Published in final edited form as:
J Neuroimmune Pharmacol. 2013 June ; 8(3): 608–620. doi:10.1007/s11481-013-9445-9.

Cannabinoid receptor 2: Potential role in immunomodulation and neuroinflammation Review

Slava Rom[1,*] and **Yuri Persidsky**[1,*]

[1]Department of Pathology and Laboratory Medicine, Temple University School of Medicine, Philadelphia PA (USA)

Abstract

An accumulating body of evidence suggests that endocannabinoids and cannabinoid receptors type 1 and 2 (CB_1, CB_2) play a significant role in physiologic and pathologic processes, including cognitive and immune functions. While the addictive properties of marijuana, an extract from the Cannabis plant, are well recognized, there is growing appreciation of the therapeutic potential of cannabinoids in multiple pathologic conditions involving chronic inflammation (inflammatory bowel disease, arthritis, autoimmune disorders, multiple sclerosis, HIV-1 infection, stroke, Alzheimer's disease to name a few), mainly mediated by CB_2 activation. Development of CB_2 agonists as therapeutic agents has been hampered by the complexity of their intracellular signaling, relative paucity of highly selective compounds and insufficient data regarding end effects in the target cells and organs. This review attempts to summarize recent advances in studies of CB_2 activation in the setting of neuroinflammation, immunomodulation and HIV-1 infection.

Keywords

cannabinoid receptor; endocannabinoids; medical marijuana; HIV-1; monocyte/macrophage; endothelial cells

Introduction

The cannabinoids are a group of terpenophenolic compounds present in the marijuana plant, *Cannabis sativa*. At present, three general types of cannabinoids have been identified: phytocannabinoids present uniquely in the cannabis plant, endogenous cannabinoids produced in humans and animals, and synthetic cannabinoids generated in a laboratory (Sarfaraz et al., 2008). It is worth noting that *Cannabis sativa* produces over 80 cannabinoids (Console-Bram et al., 2012). The broader definition of cannabinoids refers to a group of substances that are structurally related to Δ^9-tetrahydrocannabinol (Δ^9-THC) or that bind to cannabinoid receptors. The chemical definition includes a variety of distinct chemical classes: the classical cannabinoids structurally related to THC, the non-classical cannabinoids, the aminoalkylindoles, the eicosanoids related to the endocannabinoids, quinolines and arylsulphonamides (Woelkart et al., 2008; Console-Bram et al., 2012), and additional compounds that do not fall into these standard classes, but bind to cannabinoid receptors (CB). Multifaceted effects of marijuana can be singled out aiming at evaluation of

*Corresponding authors: Yuri Persidsky, Department of Pathology and Laboratory Medicine, Temple University, School of Medicine, 3401 N. Broad St., Philadelphia, PA 19140, yuri.persidsky@tuhs.temple.edu, Phone: (215) 707-4353 Fax: (215) 707-2781 and, Slava Rom, Department of Pathology and Laboratory Medicine, Temple University, School of Medicine, 3401 N. Broad St., Philadelphia, PA 19140, srom@temple.edu, Phone: (215) 707-2543 Fax: (215) 707-2781.

the potential medical value of marijuana and cannabinoids in specific human diseases, with minimal undesired side effects.

Nomenclature of cannabinoid receptors (CB)

There are two well-characterized CB with distinctly different physiological properties. The psychoactive effects of cannabinoids are associated with the CB_1 receptor; the CB_2 receptor mainly mediates anti-inflammatory and immunomodulatory actions (Miller and Stella, 2008).

CB_1

Cannabinoid receptor 1 (CB_1) was discovered by Devane et al. in 1988 (Devane et al., 1988). CB_1 cDNA was cloned by Matsuda et al. (Matsuda et al., 1990) from rats using a homology approach to G-protein-coupled receptors (GPCR). Subsequently, CB_1 receptors have been found in other vertebrates as well. The genes for mouse, rat and human CB_1 receptors (CNR1) are located on chromosomes 4, 5 and 6, respectively. The CB_1 receptor is widely distributed throughout the brain regions, especially the frontal cortex, the limbic system, including the hippocampus and amygdala, sensory and motor areas, hypothalamus, pons and medulla (Ashton et al., 2007; Ashton and Moore, 2011). Human CB_1 shares 94% amino acid sequence identity with rodent CB_1 (Anday and Mercier, 2005). In addition to full-length CB_1 receptor, two splice variants, human CB_1a and human CB_1b, have been described. Both splice variants have altered ligand binding and activation properties compared to full length CB_1, and are expressed at very low levels in different tissues (Ryberg et al., 2005). In contrast, another group demonstrated no significant differences between the three variants (full-length and two splice-variants) in pharmacological characteristics, such as binding affinity, functional potency, and efficacy of several CB_1 agonists (Xiao et al., 2008). The functional significance of different human cannabinoid CB_1 receptor variants remains to be clarified.

The majority of high CB_1-expressing cells are GABAergic (gamma-aminobutyric acid) neurons belonging mainly to the cholecystokinin-positive and parvalbumin-negative type of interneurons (basket cells) and, to a less extent, to the mid-proximal dendritic inhibitory interneurons. Only a fraction of low CB_1-expressing cells is GABAergic. In the hippocampus, amygdala and entorhinal cortex areas, CB_1 mRNA is present at low but significant levels in many non-GABAergic neuronal cells (Marsicano and Lutz, 1999).

CB_2

The second cannabinoid receptor (CB_2) was isolated from human myeloid cells in 1992 (Munro et al., 1993). Soon afterwards, CB_2 was identified in other species, such as mouse, rat, bovine and zebra fish. The CB_2 gene is located on chromosome 1 and 4 in humans and mice, respectively. Unlike the mouse CB_2 gene, which is intron-less, the human gene has been reported to have two splice variants (Liu et al., 2009). The longer variant human CB_2a, comprised of exon 1a, exon 1b and exon 3, is mostly expressed in testis (about 100-fold more compared to spleen or leukocytes). It is also detected in various regions of the brain (Liu et al., 2009). The shorter variant, human CB_2b, consisting of exon 2 and exon 3, is expressed mostly in the spleen and leukocytes, 100-and 30-fold higher, respectively, when compared to CB_2b expression in the brain (Liu et al., 2009). Immune cells express high levels of CB_2 and there is a hierarchy of CB_2 expression within the immune system (B cells > natural killer cells > monocytes > neutrophils > CD8 lymphocytes > CD4 lymphocytes) (Nunez et al., 2004; Yao and Mackie, 2009). The level of expression is dependent on the activation state of the cell and the type of stimuli. Stimulation of splenocytes with LPS leads to CB_2 mRNA down- regulation, whereas CD40 co-stimulation results in CB_2 mRNA up-

regulation (Lee et al., 2001). CB_2 receptor sequences are less conserved throughout evolution compared to CB_1 receptors. CB_2 and CB_1 receptors share about 44% identity in humans at the level of amino acids (Liu et al., 2009; Console-Bram et al., 2012). Humans and mice share about 82% identity in amino acid sequence of CB_2 (Anday and Mercier, 2005), whereas mice and rat share 93% identity. Human, rat and mouse sequences diverge at the C-terminus. The mouse protein is 13 amino acids shorter, while the rat protein is 50 amino acids longer than the human (Console-Bram et al., 2012). Diversity in CB_2 sequences between species can possibly explain differential effects of CB_2 agonists in human and rodent models (Figure 1).

Structural similarities and differences of CB_2 and CB_1

Cannabinoid receptors are integral membrane proteins whose amino acid sequences are characterized by seven hydrophobic segments containing -helical patterns. CBs belong to α group of rhodopsin-like seven-transmembrane (7TM) GPCRs (Palczewski et al., 2000). CBs share the structural characteristics of other GPCRs: an extracellular N-terminus that is glycosylated, seven transmembrane α-helixes with extracellular and intracellular loops, and an intracellular C-terminus (Ahn et al., 2009). While CB_1 and CB_2 are encoded by different genes, they exhibit 44% amino acid identity throughout the whole protein (Figure 1). Since CBs are intrinsic membrane proteins, they are difficult to crystallize for X-ray study. Although their crystal structures are not available, homology models built by using bovine rhodopsin as a template, along with resolution NMR and computer modeling, have revealed that the most important differences between CB_1 and CB_2 with respect to their interaction with the surrounding lipid environment are located in theTM7 juxtamembrane domain (Dainese et al., 2008). CB_1 presents a hydrophobic surface at the helix I-helix VII interface that is suitable for a specific interaction with cholesterol and palmitic acid, whereas CB_2 displays a negatively charged region within TM7, which is rather unfavorable for any interaction with lipids. Arg302 in CB_2 TM7 (homologous to Arg401 in CB_1) is not available for any interaction with cholesterol or with G-proteins (Xie and Chen, 2005). Despite the ability to be activated by the same endocannabinoids and trigger the same signaling pathways, CB_2 is independent of membrane cholesterol content (Bari et al., 2006).

In every TM domain, there are residues that have been probed for their importance in ligand binding or signal transduction. CB_2 helix III is in contact with the other helixes and plays an important role in the regulation of cannabinoid activities (Xie et al., 2003). CB_2 Ser112 has been shown to be crucial for recognition of several cannabinoid ligands (based on mutagenesis studies)(Tao et al., 1999). The homologous residue in the CB_1 TM3 is Gly195 (Xie et al., 2003). Unlike the CB_1 receptor, in which Lys192 plays an important role for the binding of most cannabinoid ligands, the homologous residue Lys109 in CB_2 TM3 does not appear to be a key residue (Tao et al., 1999; Xie et al., 2003). Mutagenesis studies indicate that the TM4 domain in CB_2 plays a more important role for the recognition of the cannabinoid ligand that in CB_1. The CB_2-selective antagonist, SR144528, completely fails to antagonize the CB_2 in S161A or S165A mutants, which is explained by existence of alanines in the homologous two sites of TM4 of the CB_1 receptor (Xie et al., 2003). To date, studies of the CB_2 have been limited by the lack of a three dimensional structure, leaving many unanswered questions about interactions at the molecular level with its ligands as well as the nature of the receptor active site(s) (Xie and Chen, 2005). Knowledge of the three-dimensional structure of CB_2 should greatly aid in the rational design of specific CB_2 ligands possessing therapeutic activities, but devoid of undesirable side effects. The dissimilarities between the two receptors could be exploited to design CB_2-specific ligands in the future.

Medical marijuana

Medical marijuana refers to the parts of the cannabis plant used as a physician-recommended form of herbal therapy or as medicine (synthetic analogs of cannabinoids). In the US recently, use of medical marijuana has surged in the 16 states and the District of Columbia that permit its use. The cannabis plant has a long history of use as a medicine dating back to 2737 B.C. (Ben Amar, 2006). *Cannabis sativa* contains over 80 different chemical constituents (Molina et al., 2011; Console-Bram et al., 2012). There are three phytocannabinoids (Figure 2): THC, cannabidiol (CBD) and cannabinol (CBN). THC is a primary component of cannabis, responsible for the psychoactive effects of the plant; it has mild analgesic and antioxidant activities (Hampson et al., 1998; Molina et al., 2010). CBD represents up to 40% of the extract of medicinal cannabis. It has been shown to relieve cough, congestion and nausea, convulsion, inflammation and anxiety, and has been shown to be effective in treating multiple sclerosis (MS), anxiety attacks and schizophrenia (Crippa and Zuardi, 2006; Zuardi et al., 2006a; Zuardi et al., 2006b; Kogan et al., 2007; Mechoulam et al., 2007; Lakhan and Rowland, 2009). CBN is a breakdown product of THC and acts as a weak agonist of CB_1 and CB_2 because of lower affinity than THC toward receptors (Mahadevan et al., 2000).

There are several medications (plant extracts or synthetic cannabinoid analogues of THC alone or in combination with CBD) that have been approved for medicinal use. Nabilone® was approved in the US and Canada in 1985 as an anti-nausea treatment for patients undergoing cancer chemotherapy (Todaro, 2012). Sativex® was approved in Canada and the UK for treatment of neuropathic pain in MS and pain associated with cancer (Russo et al., 2007; Sastre-Garriga et al., 2007). Sativex® has been also approved for MS-associated spasticity in many countries, such as New Zealand, UK, Austria, Czech Republic, Denmark, Germany, Sweden, Israel, Italy and Spain (Leussink et al., 2012). In the majority of cases, administration of cannabinoids (natural or synthetic analogs) results in improvement of symptoms of MS, especially spasticity, muscle pain, tremors and bladder control. However, there were differences in the actions of orally administered versus inhaled cannabinoids, probably due to variable absorption of orally administered cannabinoids (Maurer et al., 1990; Russo et al., 2007). Several studies have been done on the pharmacokinetics of cannabinoids during different routes of drug administration. Smoking, the major route, provides a rapid and efficient method for drug delivery from the lungs to the brain, contributing to its abuse potential. The number, duration and spacing of the puffs, as well as holding time and inhalation volumes, greatly affect the degree of exposure (Heishman et al., 1989; Azorlosa et al., 1992). Synthetic THC, Dronabinol (Marinol®), is usually taken orally, but may also be administered rectally. In addition, abuse is being common by the oral route. Absorption is slower when cannabinoids are ingested, with lower and delayed peak THC concentrations (Law et al., 1984; Huestis, 2007). To prevent first-pass metabolism by the liver, Sativex® is administered sublingually, via the oromucosal route. The oromucosal form of administration has the advantage that the first-pass effects are reduced and, compared to smoking, the maximum plasma THC levels are lower and increase more slowly (Karst et al., 2010; Leussink et al., 2012). Because MS is considered to be a relapsing chronic inflammatory disease of the CNS, beneficial anti-inflammatory effects of cannabinoids would provide much needed MS symptom relief. Clinical benefits for the use of cannabinoids in MS patients were also supported by studies in animal models of MS (Baker et al., 2001; Pryce and Baker, 2005; Mestre et al., 2009).

CB_1 antagonists (AM251 and SR141716A) lead to changes in efflux of noradrenaline and dopamine in various brain regions, leading to antidepressant effects (Tzavara et al., 2003; Witkin et al., 2005). These studies suggest the possibility of use of cannabinoids in the

treatment of mood disorders, such as depression, anxiety, manic depression and posttraumatic stress (Ashton and Moore, 2011).

Marinol® (Dronabinol) was approved by the US and Canada in 1985 for patients undergoing cancer chemotherapy to relieve nausea associated with treatment. In 1992, it was approved for treatment of anorexia associated with AIDS-related weight loss (Dejesus et al., 2007; Todaro, 2012).

Cannabinoids are increasingly being used for treatment of migraine headaches in patients that are not responding to the serotonin 1D agonist, sumatripan (Krymchantowski and Jevoux Cda, 2007). Dronabinol has been shown to inhibit the 5-HT3 receptor involved in emetic and pain responses (Fan, 1995). A patent has been filed for the use of Dronabiniol for treatment of migraines (Barbato, 2007). Canasol® is used for reduction of intraocular pressure in glaucoma (Crandall et al., 2007). Cannabinoid compounds applied topically show hypotensive and neuroprotective effects in mice and monkeys (Williams et al., 2007; Yazulla, 2008). In 1992, the FDA approved the use of Dronabinol to stimulate appetite in AIDS patients suffering from wasting syndrome. This triggered further interest in the development of cannabinoid antagonists that would reduce appetite. Rimonabant has been shown to suppress appetite, producing reduction in body weight (Pi-Sunyer et al., 2006). Dronabinol has been shown to be a potent drug for treatment of HIV-associated sensory neuropathy, the most common peripheral nerve disorder complicating HIV-1 infection (Abrams et al., 2007).

Synthetic cannabinoid agonists

Synthetic cannabinoids are functionally similar to THC, the active part of cannabis. Like THC, they bind to the same cannabinoid receptors in the brain and other organs as the endogenous ligands, anandamide and 2-arachidonylglycerol, which interact with both CB_1 and CB_2 receptors. More correctly designated as cannabinoid receptor agonists, synthetic cannabinoid agonists were developed over the past forty years as therapeutic agents, often for the treatment of pain. However, it proved difficult to separate their desired properties from unwanted psychoactive effects.

Although often referred to simply as synthetic cannabinoids, many of the substances are not structurally related to the so-called “classical” cannabinoids, i.e., compounds like THC, based on dibenzopyran. The cannabinoid receptor agonists form a diverse group, but most are lipid soluble, non-polar, and consist of 22 to 26 carbon atoms; they would therefore be expected to volatilize readily when smoked. A common structural feature is a side-chain, where optimal activity requires more than four and up to nine saturated carbon atoms. The first figure shows the structure of THC, while the others show examples of synthetic cannabinoid receptor agonists, all of which have been found in “Spice” or other smoking mixtures. The synthetic cannabinoids fall into seven major structural groups (Figure 2):

1. Naphthoylindoles (e.g., JWH-018, JWH-073, JWH-398).
2. Naphthylmethylindoles. (e.g., JWH-175, JWH-195, JWH-197).
3. Naphthoylpyrroles.(e.g., JWH-030, JWH-156, JWH-243).
4. Naphthylmethylindenes (e.g., JWH-176).
5. Phenylacetylindoles (i.e., benzoylindoles, e.g., JWH-250, JWH-253, JWH-313).
6. Cyclohexylphenols (e.g., CP 47,497 and homologs of CP 47,497).
7. Classical cannabinoids (e.g., HU-210).

Compounds in groups 1–5 (JWH compounds) were largely synthesized by Huffman et al. over the past fifteen years (Huffman et al., 1996; Wiley et al., 1998; Huffman et al., 2000; Huffman et al., 2003; Huffman et al., 2005; Huffman et al., 2006; Huffman et al., 2010). The sixth group, cyclohexylphenols, were developed by Pfizer during the 1970's and 1980's (Carissimi et al., 1976). The classical cannabinoids (seventh group) is the oldest; synthesis began in the 1960's following the identification of the chemical structure of THC (Mechoulam and Gaoni, 1965, 1967; Gaoni and Mechoulam, 1971). In general, the agonists show little selectivity between CB_1 and CB_2, while antagonist compounds are highly selective (>1000 fold selective for CB_1 vs. CB_2 and vice versa with nanomolar affinity at the relevant receptor)(Console-Bram et al., 2012). The selectivity of these antagonists allows the discrimination of CB_1- vs. CB_2-mediated effects *in vitro* and *in vivo*. Despite the existence of numerous non-selective agonists, there are some that exhibit selectivity. For example, HU-308 is a highly selective CB_2 agonist with nanomolar affinity at CB_2 and >1000 fold selectivity for CB_2 vs. CB_1. If a compound shows >100 fold selectivity, it is classified as a selective agonist (Console-Bram et al., 2012).

CB signaling – CB-ligand interactions

CB_2, as with the rest of the GPCRs, signals through the three main components of the MAPK pathway, namely, ERK, JNK and p38 (Howlett et al., 2002). However, activation of CB receptor coupling to MAPKs is dependent on cellular content. A wide range of activation and inhibition has been observed dependent on cell type, cell differentiation status and co-modulators of MAPK cascades (Howlett, 2005). CB_2 agonism decreased CXCR4-activation mediated G-protein activity and MAPK phosphorylation. Furthermore, CB_2 agonists altered cytoskeletal reorganization, by decreasing F-actin levels, impairing productive infection (Costantino et al., 2012). Signaling events associated with inflammatory responses in endothelial cells and monocytes are complex; few studies have addressed potential mechanisms of anti-inflammatory CB_2 stimulation. Gertsch et al. investigated intracellular signaling pathways triggered in monocytes by LPS-triggered TNFα and IL-1β production that were suppressed by CB_2 agonist. LPS treatment of human monocytes led to a rapid phosphorylation of p38 and JNK1/2 (Gertsch et al., 2008), and CB_2 agonist reduced Erk1/2 and JNK1/2 activation (phosphorylation)(Gertsch et al., 2008). Whether or not CB_2 modulates ion channels has been controversial. Felder and colleagues (Felder et al., 1995) suggested that CB_2 does not modulate potassium or high-voltage calcium channels. More recent work indicates that CB_2 agonists do modulate these channels and earlier results were due to functional selectivity of the ligands used (Atwood et al., 2012). CB_2 affects additional signaling pathways, including activation of phospholipase C, leading to release of calcium (Shoemaker et al., 2005), regulation of small G proteins (such as Rho, Rac and cdc42) (Kurihara et al., 2006), and activation of JNK via the phosphatidyl inositol 3 kinase (PI3K)/Akt pathway (Viscomi et al., 2009; Atwood et al., 2012). In human umbilical vein endothelial cells, cannabidiol could evoke phosphorylation of p44/42 MAPK and PKB/Akt via PI3K pathway (Offertaler et al., 2003).

GPCRs are regulated at many levels. In general, once they are activated by ligand binding, their signaling is attenuated by a desensitization process, followed by internalization. Desensitization involves G protein receptor kinase-mediated phosphorylation of multiple serine or threonine residues of GPCR, followed by binding of the β-arestins. This protein complex is usually localized to clathrin-coated pits or caveoli where it is internalized. The internalized receptor might be transported to endosomes for dephosphorylation and returned to the plasma membrane for the next signaling event or directed to lysosomes for degradation (Atwood et al., 2012). Little is known about the processes involved in CB_2 adaption to chronic activation. Most of the knowledge comes primarily from cell lines over expressing CB_2. Although the levels of expression in those systems significantly exceed *in*

vivo CB_2 levels, the use of expression systems provides very valuable information (Atwood et al., 2012).

CB_2 receptor expression in immune and neuroimmune cells

Anti-inflammatory effects of cannabinoids

The two well-characterized CBs possess different physiological properties. The psychoactive effects of cannabinoids are associated with CB_1; CB_2 mainly mediates anti-inflammatory and immunomodulatory actions (Miller and Stella, 2008). Identification of CB_1 and CB_2 resulted in the recognition of endogenous cannabinoids (endocannabinoids) (Pacher et al., 2006). Two endocannabinoid metabolizing enzymes were identified: fatty acid amine hydrolase (FAAH) and monoacylglycerol lipase. CB_1 agonists possess neuroprotective properties via diminishing excitotoxicity in postsynaptic neurons (Marsicano et al., 2003), enhancing vasodilation through CB_1 in vascular smooth muscle and the inhibition of endothelin-1 (Ronco et al., 2007), and decreasing the release of pro-inflammatory mediators including nitric oxide (NO) and tumor necrosis factor (TNFα) in the acute phase of injury (Fernandez-Lopez et al., 2006; Panikashvili et al., 2006). Besides their involvement in controlling exitotoxicity and inflammation, compelling evidence shows that CB_1 receptors in the CNS play an important role in neuroprotection (Sanchez and Garcia-Merino, 2011). CB_1-deficient mice were more susceptible for experimental autoimmune encephalomyelitis (EAE), had more neurodegeneration and had worse recovery, compared to wild type mice (Maresz et al., 2007). The presence of CB_1 in neurons, but not in T-cells, was an essential requisite for cannabinoid-mediated neuroprotection in EAE. The therapeutic limitations of CB_1 agonists are related to the very short window of their beneficial actions and psychoactive effects at effective doses. CB_2 agonists are devoid of psychoactive activities. Because neuroinflammation plays a significant role in essentially all neurodegenerative processes, CB_2 stimulation became an attractive target for development of neuroprotective therapies. CB_2 is expressed in different types of leukocytes mediating cannabinoid anti-inflammatory effects and immunomodulation (McKallip et al., 2002b; McKallip et al., 2002a).

Cannabinoid induced immunomodulation

The mechanism of cannabinoid-induced immunomodulation has been studied in both *in vitro* and *in vivo* systems; however, still many questions remain unanswered. It is known that cannabinoids bind to CB_1 and CB_2 inhibiting adenylate cyclase activity and preventing forskolin-stimulated cAMP activation. This process leads to decreased activity of protein kinase A and subsequently lesser binding of transcription factors to CRE consensus sequences (Condie et al., 1996; Rieder et al., 2010). Cannabinoids clearly modulate immune responses during inflammatory processes and their immunomodulatory effects have been studied in many disease models such as MS, diabetes, septic shock, rheumatoid arthritis, and others (Table 1 and Table 2). Results of animal studies show that cannabinoids exert their immunomodulatory properties in four ways: i) induction of apoptosis, ii) suppression of cell proliferation, iii) inhibition of pro-inflammatory cytokine/chemokine production and increase in anti-inflammatory cytokines, and iv) induction of regulatory T cells (Rieder et al., 2010). It has been shown that THC inhibits proliferation of human lymphocytes in culture (Schwarz et al., 1994) and leads to apoptosis of murine macrophages and T cells (Zhu et al., 1998). McKallip and colleagues showed that THC affects naïve lymphocytes to a greater degree than activated lymphocyte (McKallip et al., 2002b). It was also noted that activated lymphocytes had less expression of CB_2, thereby explaining the decreased sensitivity of activated lymphocytes to THC (McKallip et al., 2002b). JWH-015, a CB_2 synthetic agonist, inhibited proliferation in T and B cells in a dose-dependent manner and induced apoptosis in splenocytes and thymocytes (Lombard et al., 2007; Rieder et al., 2010).

Derocq and colleagues (Derocq et al., 1995) demonstrated that the activity of cannabinoids is not restricted to immunomodulation, and in nanomolar concentrations of synthetic (CP55,940 and WIN55212-2) and natural (THC) cannabinoids increase proliferation of B cells co-stimulated with anti-CD40-antibodies, while at micromolar range (1–100 M) the same cannabinoids inhibited proliferation (Derocq et al., 1995). Interestingly, cannabinol and THC protected human B lymphoblastoid cell line from serum-deprived cell-death and oxidative stress in sub-micromolar concentrations (Chen and Buck, 2000). The fact that activation of CB_2 triggers apoptosis in immune cells suggests that targeting CB_2 may be a novel approach to the treatment of inflammatory and autoimmune diseases.

CB_2 expression in CNS and its mediated anti-neuroinflammatory responses

CB_2 expression has been detected in microglia. Carlisle et al. (Carlisle et al., 2002) found CB_1 and CB_2 mRNA in brain tissue and in primary rat microglia cultures. CB_2 expression in microglia is up-regulated during activation. Neuroprotective effects of CB_2 agonists are associated with suppression of microglia activation (Klegeris et al., 2003; Eljaschewitsch et al., 2006) via inhibiting the release of neurotoxic factors and by decreasing neuronal cell damage in cell or tissue culture models.

Increased expression of CB_2 under neuroinflammatory conditions was found in human brain (Benito et al., 2008). Prominent CB_2 upregulation was reported in brain tissues affected by MS, amyotrophic lateral sclerosis (ALS), Down syndrome and Alzheimer's disease, AD (Benito et al., 2005; Yiangou et al., 2006; Benito et al., 2007; Benito et al., 2008; Solas et al., 2012). Enhanced CB_2 expression was detected on microglia, perivascular macrophages and T cells in simian immunodeficiency virus encephalitis, model of HIV-1 infection that paralleled FAAH over-expression in astrocytes (Benito et al., 2005). Increased expression of CB_2 on microglia/perivascular macrophages and brain microvascular endothelial cells were found in HIV-1 encephalitis, brain endothelium in HIV^+ cases (without encephalitis) and very little in non-infected control human brains (Persidsky et al., 2011). CB_2 agonist prevented neuronal injury during neuroinflammation via upregulation of mitogen-activated protein kinase phosphatase-1 that resulted in Erk1/2 inhibition (Eljaschewitsch et al., 2006). CB_2 stimulation specifically reduced iNOS production via inhibition of ERK-1/2 phosphorylation in microglia during CNS inflammation (Merighi et al., 2011). These findings have relevance to anti-inflammatory effects of CB_2 stimulation in brain. CB_2-mediated regulation of this pathway could be very important for cannabinoid regulation of neuroinflammation. Anti-inflammatory and neuroprotective effects of cannabinoids have been confirmed in animal models for MS, AD, stroke, ALS, and other animal models of diverse inflammatory diseases (summarized in Table 2). In summary, cannabinoids can be neuroprotective via their immunomodulatory properties, which have been mainly attributed to CB_2 receptors.

CB receptors in leukocytes and endothelium: relevance to HIV infection

Cannabinoids can affect different pro-inflammatory events associated with HIV infection, such as: i) chemotaxis of immune cells, ii) activation of endothelium and leukocyte infiltration into tissues, iii) injury of endothelial barriers, like the blood brain barrier (BBB), and iv) HIV-1 infection of susceptible cells.

CB_2 appears to play a major role in leukocyte migration. Human monocytes treated with the synthetic CB_2 agonist, JWH-015, showed diminished migration in response to the chemokines, CCL2 and CCL3, via down-regulation of their receptors and inhibition of IFNγ-induced ICAM-1 expression. Leukocyte migration mediated by RhoA activation was inhibited by CB_2 agonists (Kurihara et al., 2006). Cannabinoids could reduce inflammation by interfering with the action of other chemoattractants (Montecucco et al., 2008).

Cannabinoids have been reported to inhibit chemokine-induced chemotaxis of various cell types including neutrophils, lymphocytes, macrophages, monocytes and microglia (Miller and Stella, 2008). Recently, it has been shown that CB_2 agonist affected dendritic cell migration *in vitro* and *in vivo*, primarily through the inhibition of matrix metalloproteinase-9 expression (Adhikary et al., 2012).

In addition to putative effects on leukocytes, anti-inflammatory properties of CB agonists may be related to their actions on the endothelium. CB_2 has been found in brain endothelium (Golech et al., 2004; Lu et al., 2008) and in endothelial cells from other organs (Rajesh et al., 2007). Synthetic CB_2 agonists (JWH-133, HU-308) reduced TNFα-induced activation of human coronary artery endothelial cells *in vitro*. CB_2 agonists reduced secretion of MCP-1 and attenuated monocyte transendothelial migration (Rajesh et al., 2007). Those results are relevant to CB_2 anti-inflammatory effects.

Lu and colleagues (Lu et al., 2008) demonstrated that HIV-1 gp120 caused secretion of substance P and dysfunction of brain endothelial cells (Ca^{2+} influx, decreased barrier tightness, decrease in tight junction (TJ) expression) that was prevented by non-selective CB_1/CB_2 or CB_1 agonists. These compounds diminished monocyte migration across endothelial monolayers pretreated with gp120. The molecular mechanism underlying the beneficial effects of CB agonists was not investigated. Because CB_2 agonists modulate immune cell migration, they represent a promising pharmacological approach for development of anti-inflammatory therapeutics. Fraga and colleagues (Fraga et al., 2011) found that CB_2 inhibited migration of microglial cells toward HIV Tat protein in a mouse BV-2 microglia-like cell model. In addition, the level of the β-chemokine receptor CCR3 was reduced and its localization was altered.

Our group investigated the effects of CB_2 receptor agonists (JWH133 and O1966) on leukocyte-brain endothelial cell interactions. Using primary human brain microvascular endothelial cells and human monocytes, we showed diminished adhesion of leukocytes to activated endothelium and down-regulation of adhesion molecules. In an animal model of systemic inflammation, leukocyte adhesion was significantly attenuated in postcapillary venules in animals treated with CB_2 agonists. BBB injury and increased permeability were prevented (Ramirez et al., 2012).

The CB_2 agonist, JWH133, was shown to inhibit HIV-1 infection in primary CD4+ T lymphocytes by altering T cell actin dynamics via inactivation of the actin-modulating protein, cofilin. Actin rearrangements are essential for productive infection. CB_2 agonist did not alter CXCR4 expression, T cell activation or viral fusion (Costantino et al., 2012). We showed that CB_2 agonists diminished HIV replication and HIV LTR activation in monocyte-derived macrophages (personal communication). Cannabimimetic drugs are of particular relevance to HIV-1 associated neurocognitive disorders (HAND) because of their clinical and illicit use in patients with AIDS. The cannabinoid receptor agonist, Win55,212–2, inhibited HIV-1 gp120-induced IL-1β production and synapse loss in a manner reversed by CB_2 receptor antagonist. In contrast, Win55,212–2 did not inhibit synapse loss elicited by exposure to the HIV-1 protein Tat. These results indicate that cannabinoids prevent the impairment of network function produced by gp120 and, thus, might have therapeutic potential in HAND (Kim et al., 2011). Molina and colleagues (Molina et al., 2010; Molina et al., 2011) demonstrated that that chronic Δ^9-THC treatment decreased plasma and cerebrospinal fluid (CSF) viral load and tissue inflammation significantly reducing morbidity and mortality of simian immunodeficiency virus-infected macaques. THC treatment led to better maintenance of body weight without any alterations in immune responses (Molina et al., 2010). Win55,212–2 has been shown to inhibit HIV-1 expression in $CD4^+$ lymphocytes and microglial cell cultures in a time- and dose-dependant manner

(Peterson et al., 2004). Taken together, these studies point to potential therapeutic benefits of CB stimulation in treatment of HIV-1 infection.

Summary

The full potential of CB_2 agonists as therapeutic agents remains to be realized. Despite some inadequacies of preclinical models to predict clinical efficacy in humans and differences between the signaling of human and rodent CB_2 receptors, the development of selective CB_2 agonists may open new avenues in therapeutic intervention. Such interventions would aim at reducing the release of pro-inflammatory mediators particularly in chronic neuropathologic conditions such as HAND or MS. Further studies are needed to delineate the therapeutic effects of CB_2 agonists in current efforts to legalize the use of marijuana for medical purposes.

Acknowledgments

This work was supported in part by NIH Grants AA017398, MH065151, DA025566, and AA015913 (Y.P.). The authors express their grateful acknowledgement for proofreading and editing to Nancy L. Reichenbach.

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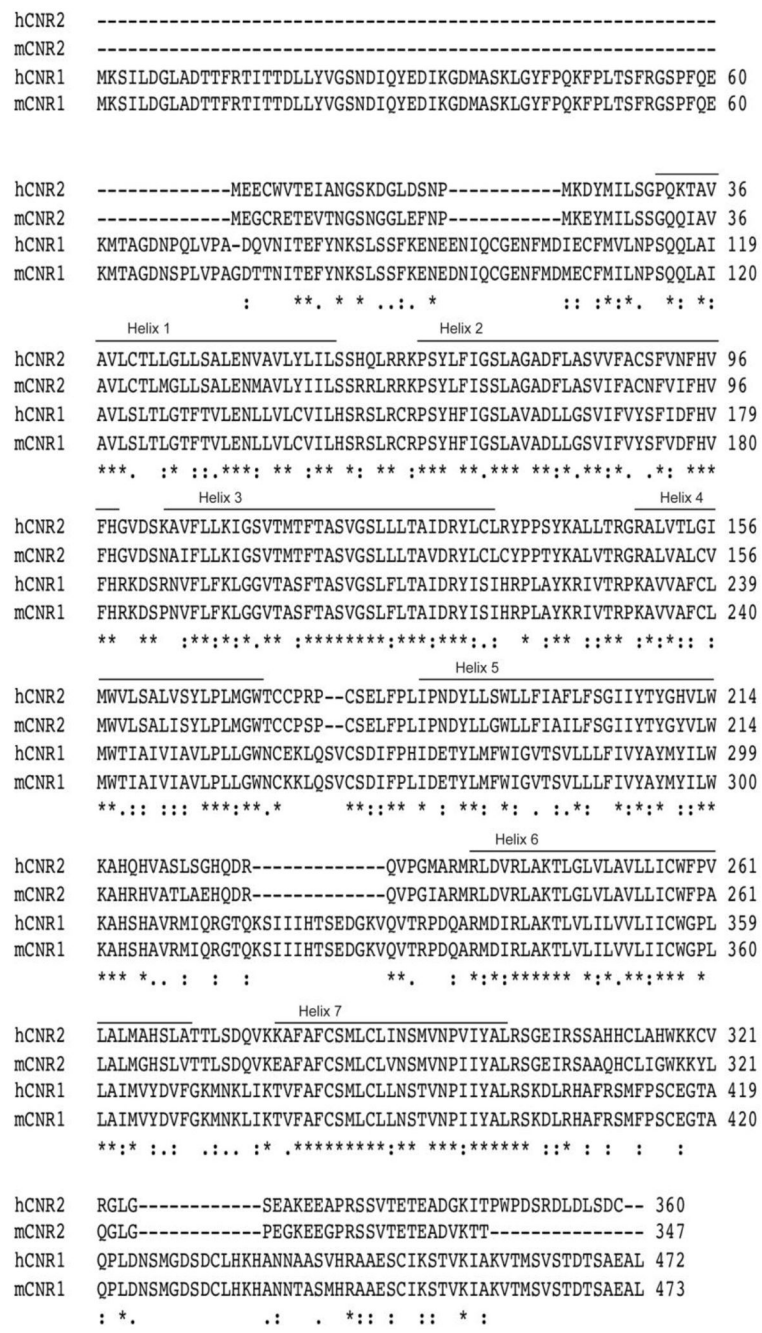

Figure 1.

Multiple alignment and homology of human and mouse CB_1 and CB_2 receptors. Sequence alignment was performed using ClustalW tool (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The following symbols denote the degree of conservation observed in each column: "*" means that the residues are identical in all sequences, ":" means that conserved substitutions have been observed, and "." means that semi-conserved substitutions are observed.

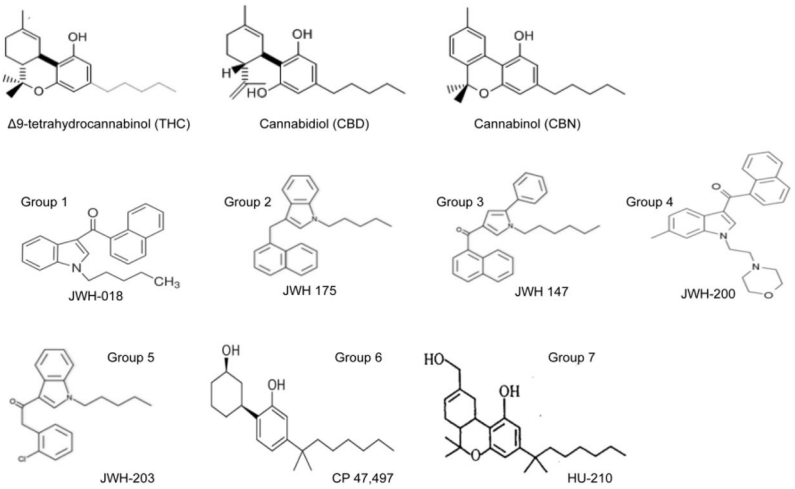

Figure 2.
Prototypical structures of natural and synthetic cannabinoids.

Table 1

Anti-inflammatory effects of CB_2 activation on immune and neuroimmune cells

Cell type	Effect of CB_2 activation	Reference
Immune cells		
Macrophages	diminished release of NO, IL-12p40 and TNFα	(Chuchawankul et al., 2004)
Macrophages	prevented ROS production and secretion of TNFα and CCL2	(Han et al., 2009)
B and T cells	affected B and T cell differentiation, and the balance of pro-inflammatory to anti- inflammatory cytokines	(Ziring et al., 2006)
Macrophages	increased secretion of the anti- inflammatory cytokine, IL-10	(Correa et al., 2009)
Macrophages (Kupffer cells)	inhibited LPS-induced NF-kB activation	(Louvet et al., 2011)
Neuroimmune		
Microglia	diminished levels of IL-1β, IL-6 and TNFα	(Puffenbarger et al., 2000)
Microglia	inhibited release of TNFα	(Facchinetti 2003; Ramirez et al., 2005) et al.,
Microglia	diminished expression of CD40	(Engelhardt and Ransohoff, 2005)
Glia	enhanced release of the anti-inflammatory factors, IL-4 and IL-10	(Molina-Holgado et al., 1998)
Microglia	inhibited TNFα production, p38 MAPK activation and NADPH oxidase (NOX) activation	(El-Remessy et al., 2008)
Microglia	interfered with expression of CCR2 and iNOS	(Racz et al., 2008)
Microglia	inhibited migration of microglial cells to HIV Tat protein	(Fraga et al., 2011)

Table 2

Anti-inflammatory effects of CB_2 activation in animal models

Animal model for disease	Reference
Inflammatory pain and ischemic stroke	(Yu et al., 2010; Pini et al., 2012)
Alzheimer's disease (AD)	(Martin-Moreno et al., 2012)
Colitis	(Storr et al., 2009)
Acute hind paw inflammation	(Berdyshev et al., 1998)
Acute lung injury	(Conti et al., 2002)
Sepsis	(Tschop et al., 2009)
Ischemic injury	(Fernandez-Lopez et al., 2006)
ALS	(Kim et al., 2006)
Viral MS model	(Mestre et al., 2009)
Stroke (ischemia and reperfusion)	(Zhang et al., 2007; Zhang et al., 2009; Murikinati et al., 2010; Tuma and Steffens, 2012)
EAE	(Docagne et al., 2007)
Alcoholic liver disease	(Louvet et al., 2011)