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Therapeutic Potentials and uses of Cannabinoid Agonists in Health and Disease Conditions

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Abstract: Cannabis and its derivatives have great therapeutic potential and have been used for centuries for medicinal purposes. The side effects of cannabinoids include euphoric mood changes, acute psychotic episodes, initiation and exacerbation of schizophrenic psychosis in predisposed persons, impaired cognitive and psychomotor performance, tachycardia and hypotension. The production of complex behavioural effects by cannabinoids are mediated by cannabinoid receptors (CB_1 and CB_2) and by interactions with other neurochemical systems. It has been shown that the therapeutic and physiological effects of cannabinoids are dependent upon whether the administration is acute or chronic and on the route of administration. The physiological effects of cannabis and its derivatives include: reduction in psychomotor coordination and performance, alterations in thermoregulation, endocrine and reproductive functions and gut motility. There is also evidence of agonist selectivity for CB_1 receptors coupled to different subtypes of G_i proteins or to G_i versus G_o proteins. Cannabinoid-activated receptors distinct from CB_1 or CB_2 exist in the central nervous system. Cannabinoids are known to inhibit GABA-mediated inhibitory postsynaptic currents in the hippocampus via a presynaptic action at CB_1 receptors located on GABAergic terminals. CB_1 receptors have also been implicated in the inhibition of glutamatergic excitatory postsynaptic currents. The synthetic cannabinoid, Win 55,212-2, a mixed CB_1-CB_2 cannabinoid receptor agonist, was found to attenuate hyperalgesia in a rat model of neuropathic pain and suppress opioid-induced emesis in ferrets.

Key words: Cannabis, Cannabinoid, Cannabinoid (CB_1) receptor, Cannabinoid (CB_2) receptor, Cannabinoid agonist, G-protein coupled receptor

INTRODUCTION

Cannabinoids are a group of terpenophenolic compounds present in *Cannabis sativa L.* and are made up of three types, namely natural or herbal cannabinoids, synthetic cannabinoids and endogenous cannabinoids (ElSohly and Slade, 2005). Cannabinoids were first characterised in the 1930s and 1940s as active ingredients of the cannabis plant (marijuana plant) from where the name was derived (Gardner, 2006). Some 68 natural plant cannabinoids have been described to date with Δ^9-tetrahydrocannabinol being shown as the main psychoactive component (ElSohly and Slade, 2005). Since the 1970s, many synthetic compounds have been developed by various laboratories to exert their effects like those of the plant cannabinoids. These are the synthetic agonists and to block such effects are the antagonists (Gardner, 2006). Three main cannabinoids found in the cannabis plant are Cannabidiol (CBD) (Baker *et al*., 1980; Lewis *et al*., 2005), Δ^9-tetrahydrocannabinol (Δ^9-THC) (Gaoni and Mechoulam, 1964; Stefanidou *et al*., 1998) and cannabinol (CBN) (Gambaro *et al*., 2002). The amount and relative abundances of these three main cannabinoids has been used to characterize cannabis and this also varies according to geographical origin (Hillig and Mahlberg, 2004). It was believed that Δ^9-THC produced its effect by perturbing neuronal cell membranes due to its lipid-soluble, hydrophobic nature (ElSohly and Slade, 2005). However, the structural and steric selectivity of the actions of Δ^9-THC and its synthetic analogues suggested the involvement of receptors (Begg *et al*., 2005) This was later demonstrated by studies that documented the existence of saturable, stereo-selective, high affinity membrane-binding sites for cannabinoids in the mammalian brain (Begg *et al*., 2005).

Several chemicals in the body were identified as acting similarly and are called endocannabinoids or endogenous cannabinoids. Cannabinoid receptors are concentrated mainly in the cerebellum and the basal ganglia, the areas of the brain responsible for motor

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control which may help explain why marijuana eases muscle spasticity in disorders like multiple sclerosis, as well as in the hippocampus, which is responsible for storage of short-term memory and the amygdala, which is part of the limbic system involved in emotional control, memory of fear and memory of pain (Gardner, 2006; Begg *et al*., 2005). These receptors are now known as CB_1 receptors and are predominant in the central nervous system (Matsuda *et al*., 1990; Soderstrom and Johnson, 2000).

A second cannabinoid receptor was initially detected in spleen cells, white blood cells and other tissues associated with the immune system. This second receptor is called the CB_2 receptor and is mainly present in the peripheral system (Munro *et al*., 1993). The original endogenous cannabinoid called Arachidonyl Ethanolamine (AEA) was named "Anandamide" (Devane *et al*., 1992). The AEA is a brain-derived lipid that binds to cannabinoid receptors and mimics the biological effects of Δ^9-THC (Begg *et al*., 2005). The second endocannabinoid was isolated from the intestinal tract and brain, called 2-rachidonoylglycerol (2-AG) (Sugiura *et al*., 1995). Subsequently, several other related lipids with endocannabinoid properties have been identified.

The biological effects of endogenous, plant-derived and synthetic cannabinoids are mediated through specific G protein coupled cannabinoid (CB) receptors. The CB_1 receptor is highly conserved in mice, rats and humans while the CB_2 receptors are more divergent (Begg *et al*., 2005). Both CB_1 and CB_2 receptors are coupled through $G_{i/o}$ proteins to inhibit adenylyl cyclase and regulate calcium and potassium channels (Begg *et al*., 2005; Mackie, 2006). In tissues naturally expressing CB receptors and in transfected cell lines, CB_1 and CB_2 receptors have been shown to have a high level of ligand-independent activation (Begg *et al*., 2005). It has been shown that in the population of wild-type CB_1 receptors, only about 30% exist in the activated form while 70% are inactive (Kearn *et al*., 1999; Begg *et al*., 2005; Carter and Weydt, 2002). Some of the CB_1 receptors exist in inactivated form within the cytosol and are in GDP-bound state while some exist in a tonically activated state and are coupled to active G-proteins within the plasma membrane in their GTP-bound state (Vásquez and Lewis, 1999; Nie and Lewis, 2001). It has been shown that in their activated state, the receptors have a higher affinity for the cannabinoid agonists (Vásquez and Lewis, 1999; Nie and Lewis, 2001). Therefore, the cannabinoid receptors exist predominantly in two states: the activated and inactivated forms, of which they show differences in their affinities to their agonists and ligands (Kearn *et al*., 1999; Nie and Lewis, 2001). Both CB_1 and CB_2 receptors are the primary targets of endogenous cannabinoids and they play important role in many processes, including metabolic regulation, craving, pain, anxiety, bone growth and immune function (Mackie, 2006). The aim of this present review is to explore the therapeutic potentials and the roles of cannabinoid receptors and agonists in health and disease conditions.

THERAPEUTIC POTENTIAL OF CANNABINOIDS

Cannabis and its derivatives have great therapeutic potential and have been used for centuries for medicinal purposes. However, cannabinoid-derived drugs on the market today lack specificity and produce many side effects, thus limiting their therapeutic usefulness (Pertwee, 2008). These side effects include euphoric mood changes, acute psychotic episodes, initiation and exacerbation of schizophrenic psychosis in predisposed persons, impaired cognitive and psychomotor performance, tachycardia and hypotension (Pertwee, 2008). The production of complex behavioural effects by cannabinoids are mediated by cannabinoid receptors (CB_1 and CB_2) and by interactions with other neurochemical systems (Adams and Martin, 1996; Carter and Weydt, 2002). It has been shown that the therapeutic and physiological effects of cannabinoids are dependent upon whether the administration is acute or chronic and on the route of administration (Halpin *et al*., 1998; Fride *et al*., 2004).

The physiological effects of cannabis and its derivatives include: reduction in psychomotor coordination and performance, alterations in thermoregulation, endocrine and reproductive functions and gut motility (Martin *et al*., 2006; Fride *et al*., 2004; Jackson *et al*., 2004). The therapeutic uses of some cannabinoid agonists and antagonists are shown in Table 1.

The active ingredient of cannabis, Δ^9-THC and other cannabinoids and their derivatives are being used to treat a variety of disorders (Baker *et al*., 2003). Drugs which selectively activate CB_1 and CB_2 receptors which include dronabinol and nabilone (synthetic analogues of Δ^9-THC) are used for the treatment of nausea and vomiting caused during cancer chemotherapy treatments (Martin *et al*., 2006). Many cannabinoids produce inhibition of pain responses and there is laboratory evidence to support the analgesic effect of cannabinoids (Martin *et al*., 2006; Martinez-Orgado *et al*., 2003). Other therapeutic uses of cannabinoid receptor agonists may include the suppression of some symptoms associated with multiple sclerosis, with spinal injury and with certain other movement disorders such as muscle spasticity and spasm and the management of glaucoma, bronchial asthma, pain and inflammatory disorders (Halpin *et al*., 1998; Adams and Martin, 1996; Martin *et al*., 2006; Garcia-Arencibia *et al*., 2007).

The CB_1 receptor antagonist, SR141716A (rimonabant; Acomplia®), may have therapeutic potential in reducing memory deficits associated with ageing or neurological diseases (Pertwee, 1997; Halpin *et al*., 1998).

Table 1: Therapeutic uses of cannabinoid agonists and antagonists

Agonist	Antagonist
Dronabinol (Marinol), an analogue of Δ^9-THC as anti-emetic agents in cancer therapy	SR141716 (Rimonabant), a selective cannabinoid (CB_1) receptor antagonist used as an anti-obesity drug
Nabilone (Cesamet), a synthetic cannabinoid and an analogue of Marinol reduces pain, an appetite stimulant and increases general well being in AIDS	SR141716 (Acomplia) is also used for the treatment of tobacco addiction
Sativex, a cannabinoid extract oral spray containing both Δ^9-THC and CBD, use for neuropathic pain and spasticity	SR141716A attenuated Δ^9-THC- or anandamide-induced memory impairment and attenuated the anandamide-induced impairment of performance
Cannabichromene (CBC), an anti-inflammatory agent	
Cannabidiol(CBD), a major anti-convulsant, anti-spasmodic, anti-asthmatic and anti-glaucoma agent	
Other effects of CB agonist include antinociceptive effects in animal models of acute inflammatory and neuropathic pain	

There is evidence that cannabinoids are effective in relieving spasticity, tremor and pain caused by multiple sclerosis or spinal injury (Croxford, 2003; Pertwee, 2005, 2008). Animal experiments have shown that cannabinoid receptor agonists suppress spinal reflexes, produce marked behavioural changes in motor function, for example hypokinesia and catalepsy and have significant efficacy in standard tests of antinociception (Halpin *et al*., 1998; Martin *et al*., 2006). In a clinical trial in healthy volunteers, using 2, 4 and 8% Δ^9-THC by weight on pain induced by capsaicin injected 5 and 45 minutes after drug exposure, pain and hyperalgesic effects were assessed. The results show that by 45 minutes after cannabinoid exposure, there was a significant decrease in capsaicin-induced pain with the medium dose and a significant increase in capsaicin-induced pain with the high dose (Wallace *et al*., 2007).

The effects on motor function are mediated by large populations of cannabinoid CB_1 receptors that are present in the basal ganglia of the brain, but whether they produce their putative antispasticity effect by acting at these brain sites remain to be established (Halpin *et al*., 1998; Neitzel and Hepler, 2006). Experiments have shown that Δ^9-THC can delay the onset and reduce the intensity of the clinical signs of experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis. It has also been shown that the synthetic cannabinoid receptor agonist, Win55212-2, can decrease the severity of dystonia in mutant Syrian hamsters with primary generalized dystonia (Herzberg *et al*., 1997). These indicate that cannabis, Δ^9-THC or nabilone can reduce the intensity of some signs and symptoms of multiple sclerosis or spinal injury, particularly spasticity, pain, tremor and nocturia (Baker *et al*., 2003). There is substantial evidence to suggest that various cannabinoids possess analgesic properties, but most of this evidence is based on experiments in laboratory animals. Results of studies which have employed clinically relevant models of inflammatory or neuropathic pain are now appearing and generally support the concept of cannabinoid-induced analgesia (Herzberg *et al*., 1997; Martin *et al*., 2006; Gilbert *et al*., 2007; Jackson *et al*., 2004).

Cannabinoids can alleviate tremor and spasticity in animal models of multiple sclerosis and clinical trials of the use of these compounds for these and other symptoms are continuously in progress (Croxford, 2003; Felder *et al*., 2006; Makriyannis, 2007). Anecdotal evidence has shown that patients with disorders such as multiple sclerosis smoke cannabis to relieve disease-related symptoms and pains (Croxford, 2003; Díaz-Laviada and Ruiz-Llorente, 2005). Evidence has also shown that cannabinoids may prove useful in Parkinson's disease by inhibiting the excitotoxic neurotransmitter, glutamate and counteracting oxidative damage to dopaminergic neurons (Halpin *et al*., 1998; Russo and McPartland, 2003). The inhibitory effect of cannabinoids on reactive oxygen species, glutamate and tumour necrosis factor suggests that they may be potent neuroprotective agents (Croxford, 2003; Croxford and Miller, 2003). Dexanabinol (HU211), a synthetic cannabinoid is being assessed in clinical trials for traumatic brain injury and stroke (Croxford, 2003). Animal models of mechanical, thermal and noxious pain suggest that cannabinoids may be effective analgesics (Croxford, 2003; Dikic *et al*., 2003; Docagne *et al*., 2007). Cannabinoids have proved more effective than the placebo in clinical trials of post operative and cancer pain and pain associated with spinal cord injury. However, they may be less effective than existing therapies (Russo, 2001; Docagne *et al*., 2007). Dronabinol, a commercially available form of Δ^9-THC, has been used successfully for increasing appetite in patients with HIV wasting disease and cannabinoid receptor antagonists may reduce obesity (Russo and McPartland, 2003).

Acute adverse effects following cannabis usage include sedation and anxiety. These effects are transient and may be less severe than those that occur with existing therapeutic agents (Russo, 2003, 2001), have shown that the use of nonpsychoactive cannabinoids such as cannabidiol and dexanabinol may allow the dissociation of unwanted psychoactive effects from potential therapeutic benefits. Rog *et al.* (2007) and Nurmikko *et al*. (2007) have demonstrated the analgesic property and efficacy of Sativex, an oro-mucosal Δ^9-THC/CBD endocannabinoid modulator compound against neuropathic pain caused by multiple sclerosis in humans.

The existence of additional cannabinoid receptors may provide novel therapeutic targets that are independent of CB_1 receptors and the development of compounds that are not associated with CB_1 receptor-mediated adverse effects (Russo, 2001). In a study to investigate the therapeutic benefits and adverse effects of prolonged use of medical marijuana in a cohort of chronically ill patients, the result demonstrated clinical effectiveness in these patients in treating glaucoma, chronic musculoskeletal pain, spasm and nausea and spasticity of multiple sclerosis (Russo, 2001, 2003). There has been substantial evidence from experiments with animals, healthy human subjects and patients with primary open-angle glaucoma that cannabinoids can lower intra-ocular pressure (Green, 1998; Halpin *et al*., 1998 ; Russo, 2003).

Cannabinoids have shown great promise for the treatment of early phase response of asthma (Halpin *et al*., 1998). This is because they can significantly dilate the bronchioles of both healthy and asthmatic subjects and seem to be no less effective than conventional drug treatment of asthma (Hollister, 1986; BMA, 1997; Halpin *et al*., 1998). It has been shown that both cannabis and individual cannabinoids are active when taken orally or when inhaled, either in smoke or in an aerosol produced by a nebulizer inhaler (Hollister, 1986; BMA, 1997). The mechanism behind the bronchodilator effect of cannabinoids remains to be established. However, only cannabinoids with psychotropic properties have been found to produce bronchodilation, indicating that the effect may be mediated through cannabinoid CB_1 receptors.

Cannabis and cannabinoids, like all other drugs, have unwanted effects, as reported by Pertwee (1997), in a clinical study with 34 cancer patients and include dizziness, sedation and dry mouth, blurred vision, mental clouding, ataxia, disorientation, disconnected thought, slurred speech, muscle twitching and impaired memory. Cannabis on its own may sometimes induce transient confusion, panic attacks, depersonalization, paranoid delusions and hallucinations (Chaudry *et al*., 1991) and has been reported to produce a subtle impairment of postural control (Pertwee, 1997). Cannabis may aggravate existing psychoses and can elevate heart rate; hence it would be unwise to give psychotropic cannabinoids to patients with schizophrenia, coronary arteriosclerosis or congestive heart failure (Hollister, 1986; Pertwee, 1997). The list of some known cannabinoid agonists and antagonists used in experimental studies is shown in Table 2.

It has been shown that various intracellular kinases, including the Mitogen-Activated Protein Kinases (MAPK), Extracellular Signal-Regulated Kinases type 1 and 2 (ERK 1 and 2), C-Jun N-Terminal Kinase (JNK), focal adhesion kinase and protein kinase B/Akt, are also activated by both CB_1 and CB_2 receptors (Derkinderen *et al*., 200, 2003; Bouaboula *et al*., 1999). The expression of the CB_2 receptor is more restricted and is limited primarily to immune and haematopoietic cells (Munro *et al*., 1993). The human CB_2 receptors show 68% amino acid homology with the CB_1 receptor in the trans-membrane domains and a 44% overall homology (Munro *et al*., 1993; Begg *et al*., 2005). However, despite the low level of homology between the two receptors, their pharmacology is similar, with most plant-derived, endogenous and classical synthetic cannabinoids having similar affinities for the two receptors (Showalter *et al*., 2005; Begg *et al*., 2005), although synthetic agonists with greater than 100-fold affinity for CB_1 or CB_2 receptors have been developed (Hillard *et al*., 1997; Malan *et al*., 2001). The CB_1 receptors are highly conserved in mice, rats and humans while the CB_2 receptors are much more divergent. The amino acid homology of CB_2 between mouse and rat is 93% while that between rat and human is only 81% (Munro *et al*., 1993; Griffin *et al*., 2000).

Table 2: List of some known cannabinoid agonists and antagonists used in experimental studies

Type	Agonist	Antagonist
CB_1	Win 55,212 mesylate	SR141716A
	CP 55940	AM 281
	HU-210	AM 251
	Δ^9-tetrahydrocannabinol (Δ^9-THC)	Tetrahydrocannabivarin (THCV)
	Δ^8- tetrahydrocannabinol (Δ^8-THC)	LY 320135
	Cannabinol (CB)	
	Leelamine hydrochloride	
	Arachidonoylethanolamide (Anandamide or AEA)	
	2-arachidonylglycerol (2-AG)	
	arachidonyl-2-chloroethylamide (ACEA)	
	methanandamide	
	Arachidonylcyclopropylamide (ACPA)	Cannabidiol (CBD)
CB_2	AM-1241	SR144528
	JWH-015	AM 630
	JWH-133	
	CB 65	JTE 907
	L-759, 633	

There is also evidence of agonist selectivity for CB_1 receptors coupled to different subtypes of G_i proteins or to G_i versus G_o proteins (Howlett, 2004). Cannabinoid-activated receptors distinct from CB_1 or CB_2 have been postulated to exist in the central nervous system (Begg *et al*., 2005; Cavanaugh, 2006).

Cannabinoids are known to inhibit GABA-mediated inhibitory postsynaptic (IPSCs) in the hippocampus via a presynaptic action at CB_1 receptors located on GABAergic terminals (Wilson *et al*., 2001). CB_1 receptors have also been implicated in the inhibition of glutamatergic excitatory postsynaptic currents. The synthetic cannabinoid, Win 55, 212-2, a mixed CB_1-CB_2 cannabinoid receptor agonist, was found to attenuate hyperalgesia in a rat model of neuropathic pain and suppress opioid induced emesis in ferrets (Bridges *et al*., 2001; Simoneau *et al*., 2001).

NEUROPROTECTION BY CANNABINOIDS

Cannabinoids have been shown to provide neuroprotection in acute and chronic neurodegeneration (Lastres-Becker *et al*., 2004; Jentsch *et al*., 1998). In a study to examine the effect of cannabinoids against the toxicity caused by 6-hydoxydopamine both *in vivo* and *in vitro*, it was found that the non-selective cannabinoid agonist HU-210 increased cell survival in cultures of mouse cerebellar granule cells exposed to the toxin. However, the effect was significantly less when cannabinoids were directly added to neuronal cultures than when these cultures were exposed to conditioned medium obtained from mixed glial cell cultures treated with HU-210, suggesting that the cannabinoid exerted its major protective role by regulating glial influence to neurons (Lastres-Becker *et al*., 2004; Pryce *et al*., 2003 ; Drysdale *et al*., 2006).

Cannabinoids may also be neuroprotectant in Parkinson's Disease (PD), a motor neurodegenerative disorder characterised by progressive death of nigrostriatal dopaminergic neurons that mainly results in bradykinesia or slowness of movement, rigidity and tremor as major motor abnormality (Sethi, 2002; Lastres-Becker *et al*., 2005). In an experiment to investigate if cannabinoids might provide neuroprotection in PD, Lastres-Becker *et al*. (2005), conducted two sets of experiments to demonstrate that cannabinoids are effective against the *in vivo* and *in vitro* toxicity of 6-hydroxyl dopamine, a toxin currently used to generate Parkinsonism in laboratory animals. In the first experiments, Lastres-Becker *et al*. (2005), examined the ability of Δ^9-THC and Cannabidiol (CBD), to alter *in vivo* the progress of neurodegeneration in rats subjected to unilateral injections of 6-hydroxydopamine into the medial forebrain bundle. In the second experiments, Lastres-Becker *et al*. (2005), evaluated whether the termination of Δ^9-THC administration to 6-hydroxdopamine-lesioned rats after 2 weeks would result in re-initiation of the process of neuronal injury during two subsequent weeks. This experiment also examined whether the potential effects of Δ^9-THC against *in vivo* toxicity of 6-hydroxydopamine are mainly neuroprotective because they do not disappear after discontinuation of cannabinoid treatment. The results from the studies by Lastres-Becker *et al*. (2005), showed that the daily administration of Δ^9-THC for 2 weeks produced a significant increase in dopamine content and tyrosine hydroxylase activity in the lesioned striatum and these were accompanied by an increase in tyrosine hydroxylase-mRNA levels in the substantia nigra. This suggests a potential neuro-protective action of cannabinoids against the *in vivo* and *in vitro* toxicity caused by 6-hydroxydopamine, which may be relevant in Parkinson's disease.

Cannabinoids have been shown to protect against neurotoxicity in a number of different cellular, animal and human experimental models (Davies *et al*., 2002; Fride and Shohami, 2002; Mechoulam *et al*., 2002; Pryce *et al*., 2003; Zhuang *et al*., 2005). Zhuang *et al*. (1999) had earlier demonstrated that cultured rat hippocampal neurons were protected from excitotoxic insults by pre-treatment with either Δ^9-THC or Win 55, 212-2 and that these compounds were effective in preventing cell death even if administered prior to the neurotoxin exposure. Cannabinoids have been demonstrated to be protective *in vivo* with respect to neurodegeneration resulting from experimental ischaemia (Zhuang *et al*., 2005; Molina-Holgado *et al*., 2005). Leker *et al*. (2003) have shown that *in vivo* administration of CB_1 agonist HU-210, was able to significantly reduce motor disability and infarct volume after focal irreversible cerebral ischaemia.

The mechanisms involved in the neuroprotective properties of cannabinoid have not yet been fully characterised (Mechoulam *et al*., 2002; Baker *et al*., 2003; Zhuang *et al*., 2005; Cavanaugh, 2006). Zhuang *et al*. (2001) have shown that cannabinoid receptor-mediated neuroprotection is sensitive to intracellular calcium levels. In their work, Zhuang *et al*. (2002), presented a detailed analysis of how cannabinoids act to reduce or block release of intracellular calcium $[Ca^{2+}]i$ under neurotoxic conditions. They demonstrated that such neuro- protection is based on cannabinoid CB_1 receptor-mediated decreases in cAMP-dependent Protein Kinase (PKA), an effect that alters the sensitivity of particular intracellular calcium channels. Several possible alternative signalling pathways were also investigated and systematically ruled out on the basis that they did not block the NMDA provoked increase in $[Ca^{2+}]i$ in the same manner as CB_1 receptor activation (Ryan *et al*., 2007). It has been shown that there is a similar time course for the protective effect on cultured neurons, the blockade of intracellular calcium release and the inhibition of PKA (Zhuang *et al*., 2005). Common factors underlying these changes are the alteration in sensitivity of type-II ryanodine receptor (RyR)-coupled intracellular calcium channels and the decrease in cAMP due to cannabinoid inhibition of adenylyl cyclase, as originally demonstrated by Howlett *et al*. (1990).

Most of these protectant effects appear to be mediated by activation of the cannabinoid CB_1 receptor subtype (Parmentier-Batteur *et al*., 2002), although additional mechanisms may be involved (Lastres-Becker *et al*., 2005). It has been shown that the sameneuroprotective effect is also produced by Cannabidiol (CBD), another plant-derived cannabinoid, with negligible affinity for cannabinoid CB_1 receptors (Pertwee, 1999; Ryan *et al*., 2006), suggesting that antioxidant properties of both compounds might be involved in these *in vivo* effects. However, an alternative

explanation might be that, the neuroprotection exerted by both compounds is due to their anti-inflammatory potential (Lastres-Becker *et al*., 2005; Jentsch *et al*., 1998; Jackson *et al*., 2004).

Cannabinoids' anti-inflammatory properties are likely related to their ability to modulate glial influence on neurons (Walter and Stella, 2004; Lastres-Becker *et al*., 2005; Marchalant *et al*., 2007). These anti-inflammatory properties might be important in Parkinson's Disease (PD) since nigral cell death is accompanied by astrocyte proliferation and reactive microgliosis at the sites of neurodegeneration. Microglial activation may play important role in the initiation and early progression of the neurodegenerative process especially in regions which are particularly rich in microglia and other glial cells (Gao *et al*., 2002; Lastres-Becker *et al*., 2005; Louw *et al*., 2000).

It is shown that activated microglia produce a wide array of cytotoxic factors, including tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), eicosanoids, nitric oxide and reactive oxygen species that impact on neurons to induce neurodegeneration and that some of them have been reported to be increased in the substantia nigra and the caudate-putamen of PD patients (Gao *et al*., 2002).

THE ROLE OF CANNABINOIDS IN NEUROINFLAMMATION

Inflammation is a complex biological response and active defence reaction of tissues against harmful stimuli and insults (Walter and Stella, 2004). Inflammation is designed to remove or inactivate noxious agents and to inhibit their detrimental effects (Walter and Stella, 2004). It has been shown that inflammation, even though it serves a protective function in controlling infections and promoting tissue repair, can also cause tissue damage and disease (Walter and Stella, 2004; Lu *et al*., 2005). There is growing evidence that a major physiological function of cannabinoid signalling is to modulate neuroinflammation. The anti-inflammatory properties of cannabinoids have been studied at the molecular, cellular and whole animal levels, by examining the evidence for anti-inflammatory effects of cannabinoids obtained using *in vivo* animal models of clinical neuroinflammatory conditions, especially rodent models of multiple sclerosis (Walter and Stella, 2004; Jackson *et al*., 2004; Marchalant *et al*., 2007).

CNS inflammations occur in myelin degenerative disorders such as Multiple Sclerosis (MS), Alzheimer's disease, HIV encephalopathy, ischaemia and traumatic brain injury (Martino *et al*., 2002). Experimental Autoimmune Encephalopathy (EAE) has been shown to be a widely used animal model to study MS owing to histological similarities (Zamvil and Steinman, 1990). The EAE is initiated and maintained as a result of T lymphocytes becoming sensitized to myelin proteins and eliciting a cell-mediated immune response. The pathological changes involve demyelination and a progression of inflammation in the CNS (Walter and Stella, 2004). Cannabinoid administration has been shown to influence the course of the disease progression on several studies performed on various rodent models of MS (Matsuda *et al*., 1990; Matsuda, 1997; Munro *et al*., 1993). In animals administered with Δ^9-THC prior to inoculation with lymphocytes from CNS of animals with acute EAE, full clinical development of EAE was prevented, suggesting that that Δ^9-THC suppressed the immune system (Lyman *et al*., 1989). In animals given Δ^9-THC after inoculation with the lymphocytes, onset of symptoms was delayed and clinical index was lowered, while histological examination of spinal cords showed significantly less inflammation in Δ^9-THC treated animals (Lyman *et al*., 1989).

Wirguin *et al*. (1994), administered delta-8-tetrahydrocannabinol (Δ^8-THC), a minor form of THC, daily to rats with EAE beginning several days prior to symptoms onset. The Δ^8-THC treated animals had a delayed symptom onset, lowered incidence of EAE and a shorter mean duration of EAE but not a lower mean severity. In mice with EAE, a synthetic cannabinoid receptor agonist (Win 55212-2), THC, methanadamide and selective CB_2 receptor agonist (JWH-133), but not cannabidiol, relieved the spasticity and tremor symptoms within 1 to 10 minutes (Baker *et al*., 2000). The effects of Win 55212-2 were reversed by treatment with the selective cannabinoid receptor antagonists SR141716A (CB_1) and SR144528 (CB_2) and these two compounds administered alone worsened symptoms (Baker *et al*., 2000). Since the CB_2 receptor agonist and a CB_2 receptor antagonist influenced these symptoms, these results point towards an anti-inflammatory effect mediated via CB_2 receptors, which are expressed mainly on immune cells (Walter and Stella, 2004).

The effects of cannabinoid compounds over several weeks were studied on a model of human Multiple Sclerosis (MS) in which the cannabinoid agonists Win 55212-2, Arachidonyl-2-Cloroethylamide (ACEA) and a CB_2 selective agonist JWH-015 were administered daily for 10 days following Theiler's Murine Encephalomyelitis Virus (TMEV) infection of the CNS. This induces an immune-mediated demyelinating disease in susceptible mouse strains, but prior to symptoms (Walter and Stella, 2004). These drugs improved motor function, decreased the number of activated microglia in the spinal cord, decreased Major Histocompactibility Complex class II (MHC II) expression, decreased the number of CD4 T cells in spinal cord and promoted spinal cord remyelination (Arevalo-Martin *et al*., 2003; Walter and Stella, 2004). Also, Win 55212-2 was administered daily

for 5 days to mice with TMEV either prior to symptoms, at onset of symptoms, or several days after symptom onset (Croxford and Miller, 2003). The results showed that clinical disease symptoms are decreased under all these conditions, while Win 55212-2 increased the susceptibility of mice to TMEV infection, suggesting an immunosuppressive effect, although it had no effect on splenic cell populations (Croxford and Miller, 2003). Win 55212-2 also decreased CNS mRNA encoding for proinflamatory cytokine tumour necrosis factor α (TNFα), interleukin (IL)-1β and IL-6 in these mice (Croxford and Miller, 2003; Walter and Stella, 2004; Lu *et al*., 2005). However, in EAE animals there was increased CB_1 receptor activity in the cerebral cortex and caudate-putamen, suggesting that the remaining receptors in these areas may be efficiently coupled to G protein-mediated signalling mechanisms (Berrendero *et al*., 2001; Walter and Stella 2004). These results show that cannabinoid agonists ameliorate symptoms both acutely and over several weeks in EAE and TMEV models of MS and the CB_1 receptor expression and function change in EAE, while the absence of CB_1 receptors worsens symptoms of EAE (Simonds, 2003). Although these studies provide insights into the effects of cannabinoids on neuroinflammation, the mechanism of action of these drugs is still incomplete due to differences in the compounds, animal models of MS, rodent species, routes of drug administration and dosing schedules used (Walter and Stella, 2004).

Microglia have been shown to regulate the initiation and progression of immune responses in the CNS (Carson and Sutcliffe, 1999; Walter and Stella, 2004). Primary cultures of rat and mouse microglia express both CB_1 and CB_2 receptor mRNA and protein (Walter *et al*., 2003). Human microglia express both CB_1 and CB_2 receptor mRNA, while primary mouse microglia express CB_2 receptors at the leading edges of lamellipodia and micro spikes, suggesting a function in motility (Walter *et al*., 2003; Walter and Stella, 2004). The proinflamatory cytokine interferon-gamma (IFN-γ), which is produced by T-helper (T_H) 1 cells and Natural Killer (NK) cells in MS and EAE, increases CB_2 receptor mRNA and protein in rat microglia (Carlisle *et al*., 2002).

Astrocytes are the main non-neuronal supporting glial cells in the brain which help to regulate aspects of inflammation in the CNS and may be involved in the pathogenesis of MS. While some evidence of CB_1 receptor expression by astrocytes has been found, it has not been found by all workers (Salio *et al*., 2002; Walter and Stella, 2004). These conflicting results may indicate variations in CB_1 receptor expression due to differences in species, culture systems, CNS structures from which cultures are derived, ages of cultures, or activation levels of cells, while CB_2 receptor expression by astrocytes has not been found (Walter and Stella, 2003, 2004). Oligodendrocytes, which undergo degeneration during MS and EAE, also express CB_1 and CB_2 receptors (Walter and Stella, 2004). Some of the major types of glial cells expressing cannabinoid receptors may account for some of the anti-inflammatory effects by cannabinoids in rodent models of MS (Walter and Stella, 2004). It is known that cannabinoid receptor expression is modulated by cytokines in microglial cells but it is not known if cannabinoid receptor expression is modulated in astrocytes or oligodendrocytes (Carlisle *et al*., 2002; Walter and Stella, 2004).

Neuroinflammation induces a complex and dynamic change in glial cell phenotypes. Microglial cells are one of the first cell types to respond by retracting their processes and migrating towards the site of injury where they release proinflamatory cytokines such as IL-1β, TNFα and IL-6 (Becher *et al*., 2000). In primary cultures of mouse microglia, 2-AG induces cell migration and this is reversed by the selective CB_2 antagonist SR144528, cannabinol and cannabidiol (Walter *et al*., 2003). This suggests that under neuroinflammatory conditions, neurons or astrocytes produce endocannabinoids as a means of recruiting microglia (Walter *et al*., 2003; Walter and Stella, 2003).

Nitric Oxide (NO) production by glial cells is also associated with immune-mediated cellular cytotoxicity and pathogenesis of MS and EAE (Parkinson *et al*., 1997). The cannabinoid agonist CP55940 inhibits NO production in IFN-γ- and Lipopolysaccharide (LPS)-stimulated rat microglia (Waksman *et al*., 1999; Cabral *et al*., 2001). Primary cultures of rat microglia, when activated by LPS, release TNFα, which is inhibited by cannabinoid agonists: anandamide, 2-AG, Win 55212-2, CP55940 and HU210. However, the antagonists SR141716A, AM251 and SR144528 do not alter the effects of Win 5212-2 on the microglia, suggesting non $CB_{1/2}$-mediated effects (Facchinetti *et al*., 2003). Δ^9-THC reduces IL-1β, IL-6 and TNFα production in LPS-stimulated rat microglia (Puffenbarger *et al*., 2000). The selective CB_2 agonistJWH-015 treatment reduces toxicity of human microglia towards neurons (Klegeris *et al*., 2003). When these results are compared, they showed that cannabinoid decrease neurotoxicity and release of proinflamatory cytokines from microglia. However it is not known whether these effects are mediated through cannabinoid receptors or other mechanisms (Facchinetti *et al*., 2003; Klegeris *et al*., 2003; Cavanaugh, 2006).

Cannabinoids may suppress the immune response and hence the inflammatory response by modulating proliferation or inducing apoptosis in lymphocytes (Malfait *et al*., 2000). An increase in the number of lymphocytes is crucial for an inflammatory response to occur. The Δ^9-THC induces apoptosis in macrophages (Zhu *et al*., 1998). Cannabidiol causes a dose-dependent suppression of lymphocyte proliferation (Malfait *et al*.,

2000). Δ^8-THC, CP55940 and anandamide also suppress T- and B - cell proliferation (Schwarz *et al*., 1994), while CP55940 enhances proliferation of B cells an effect blocked by the antagonist SR144528 (Carayon *et al*.,1998; Walter and Stella, 2004). Also Δ^9-THC inhibits Nitric Oxide (NO) production in LPS/IFN-γ-stimulated mouse macrophages and in LPS-stimulated RAW 264.7 macrophages (Coffey *et al*., 1996; Jeon *et al*., 1996). Win inhibits the LPS-induced release of NO in macrophages, an effect blocked by the antagonist SR144528 (Ross *et al*., 2000). The agonist CP55940, reduces NO production from IFN-γ/LPS-stimulated feline macrophages and this is reversed by antagonists SR141716A in CB_1 receptors and SR144528 in CB_2 receptors in both macrophages and primary dorsal root ganglion cells (Ponti *et al*., 2001; Ross *et al*., 2000). Plant and synthetic cannabinoids inhibit NO production from immune cells, while the endogenous cannabinoids induce it (Walter and Stella, 2004). Anandamide increases NO production in human monocytes and macrophages (Stefano *et al*., 1998).

Anandamide stimulates Arachidonic Acid (AA) release in monocytes and J774 mouse macrophages, an effect blocked by pertussis toxin, an inhibitor of Gi/o proteins (Berdyshev *et al*., 1996; Di Marzo *et al*., 1997). Anandamide also induces AA release in cells that do not express CB_1 or CB_2 receptors (Felder *et al*., 1996; Felder and Glass, 1998). Δ^9-THC induces AA release in RAW 264.7 mouse macrophages and this is likely to be mediated by the CB_2 receptor (Hunter and Burstein, 1997; Felder *et al*., 2006). The effects of cannabinoids on AA release indicate a proinflamatory influence on peripheral immune cells. It has been shown that cells and tissues involved in neuroinflammation produce and degrade endocannabinoids and anandamide and 2-AG levels are differentially regulated in cells (Walter and Stella, 2004).

CONCLUSION

Cannabis and cannabinoids have great therapeutic potential and physiological effects which are dependent upon whether the administration is acute or chronic and on the route of administration. The active ingredient of cannabis, Δ^9-THC and other cannabinoids and their derivatives such as dronabinol and nabilone are used in the treatment of nausea and vomiting caused during cancer chemotherapy treatments. Many cannabinoids and cannabis derivatives produce analgesic effects. Other therapeutic uses of cannabinoid receptor agonists include the suppression of some symptoms associated with multiple sclerosis, with spinal injury and with certain other movement disorders such as muscle spasticity and spasm and the management of glaucoma, bronchial asthma, pain and inflammatory disorders and reduction in memory deficits associated with ageing and neurological diseases. Cannabinoids are effective in relieving spasticity, tremor and pain caused by multiple sclerosis or spinal injury. Cannabinoids receptor types are coupled to intracellular effectors via $G_{i/o}$-proteins, modulating cAMP levels, K^+ and Ca^{2+} channel activities and MAP kinase phosphorylation, indicating that the systems may interact at the post receptor level which might open-up new therapeutic opportunities.

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