

## Cannabinoid receptors: A brief history and “what’s hot”.

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## 1. ABSTRACT

Our understanding of the complexity of the endocannabinoid system has evolved considerably since the cloning of the receptors in the early 1990s. Since then several endogenous ligands have been identified and their respective biosynthetic pathways unravelled. This research has revealed the involvement of the cannabinoid system in a number of important physiological processes including the regulation of neurotransmitter release, pain and analgesia, energy homeostasis, and control of immune cell function. All of these events are mediated by two similar receptors, CB1 and CB2, which were initially thought to possess mutually exclusive expression profiles. Recent advances have begun to dissolve such absolutes with the discovery of CB2 in brain tissue and identification of a range of functions for CB1 in peripheral tissues. With improved understanding of the cannabinoid system comes the illumination of various roles in disease pathologies and identification of potential therapeutic targets. This review provides an overview of the current understanding of the endocannabinoid system, and then focuses on recent discoveries that we believe are likely to shape the future directions of the field.

## 2. INTRODUCTION

The purpose of this review is to provide the reader with an overall understanding of the current state-of-play in the cannabinoid field, with a historical perspective of the road travelled thus far. The scope of the cannabinoid system has grown considerably in recent years and some areas are too complex to extensively review herein, however, recent reviews on specific aspects of cannabinoid biology are available (e.g. 1,2,3). The meaning and origin of the different components comprising the cannabinoid system covering the major endocannabinoid ligands, their endogenous receptors, and enzymology of ligand biosynthesis will be discussed along with several ‘hot topics’ that have emerged within the field in the last few years. Several examples of promising new therapeutic targets will be highlighted in addition to possible future directions.

## 3. THE CANNABINOIDS: A BRIEF HISTORY

### 3.1. Cannabinoids and the discovery of cannabinoid receptors

The major psychoactive ingredient of cannabis was first isolated as a “toxic red oil” in 1964 (4) and then

subsequently identified as  $\Delta$ -9-tetrahydrocannabinol (THC) in 1967 (5). This finding launched the search for the cannabinoid receptors. The highly lipophilic nature of THC made binding assays impossible, therefore many laboratories focused on signalling and behavioural assays to try to establish the existence of a receptor. Amongst the most compelling evidence for specific cannabinoid receptors was the stereoselectivity of THC-mediated effects (6,7) and inhibition of adenylate cyclase through a pertussis toxin sensitive mechanism (8-11). These two lines of evidence are characteristic of a cell surface receptor, and more specifically of a G-protein coupled receptor (GPCR) linked to Gi/Go type G-proteins.

In the mid 1980s Pfizer developed a series of potent synthetic cannabinoids based on the structure of THC, the most well characterised being CP55,940 (12). This compound allowed for the development of classical ligand binding assays (8) and the mapping of a putative receptor (13). Around this time a novel GPCR receptor was cloned by Matsuda *et al* (14) on the basis of its homology to bovine substance K receptor. A comparison of the distribution of this receptor's mRNA with the binding of CP55,940 suggested a substantial overlap (15), and subsequent transfection and screening showed that the novel receptor responded to classical cannabinoids and it was termed CB1 (14).

The receptor has now been mapped in many species through classical autoradiographic methods, immunohistochemistry and *in situ* hybridisation. CB1 is highly expressed in the brain, predominantly on neurons. The distribution of receptors appears well conserved between species and correlates well with the known behavioural effects of cannabinoids. Thus high levels of receptor are observed in the hippocampus, in basal ganglia output nuclei (globus pallidus and substantia nigra), the cerebellum, and cortical association regions, while moderate levels are observed in primary cortical regions, thalamic nuclei, striatum, and low levels in brain stem and spinal cord regions. This distribution of CB1 is consistent with the known physiological effects of cannabinoids, including impairment of short term memory formation, altered motor activity and anxiety (16,17). Detailed immunohistochemical studies have shown that CB1 is predominantly expressed presynaptically on axon terminals (18,19) and has a highly localised cellular distribution. Low receptor expression in a region may not represent a low level of activity in this region as it has been demonstrated in rat brain that greater amplification in signal transduction through the G-proteins may exist in some regions with relatively low expression (20).

The discovery of a specific CB1 receptor naturally led to the search for further possible members of this family. CB2 receptors were first cloned from immune cells (21), and the distribution of these receptors for many years was assumed to be exclusively on peripheral immune cells. More recently it has become clear that the receptor is expressed on a small population of neurons (22), enterocytes, osteoclasts and osteoblasts (23), as well as liver cells (24), and this list seems likely to grow.

Intriguingly, expression of CB2 appears to be highly inducible following injury or activation, which may account for the range of contradictory reports on CB2's presence or absence.

Additional cannabinoid receptors have been proposed. For example ( $^{35}$ S-GTP $\gamma$ S) binding was observed in response to WIN55,212-2 and anandamide in brains from CB1/CB2 double knock-out mice (25). More recently GPR55 has been identified as a putative cannabinoid receptor through a study which mined patents (26). Analysis of its mRNA expression suggests that this receptor has a widespread distribution, including the CNS and adipose tissue (27). Whether or not this receptor is indeed a true cannabinoid receptor is still a controversial issue (28,29).

### 3.2. Endocannabinoids

Following the discovery of cannabinoid receptors attention naturally turned to the search for endogenous ligands (endocannabinoids). The first identified endocannabinoid was N-arachidonylethanolamide (30), which was named anandamide, for its structure and from "ananda" which is Sanskrit for "bliss". Anandamide consists of an arachidonic acid backbone, with a polar headgroup (ethanolamine) and this structure has been largely conserved in the subsequent identification of cannabinomimetic compounds. The second endocannabinoid to be described was 2-arachidonoyl glycerol (2AG) (31,32). 2AG is detected at considerably higher levels in the brain than anandamide. The relevance of detected levels of endocannabinoids (usually from post mortem tissues) to cannabinoid activity is not clear, as in contrast to classical neurotransmitters, neither compound is stored in vesicles, rather both compounds are synthesised on demand, therefore their functional concentrations remain unknown. Furthermore, 2AG is an intermediate in a range of synthetic pathways. Other compounds which can interact with cannabinoid receptors have been identified including 2-arachidonoylglycerol ether (nolandin ether) (33) and O-arachidonylethanolamine (virodhamine) (34), however whether or not these represent genuine neurotransmitters is still unclear. The enzymes involved in the synthesis and breakdown of the endocannabinoids has produced many new therapeutic targets in addition to the receptors, and are therefore described in more detail below.

### 3.3. Synthesis and degradation of endocannabinoids

#### 3.3.1. Anandamide

Anandamide is synthesised by the hydrolysis of N-arachidonoyl phosphatidyl ethanolamine (NAPE) by a specific phospholipase D (35,36). NAPE is generated by the transfer of arachidonic acid from the sn-1 position of rare phospholipids to the sn-3 position of phosphatidylethanolamine. This calcium and cAMP dependent step, catalysed by an N-acyl transferase may well be the rate limiting step in anandamide synthesis (37,38). A specific calcium dependent enzyme then hydrolyses NAPE's phosphodiester bond, releasing anandamide. Termination of anandamide signalling occurs following uptake, possibly by a specific transporter, followed by enzymatic hydrolysis into arachidonic acid and

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ethanolamine by cytoplasmic fatty acid amide hydrolase (FAAH) (39). FAAH has been extensively characterised and appears to tightly regulate the concentration of free anandamide. It is considered an attractive drug target for modulating levels of anandamide and is the subject of much pharmacological investigation (40). This enzyme is not specific for anandamide however, hydrolysing a number of other fatty acid amides and ethanolamides, therefore the specificity of such a therapeutic approach remains to be proven.

### 3.3.2 2-Arachidonoyl glycerol

2-AG is generated from diacylglycerol (DAG) by DAG lipase selective for the sn-1 position. DAG, an intracellular second messenger that activates protein kinase C, can be generated from phosphoinositides by a phosphoinositide specific phospholipase C or from phosphatidic acid by phosphatidic acid phosphohydrolase (41). Two DAG lipase isozymes, alpha and beta, have been cloned (42). In the adult brain they are localised in the postsynaptic plasma membrane (43), in line with their putative role in generating 2-AG involved in retrograde transmission (44). 2-AG can be metabolised by FAAH, indeed at a faster rate than AEA (45,46). However, in the brain, the enzyme monoacylglycerol lipase (MAGL) is a more important metabolic enzyme for this endocannabinoid (47,48). A recent proteomic approach in mouse brain has suggested that approximately 85% of brain 2-AG hydrolase activity can be ascribed to MAGL, and that the remaining 15% is mostly catalyzed by two previously uncharacterized enzymes, ABHD6 and ABHD12 (49). Each of these enzymes display distinct subcellular distributions, suggesting that they may control different pools of 2-AG in the nervous system, and their precise roles remain to be elucidated.

### 3.4. Cannabinoid receptor mediated signal transduction

Both CB1 and CB2 belong to the superfamily of G-protein coupled receptors. Both receptors are linked to the pertussis toxin sensitive Gi/o proteins. Activation of adenylate cyclase also occurs in the presence of pertussis toxin, or when CB1 and dopamine D2 receptors are simultaneously activated (50) probably as a result of heterodimerisation of these two receptors (51). The agonist WIN55,212-2, but not other cannabinoids, has also been shown to promote activation of Gq by CB1 (52). Cannabinoids acting on CB1 also inhibit calcium channels (53,54) and activate inwardly rectifying potassium channels via G-protein  $\beta\gamma$  subunits (55). CB1 activation has also been demonstrated to activate all three families of mitogen activated protein (MAP) kinases, including p44/42 MAP kinase (56,57), p38 kinase (58,59) and JUN-terminal kinase (58,60). These effects could be via G-proteins (57,61) or other independent pathways (62) and appear to be highly cell specific.

In contrast to CB1, which has been extensively studied, considerably less detail is known about CB2 mediated signalling. Similar to CB1, CB2 receptors can modulate adenylate cyclase and MAP kinase activity (63,64). CB2 however, did not couple to ion channels in either transfected AtT20 cells (63), or transfected *Xenopus*

oocytes (65), nor has there been any evidence for coupling to Gs (50,66). The lack of selective cannabinoid agonists and co-expression of CB1 and CB2 receptors in immune cells has further complicated the investigations into CB2 mediated signalling. However, SR144528-sensitive activation of PI3K/PKB, and subsequent translocation of Raf-1 and phosphorylation of p42/44 MAP kinase (67) has been observed in human prostate epithelial PC-3 cells, while activation of phospholipase C and subsequently increased intracellular calcium (68) has been shown in calf pulmonary endothelial cells. CB2-receptor activation attenuates TNF $\alpha$ -triggered NF- $\kappa$ B and RhoA activation, upregulation of adhesion molecules ICAM-1 and VCAM-1, expression of monocyte chemoattractant protein, TEM of monocytic THP-1 cells, and monocyte-endothelial adhesion in HCAECs (69). Importantly, considerably more species differences exist between rat, human and mouse CB2 than within CB1, so care must be taken in extrapolating results between the species (see below).

### 3.5. Cannabis based drugs

For many years extracts from the *Cannabis sativa* plant have been used for the management of pain and other conditions. Sativex is a preparation from *sativa* comprised of THC and cannabidiol and was approved in 2005 for the treatment of neuropathic pain in Canada. Marinol and Cesamet are THC-based prescription drugs which have been approved as appetite stimulants and for the treatment of nausea ([www.marinol.com](http://www.marinol.com), [www.cesamet.net](http://www.cesamet.net)). These are legal drugs available through prescription in some countries whereas smoked cannabis is illegal in most countries and is a schedule-I drug in the US. Cannabis plants have typically been bred for high THC content as this is the primary constituent that provides the euphoric high. There is now interest in selecting high cannabidiol containing plants as this component does not evoke the undesirable effects of THC, including tachycardia, confusion and psychosis. Thus the objective of developing these compounds is to obtain the therapeutic benefit without the euphoric high.

## 4. EXCITING NEW DEVELOPMENTS IN CANNABINOID RESEARCH

### 4.1. CB1 as a potential target for the treatment of obesity

For a considerable amount of time anecdotal evidence has suggested that activation of the cannabinoid system can lead to the stimulation of appetite. This was subsequently proven in animal models where cannabinoid agonists were able to increase food intake (70-72) whilst the CB1-specific antagonist SR141716A (Rimonabant, Sanofi Reschercher) blocked these effects (70,73). Moreover, when given alone SR141716A was capable of reducing body weight (74,75), adipose tissue mass and positively altered the level of several critical biochemical markers associated with increased risk of developing diabetes or metabolic syndrome (see 2,76). In fact some authors have suggested that obesity and metabolic diseases are conditions strongly correlated with over-activation of the endocannabinoid system in central brain circuits, adipose tissue, liver and muscle (reviewed by 77).

Diabetes and obesity, (often termed 'diabesity' by researchers in the field) are massive health-care burdens globally. With the pharmaceutical value of effective anti-obesity drugs currently in the range of US\$1 billion per annum, and with 'diabesity' consuming over US\$100 billion of the national health budget (US Health Statistics), it is obvious why the anti-obesity properties of CB1 are very hot intellectual property. Following promising Phase III clinical trials, Rimonabant was granted license in Europe in 2006 and is marketed as Acomplia® by Sanofi-Aventis. However, following the United States RIO (Rimonabant in Obesity) clinical trials which highlighted the risk of psychiatric side effects, Rimonabant has not yet been licensed in the United States. Rimonabant is the first generation of CB1 antagonist from the Sanofi-Aventis stable and is unlikely to be the last cannabinoid-based obesity drug.

The endocannabinoid system has also been reported to be unbalanced following long term tobacco use and may be involved in the addictive properties of nicotine (reviewed in 78). A major concern of smokers is the subsequent weight gain that follows successful abstinence (79). Therefore, the CB1 receptor was proposed as a potential therapeutic target to combat the desire to smoke and potentially suppress the craving to eat. To date several clinical trials using Rimonabant have been completed (Stratus-EU 2006, Stratus-US 2006, and Stratus-WW 2006). The Stratus-EU and Stratus-US studies evaluated Rimonabant for smoking cessation, whereas the Stratus-WW investigated smoking relapse. The findings of these studies have not been published in full (see Cochrane review 80). The Cochrane review describes in detail the objectives of the clinical trials and the published outcomes currently available. In summary, it appears from across the three clinical trials that 20 mg Rimonabant was effective at increasing the odds of long-term cessation by 1.5-fold. However, the likelihood of current CB1 antagonist strategies reaching market for smoking cessation anytime soon is confounded by the same adverse neurological issues affecting the use of Acomplia® for obesity treatment.

### 4.2. CB1 and neurodegeneration

The anatomical localisation of CB1 receptors in the brain has been extensively studied by several groups (17,81-83). These studies have shown that CB1 is predominantly located in the pre-synaptic membrane of GABAergic and glutamatergic synapses. Within these sites CB1 controls the release of the respective neurotransmitter (84,85) from the pre-synaptic boutons. CB1 is abundantly located in basal ganglia nuclei and this region is particularly susceptible to the neuronal cell death that occurs during the progression of Huntington's disease (HD). It is now well recognised that loss of CB1 in the globus pallidus and striatum are amongst the earliest molecular changes known in human HD (86). Combined with the known neuroprotective effects of cannabinoid agonists and the consequence of CB1 loss during excitotoxicity it was proposed that neurons that lose their protective CB1 receptors during HD may be more vulnerable. Thus it has been hypothesised that if these receptors were rescued or their loss prevented that this may

be advantageous and delay neuronal death. This hypothesis has yet to be proven and the underlying molecular mechanisms that mediate the selective loss of CB1 are not known.

Evidence is also accumulating which supports the hypothesis that the cannabinoid system may be important in the progression of other neurodegenerative conditions including multiple sclerosis (MS), epilepsy, Parkinson's disease, Alzheimer's and several other conditions where neuro-inflammatory components exacerbate progression (87-91). Interesting work from Ramirez and colleagues has highlighted abnormal CB1 and CB2 receptor expression in close proximity to senile plaques in the brains of individuals with Alzheimer's disease (90). CB1 expression was reduced as a function of neuronal cell loss around the plaques whereas CB2 expression was increased in cells identified as microglia, with CB1 neuronal loss greater in areas of high numbers of activated microglial cells (90). This insightful correlation was probed further using a rat Alzheimer's model where cannabinoid agonists were successful at preventing microglial activation and the cognitive impairment associated with the neuronal cell death in the disease. These are promising findings and much work has yet to be conducted to understand the roles of cannabinoids in the early stages of brain damage especially with regard to effects on vascular blood flow, modulation of brain glial cells, regulation of cannabinoid receptor expression (loss of CB1 and elevation of CB2) and production of the endocannabinoid ligands proximal to the damaged tissue.

### 4.3. CB1 receptor oligomerisation

The GPCR field has been revolutionised with the realisation that many GPCRs oligomerise with other receptors of the same type (homodimerisation) or with distinct receptor types (heterodimerisation). This is now well characterised for a number of GPCRs with researchers in the field anxious to assimilate data on the physiological relevance of oligomeric receptor complexes. The CB1 receptor has been shown to oligomerise with the dopamine D2 receptor following dual agonist stimulation, which appears to promote the rapid complexing of D2 with CB1 and also switches the signalling from Gi/o to Gs (51). CB1 is also thought to complex with the orexin-1 receptor (92). There are several obvious pharmacological and physiological implications of having receptor complexes comprised of different separate receptor monomers, analogous to a complex structure comprised of different subunits. In a pharmacological context, of uppermost consideration is the effect or influence different receptor subunits have on receptor pharmacology including ligand binding, trafficking, and of course coupling and signalling through G-proteins. These receptor complexes hold particular interest for pharmaceutical drug targeting to create designer ligands that will recognise the specific oligomer. It is easy to envisage that such ligands may bind a site present only in the oligomer created by its unique conformations. An alternative strategy is to create molecules that comprise two separate ligands, one for each receptor that are linked by a spacer bridge (termed bivalent ligands). This will require considerable optimisation of the

molecular chemistry to create the correct pharmacophores but some success in this area has been reported for kappa and delta opioid receptors co-expressed in HEK cells (93).

In a physiological context CB1 is considered one of the most widespread GPCRs in the central nervous system. It is involved in the regulated release of several neurotransmitter molecules including GABA and glutamate and is located in other brain regions involving the dopaminergic and serotonergic systems. Therefore, the scope for CB1 receptors to be expressed in neurons and other cells types (e.g. astrocytes, immune cells and adipocytes) that express a myriad of other receptors is quite large. In some immune cells (B and T lymphocytes) CB1 and CB2 are known to be co-expressed albeit at considerably different levels. To our knowledge the concept of whether CB1:CB2 dimers exist has not been investigated. This area of novel ligand design and pharmacotherapy to target specific populations of receptors is an attractive avenue for CB1 compounds as they may potentially avoid some of the psychotropic effects of some CB1 ligands. This is an area that we predict will certainly grow in the next decade.

A closely related topic which has attracted considerable interest for other GPCRs is the elucidation of the receptosome complex. This includes the identification of the cytoplasmic adaptor and scaffolding proteins that control the trafficking of the receptor. Importantly, these proteins control receptor targeting. Neurons are highly polarised cells with compartmentalised cell membranes. Therefore the delivery of CB1 to the appropriate membrane location likely involves communication and transport with as yet unidentified trafficking proteins. To date, only two fate-controlling trafficking proteins have been identified that bind CB1 (94,95). CB1-interacting protein (CRIP) was identified in a yeast-2-hybrid screen and appears to modulate aspects of CB1 signalling (95), whereas GASP-1 was found to drive CB1 into a degradation pathway following agonist stimulation (94,96). Both of these proteins recognise domains within the receptor's extreme carboxy-terminus. The specificity of CRIP and GASP-1 for CB1 is not fully understood but these examples are probably just the beginning.

#### **4.4. CB2 expression in the central nervous system: a new anti-inflammatory target?**

The discovery in the past few years of the 'peripheral' CB2 receptor in the brain of rodents and humans under specific pathological conditions but not in normal healthy adult brain may prove to be amongst the most important discoveries in the cannabinoid field in the past 10 years. The primary reason why CB2 expression in the brain may be extremely important is the hypothesis that its function is associated with suppressing the activity of resident brain immune cells (microglia and astrocytes) and as such has been suggested as a new immuno-modulatory target for the clinical treatment of several neurological states (reviewed by 3,97). This hypothesis is supported by several experimental studies, which will be discussed in more detail below.

The presence of CB2 in the central nervous system was first reported in the spinal cord of rats with chronic neuropathic pain associated with peripheral nerve damage (98). More recent studies have measured CB2 receptor protein levels in several mouse (99-101) and rat brain injury and disease models (88,102). All of these studies indicated that the up-regulation of CB2 was in cells with a microglial phenotype. In humans, CB2 is up-regulated in cells with a microglial phenotype in individuals with Alzheimer's disease, Down's syndrome, HIV, multiple sclerosis and in glioma tumours. There have been a number of cell-based studies that have demonstrated that activation of CB2 receptors regulates microglial proliferation, migration, chemotaxis, and inflammatory functions (103-105). In addition, two *in vitro* models employing co-cultures of microglia with neurons (106) or with organotypic hippocampal slice cultures (107) have convincingly demonstrated the neuroprotective properties of microglial CB2 receptors.

It is important to note that in most *in vivo* studies the expression of CB2 in the brain has been associated with microglia on the basis that these cells co-express CD68 and/or HLA-DR. However, these are not definitive cell-specific markers of microglia as CD68 is expressed by macrophages and monocytes, whereas HLA-DR antigen is expressed by a number of peripheral immune cells. As these cells are capable of crossing the blood-brain-barrier of compromised brain tissue in several pathological conditions (e.g. stroke, autoimmune diseases, MS, lesion models) better definitive markers of microglia are required to distinguish between resident brain microglia and infiltrating immune cells.

The current surge of interest in CB2's roles in the brain is likely to increase following the demonstration of a 30% reduction of infarct volume by a CB2 agonist in a rodent occlusion model of stroke (108). Although the underlying cellular mediators involved are not known at present it may involve CB2 expressed by brain microglia. The potential of cannabinoid system as a therapeutic for treating neuronal damage was further demonstrated by the same group who investigated co-administration of a CB1 antagonist at the same time as the CB2 agonist in the occlusion model. Surprisingly, the CB1 antagonist SR141617 was protective and along with the CB2 agonist reduced the infarct volume by about 90%.

Current evidence suggests that with specific exceptions CB2 is not expressed in mature brain neurons (22). CB2 expression in neural progenitor cells has been in stark contrast to the lack of expression in neighbouring mature neurons (109). In these well controlled studies, microglia or neural progenitors are conspicuously the only CB2-positive cells present. CB2 has also been identified in astrocytes in MS (110) and in astroglial tumours (111). The regulation and function of CB2 by astrocytes is less well defined and an area in humans that requires further detailed investigation.

### 4.5. CB2 and multiple sclerosis

Non-selective cannabinoids such as dronabinol and Sativex have been under investigation for the control of symptoms of multiple sclerosis for some time. The endocannabinoid system is involved in MS pathogenesis. For example, Eljaschewitsch *et al.* (112) found that anandamide levels were significantly increased in active lesions taken from human patients with MS.

The rationale for the use of cannabinoids in MS treatment is to target CB1 receptors in the central nervous system that control motor output (such as the basal ganglia), and reduce spasticity and tremor. A secondary outcome has been the reduction of MS-related neuropathic and paroxysmal pain. For example, Svendsen *et al.* (113) found that central pain was significantly reduced by dronabinol in patients with MS. A similar drug, Nabilone was tested for spasticity related pain in MS by Wissel *et al.* (114), who found that pain was reduced by the drug and that adverse effects were generally mild. Trials using Sativex, such as by Rog *et al.* (115), and Wade *et al.* (116), have found modest improvements in MS symptoms. Similar results were obtained in the “Cannabinoids in Multiple Sclerosis (CAMS)” trial, which studied the effects of oral administration of cannabis oil capsules (Cannador) which contained, with other constituents of cannabis, 2.5 mg of  $\Delta$ -9-THC and 1.25 mg of cannabidiol (117,118).

Targeting CB2 with potent CB2-selective agonists has the promise to modify disease progression in MS. CB2 is expressed in activated microglia and infiltrating macrophages, and in T cells in animal models of MS, such as the experimental autoimmune encephalomyelitis (EAE) model. Maresz *et al.* (100) found that after induction of EAE in mice, spinal cord CB2 expression peaked at 10 days and then declined. The authors were able to sort CB2-expressing cells into resting microglia, activated microglia, macrophages, and T-cells. CB2 was expressed in particularly high levels by activated microglia and macrophages at 10 times the level of CB2 as resting microglia or T-cells.

In a major recent study, the same group (119) used the EAE model to investigate the role of both CB1 and CB2 in disease progression. Interestingly, they found that CB1 expressed by neurons, but not immune cells, was required for disease retardation by cannabinoids. However, CB2 expressed by encephalitogenic T cells were critical for controlling inflammation. CB2-deficient T cells in the CNS during EAE resulted in more severe disease. Although this would appear to implicate both CB1 and CB2 in disease progression during MS, strong activation of CB2 with CB2-selective agonists may allow for a greater therapeutic effect with higher drug doses by avoiding the psychoactivity associated with CB1 activation.

### 4.6. CB2 and neuroprotection following ischaemia

The endocannabinoid system has been known to be involved in neuroprotection during ischemia for a number of years. Both CB1 and CB2 receptors appear to mediate the neuroprotective effects. For example, Martinez-Ogado *et al.* (120) found that both CB1 and CB2

activation with WIN55,212-2 was important for reducing neuronal death following hypoxia-ischaemia (HI) in rats.

CB2 is expressed in rat brains following both HI and middle cerebral artery occlusion (MCAO) (1). It also appears that CB2 agonists can be neuroprotective following MCAO. Zhang *et al.* (108) have shown that pre-treatment with a CB2 agonist can reduce brain injury during the acute stage of MCAO. Which cells the CB2 agonist targets for these effects (e.g., microglia or blood-borne immune cells) remains to be determined. Even more promising are preliminary results on the combination of a CB2 agonist and CB1 antagonist (Rimonabant) using the MCAO model, presented by Zhang *et al.* at the 2007 International Cannabinoid Research Society Symposium (121). The combined drugs decreased infarct size by considerably more than each drug administered alone. However, although these results are encouraging, clinical trials for neuroprotectants during ischaemia have shown that most drugs are not effective if administered more than a few hours after the event. Therefore, it will be interesting to determine if CB2 agonists are protective with post-event delivery.

### 4.7. CB2 in the spinal cord: regulation of neuropathic pain

Approximately 5 to 8% of adults in developed countries experience neuropathic pain. Current treatments include anti-convulsants, tricyclic antidepressants, and NMDA receptor antagonists. All have a poor therapeutic ratio, and the development of new drugs to treat neuropathic pain is of urgent clinical concern. Major reviews or meta-analyses that have appeared (122,123) have all reported that cannabinoids are useful adjuvants for the treatment of neuropathic pain. Iskedjian *et al.* carried out a meta-analysis of the efficacy and safety of cannabinoids for neuropathic pain (123). Four studies used Sativex, five cannabidiol, and three dronabinol. The best performed drug, Sativex, decreased pain by 1.7  $\pm$  0.7 points on an 11-point scale. Many average baseline scores in the trials were around 50-65% of the maximum possible pain. This corresponds to an approximate 28% decrease in pain.

Iskedjian *et al.* (123), also found that pain reduction was twice as great in subjects receiving a cannabinoid at 6-10 weeks compared with earlier times. Possibly the effect is due to increases in dose with time. In most of the higher quality trials, patients gradually increased their dose over several weeks, suggesting that increasing the dose increased the pain-relieving effects. However, this would also increase psychoactive side effects, and the challenge for improving cannabinoid analgesia is to target cannabinoid receptors in the pain pathway, alone. These delayed effects may also reflect time taken to reach drug steady state concentrations.

One way in which this might be achieved is by targeting spinal CB2 receptors, which are involved in the regulation of pain in some models (124). Selective CB2 agonists are effective in some of these models, particularly of inflammatory pain (125). CB2 agonists may reduce

neuropathic pain via CB2 receptors in microglia in the dorsal root ganglion and in the spinal cord dorsal horn. Microglia in the dorsal horn regulate the excitability of nerve fibres in the ascending nociceptive pathway (126), and CB2 is expressed in the rat spinal cord dorsal horn (101). Expression of CB2 is up-regulated in these cells in models of neuropathic pain (98). CB2 agonists suppress inflammation in spinal cord slices that have been treated with capsaicin (101), and suppress spinal Fos protein expression in a carrageenan model of inflammation (127). CB2 agonists also inhibit electrically evoked responses in slow conduction C-Fibres (128). It has also been shown that intrathecal injection of the selective CB2 agonist JWH-015 can reduce post-operative hyperalgesia in the ipsilateral paw in rats (124). Another CB2-selective agonist PRS-211,375 (Cannabior, Pharmos Corporation) is currently in Phase II clinical trials as an analgesic and the company reports that next-generation CB2-selective compounds are also being developed ([www.pharmos.com](http://www.pharmos.com)).

### 4.8. Cannabinoids in arthritis and osteoporosis

*In vitro* experiments and preclinical experiments using animal models have suggested that cannabinoids may be of use in treating rheumatoid arthritis. In a murine model of arthritis<sup>1</sup> HU-320 reduced both disease progression and symptoms. HU-320 inhibited TNF-alpha production by macrophages, and regulated plasma levels of TNF-alpha following treatment with LPS (129). In another experiment, it was found that both WIN 55,212 and anandamide inhibited production of NO in bovine articular chondrocytes following stimulation with pro-inflammatory IL-1. Both drugs also decreased the amount of cartilage in the bovine nasal cavity that was degraded by proteoglycan. Intriguingly, neither a CB1 nor a CB2 receptor antagonist (AM281 and AM630) reduced the protective effect of the cannabinoids, but instead increased it. In related experiments, WIN 55,212 and anandamide suppressed the release of sulphated glycosaminoglycans in bovine cartilage, suggesting that cannabinoids may help reduce cartilage resorption and joint destruction (130,131).

Ajulemic acid, a synthetic analog of the THC metabolite THC-11-oic acid given to rats with adjuvant-induced polyarthritis reduced both inflammation and joint damage (132). Ajulemic acid also suppressed the release of interleukin-1beta (IL-1beta), a critical mediator of rheumatoid arthritis, in LPS-treated monocytes that had been extracted from human synovial fluid (133), and induced apoptosis in synovial T cells (134). Sativex is currently under investigation for the reduction of arthritic pain (135). In clinical trial, Sativex produced statistically significant reductions in pain in comparison with placebo, and was well tolerated with mostly mild adverse effects. Promisingly, patients using Sativex showed a small but statistically significant reduction in disease progression scores (135).

One of the most promising uses of cannabinoids may be for the treatment of osteoporosis. The role of the endocannabinoid system in bone growth and maintenance has only recently been discovered (136). Activation of cannabinoid receptors in bone tissue is crucial for

maintaining bone mass. CB2 is expressed by both bone-producing osteoblasts and bone-removing osteoclasts (137), and both osteoblasts and osteoclasts produce anandamide and 2-AG (23). Mice that lack CB2 receptors lose bone mass with age faster than wild type mice. One research team has therefore suggested that bone is the main tissue that is regulated by CB2 (136). CB2-selective ligands appear particularly promising, as mutations in the gene that encodes CB2, leads to low bone density in women (138). However, CB1 is also involved in the endocannabinoid regulation of bone mass. CB1 in sympathetic nerve terminals in bone inhibits noradrenaline release, which results in increased bone growth (139).

### 4.9. The development of CB2-selective agonists

It is evident that selective CB2 receptor agonists have the potential to help improve many disease outcomes without the psychoactive effects of CB1-selective or non-selective cannabinoid receptor agonists. The side-effect profile for CB2 agonism is benign, with no adverse effects known. However, there currently exist no highly selective CB2 full agonists that are widely available, and the development of CB2-selective agonists is a subject of intense research in the fields of cannabinoid treatment of inflammation and pain.

CB1 is highly conserved between mammalian species, but CB2 has evolved far more rapidly (140) such that there is only 81% sequence identity between rat and human CB2, increasing to 87% identity in the critical transmembrane regions (141). As a result, rodent models may not reliably predict the performance of a CB2 agonist for human receptors. This has been an overlooked theme in cannabinoid research until relatively recently (142).

Mukherjee *et al.* (142) have shown that cannabinoids can vary a great deal in their selectivity for CB2 depending upon the species. This is due partly to varying affinity for CB2 of different species. However, differences can also result from a compound having a much lower affinity for CB1 in a particular species. For example, Valenzano *et al.* (125) have characterized the CB2-selective partial agonist GW405833 at both human and rat CB1 and CB2. GW405833 is an analogue of WIN55,212-2 which has very high selectivity for human CB2 over human CB1 (1223-fold). However, it is much less selective for rat CB2 over rat CB1 (76-fold). The difference in selectivity is due to a loss of affinity for human CB1 compared with rat CB1. Understanding why this occurs could potentially help in the design of CB2 agonists with high selectivity at the human receptor. For many compounds the data on relative affinities for human versus mouse or rat CB2 is lacking. Some compounds have been tested only using rodent receptors or else by using a different species for each receptor (CB1 or CB2). It seems possible that selective and potent CB2 agonists for humans might already exist, but have been missed due to screening only of rodent receptors. Therefore, many existing cannabinoids need to be assayed using human receptors, and accurate comparisons between human and rodent receptor affinities should be carried out (142).

## 5. SUMMARY AND FUTURE PERSPECTIVES

The advances and discoveries in the cannabinoid field in the last 15 years have paved the way for a very exciting future for cannabinoid research and cannabinoid-based therapies to potentially treat a variety of conditions from obesity to neurodegeneration. With improved molecular tools it has been possible to look deeply into the complex world of cannabinoid receptor signalling, receptor expression and understand the enzymatic processes involved in the biosynthesis of the endocannabinoid ligands. Yet with all this knowledge there is much to learn and many questions to be answered. The development of more selective ligands will undoubtedly facilitate the pharmacotherapy of the fore-mentioned conditions and increase the number of cannabinoid based drugs in the market place.

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**Footnote:** <sup>1</sup>DBA/1 mice immunized with type II bovine collagen

**Key Words:** Cannabinoid; Endocannabinoid; Anandamide; Multiple Sclerosis; Ischemia; Pain; Arthritis; Immune Cell; Oligomerisation; Receptor Signalling, Review

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