neuroprotection, ocular inflammation, pain, phytocannabinoids, proliferative vitreoretinopathy, spasticity, Sativex®

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Preface

Melanie E. M. Kelly

Cannabis use globally, both for recreational and medical purposes, currently outstrips other drugs (Bostwick, 2012). As cannabis moves into a new era of legalization in many parts of the world, it becomes imperative to carry out and disseminate basic and clinical research that provides a deeper understanding of the actions of this complex plant. In particular, information on the use of cannabis for therapeutic purposes, including its individual constituent phytocannabinoids, as well as synthetic cannabinoid derivatives, is critical to establish the potential for cannabis and cannabinoid drugs to be effectively used to alleviate human disease and suffering.

The cannabis plant contains a plethora of bioactive phytochemicals including >100 phytocannabinoids (Russo, 2007). The primary phytocannabinoid responsible for the psychoactive effects of cannabis following ingestion is Δ^9-tetrahydrocannabinol. Two cannabinoid receptors, cannabinoid type 1 receptor (CB_1R) and cannabinoid type 2 receptor (CB_2R) mediate many of the actions of Δ^9-tetrahydrocannabinol, with CB_1R responsible for the pyschoactivity of Δ^9-tetrahydrocannabinol (reviewed in, Mechoulam et al., 2014; Pertwee, 2010). The endogenous ligands for cannabinoid receptors are lipids called endocannabinoids that are produced in a Ca^{2+}-dependent manner by biosynthetic enzymes and released "on-demand" before being rapidly broken down by degradative enzymes. This system of endogenous ligands, biosynthetic and degradative enzymes, and cannabinoid receptors has been coined the endocannabinoid system (reviewed in Hillard, 2015; Pertwee, 2015; Maccarone et al., 2015).

Substantive evidence now indicates that the endocannabinoid system is an important modulator of numerous biological systems including the immune system, where activation of the endocannabinoid system, particularly CB_2R, may be a sentinel against inflammation (reviewed in, Turcotte et al., 2016; Chiurchiu et al., 2015; Cabral et al., 2015a). Elements of the endocannabinoid system, including endocannabinoids and cannabinoid receptors, are present in a diverse array of circulating and resident immune cells, and activation of cannabinoid receptors on immune cells by exogenous cannabinoids or endocannabinoids results in alterations in immune function (reviewed in Turcotte et al., 2016). Evidence indicates that in contrast to the more ubiquitous CB_1R expression, CB_2R expression is, for the most part, highly localized to immune cells. In addition to Δ^9-tetrahydrocannabinol, research also supports immunomodulatory actions of other phytocannabinoids in cannabis including the major non-pyschoactive phytocannabinoid, cannabidiol, which

may act differentially at cannabinoid receptors as well as non-cannabinoid receptors to produce its anti-inflammatory actions (Burstein, 2015).

The last few decades have seen significant advances in our understanding of the endocannabinoid system. Armed with this knowledge, the research community has begun to decipher the actions of the endocannabinoid system in regulating important biological functions. Furthermore, we are now beginning to have a better understanding of the complex pharmacology of compounds that modulate the endocannabinoid system, including plant cannabinoids, and endocannabinoids. The present book highlights several key areas where this information may be applied to develop new endocannabinoid system targeted therapeutics that could help to both understand and alleviate human disease.

CHAPTER 1

Inflammation and the Endocannabinoid System

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Abstract

Local inflammation is launched by trigger events such as microbial invasion or environmental factors and results in immune cell recruitment at the primary site of injury. In the case of systemic inflammation, the immune response is dysregulated, and the activation of endothelial cells and leukocytes occurs at multiple sites. The endocannabinoid system plays an important role in the modulation of the immune response. Increasing evidence supports upregulation of cannabinoid type 1 and type 2 (CB_1R and CB_2R) receptors and release of endocannabinoids from macrophages, dendritic cells, platelets and parenchymal cells in response to inflammatory stimuli. This chapter will summarize current knowledge regarding involvement of cannabinoid receptors and their ligands in both local and systemic inflammation.

Key Words

immune system, inflammation, infection, cannabinoid type 1 receptor, cannabinoid type 2 receptor

1.1 INTRODUCTION

The cardinal signs of inflammation—heat, redness, swelling, and pain—represent basic processes that define local inflammation. Inflammation is launched by trigger events such as cytokine secretion by cells challenged by microbes or microbial products, or environmental factors that result in degranulation of certain leukocytes. The initial wave of mediator release is followed by vasodilatation, increased vascular permeability, leukocyte margination, extravasation and tissue infiltration, and activation of proteases that cleave bradykinins. Vasodilation in the microvasculature contributes to the heat and redness, while plasma and cells accumulating in the tissue contribute to the swelling. Pain is a product of the kinins, which also modulate other aspects of inflammation (Moreau et a., 2005). In the case of systemic inflammation, the same events are occurring but without the

road map provided by chemokines or anaphylatoxins from local tissue sites. Consequently, endothelial cell and leukocyte activation become systemic and leukocytes marginate in multiple sites. This margination is mediated by specific adhesion molecule and integrin interactions between the endothelial cells and leukocytes though there may not be directed extravasation.

One of the prototypes of inflammatory triggers during local and systemic inflammation, is lipopolysaccharide, which, in turn, is primarily detected through Toll-like receptor 4 (TLR4) on multiple cell types. Thus, the activation of cells through TLR4 has continued to draw considerable attention as a means to understanding the inflammation and impact of inflammation on the immune system (Rosadini and Kagan, 2017). Notwithstanding the importance of the TLR4 response, there is great redundancy and synergy among the different mediators and cascades that become activated during inflammation, and which lead to cell death and further activation, that has made dissecting the pathophysiology and immune activation so elusive (Delano and Ward, 2016; Mira et al., 2016; Napier et al., 2016). The impact of the endocannabinoid system in this network offers a new perspective in the control of inflammatory processes.

1.2 CANNABINOID TYPE 1 RECEPTOR

Cannabis sativa has been used for recreational, religious and medicinal properties for several thousand years (reviewed in Russo, 2007). The cannabis plant contains >100 phytocannabinoids including Δ^9-tetrahydrocannabinol (THC) and cannabidiol. The first phytocannabinoids, cannabinol (CBN) and cannabidiol (CBD) were isolated prior to the 1950's (reviewed in Mechoulam and Hanus, 2000), however it was not until 1964 that THC, the primary phytocannabinoid responsible for the psychoactive effects seen after cannabis ingestion, was isolated by Gaoni and Mechoulam (1964). Subsequent to this, it was discovered that cannabinoids exert behavioral effects via high affinity binding to a receptor in the central nervous system (Devane et al., 1988). This receptor named the cannabinoid 1 receptor (CB_1R) was found to be a member of the 7 transmembrane Family A G-protein coupled receptors that transduce their actions via coupling to a G protein (Howlett et al., 2002). Development of high affinity synthetic cannabinoids (Table 1.1) was a key contributory factor in the identification and cloning of CB_1R (Matsuda et al., 1990). CB_1R is highly expressed in the brain and throughout the nervous system with expression also in peripheral tissues.

Following the identification of the "THC receptor," high-affinity endogenous ligands for CB_1R were discovered. These included arachidonoyl ethanolamide (AEA) (Devane et al., 1992) and 2-arachidonoyl–glycerol (2-AG) (Mechoulam et al., 1995). AEA and 2-AG both bind and activate CB_1R with 2-AG being a full agonist (Howlett et al., 2002). Cumulative research has now indicated that endocannabinoids are generated "on-demand" from cells, including immune cells, via enzymatic production from membrane lipids (Figure 1.1; reviewed in Hillard, 2015). The lifetime of endocannabinoids is limited by degradative enzymes including fatty acid amide hydrolase (Cravatt et al., 2001).

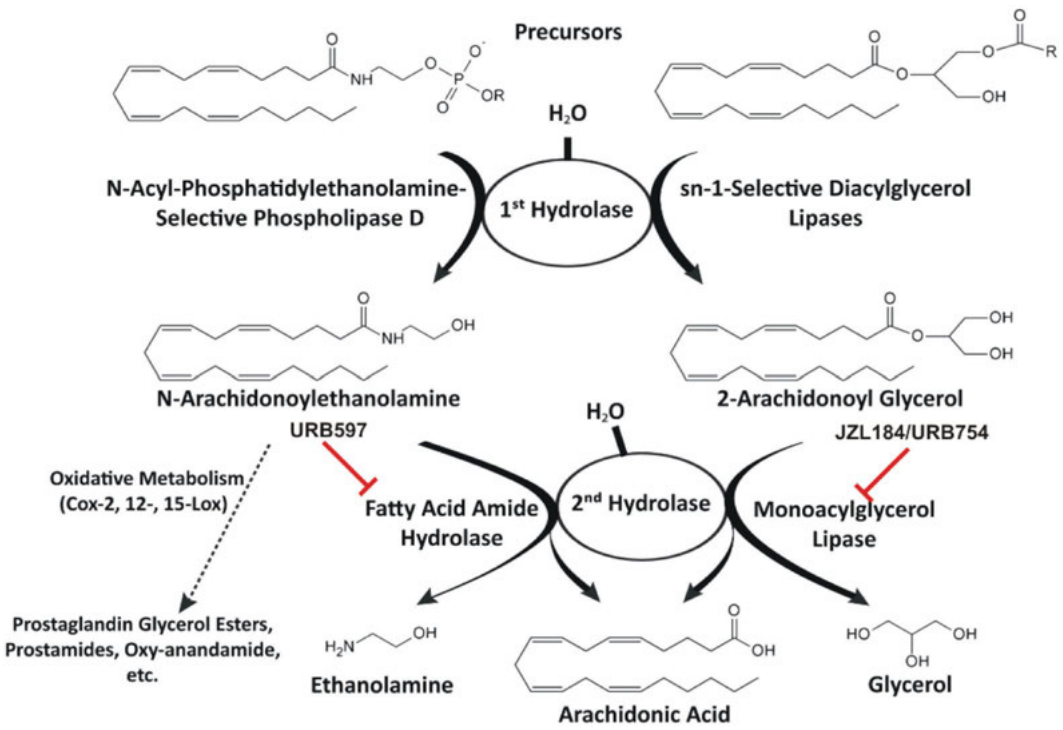

FIGURE 1.1: Biosynthetic and degradative pathways for AEA and 2-AG. URB597 inhibits fatty acid amide hydrolase (FAAH) while JZL184/URB754 inhibits monoacylglycerol lipase (MAGL) (adapted from Di Marzo et al., 2009).

Central nervous system (CNS) activation of CB_1R is associated with signaling via $G_{\alpha i}$-coupled downstream signaling pathways including adenylyl cyclase and cAMP and mitogen activated protein kinase (reviewed in Pertwee, 2010). In the CNS, activation of CB_1R by endocannabinoids or exogenous phyto- or synthetic cannabinoids is associated with a reduction in neurotransmitter (NT) release at central synapses via a retrograde signaling mechanism involving inhibition of presynaptic voltage-dependent Ca channels (Hillard, 2015). Both pre- and postsynaptic neuronal CB_1R activation has been demonstrated to be neuroprotective in various neurodegenerative CNS disorders and may involve, in part, a reduction in excitotoxic NT release, modification in glial release of pro-inflammatory mediators and improved blood flow to the damaged brain (Golech et al., 2004; Fernández-Ruiz et al., 2015). Validation of anti-inflammatory and neuroprotective effects in the CNS is supported by research using CB_1R antagonists or genetic loss of CB_1R. However, despite evidence of decreased neuroinflammation and neuroprotective efficacy, there are a few issues related to targeting CB_1R for CNS neuroinflammatory and neurodegenerative disease. Namely, the

behavioral actions of ligands that modulate CB_1R and the loss of neuronal CB_1R in neurodegenerative disorders (Fernández-Ruiz et al., 2015; McCaw et al., 2004).

1.3 CANNABINOID TYPE 2 RECEPTOR

Cloning of a second cannabinoid receptor, cannabinoid 2 receptor, with 44% amino acid homology to CB_1R was reported by Munro et al. (1993) and as the expression of this receptor, unlike CB_1R, was highly localized to immune cells, CB_2R was referred to as "the peripheral receptor". However, later studies have also indicated that this receptor may also be found in select areas of the CNS (Van Sickle et al., 2005). CB_2R is activated by the phytocannabinoid, THC, synthetic cannabinoids, and also by endocannabinoids such as 2-AG (Howlett et al., 2002; Pertwee, 2010). A number of other phytocannabinoids, including cannabidiol, activate CB_2R and have reported immunomodulatory actions (Pertwee, 2010). Additionally, cannabidiol has been reported to antagonize the actions of THC and other cannabinoids that act at CB_1R and can also bind to non-cannabinoid receptors including serotonin 1A (5-HT1A), as well as transient receptor potential receptor 1 (TRPV1) (Devinsky et al., 2014). The collective actions of constituent phytocannabinoids from cannabis is sometimes referred to as the "entourage effect," a description used to describe the effects of interactions between cannabis constituents (Russo, 2011). Several cannabinoid derivatives have now been developed that selectively activate CB_2R and alter immune function (Table 1.1).

TABLE 1.1: CB_2R agonists and antagonists (Turcotte et al., 2016)

Agonists	Ki (nM)	Other targets	References
AM 1241	3.4	TRPA1	Ibrhim et al., 2003; Akopian et al., 2008
JWH 133	3.4	TRPV1	Huffman et al., 1999; McDougall et al., 2008
GW 405833	3.6–3.92		Valenzano et al., 2005
JWH 015	13.8		Showalter et al., 1996
HU 308	22.7		Hanus et al., 1999
L-759,633	6.4		Ross et al., 1999
L-759,656	11.8		Ross et al., 1999
SER 601	6.3		Pasquini et al., 2008
GP 1a	0.037		Murineddu et al., 2006
GP 2a	7.6		Murineddu et al., 2006
CB 65	3.3		Manera et al., 2006
HU 210	0.061–0.5	CB_1R, GPR55, 5-HT2	Felder et al., 1995; Ryberg et al., 2007; Cheer et al., 1999
CP 55,940	0.6–5.0	CB_1R, GPR55	Ryberg et al., 2007; Thomas et al., 1998
WIN 55, 212-2	62.3	CB_1R,TRPA1	Akopian et al., 2008; Felder et al., 1995; Thomas et al., 1998

Antagonists	Ki (nM)	Other targets	References
SR144528	0.6–4.1		Ross et al., 1999; Rinaldi-Carmona et al., 1998
AM 630	5.6–31.2	TRPA1	Ross et al., 1999; Patil et al., 2011
JTE907	35.9		Buckley et al., 2000

In contrast to CB_1R, activation of CB_2R by phytocannabinoids and synthetic cannabinoid ligands is non-psychoactive. Activation of CB_2R promotes coupling of the receptor to $G_{\alpha i}$-signaling pathways, resulting in inhibition of adenylate cyclase (AC) and decreased cAMP, together with activation of mitogen-activated protein kinase (MAPK) signaling (Devane et al., 1988). CB_2R agonists attenuate the inflammatory response by inhibiting production of pro-inflammatory mediators and decreasing neutrophil chemotaxis and extravasation (Fernández-Ruiz et al., 2015; Cabral et al., 2015). In injury models, levels of CB_2R expression, along with endogenous cannabinoid levels (see Table 1.2), are increased, suggesting that this receptor may function in an "auto-protective role" to limit inflammation (Rom and Persidsky, 2013; Steffens and Pacher, 2012; Toguri et al., 2016). Accordingly, in preclinical models, activation of CB_2R has been associated with a reduction in inflammation (Toguri et al., 2015, 2014; Gómez-Gálvez et al., 2016; Wright et al., 2008).

TABLE 1.2: CB_2R-mediated effects of endocannabinoids on immune cell functions (Turcotte et al., 2016)

Cell type	Species	Endocannabinoid	Effects	References
Anti-inflammatory Effects				
Astrocytes	Rat	AEA	↓TNF	Ortega-Gutiérrez et al., 2005
Dendritic cells	Human	AEA	↓IL-6, IL-12, IFN	Chiurchiù et al., 2013
Microglia	Mouse	AEA	↓NO	Elijaschewitsch et al., 2006
	Mouse	AEA	↑IL-10, IL-12p70, IL-23	Correa et al., 2011
	Rat	AEA	↑NO	Malek et al., 2015
Neutrophils	Human	2-AG	↓ migration	Kurihara et al., 2006
Splenocytes	Human	AEA	↓antibody formation	Eisenstein et al., 2007
T cells	Human	AEA	↓ proliferation	Cencioni et al., 2010
		2-AG	↓ migration	Coopman et al., 2007
CD4+ T cells	Human	AEA	↓ IL-17, IFN, TNF	Cencioni et al., 2010
CD8+ T cells	Human	AEA	↓ IFN, TNF	Cencioni et al., 2010
	Human	AEA	↓ migration	Joseph, et al., 2004

Cell type	Species	Endocannabinoid	Effects	References
Pro-inflammatory Effects				
B cells	Human	2-AG	↑migration	Rayman et al., 2004
	Mouse	2-AG	↑migration	Tanikawa et al., 2007
Dendritic cells	Human	2-AG	↑migration	Maestroni, 2004
Eosinophils	Human	2-AG	↑migration	Kishimoto et al., 2006
	Human	2-AG	↑migration, LTC4, EXC4	Larose et al., 2014
Macrophages	Mouse	2-AG	↑phagocytosis	Shiratsuchi, et al., 2008
	Human	2-AG	↑actin polymerization	Kishimoto et al., 2003
Microglia	Mouse	2-AG	↑migration	Walter et al., 2003
Monocytes	Human	2-AG	↑migration, adhesion	Kishimoto et al., 2003; Gokoh et al., 2005
NK cells	Human	2-AG	↑migration	Kishimoto et al., 2005
T cells	Human	2-AG	↑adhesion, transmigration	Gasperi et al., 2014

1.4 OTHER RECEPTORS AND LIGANDS

Several other GPCRs and nuclear receptors have also been proposed as targets for cannabinoids that exert immunomodulatory effects. These include GPR55 as well as PPARγ respectively (Pertwee, 2015). The putative roles of these non-cannabinoid receptors have been outlined in several excellent comprehensive reviews (Pertwee, 2010; Golech et al., 2004; Fernández-Ruiz et al., 2015; McCaw et al., 2004; Munro et al., 1993; Van Sickle et al., 2005; Devinsky et al., 2014; Russo, 2011; Cabral et al., 2015; Rom and Persidsky, 2013; Steffens and Pacher, 2012; Toguri et al., 2016; Toguri et al., 2015; Toguri et al., 2014; Gómez-Gálvez et al., 2016; Wright et al., 2008; Pertwee, 2015; Macki and Stella, 2006). GPR55 was originally thought to be a third putative cannabinoid receptor but is now known to be the receptor for the endogenous ligand, Lysophosphatidylinositol (LPI). Additionally, a number of endogenous fatty acid ethanol amides and fatty amino-acid amides have been found to either bind to cannabinoid receptors, or their actions are blocked in cannabinoid receptor null mice, implying that interactions with cannabinoid receptors contribute to their actions. These interactions may be mediated via allosteric binding to binding sites at cannabinoid receptors that are distinct from the orthosteric site that endocannabinoids, synthetic cannabinoids, or THC bind to. It may even occur via interactions with distinct receptors that may form dimerized complexes with cannabinoid receptors with resultant allosteric modulation of cannabinoid receptor signaling when activated by orthosteric ligands (Hudson et al., 2010; Laprairie et al., 2015).

Extensive preclinical research has demonstrated that cannabis and cannabinoids have therapeutic potential in ameliorating symptoms for several diseases (reviewed in Pertwee, 2015). However, in contrast to preclinical studies, the number of clinical trials to determine safety and efficacy of cannabis and cannabinoids at this time still remains relatively limited. One area that shows considerable promise for the development of cannabinoid therapeutics is for inflammatory disease, as described below.

1.5 MODULATION OF THE INFLAMMATORY IMMUNE RESPONSE BY THE ENDOCANNABINOID SYSTEM

The endocannabinoid system (ECS) plays an important role in immune system modulation, and increasing evidence supports upregulation of the ECS during both local and systemic inflammation. Endocannabinoids released from macrophages, dendritic cells, platelets, and parenchymal cells in response to inflammatory stimuli and oxidative stress, are present in elevated levels in the sera of patients and animals with systemic inflammation (Varga et al., 1998; Wagner et al., 1998; Pacher et al., 2005; Orliac et al., 2003).

Examination of cannabinoid receptors function has revealed that CB_2R are present on macrophages, neutrophils, and lymphocytes, and activation of these receptors has generally been associated with anti-inflammatory effects, including reduced macrophage and neutrophil numbers at the site of infection and decreases in pro-inflammatory cytokines (Caldwell et al., 2010). The use of CB_2R agonists in experimental models of systemic inflammation and infection reduced the continued recruitment of neutrophils to the site of infection, while increasing phagocytosis and clearance of bacteria (Tschöp et al., 2009).

With respect to the contribution of CB_1R to inflammation and infection, several studies suggested that activation of CB_1R located on the presynaptic terminals of autonomic nerves or the vascular walls may contribute to vasodilation (Varga et al., 1998; Wagner et al., 1998; Pacher et al., 2005; Orliac et al., 2003).

Additionally, a number of *in vitro* studies have examined the effects of endocannabinoids on the levels of pro-inflammatory cytokines, including: TNF-α, interleukin-1beta (IL-1β), interleukin-6 (IL-6), and interleukin-2 (IL-2). Both the endocannabinoids, 2-AG and AEA, decreased LPS-mediated increases of pro-inflammatory cytokines, including TNFα from macrophages and microglial cells and 2-AG inhibited IL-2 secretion in activated murine splenocytes (Facchinetti et al., 2003; Gallily et al., 2000; Ouyang et al., 1998). Consistent with these findings, a study using the selective fatty acid amide hydrolase (FAAH) enzyme inhibitor, URB597, to augment levels of endogenous AEA, reported a reduction in LPS-stimulated microglial expression of inflammatory mediators, including nitric oxide, in LPS-stimulated rat cortical microglia (Tham et al., 2007). URB597 treatment also attenuated levels of pro-inflammatory cytokines, TNFα and IL-1β, in LPS-treated paws in a rat endotoxemia model of inflammatory pain (Naidu et al., 2010).

1.6 CONCLUSIONS

The ECS is upregulated in local inflammation and during systemic inflammatory responses. There is an increasing body of evidence on how endocannabinoids affect inflammatory reactions, which cannabinoid receptor subtypes and cell targets are involved, and the functional outcomes of modulating endocannabinoid signaling during different stages and severity grades of inflammation (see Figure 1.2).

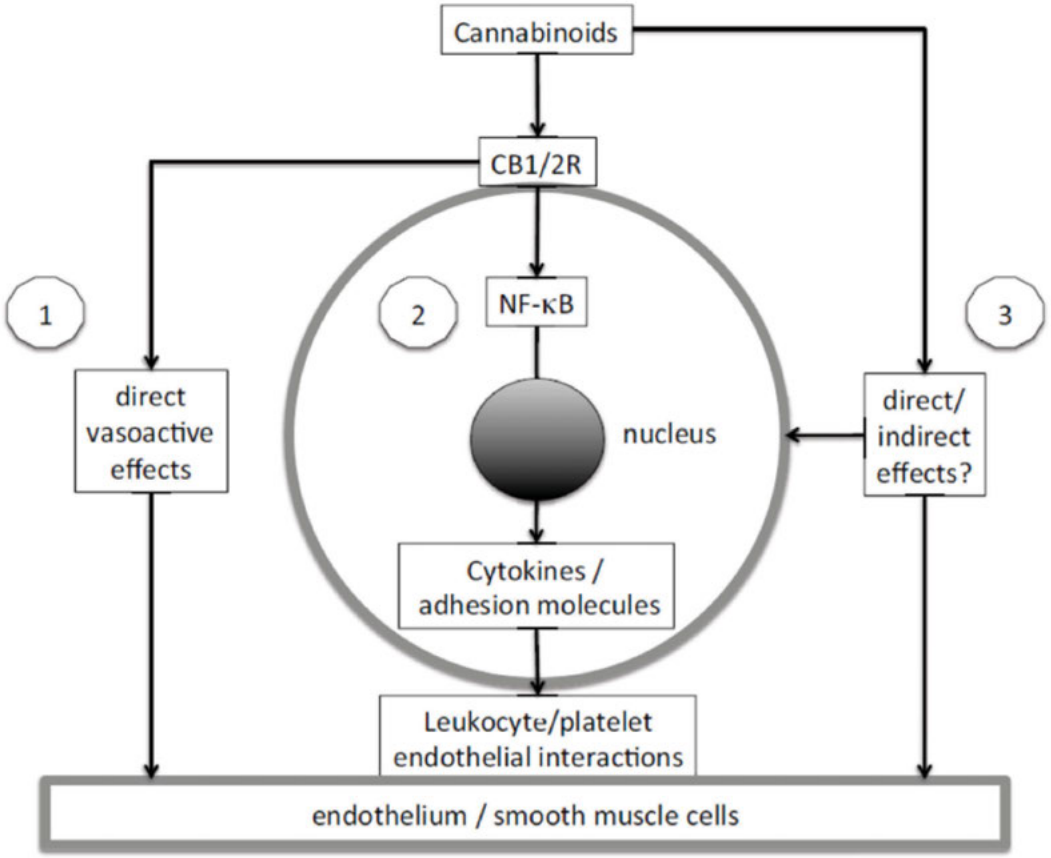

FIGURE 1.2: Hypothesized mechanisms. (1) direct vasoactive effects (vasodilation/vasoconstriction); (2) effects on cytokine and adhesion molecule expression, e.g. via NF-kB; and (3) direct/indirect effects mediated by non-CB_1R/CB_2R receptors, e.g. PPARγ, GPR55, or GPR18.

CHAPTER 2

Cannabinoids in Multiple Sclerosis

Contributing Author
Anna-Maria Szczesniak

Abstract

Multiple sclerosis (MS) is an autoimmune and neurodegenerative disorder of the central nervous system (CNS) that predominantly affects young adults. The current treatments for MS are not always effective in the management of symptoms and disease progression, may produce significant side effects, and are also not curative. The endocannabinoid system has been shown to modulate inflammatory and neurodegenerative processes in a number of CNS pathologies, including MS. In experimental autoimmune encephalomyelitis (EAE), an animal model of MS, both endogenous and exogenous cannabinoids reduce symptomatic features associated with the disease, and are neuroprotective. This effect is primarily mediated by the CB_1R, since pharmacological inhibition or genetic deletion of this receptor results in a more severe disease. While the clinical evidence for the effectiveness of cannabinoids is less conclusive, there is sufficient evidence for symptomatic relief of spasticity and pain that are associated with MS. This chapter reviews the clinical and experimental data on the efficacy of cannabinoids in the treatment of MS.

Key Words

Dronabinol, endocannabinoids, experimental autoimmune encephalomyelitis, multiple sclerosis, Nabiximols, neuroinflammation, neuroprotection, pain, spasticity, Sativex®

Abbreviations

2-AG	2-arachidonoylglycerol
AEA	N-arachidonoylethanolamine; anandamide
CB_1R	cannabinoid type 1 receptor
CB_2R	cannabinoid type 2 receptor
CBD	cannabidiol
CNS	central nervous system
EAE	experimental autoimmune encephalomyelitis
ECS	endocannabinoid system
FAAH	fatty acid amide hydrolase

MAGL	monoacylglycerol lipase
MS	Multiple Sclerosis
OEA	N-oleoylethanolamide
PEA	N-palmitoylethanolamide
PPMS	primary-progressive multiple sclerosis
RRMS	relapsing-remitting multiple sclerosis
SPMS	secondary-progressive multiple sclerosis
Δ^9THC	Δ^9 tetrahydrocannabinol
TNF-α	tumor necrosis factor -alpha

2.1 MULTIPLE SCLEROSIS

Multiple Sclerosis (MS) is the most common autoimmune, inflammatory disorder of the central nervous system (CNS), affecting around 2–3 million people worldwide (Browne et al., 2014). The onset of the disease usually presents itself in young adults (20–40 years of age), and is more common in females than males, with a ratio approaching 3:1. Historically, a higher incidence of MS has been reported in people of northern European background (MS Society, Canada; Compston and Coles, 2008), with the highest incidence of MS reported in Northern hemispheres and Southeastern Australia. However, more current epidemiological studies suggest that geographic location and incidence of MS are not as well correlated as previously thought, and other factors, including environmental influence and lifestyle, play a prominent role in the development of MS (Koch-Henriksen and Sorensen, 2010).

2.1.1 ETIOLOGY

There is evidence of genetic and environmental components that underlie the susceptibility of the development of MS. The involvement of genetic components is supported by the higher occurrence of MS in monozygotic twins as compared to dizygotic twins (Hansen et al., 2005), with concordance of approximately 25–30% (Ebers et al., 1986, 1996; Hansen et al., 2005). Furthermore, the genetic susceptibility to MS appears to be polygenic, with a number of loci affected. The association of major histocompatibility complex type II alleles, particularly HLA-DRB1*1501 and HLA-DRB5, is well established (International Multiple Sclerosis Genetics Consortium et al., 2007; Jersild et al., 1973; Okuda et al., 2009). The environmental factors that have been associated with CNS autoreactivity and development of MS includes infection with Epstein-Barr virus (Handel et al., 2010b), which has been detected in the majority, if not all, patients with MS (Ascherio and Munger, 2007), deficiency in sunlight/vitamin D, which affects immune responses (Smolders et al., 2008, 2011; Smolders, 2010), and cigarette smoking (Hedstrom et al., 2009). The link between environment and genetics can be partially explained by epigenetic modification, where gene expression is altered in a

heritable, but reversible, manner with environmental or biological factors, with no effect on DNA sequence. With respect to MS, unique alterations in DNA methylation have been shown in cell-free plasma DNA (cfpDNA) from patients with relapse and remitting multiple sclerosis (RRMS), as compared to healthy individuals (Liggett et al., 2010). Because HLA-DRB1*1501 is correlated to the clinical course of MS (Okuda et al., 2009), Handel and colleges (2010a) investigated whether the disease severity is correlated with DNA methylation status for HLA-DRB1*1501 and HLA-DRB5, but found no evidence for it. However, DNA hypomethylation for the key regulatory genes involved in the immune response and cell differentiation have been reported (Janson et al., 2011). As the field of epigenetic contribution to neurodegenerative disorders is still in the early stages, future research will shed more light on the impact it plays in the development and progression of diseases, including MS.

2.1.2 PATHOLOGY AND SYMPTOMS OF MULTIPLE SCLEROSIS

The pathology of MS is characterized by mononuclear cell infiltration of the CNS. The primary cells involved in MS are peripheral CD4+ autoreactive lymphocytes (T-helper 1; Th1), which are activated by autoantigenic peptides, including myelin basic proteins. Then, the Th1 cells trans-migrate into CNS and initiate a cascade of events that cumulate in inflammatory response and neurodegeneration. This pathogenic process involves the release of proinflammatory cytokines and chemokines, as well as the recruitment of other cells of innate and adaptive immunity, including CD8+ lymphocytes, CD4+ Th17 cells, antibody producing B cells, and monocytes. The activation of microglia and macrophages and the release of the inflammatory mediators further potentiate and sustain the inflammation. The consequence of the inflammatory cascade is the loss of oligo-dendrocytes and myelin sheath, as well as axonal loss and astrocyte proliferation. The formation of MS lesions (or plagues) affects neuronal transmission and results in the clinical manifestation of the disease.

The neurological deficits in MS can involve any part of the CNS and affect autonomic, sensory and motor functions. Furthermore, the symptoms can resolve completely, partially, or not at all. Initially, the majority of MS patients present themselves with an episode of acute neurological symptoms (defined as clinically isolated syndrome), with the most common symptoms including optic neuritis and/or incomplete myelitis (which may present itself with muscle weakness, lower back pain, abnormal sensations in toes and feet, and may progress to paralysis)(Milo and Miller, 2014; Miller et al., 2005). The common chronic symptoms of MS include spasticity, which affects movement and results in reduced walking ability (Oreja-Guevara et al., 2013) and falls (Gunn et al., 2013), bladder dysfunction, and pain (Pollmann and Feneberg, 2008). Interestingly, in patients where sensory symptoms predominate, complete recovery during remission is common. On the other hand, those presenting with motor impairments have much poorer prognosis (Eriksson et al.,

2003). As the disease progresses, the symptoms tend to worsen, which increases the burden of the disease for MS patients as well as their caregivers.

In terms of the clinical course of MS, in the majority of patients (approximately 80%), the disease initially presents itself with episodes of relapse and remission (RRMS), which is more common in early adulthood and often advances to a secondary progressive phase (SPMS). Another 10-15% of MS patients are diagnosed with primary progressive MS (PPMS), with a sustained neurodegenerative course of the disease; although minor and transitory improvement may occur (Milo and Miller, 2014). The onset of PPMS usually occurs later in life, and affects approximately equal numbers of women and men. PPMS is characterized by fewer brain lesions and much less inflammation as compared to RRMS and SPMS (Miller and Leary, 2007).

2.1.3 CURRENT TREATMENTS FOR MULTIPLE SCLEROSIS

The pathologic features associated with MS have been recognized for centuries, but only in the early 1990s did immune response-modifying therapies become available. Although they do not offer a cure for MS, they do modify the disease progression and improve symptoms in the majority, but not all, of patients (Wingerchuk and Weinshenker, 2016). Immune-modifying therapies are aimed at the relapsing stages of MS and have immunomodulatory/ immunosuppressive properties. First line treatments include interferon β (IFN-β) and glatiramer acetate, both of which induce a switch from pro-inflammatory Th1 leukocytes to anti-inflammatory Th2 phenotype, and also result in an increased number of regulatory T cells (for review, please see Wingerchuk and Weinshenker, 2016). One of the most potent drugs available is natalizumab, a monoclonal antibody directed against α4β1 integrin, expressed on the surface of lymphocytes that is critical for lymphocyte vascular endothelial adhesion and migration into the CNS (Polman et al., 2006). Natalizumab reduces the occurrence of relapses by 68% and decreases the rate of new CNS lesions by 83% (Butzkueven et al., 2014; Spelman et al., 2016). However, natalizumab is associated with numerous serious complications, including progressive multifocal leukoencepalitis and increased risk of opportunistic infections, which result in a higher morbidity and mortality rates (Butzkueven et al., 2014; Langer-Gould et al., 2005). Other, more currently approved therapies that aim to suppress the immune system include fingolimod (Gilenya), which sequesters autoreactive T and B cells within lymph nodes, and terifluonamide (Aubgio), which decreases T and B cell activation and proliferation. Tecfidera (BG-12) is another drug used in MS patients, and is thought to activate antioxidative pathways and be neuroprotective. Each of these medications comes with its own set of side effects (for review, please see McCoyd, 2013), and none of them cure the disease, or directly address symptoms such as spasticity, pain, gait problems, or tremor. Therefore, other pharmaceuticals are included and required in the treatment of MS in order to alleviate these symptoms and improve patient quality of life.

The current treatments for spasticity in MS patients include drugs such as baclofen, tizanidine gabapentin, or botulinum toxin, but many patients do not respond adequately, or become resis-

tant to these agents (Beard et al., 2003). Pain is treated with antiepileptic, tricyclic antidepressants, opioid analgesics, and anaesthetics (Pollmann and Feneberg, 2008; Solaro and Messmer Uccelli, 2010), but yet again, in many patients, the pain control is inadequate (Nick et al., 2012) and results in significant side effects. Finally, while patients diagnosed with RRMS respond well to immunomodulatory therapies, those with SPMS and PPMS are often refractory to these treatments (Miller and Leary, 2007; Solaro and Messmer Uccelli, 2010). Therefore, there is an urgent need for new pharmacotherapeutics, especially for those who do not respond well to current agents.

2.2 CANNABINOIDS IN MULTIPLE SCLEROSIS

Cannabinoids are chemical compounds that act at the receptors of the endocannabinoid system (ECS). The ECS consists of two G protein-coupled receptors, CB_1Rs and CB_2R; endogenous lipid ligands (anandamide (AEA) and 2-arachidonoyl glycerol (2-AG)) and enzymes responsible for endocannabinoid synthesis and degradation. Exogenous cannabinoids include phytocannabinoids, derived from the plant *Cannabis sativa*, and synthetic compounds that bind and modulate the activity of cannabinoid receptors (Pacher et al., 2006). CB_1R is expressed in the CNS and periphery, and its activation modulates synaptic transmission, while CB_2R is primarily located on the cells of the immune system, and plays a role in innate and adaptive immunity.

A growing body of evidence now indicates neuroprotective and immunomodulatory roles for cannabinoid compounds in the treatment of CNS pathologies, including MS. Both MS patients and animals induced with experimental autoimmune encephalomyelitis, when treated with cannabinoid agonists, show improvements in symptoms associated with the disease, supporting the use of cannabinoids in the treatment of MS (Pryce et al., 2003; Pryce and Baker, 2015).

2.2.1 EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS ANIMAL MODELS

Experimental autoimmune encephalomyelitis (EAE) is the most widely used animal model of MS. It is also the major preclinical model used for developing therapeutic strategies and testing novel pharmacological treatments for MS prior to clinical studies (Baker et al., 2000, 2001). EAE is induced by a variety of immunological and neuropharmacological interventions, ultimately resulting in the development of a disease phenotype and in many aspects resembles the human condition (Constantinescu et al., 2011). Animals in the chronic phase of EAE show neurodegeneration, inflammatory lesions, loss of neuronal function, as well as experience tremor, hind-limb spasticity, and paralysis (Baker et al., 2000). Importantly, in the chronic phase of EAE, animals have increased levels of endocannabinoids, including AEA, 2-AG, and PEA, in the CNS as compared to control animals (Baker et al., 2001), an effect that is thought to be neuroprotective.

The beneficial effect of endocannabinoids is supported by the pharmacological inhibition of hydrolytic enzymes responsible for their degradation, and consequent elevation in endocannabi-

noids levels. For example, EAE-induced spasticity can be decreased by the inhibition of fatty acid amide hydrolase (FAAH) and consequent increase in AEA—an effect which is mediated by the activation of CB_1R, since CB_1R inverse agonist SR141716A (Rimonabant®) negates the anti-spastic action of FAHH inhibitor. This finding is further confirmed with the use of FAAH deficient animals, where the beneficial effect of enzyme inhibition is lost (Pryce et al., 2013). Beneficial effects of increased AEA levels have also been reported with a selective AEA uptake inhibitor, UCM-707, which reduces the symptoms of the EAE, and decreases microglia activation and immune cell infiltration into the CNS (Ortega-Gutierrez et al., 2005), or by the inhibition of the AEA transporter (de Lago et al., 2004, 2006; Ligresti et al., 2006). Inhibition of monoacylglycerol lipase (MAGL) has also been reported to ameliorate the disease progression of EAE, an effect that is accompanied by increased levels of 2-AG in the spinal cord of animals, as well as decreased leukocyte migration and microglia activity (Hernandez-Torres et al., 2014). Likewise, administration of the exogenous cannabinoid agonists, WIN55,212-2 or CP55,940, also reduce the symptoms of EAE and reduce neurodegenerative processes (Pryce et al., 2003). This latter effect is mediated by activation of CB_1R, since administration of SR141617A (Rimonabant®) increases hindlimb spasticity (Pryce et al., 2003; Pryce and Baker, 2007). In support of the involvement of CB_1R, the induction of EAE in mice lacking CB_1R leads to more pronounced neuronal damage than in wild-type (WT) animals and faster and more severe progression of EAE, including more prominent immobility and permanent hidlimb paralysis. In addition, higher mortality rates in CB_1R deficient mice are observed (Pryce et al., 2003).

The reduction in neuroinflammation and symptomatic relief produced by cannabinoids in EAE are mediated in part by the activation of CB_1R; Maresz et al. (2007) showed that the beneficial effects of Δ^9-THC on the clinical symptoms and the onset of EAE are abolished in knockout mice with neuronal CB_1R deficiency. CB_1Rs are expressed on the presynaptic terminals, and their activation regulates Ca^{2+} channels (Twitchell et al., 1997), and modifies the synaptic input through the inhibition of glutamate release, dampening the excitotoxic damage. In *in vitro* experiments, Pryce et al. (2003) showed that when cerebellar neurons obtained from either WT or CB_1R knockout animals were stimulated with NMDA agonists, the Ca^{2+} influx was more pronounced in CB_1R deficient cells. Thus, suggesting that CB_1R modulates NMDA glutamate receptor activity, an effect that has been reported by others (Nagayama et al., 1999).

The key players in the pathology of MS, neurodegeneration and inflammation, are T cells and microglia, both of which express CB_2Rs that are up-regulated during inflammatory states (Sagredo et al., 2009). The activation of CB_2Rs modulates the behavior of infiltrating T cells, as well as microglia. Maresz et al. (2007) showed that adoptive transfer of CB_2R deficient T cells into WT EAE animals results in a higher infiltration rate of these cells into inflamed CNS, and increased pro-inflammatory cytokines production, including IL-2 and IFN-γ. *In vitro* assays are consistent with these findings and show that the activation of CB_2R with the selective agonist JWH-133 reduces T cell proliferation and cytokines production, an effect which was absent in CB_2R deficient

T cells (Arevalo-Martin et al., 2003). Consistent with these findings, CB_2R deficient mice are more prone to disease induction, develop more severe EAE symptoms, and have higher mortality rates than WT controls (Maresz et al., 2007). The exacerbated EAE symptoms in CB_2R deficient mice are accompanied by axonal loss, and T lymphocyte and microglia activation (Palazuelos et al., 2008). The activation of microglia is associated with the increased levels of proinflammatory cytokines, including IL1β, IL-6, IFNγ and TNFα (Muzio et al., 2007), as well as generation of reactive oxygen species, and may account for the increased levels of cytokines in the CSF fluid of MS patients (Baraczka et al., 2003, 2004; Rovaris et al., 1996) and in the CNS of EAE animals (Murphy et al., 2010). TNFα released from microglia is inhibited by cannabinoid agonists, and therefore cannabinoids may modulate disease progression (Ortega-Gutierrez et al., 2005). Taken together, the experimental data in EAE demonstrates that the cannabinoid agonists are effective as neuroprotective and immunosuppressive agents, effects that are largely mediated through the activation of CB_1R; although CB_2R modulates some of the inflammatory responses.

2.2.2 CLINICAL DATA

Alterations in endocannabinoid levels have been reported in MS. Di Filippo et al. (2008) showed that the endocannabinoids levels, including AEA, 2-AG, PEA, and OEA are decreased in CSF of patients with MS, as compared to controls. Interestingly, the same study also showed that in the patients with RRMS, AEA and PEA levels, although still below control levels, are increased during relapse phase (Di Filippo et al., 2008). Contrary to these findings, Centonze et al. (2007) reported a significant increase in the level of AEA, but not 2-AG, in the CSF of relapsing MS patients. This latter study also showed elevated synthesis and reduced degradation of AEA in lymphocytes derived from these patients, suggesting that inflammatory cells infiltrating the active lesions may be the source of increased AEA in the CNS (Centonze et al., 2007). These results are also in line with another report by Jean-Giles et al. (2009), which showed a significant increase in levels of AEA in plasma from RRMS, SPMS, and PPMS patients, as compared to control subjects. In addition, in the SPMS group, PEA and OEA were also elevated. This study also reported a reduction in the mRNA expression for FAAH, an enzyme responsible for degradation of AEA, in SPMS, but not in RRMS or PPMS groups (Jean-Gilles et al., 2009). While findings from these clinical studies are inconsistent, the results likely reflect the different MS disease subtypes, i.e., relapse vs. remission phases, or methodological variables. Nevertheless, the alterations in the endocannabinoid system reported may reflect a neuroprotective role for endocannabinoids in MS disease progression, especially in patients diagnosed with PPMS. In keeping with this, dysfunction in ECS may add to disease severity.

Spasticity and pain are the most common symptoms in patients with MS, and are inadequately controlled with current pharmacological therapies. As new treatments have emerged, cannabinoid preparations have shown therapeutic benefits in symptom alleviation, and have now

been introduced into MS treatment in a number of countries, including Canada. Nabiximols (Sativex®), an oromucosal spray of an approximate 1:1 ratio of Δ^9-THC and cannabidiol (CBD), and Dronabinol, a synthetic Δ^9-THC, have been approved for the treatment of patients with moderate to severe MS, who do not respond well to standard therapies. However, as the clinical trials assess the effectiveness of cannabinoids, conflicting data emerges.

In the Cannabinoids in MS (CAMS) trial, involving over 600 patients with progressive MS, dronabinol had no significant effect on spasticity over a 15-week trial, as assessed by the Ashworth scale; although objective improvement in patients' mobility and subjective improvement in muscle spasm, pain, and sleep were reported (Zajicek et al., 2003). Interestingly, at the 1-year follow-up phase, a significant beneficial effect of dronabinol on muscle spasticity was evident (Zajicek et al., 2005). This latter finding led to the double-blind study, which investigated the neuroprotective effects of dronabinol, assessed by a degree of progression of MS in PPMS and SPMS patients with limited ability to walk, over a 3-year period (Zajicek et al., 2013). The researchers used neurological assessments of patients, as well as subjective responses to questionnaires, and showed no beneficial effect of dronabinol on the progression of the disease. However, the subgroup analysis revealed significant benefits in MS patients with lesser disability scores, quantified by the Extended Disability Status Scale (Zajicek et al., 2013). As this group of patients was relatively small, further investigation is needed in order to draw meaningful conclusions.

A number of clinical trials also evaluated the anti-spastic effect of nabiximols (Sativex®), a cannabis extract of 1:1 THC:CBD. In the enriched study design, Novotna et al, (2011) used Sativex® as an oromucosal spray and reported a reduction in spasticity, as assessed by a numeric rating scale (NRS, 0-10), as well as global improvement in function in MS patients. The beneficial effects of Sativex® on the amelioration of spasticity was also shown in the MOVE 2 Study (Flachenecker et al., 2014), SA.FE. study (Patti et al., 2016), and by the Marinelli group (2016), who, in addition to the Ashworth scale and NRS, evaluated stretch reflex, which validated the effect of the drug. As spasticity affects movement, Coghe et al. (2015) looked at the effects of the nabiximols, Sativex®, on this outcome. Objective assessment of movement function in response to nabiximols treatment, in patients who initially responded to the drug, revealed a significant improvement in speed velocity, as a function of lesser spasticity, and consequently greater and faster joint movements and improved walking ability (Coghe et al., 2015).

Another symptom that that is commonly associated with MS and responds to treatment with cannabinoids is pain, a clinical symptom experienced by 40–70% of patients (Osterberg et al., 2005; Solaro et al., 2004). The effect of dronabinol in the amelioration of pain, as a secondary outcome to spasticity, has been evaluated in a large double-blind randomized trial (Zajicek et al., 2003). Interestingly, while improvement in pain perception was reported by the majority of patients, around 20% of patients reported worsening of pain while on cannabinoid treatment (Zajicek et al., 2003). As neuropathic pain is the most common pain syndrome experienced by MS patients, effectiveness of Sativex® in neuropathic pain control has been investigated by focusing on this particular

symptom. More specifically, the beneficial effect of oromucosal nabiximols on neuropathic pain relief was reported by Rog and colleges (2007) in an open–labeled, 2-year extension trial, with mild to moderate side effects being reported. Similar findings were also reported by Russo et al. (2016) who evaluated the effect of Sativex® in the management of neuropathic pain in 20 MS patients (10 with and 10 without neuropathic pain). The study showed improvement in neuropathic pain and in the subjective and objective spasticity scores. Nabiximols (Sativex®), as an add-on therapy, was also evaluated in a larger cohort of MS patients who did not respond adequately to standard analgesics; however, the results from the two phases of the study were conflicting. The initial phase A of the study showed a large number of responders in both placebo and Sativex® treatment groups, and therefore no significant difference between those two groups; the second phase B resulted in more promising results and showed a significant improvement in pain relief, as well as quality of sleep, in the cannabinoid treated group (Langford et al., 2013).

While the majority of data support the beneficial effects of cannabinoids in the symptomatic relief of pain associated with MS, the findings from clinical trials are not as consistent as the experimental data. Among a number of different variables, the inconsistencies we see may reflect differences in study design, compound tested, or dosing schedule. Clinical trials evaluating the effectiveness of cannabinoid preparations in MS range from the uncontrolled open-label trials to trials with an initial enrichment phase, followed by second phase of a double-blind design. The enrichment phase allows the identification of "responders," who are then randomized into placebo or treatment groups, while non-responders discontinue the trial. The enriched trials that test the effectiveness of cannabinoids result in a smaller therapeutic effect, as compared to double-blind, placebo-controlled parallel trials (Collin et al., 2007, 2010; Wade et al., 2004). Furthermore, the majority of cannabinoid trials evaluat the effect of these agents in a population of MS patients with moderate to severe symptoms, who are refractory to current treatments. It is, therefore, possible that cannabinoid treatments may be more effective in the earlier stages of MS, as seen in a subgroup of patients from Zajicek et al. (2013) trial. In addition, many studies use subjective measures to assess the given outcomes, and patient expectancy of treatment effect can influence response. Indeed, the placebo effect varied significantly across the published studies ranging from 10% to as high as 50%, again, depending on the study design and compound tested, suggesting that findings from clinical trials should be evaluated with caution (Di Marzo and Centonze, 2015).

2.2.3 TOLERABILITY OF CANNABINOIDS

As the clinical trials continue to evaluate the efficacy of cannabinoids in the treatment of symptoms associated with MS, safety data for these compounds is also emerging. The retrospective analysis of risk-benefit profile for Sativex® from over 900 patients across the United Kingdom, Germany, and Switzerland, has shown that clinical outcomes, especially the sustained reduction in spasticity, outweigh the adverse effects of the drug (Fernandez, 2016). The most common adverse effects re-

ported by patients from these trials, as well as others, were nausea, dizziness, fatigue, and weakness (Fernandez, 2016; Flachenecker et al., 2014; Novotna et al., 2011; Rog et al., 2007; Zajicek et al., 2003). In addition, psychiatric events (including depression) are reported, albeit in a small number of patients. There is no evidence of tolerance or addiction/abuse of Sativex® in these studies (Fernandez, 2016; Rog et al., 2007).

2.3 CONCLUDING REMARKS AND FUTURE DIRECTIONS

The experimental and clinical data on the use of cannabinoid agonists in the treatment of MS suggests a potential benefit for these compounds in the symptomatic treatment of the disease, especially spasticity. Evidence for the neuroprotective effects, which may modulate MS progression, is supported by experimental studies, with clinical data being less conclusive. The beneficial effects of cannabinoids are mediated by the activation of both cannabinoid receptors, CB_1R and CB_2R, which modulate inflammatory and pain responses, and may confer neuroprotective benefit. As activation of CB_1R may produce side effects, including behavioral effects, another approach that may be useful in the future treatment of MS is the activation of ECS by pharmacological inhibition of degradative enzymes for AEA and 2-AG (Baker et al., 2001; Hernandez-Torres et al., 2014; Ligresti et al., 2006; Pryce et al., 2013). This strategy is especially attractive because it is localized and does not affect motor function or produce any overt psychotropic effects. Taken together, the existing preclinical and clinical data for MS suggest that activation of the ECS plays a protective role against inflammatory and neuronal damage in MS, while dysfunction in the ECS system may contribute to pathology.