The Cannabinoid CB2 Receptor as a Target for Inflammation-Dependent Neurodegeneration

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Abstract: Endocannabinoids are released following brain injury and may protect against excitotoxic damage during the acute stage of injury. Brain injury also activates microglia in a secondary inflammatory phase of more widespread damage. Most drugs targeting the acute stage are not effective if administered more than 6 hours after injury. Therefore, drugs targeting microglia later in the neurodegenerative cascade are desirable. We have found that cannabinoid CB2 receptors are upregulated during the activation of microglia following brain injury. Specifically, CB2-positive cells appear in the rat brain following both hypoxia-ischemia (HI) and middle cerebral artery occlusion (MCAO). This may regulate post-injury microglial activation and inflammatory functions. In this paper we review *in vivo* and *in vitro* studies of CB2 receptors in microglia, including our results on CB2 expression post-injury. Taken together, studies show that CB2 is upregulated during a process in which microglia become primed to proliferate, and then become fully reactive. In addition, CB2 activation appears to prevent or decrease microglial activation. In a rodent model of Alzheimer's disease microglial activation was completely prevented by administration of a selective CB2 agonist. The presence of CB2 receptors in microglia in the human Alzheimer's diseased brain suggests that CB2 may provide a novel target for a range of neuropathologies. We conclude that the administration of CB2 agonists and antagonists may differentially alter microglia-dependent neuroinflammation. CB2 specific compounds have considerable therapeutic appeal over CB1 compounds, as the exclusive expression of CB2 on immune cells within the brain provides a highly specialised target, without the psychoactivity that plagues CB1 directed therapies.

Key Words: Cannabinoid, CB2 receptor, inflammation, macrophage, microglia, neuroinflammation, brain injury.

1. INTRODUCTION: THE CANNABINOID CB2 RECEPTOR AS A BIORATIONAL TARGET FOR THE TREATMENT OF NEURODEGENERATION

1.1. Cannabinoids and the Endocannabinoid System

The first approved cannabinoid drugs were analogues of Δ^9 -tetrahydrocannabinol (Δ^9 -THC). Dronabinol is a natural isomer of THC that is found in the cannabis plant, and MarinolTM contains synthetic dronabinol. Marinol, and another analogue nabilone (CesametTM) are used to prevent nausea and vomiting after treatment with anti-cancer medicines. More recently, GW-100 (SativexTM) which combines nearly equal amounts of Δ^9 -THC and cannabidiol in a whole plant extract from cultivated cannabis, has been approved in Canada for patients with multiple sclerosis [69].

This development of relatively polar cannabimimetic compounds facilitated the characterisation of specific binding sites (cannabinoid receptors) and the development of highly potent and efficacious ligands. CP 55,940 is 40 times more potent than Δ^9 -THC, and is extremely psychoactive. HU-210, is also highly potent, and has a longer duration of action than Δ^9 -THC. WIN 55,212-2 is also a potent, non-selective cannabinoid receptor agonist [17].

There are two well characterised cannabinoid receptors with distinct physiological properties. The CB1 receptor mediates most of the psychoactive effects of cannabinoids, whereas the CB2 receptor is principally involved in anti-inflammatory and immunosuppressive actions. Therefore, selective CB2 receptor activation has the potential to provide the anti-inflammatory effects of cannabinoids without the psychoactive effects. A number of highly selective cannabinoid receptor antagonists currently exist. Of particular interest, SR141716 Rimonabant is currently in Phase 3 clinical trials for the treatment of cardiovascular risk factors. SR145528 is a highly selective CB2 receptor antagonist.

The characterisation of specific cannabinoid receptors in turn leads to the identification of endogenous cannabinoids (endocannabinoids), for which the cannabinoid receptors are the natural target. Arachidonylethanolamide (anandamide) was characterised in 1992. 2-arachidonoyl-glycerol (2-AG), was discovered later, and

in recent years, three more endocannabinoids have been suggested: 2-arachidonyl-glycero-lether (noladin, 2-AGE), O-arachidonyl-ethanolamine (viro-dhamine), and N-arachidonoyl-dopamine (NADA) [53].

With the characterisation of endocannabinoids, there has been intensive research into new potential targets for pharmacological manipulation of the endocannabinoid system other than the CB1 and CB2 receptors. Two endocannabinoid metabolising enzymes have now been identified, fatty acid amine hydrolase (FAAH) and monoacylglycerol lipase (MGL). Other potential targets include an anandamide transporter protein [33] and recently discovered endocannabinoid synthesizing enzymes [53]. However, to selectively activate either CB receptor will require the development of potent and selective agonists. Given the intense efforts being made in this direction, it is perhaps only a matter of time before such compounds are developed.

1.2. Limitations on the CB1 Receptor as a Target for Neurodegeneration

It has been known since at least 1994 that the cannabinoids can be neuroprotective [4]. Endocannabinoids are released in the brain following injury [20] and specific roles for CB1 receptors have been determined using CB1 knockout mice and selective CB1 receptor antagonists. There is an extensive literature on the role of CB1 in neuroprotection and neurodegeneration. This has been well reviewed elsewhere [37, 41]. Briefly, at least three mechanisms have been proposed for CB1-mediated neuroprotection. First, endocannabinoid activation of pre-synaptic CB1 receptors is known to reduce neurotransmitter release and, hence, excitotoxicity in post-synaptic neurons [36]. Second, CB1 is involved in the regulation of vasodilation, both directly through vascular CB1 receptors and indirectly through the inhibition of the vasoconstrictor endothelin-1 [60, 71]. Third, CB1 receptors are known to regulate the release of pro-inflammatory factors such as NO and TNF- α in the acute phase of injury [18, 55].

There have been a number of problems that have plagued the development of CB1 as a target for neuroprotection. First, at the acute phase of injury, endocannabinoids are released in large amounts and although blockade of CB1 receptors increases neuronal damage, exogenously applied cannabinoids have not had a beneficial effect to a corresponding degree. Second, the therapeutic window for CB1 targeting in brain injury is likely to be short, as

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most neuroprotective drugs that work at the acute phase are ineffective if administered more than 6 hours after stroke or brain trauma [58]. This has made such drugs impractical for clinical and emergency use. Third, drugs that activate CB1 can have unwanted psychoactive effects at moderate to high doses, whereas CB2 receptors are not psychoactive (see below).

CB2 is believed to be devoid of psychoactivity, and has significant anti-inflammatory functions. Inflammation is known to be a critical part of many types of neurodegeneration [10]. However, non-steroidal antiinflammatory drugs (NSAIDs) inhibit clot formation, and increase haemorrhaging during brain injury [58]. Because of this, novel drug targets for the control of inflammation in the brain are of considerable interest and, therefore, the CB2 receptor has recently attracted attention as a potential target for neuroprotection. The rationale for CB2 as a target for neuroinflammatory neurodegeneration will be discussed in detail in the remainder of this article.

1.3. CB2-Mediated Immunosuppressive and Anti-Inflammatory Effects

Cannabis and some cannabinoids are both immunosuppressive and anti-inflammatory. Although both CB1 and CB2 receptors have been detected in leukocyte mediators of inflammation, CB2 is widely and strongly expressed in a range of leukocytes and appears to be the key mediator of cannabinoid regulation of inflammation and immune functions [39, 40].

CB2 receptors are implicated in a range of leukocyte functions. For example, blockade of the CB2 receptor with SR145528 inhibits splenocyte proliferation and induces apoptosis *in vitro* [40]. CB2 also regulates B and T Cell differentiation, and the balance of T helper (Th1) pro-inflammatory to Th2 anti-inflammatory cytokines [82]. In macrophages, CB2 stimulation suppresses proliferation and the release of pro-inflammatory factors such as NO, IL-12p40, and TNF- α , inhibits phagocytosis, and reduces IL-2 signalling to T-cells [9]. CB2 activation also suppresses neutrophil migration and differentiation [47], but induces natural killer cell migration [31].

Taken together, studies on CB2 receptors in leukocytes are consistent with an anti-inflammatory and immuno-suppressive role. This has been supported in recent years by demonstrations that CB2 regulates inflammation in a diverse range of animal models, a small sample of which includes gastro-intestinal inflammation [38], acute hindpaw inflammation [11], and pulmonary inflammation [5]. CB2-selective agonists have been particularly promising in the treatment of inflammation-induced hyperalgesia [74].

1.4. The Potential for CB2-Mediated Psychoactive Effects

A key aspect of the attractiveness of CB2-selective agonists is the lack of psychoactive side effects from CB2 stimulation. For instance, the selective CB2 partial agonist GW 405833 has strong anti-inflammatory hyperalgesia effects in rats at less than 1 mg/kg. Ataxia and other CNS effects are not observed until 30 or even 100 mg/kg, at which point the drug is probably also activating CB1 receptors (see section 5).

Psychoactivity through CB2 would require expression of CB2 receptors in CNS neurons. The seminal work of Munro *et al.* [45], characterising and cloning the CB2 receptor, was accompanied by the report that no CB2 mRNA was detected in the brain. Most subsequent studies have confirmed this finding, and although a wealth of data has accumulated from immunohistochemistry studies on the specific distribution of CB1 receptors in the brain, this has not been the case for CB2.

Nevertheless, Skaper *et al.* [68] observed that CB2 mRNA was expressed in cerebellar granule cells *in vitro*, and in 4-week rat cerebellum cell *in situ*. However, neuronal CB2 has not been reported in the cerebellum since these results. Molina-Holgado *et al.* [43] reported that AMPA-induced neuronal toxicity *in vitro* was

blocked by 1 μ M of the CB2 antagonist AM630. However, this concentration is likely to have saturated both CB1 and CB2 receptors [34].

Convincing evidence for CNS neuronal CB2 was presented by Van Sickle *et al.* [75] who described CB2 expression in a select population of vagus nerve cells in the mouse and ferret brainstem, and demonstrated the role of CB2 in the control of emesis, which is regulated through these pathways. This may be a special case, as these CB2-positive neurons may be considered as peripheral nerves that enervate the CNS. However, this finding enhances the likely side effect profile for CB2-selective agonists.

Recently, controversial work has been published that describes widespread CB2 expression in CNS neuronal beds [24]. However, in our own work [2, 3] we have been unable to replicate these results, and have evidence only for the absence of CB2 from non-glial/non-endothelial cells in the brain. The same research group has recently published findings showing a virtual absence of CB2 mRNA from the CNS outside of the brainstem and hypothalamus [50], although they have argued for CB2 regulation of stress mediated depression [51].

On the balance of evidence, we believe that truly selective CB2 agonism is unlikely to have major and/or detrimental psychoactive effects.

2. INFLAMMATION IN CNS NEURODEGENERATION

2.1. Microglia: Key Mediators of Neuroinflammation

Inflammation was first suspected to be important in chronic neurodegenerative diseases such as Alzheimer's disease (AD) early last century [14]. However, this view lost favour, largely because of the widespread belief that the blood-brain barrier provides the CNS with a privileged exemption from blood-borne leukocytes and the immune system.

The discovery that microglia are resident immune cells in the brain led to the breakdown of this view. This was facilitated by the development of modern gene and protein expression technologies in the 1980's and 1990's, which led to the discovery that many mediators of peripheral inflammation are also involved in neurodegenerative processes, including various growth factors, inflammatory chemokines and cytokines, and nitric oxide [14].

Microglia are established in the brain early in development, although it also appears that perivascular microglia have a monocyte origin and may replenish the CNS throughout adult life [48]. Resting microglia are not dormant, but carry out a number of metabolic functions, and through a branching set of extended processes continually monitor the CNS environment. Various stimuli can then induce the microglia to assume various phenotypes (discussed below), depending on the nature and the scale of the stimuli. When fully activated, microglia are amoeboid and phenotypically indistinguishable from macrophages, and carry out the various roles that macrophages perform in the periphery, including pro- and anti-inflammatory functions, and antigen presentation to immune cells. Microglia are activated within hours of CNS injury, whereas monocytes and other cells are not able to penetrate the blood brain barrier for up to several days, at which stage blood-borne immune cells complement the microglia in the regulation of inflammation. Because of their early activation and their extensive distribution and numbers, microglia represent a critical step in the development of CNS inflammation.

2.2. Chronic Neurodegenerative Diseases

Alzheimer's disease was considered an inflammatory pathology early last century by Alzheimer and other German histopathologists. This view has been rekindled by the observation that microglia cluster at β -amyloid plaques in Alzheimer's diseased brains [14]. The distribution of reactive microglia correlates with areas of

neuronal loss in animal models of Alzheimer's disease [59, 62], and inflammation is known to be involved in the pathogenic cascade. Chemokines and cytokines produced at plaques are not blood derived, but are produced by local neurons and glia [14]. However, while microglia are involved in the metabolism and clearance of β -amyloid, it isn't known whether microglial reactivity and inflammation is a cause or consequence of β -amyloid accumulation. It is known that in animal models the build up of β -amyloid can be dissociated from neuronal damage by blocking microglial activation and that β -amyloid itself can induce microglial reactivity [59]. Therefore, it is plausible that reactive microglia are central to the neurodegenerative cascade in Alzheimer's. For instance, release of TNF- α from microglia can stimulate glutamate release from astrocytes, which may contribute to NMDA-receptor mediated neurotoxicity [61].

Evidence is also accumulating that microglia and neuroinflammation are central to other chronic neurodegenerative diseases, such as Huntington's disease and Parkinson's disease. For example, cerebral quinolinic acid injection (a chronic lesion model of Huntington's disease) stimulates microglial reactivity, and induces the expression of various pro-inflammatory factors such as IL-1 β , IL-6, TNF- α , COX-2, and iNOS [63]. Following 6-OHDA injection into the basal ganglia (a model of Parkinson's disease), microglia are activated in both the substantia nigra and the striatum. This is correlated with a loss of tyrosine hydroxylase expression in the striatum [1].

2.3. Injury induced Neuroinflammation

Brain injury results in a pathogenic cascade, which includes glutamate excitotoxicity, oxidative stress, release of neurotoxic factors from damaged cells, necrosis, and apoptosis. Injury can result from trauma, toxins, or from oxidative stress either globally (e.g., hypoxia) or locally (e.g., focal ischemia or stroke). Oxidative stress appears to be a common factor in most types of brain injury, and involves not only the generation of damaging reactive oxygen species, but also redox signalling to molecular mediators of further cell damage, including apoptotic and inflammation pathways. Injuries are characterised by a virtually immediate cascade of cell death at the focus of the injury or infarction which continues for several hours. If these cells are not rescued within 6-9 hours of the event, then damage is irreversible [58]. However, in the penumbral zone (the area surrounding the primary zone of cell death from the acute phase) cell death may continue for many days or even weeks, mediated largely through inflammation. Key early mediators in this process are microglia, and later infiltrating leukocytes such as macrophages, neutrophils, and T-cells. The volume of this secondary area of cell death can be considerable, and has the potential for pharmacological intervention. Importantly, the therapeutic window appears to extend for many hours and even days following injury. Therefore, pathways which may inhibit neurotoxic inflammatory processes (and/or promote neuroprotective immune functions) are attractive targets for drug development. One such drug, pioglitazone, has shown promising preclinical results and is currently in clinical trial [58].

Astrocytes are mediators of neuroinflammation in the acute phase of injury, and may contribute to the neuroprotective effects of cannabinoids. Sheng *et al.* [66] demonstrated that WIN 55,212-2 suppresses the release of pro-inflammatory factors such as TNF- α , CXCL10, CCL2, CCL5, and NO release by cultured astrocytes stimulated with IL-1 β . Both HU-210 and UCM707 (a selective anandamide reuptake inhibitor) inhibit the release of TNF- α , IL-1 β , and NO by LPS-stimulated astrocytes, but promote IL-6 release [52]. However, although these effects can be completely blocked by cannabinoid receptor antagonists, there have been no studies published which have used antagonists at concentrations sufficiently low enough to deduce the role of a specific receptor (CB1 or CB2)

in astrocyte-dependent inflammation. Therefore, in the remainder of this review we will focus on CB2 receptor function in microglia.

3. CB2 RECEPTORS AND MICROGLIA

3.1. In Vitro Studies

Following the microglial activation in rat primary cultures with endotoxin (*E. coli* lipopolysaccharide, LPS) and IFN- γ , Waksman *et al.* [76] showed that both CP 55,940 and its enantiomer CP 56,667 inhibited NO release. The effect was blocked by the CB1 antagonist SR141716 (0.5 μ M), though a CB2 receptor antagonist was not tested. As this concentration saturates CB1, and is at 0.5 of the K_i for CB2, CB2-mediated effects could not be ruled out. However, CB1 mRNA was detected in rat microglia cultures, but not CB2 mRNA. All subsequent studies on microglia cultures have detected CB2 mRNA and/or protein. Two studies subsequently demonstrated possible non-CB1, non-CB2 mediated effects of cannabinoids on microglia inflammatory functions. Puffenbarger *et al.* [56] demonstrated that a range of cannabinoids all reduced LPS-induced mRNA expression for IL-1 α , IL-1 β , TNF- α and especially for IL-6 in rat microglial cells. Interestingly, the effects of the cannabinoid receptor agonist levonantradol were not inhibited by either SR 141716 or SR144528. Fachinetti *et al.* [16] further demonstrated that anandamide, 2-AG, WIN 55,212-2, CP 55,940, and HU-210 all inhibited TNF- α release in LPS-activated rat cortical microglia cultures. WIN 55,212-2, but not the low-affinity stereoisomer WIN 55,212-3, inhibited TNF- α release, indicating probable receptor-mediated effects. However, consistent with Puffenbarger *et al.* [56], while CB1 and CB2 mRNA were detected, neither CB1 nor CB2 antagonists reduced the effect of WIN 55,212-2. The effect of WIN 55,212-2 was also insensitive to pertussis toxin, but was sensitive to both dibutyl cAMP and forskolin. These authors argue that these results suggest the existence of currently unidentified cannabinoid receptors in microglia.

Evidence for functional CB2 receptors in microglia was first reported by Carlisle *et al.* [7], who detected both CB1 and CB2 mRNA in brain tissue and in primary rat microglia cultures. The aim of this study was primarily to characterize the effect of varying stages of macrophage activation on CB2 expression. CB2 was not detected in peritoneal macrophages, but was expressed at high levels in thioglycolate-elicited inflammatory and IFN- γ -primed macrophages. However, LPS-activated macrophages expressed considerably lower levels of CB2. The same variation of CB2 expression was also observed in microglia at the various stages of activation. This pattern of CB2 expression in microglia has since been repeated in subsequent studies, and may have important implications for drugs targeting CB2 at various stages of microglial activation (see section 4). Most recently, Maresz *et al.* [35] have demonstrated that the combination of IFN- γ and granulocyte mononuclear colony stimulating factor (GM-CSF) induces even higher levels of microglial CB2.

A series of studies have investigated the role of CB2 in microglial migration. Franklin & Stella [21] showed that arachidonylcyclopropylamide (ACPA) induced migration of BV-2 microglia in a concentration-dependent manner. This was partly inhibited by SR141716, but almost entirely blocked by SR145528 at the highly CB2 specific concentration of 30 nM. Franklin *et al.* [20] reported that 2-AG induced microglial migration in a dibutyl cAMP sensitive manner. Anandamide and palmitoylethanolamide (PEA) in combination had the same effect, though neither compound did when alone. Also in this study, both CP 55,940 and PEA significantly inhibited forskolin induced cAMP accumulation. The effect of CP 55,940 was blocked by 300 nM of either SR141716 or SR144528. However, the PEA-mediated effect was insensitive to either compound. Lastly, Walter *et al.* [77] demonstrated that both 2-AG and anandamide induced BV-2 microglia migration in a concentration dependent manner. Cannabinol and cannabidiol pre-

vented the effect of 2-AG, by blocking CB2 and cannabinoid-sensitive receptors respectively. HEK293 cells transiently transfected with CB2 showed intense receptor expression at the migrating cells lamellipodia tips, suggesting a role for CB2 in microglial chemotaxis.

The effects of endocannabinoids on microglia proliferation were studied by Carrier *et al.* [8], who found that 2-AG, but not anandamide, stimulated a concentration-dependent increase in proliferation in microglial cells cultured in the presence of mononuclear colony stimulating factor (M-CSF). This is in contrast to McKallip *et al.* [39], who showed that cannabinoids inhibit the proliferation of cultured macrophages. This disparity might be explained by the contrast in cell phenotype studied, with non-reactive microglia assuming different functional roles than differentiated macrophages (discussed below).

The specific role of CB2 in cannabinoid-mediated inhibition of inflammatory cytokine release was investigated by Ehrhart *et al.* [13], who reported that CB2 expression was upregulated in a concentration dependent manner by IFN- γ , and that β -amyloid and CD40L induced NO and TNF- α release was inhibited by the CB2 selective agonist JWH-015. Ramirez *et al.* [59] have also demonstrated that microglia activated with fibrillar β -amyloid show a large increase in TNF- α release, and that this is almost entirely blocked by the agonists JWH-015, HU-210, or WIN 55,212-2. In the experiments of Ehrhart *et al.* [13], microglial CD40 expression was upregulated by IFN- γ , with the effect inhibited by JWH-015 and by non-selective cannabinoids. CD40L signalling is an important means by which T-cells influence microglia-mediated cytotoxicity, and CB2-mediated regulation of this pathway could be an important aspect of cannabinoid regulation of neuroinflammation. Although this study used saturating concentrations of JWH-015 for both CB1 and CB2, inhibition of cannabinoid mediated effects by CB2 siRNA strongly suggested a central role for CB2.

Two studies have now investigated the effects of cannabinoids on microglial neurotoxicity. Using co-cultures of IFN- γ /LPS activated human microglia and SH-SY5Y neuroblastoma cells, Klegeris *et al.* [32] found that both loss of neuronal cell viability and increase in neuronal cell death could be significantly inhibited by JWH-015. Importantly, although the microglia expressed both CB1 and CB2, the SH-SY5Y cells only expressed CB1. However, significant effects for JWH-015 were only seen at 5 μ M, which is a saturating concentration for both CB receptors. In a major recent study, Eljaschewitsch *et al.* [15] used organotypic hippocampal slice cultures (OHSCs) to investigate the neurotoxic effects of BV-2 microglia. Anandamide was released at moderate levels in OHSC cultures following NMDA induced injury, and in BV-2 cells alone, but at high levels in combined NMDA-stimulated OHSC/BV-2 cultures. Anandamide and WIN 55,212-2 inhibited NO release and iNOS expression in LPS-activated BV2-cells, sensitive to CB2 but not CB1 antagonism. BV-2 cells increased neuronal cell death in OHSCs, and the effect was increased by both the CB1 antagonist AM251 and the CB2 antagonist AM630. Following NMDA induced injury, AM630 but not AM251 treatment lead to even greater cell death. These results strongly implicate the endocannabinoid system, and particularly microglial CB2, in the regulation of NMDA induced neural injury. In addition, exogenously applied anandamide and WIN 55,212-2 decreased neuronal damage following either NMDA injury or oxygen/glucose deprivation (OGD). This was reduced by CB2 but not CB1 antagonism.

Complementary roles for microglial CB1 and CB2 have been suggested by Cabral & Cabral [6], who argue that CB1 is constitutively expressed in various microglial phenotypes, but that CB2 is inducible and varies with phenotype, as described above. In support of this, inoculation of microglia with *Acanthamoeba culbertsonia* induced an upregulation of CB2 expression, but not CB1 expression.

3.2. *In Vivo* Studies

The first demonstration of CB2 expression in microglia in the brain *in situ* was published by Benito *et al.* (2003), in a study using human brain tissue taken from patients who had died with Alzheimer's disease. Microglia clustering at β -amyloid plaques expressed both CB2 receptor protein and FAAH. This finding was replicated by Ramirez *et al.* [59], who also recorded that the cells express HLA-DR (indicating microglial reactivity) and co-localize with nitrotyrosine (N-Tyr), an indicator of NO activity. Consistent with this, immunoprecipitates of brain homogenates with CB1 or CB2 antibodies showed a significant upregulation of N-Tyr compared with healthy brains.

In the same study, Ramirez *et al.* [59] also investigated the role of CB2 in the pathogenesis of a mouse model of Alzheimer's disease. Mice were subject to intracerebroventricular daily injections of A β ₂₅₋₃₅ or a control peptide for 7 days. Other mice received WIN 55,212-2, (10 μ g /day) together with the peptides. Mice receiving only A β ₂₅₋₃₅ showed significantly reduced levels of the neuronal proteins calbindin and α -tubulin, matching similar findings in human Alzheimer's brains. These mice also showed a marked inability to improve on a Morris water maze test compared with controls. All of these effects were reversed by WIN 55,212-2. Intriguingly, mice receiving A β ₂₅₋₃₅ also showed a marked loss of brain CB1 receptor protein. However, this was not reversed by WIN 55,212-2.

In a rat chronic lesion model of Huntington's disease, Fernandez-Ruiz *et al.* found that CB2 expression was upregulated in subpopulations of microglia (and astroglia) in the lesioned striatum. These authors suggest that neuroprotective properties of Δ 9-THC in Huntington's disease might be mediated by CB2 [19].

CB2 expression *in situ* has also been reported in perivascular microglia in the cerebellar white matter by Nunez *et al.* [48]. This suggests the possibility that blood-borne CB2-expressing monocytes may be involved in replenishing the central nervous systems stock of CB2-responsive microglia. Ashton *et al.* [2] also reported non-neuronal CB2 expression in the cerebellar white matter, consistent with microglia.

The immune induction of CNS CB2 expression has also been demonstrated by Mukhopadhyay *et al.* [44], who showed that LPS induced an upregulation of expression of CB2 in rat brains. The first demonstration that CNS injury can induce the upregulation of CB2 expression was described by Ashton *et al.* [3]. In this study, both hypoxia-ischemia (HI) and middle cerebral artery occlusion (MCAO) induced the expression of CB2-positive microglia in rat brains. Three days following either MCAO or HI, CB2-positive cells were common on the lesioned side of the brain, coincident with areas of reactive astrocytosis, particularly in the hilus of the dentate gyrus. These cells labelled positively for MHC II and CD45 indicating a microglia/macrophage phenotype. At 7 days following MCAO, some CB2-positive cells did not express CD45 and may have been T-cells or other infiltrating immune cells, or apoptotic microglia/macrophages.

Very recently, CB2 activation with O-3853 and O-1966 (47-fold and 218-fold selective for CB2 over CB1 respectively) has been shown to reduce infarct size in mouse brains 24 hrs after induction of focal ischemia and reperfusion by up to 30% [80]. In this study, leukocyte rolling and adhesion was decreased by the drugs, but effects on microglia were not directly assessed. It will be interesting to see if future studies find a neuroprotective role for similar drugs at later times following induction of ischemia.

In this article, we are mainly reviewing the evidence for CB2-mediated effects in inflammation in the brain. However, CB2-positive microglia and macrophages in the spinal cord have now been the subject of a number studies. These studies have focused on the cannabinoid treatment of inflammatory hyperalgesia, and the

cannabinoid treatment of multiple sclerosis. These topics are of vital interest to the study of CB2 receptors in neural inflammation, and have been well reviewed elsewhere [42]. Briefly then, we will mention only one elegant study by Maresz *et al.* [35]. Following induction of experimental autoimmune encephalomyelitis (EAE) in mice, spinal cords were examined for CB2 expression over a course of 22 days. CB2 expression peaked at 10 days and then declined, though the disease score reached a peak at day 14. Briefly, using bone marrow chimeric mice, and labelling with bone marrow cells with H-2K^b, the authors employed FACS to sort CB2-expressing spinal cord cells into resting microglia, activated microglia, macrophages, and T-cells. Activated microglia and macrophages expressed approximately 10 times the level of CB2 as resting microglia or T-cells. EAE is used as a model of multiple sclerosis (MS) and Eljaschewitsch *et al.* [15] have reported that anandamide levels are significantly increased in active lesions taken from human patients with MS.

4. DISCUSSION: THE ROLES OF CB2 IN THE PATHOLOGICAL CNS.

4.1. Regulation of Microglia-Mediated Neuroprotection

Microglia may be either neuroprotective or neurotoxic, depending upon the type and extent of exogenous or endogenous stimuli they receive and the phenotype they assume [64]. For instance, in nerve transection models of glutamate injury, microglial activity is central to the healing response [65]. By contrast, IFN- γ primed microglia then treated with LPS will adopt a phenotype adapted for defensive immunity, and hence cytotoxicity. When microglia are not reactive for defensive immune functions, however, they do not release inflammatory cytokines. Stimulation of microglia by growth factors and mitogens (e.g., M-CSF) may induce a proliferative and chemotactic phenotype, prior to adopting either neuroprotective or neurotoxic phenotypes, depending upon other stimuli. Microglia are known to assume different phenotypes depending upon the concentration of stimulating factors (e.g., IFN- γ). Therefore, it has been argued that microglia may make a transition from neuroprotective to neurotoxic roles depending upon both the size and duration of a neuronal insult [81]. One implication of this is that microglia in chronic neurodegenerative conditions may assume a neurotoxic rather than a neuroprotective or supporting role.

Variations in expression of CB2, but not CB1, correlate with different microglial stimuli and phenotype *in vitro*. Cabral & Cabral [6] argue that CB2 expression is closely related to the multi-step activation of microglia. Interestingly, CB2 appears to be expressed to the greatest degree when microglia are primed to proliferate. Given that 2-AG is mitogenic for this microglial phenotype, and that cannabinoids induce microglial migration and could be critical to chemotaxis, it is possible that CB2 regulates not only the cytotoxic properties of activated microglia, but also the neuroprotective properties of microglia. To our knowledge, this has yet to be determined.

A further possibility is that release of the immune suppressive cytokine TGF- β is regulated in microglia by CB2. TGF- β is known to play a critical role in the regulation of TNF- α and IL-1 release from microglia [67] as well as in the induction of neuronal proliferation. In addition, Δ^9 -THC stimulates TGF- β production in peripheral blood lymphocytes *via* CB2, and TGF- β regulates lymphocyte CB2 receptor expression [22]. However, the role of TGF- β in CB2-mediated effects on microglia remains to be determined.

4.2. CB2 and Neurogenesis

Another potential benefit of CB2 stimulation in neurological disease is upregulation of neurogenesis and subsequent production of new neurons in the hippocampus and the SVZ-olfactory bulb system. It has been known for several years that cannabinoids stimulate neurogenesis in the adult brain [30]. Recently, Palazuelos

et al. [54] reported that CB2 receptors are highly expressed in neural progenitors and immature neurons *in vitro* and *in vivo*. Stimulation of CB2 *in vitro* induced neural progenitor cell proliferation and the formation of neurospheres. *In vivo*, dentate gyrus progenitor proliferation was increased by the CB2-selective agonist HU-308. CB2 knockout mice show greatly retarded rates of neurogenesis in normal conditions and following kainate-induced excitotoxicity [54]. Additionally, in characterising the activation of CB2-expressing microglia following global and focal oxidative stress [3], we also discovered a population of CB2-positive cells in the outer granule layer of the dentate gyrus 3 days after HI (unpublished data). These cells did not label with macrophage markers, and had smaller nuclei than the nearby (CB2-negative) neuronal nuclei. It is therefore possible that these cells were also neuronal progenitors, as stroke is known to induce increased rates of neurogenesis [29].

The findings of Palazuelos *et al.* [54] might also help to explain the observations of Skaper *et al.* [68] (section 1.4). The cerebellum shares a conserved pattern of glutamatergic neurogenesis with the hippocampus and SVZ early in development [26], and it is possible that the CB2 positive cells observed by Skaper and colleagues in the 4 week rat cerebellum may have been immature neurons.

4.3. Regulation of Microglial Neurotoxicity

Stimulation of CB2 receptors in microglia not only appears to drive their proliferation and migration when in a benign phenotype, but also to block their differentiation to a neurotoxic phenotype. This was demonstrated *in vivo* by the ability of WIN 55,212-2 stimulation of the CB2 receptor to block the expression of markers of microglial activation in mice brains following β -amyloid injections [59]. *In vitro*, cannabinoids acting through the CB2 receptor inhibit the release of pro-inflammatory and cytotoxic factors such as IL-1, NO and TNF- α in microglia previously activated for defensive immunity. It would be interesting to determine if this is due to a reversal of microglial phenotype (with pro-inflammatory factors remaining suppressed following the cessation of CB2 activation). Conversely, suppression of pro-inflammatory factors by activated microglia may be dependent upon ongoing CB2 stimulation.

CB2 receptor stimulation inhibits microglial neurotoxicity in a multitude of conditions, including in β -amyloid, CD40L, LPS, and NMDA-injury models. In addition, the work of Klegeris *et al.* [32] and Eljaschewitsch *et al.* [15] has shown that CB2 agonism not only inhibits the release of neurotoxic factors, but also inhibits neuronal cell damage in at least two cell/tissue based models.

To summarise this section, CB2 receptor activation appears to have benign effects on microglia potentially primed for adaptive immunity and neuroprotection, to block differentiation of microglia into a neurotoxic phenotype, and to inhibit the release of neurotoxic factors when microglia are activated. In addition, CB2 stimulation is pro-neurogenic in areas of adult neurogenesis.

5. THE OUTLOOK FOR CLINICAL THERAPIES

5.1. Concurrent Effects of CB2 Stimulation

For drugs targeting the CB2 receptor to be clinically useful for neuroinflammation, it is necessary that their effects on other regions of the body are benign, or at least minimally detrimental. CB2 is now known to regulate a number of processes, and in a way that appears to be uniformly benign and even beneficial.

In bone tissue, CB2 receptors stimulate osteoblast function and inhibit osteoclasts, leading to increased bone thickness [49]. Potentially useful for osteoporosis, this also has the promise to help control key mechanisms involved in the generation of pain in bone cancer [27]. CB2 stimulation also reverses various types of hyperalgesia in animal models [74], inhibits emesis [75], retards the progression of atherosclerosis in a rodent model [72], and has anti-angiogenic and anti-tumourogenic effects in several cancer models [19].

Taken together with the lack of psychoactive effects from CB2 activation, and the inhibition of inflammation through CB2 in a range of animal models of inflammation (see above), this is a clear and compelling rationale for the ongoing investigation of CB2 as a target for clinical intervention into neuroinflammatory disorders, and provides a context within which to understand the intense effort now underway to identify potent and highly selective CB2 agonists.

5.2. Current Cannabinoid Drugs and CB2 Selective Agonists

Cannabinoids that have been approved in USA or Europe include dronabinol (MarinolTM), nabilone (CesametTM), and GW-100 (SativexTM). However, all of these drugs contain Δ^9 -THC or an analogue, and are non-selective with respect to CB1 and CB2 receptors. Nevertheless, it will be interesting to see whether the long term use of Sativex by MS patients leads to any decrease in neuroinflammation. Sativex is a drug that combines Δ^9 -THC and cannabidiol derived from cultivated cannabis, and has been approved in Canada for the treatment of neuropathic pain in patients with multiple sclerosis [69]. Clinical trials for Sativex treatment of pain and spasticity in MS patients have not lead to any clear indication that this is the case [70], and it may be difficult to obtain sufficient stimulation of microglial and macrophage CB2 receptors with these drugs without causing psychoactive effects (though patients vary, and some people tolerate CB1 agonists with minimal unwanted effects). Increased stimulation of CB2 receptors with potent non-selective CB agonists such as CP 55,940 and HU-210 would lead to even greater problems. Selective upregulation of endocannabinoids by inhibitors of metabolising enzymes is one promising avenue of exploration. FAAH metabolises a range of lipid mediators, and FAAH-inhibitors may therefore have unwanted effects. However, FAAH subtypes have recently been described, and selective upregulation of endocannabinoids may become a possibility in the future [78]. In addition, ongoing work on the endocannabinoid transporter [12] and synthesising enzymes might also provide an "on-demand" upregulation of endocannabinoids for neuroinflammation, without global CB1 receptor activation.

The most promising avenue at present is the development of CB2-selective agonists. This is currently an area of intense activity. There are currently a number of potent and moderately selective CB2 agonists such as JWH-015 and JWH-133 [28], and AM1421 [79]. Although extremely useful for experimental analysis of CB receptor function, these compounds are insufficiently selective for preclinical testing. HU-308 is 440-fold selective for the CB2 receptor [25]. However, even this degree of selectivity may be insufficient for clinical use in some patients. Several alkylamides have recently been shown to have anti-inflammatory effects *via* the CB2 receptor, and are approximately 100-fold selective for CB2 over CB1 [57]. Murineddu *et al.* [46] have also shown that some tricyclic pyrazoles are potent and selective mouse CB2 receptor agonists, may represent lead compounds for CB2-selective agonists.

One promising compound is GW405833, a highly selective partial agonist for the CB2 receptor. GW405833 has a 1200-fold selectivity for human CB2 receptors (80-fold for rat CB2), and reduces forskolin-induced cAMP accumulation by approximately 45% (~75% for CP 55,940). Despite being a partial agonist, GW405833 has proven to be a potent inhibitor of hyperalgesia in a number of inflammatory and chronic pain rodent models [73]. Importantly, these effects reach a maximum at approximately 1 mg/kg in rodents, whereas CB1-mediated psychoactive effects such as loss of motor coordination (ataxia) are only observed at extremely high doses. Partial agonism is common amongst cannabinoid receptor ligands, with many high affinity cannabinoids only moderately efficacious at activating the cannabinoid receptors and their G-proteins [23]. In addition, partial agonists are often advantageous for drug therapies because they can lead to decreased levels of tolerance and desensitization compared with full agonists. GW405833

can therefore be considered a promising lead compound for therapeutic CB2 agonists.

6. CONCLUSION: THE FUTURE OF CB2 SELECTIVE AGONISTS IN NEUROLOGICAL DISEASE.

In vivo studies using CB2 selective antagonists, CB2 siRNA, or CB2 $-/-$ mice are still scarce. However, in only the last few years, a number of impressive studies have been published on the therapeutic effects of CB2 stimulation. Chief among these for neuroinflammation has been the experiments of Ramirez *et al.* [59] on the effects of CB2 stimulation in a model of Alzheimer's disease.

Cell and tissue based studies on the effects of CB2 stimulation have also only just begun to appear, and are showing promising results, consistent with research starting with Waksman *et al.* [76], which suggested neuroprotective effects from cannabinoid stimulation of microglia. Subsequent studies on CB2 have been consistent with anti-neurotoxicity, inhibition of pro-inflammatory factor release, suppression of microglial activation, and potentially benign effects in non-reactive microglia. In addition, CB2 stimulation has recently been shown to be pro-neurogenic.

As microglia and neuroinflammation are studied with ever-greater scrutiny for neuroprotective and neurodegenerative properties, critical receptor targets in microglia have become increasingly attractive. On the balance of evidence, CB2 appears to be such a target, with the potential to both help improve outcomes in chronic neuroinflammatory conditions, and to reduce secondary damage following acute injury. The side-effect profile for CB2 agonism also appears to be benign, with no adverse effects currently known. It therefore appears likely that following the eagerly awaited development of potent CB2-selective agonists, phase I clinical trials will quickly follow.

REFERENCES

- [1] Armentero, M.T., Fancellu, R., Nappi, G., Bramanti, P., Blandini, F. (2006) Prolonged blockade of NMDA or mGluR5 glutamate receptors reduces nigrostriatal degeneration while inducing selective metabolic changes in the basal ganglia circuitry in a rodent model of Parkinson's disease. *Neurobiol. Dis.*, **22**, 1-9.
- [2] Ashton, J.C., Friberg, D., Darlington, C.L., Smith, P.F. (2006) Expression of the cannabinoid CB2 receptor in the rat cerebellum: an immunohistochemical study. *Neurosci. Lett.*, **396**, 113-6.
- [3] Ashton, J.C., Rahman, R.M., Nair, S.M., Sutherland, B.A., Glass, M., Appleton, I. (2006) Cerebral hypoxia-ischemia and middle cerebral artery occlusion induce expression of the cannabinoid CB2 receptor in the brain. *Neurosci. Lett.*, **412**(2), 114-7.
- [4] Bar-Joseph, A., Berkovitch, Y., Adamchik, J., Biegon, A. (1994) Neuroprotective activity of HU-211, a novel NMDA antagonist, in global ischemia in gerbils. *Mol. Chem. Neuropharmacol.*, **23**, 125-35.
- [5] Berdyshev, E., Boichot, E., Corbel, M., Germain, N., Lagente, V. (1998) Effects of cannabinoid receptor ligands on LPS-induced pulmonary inflammation in mice. *Life Sci.*, **63**, PL125-9.
- [6] Cabral, G., Marciano-Cabral, F. (2005) Cannabinoid receptors in microglia of the central nervous system: immune functional relevance. *J. Leukoc. Biol.*, **78**, 1192-7.
- [7] Carlisle, S.J., Marciano-Cabral, F., Staab, A., Ludwick, C., Cabral, G.A. (2002) Differential expression of the CB2 cannabinoid receptor by rodent macrophages and macrophage-like cells in relation to cell activation. *Int. Immunopharmacol.*, **2**, 69-82.
- [8] Carrier, E., Kearn, C., Barkmeier, A., Breese, N., Yang, W., Nithipatikom, K., Pfister, S., Campbell, W., Hillard, C. (2004) Cultured rat microglial cells synthesize the endocannabinoid 2-arachidonyl-glycerol, which increases proliferation *via* a CB2 receptor-dependent mechanism. *Mol. Pharmacol.*, **65**, 999-1007.
- [9] Chuchawankul, S., Shima, M., Buckley, N.E., Hartmann, C.B., McCoy, K.L. (2004) Role of cannabinoid receptors in inhibiting macrophage costimulatory activity. *Int. Immunopharmacol.*, **4**, 265-78.
- [10] Clarkson, A.N., Rahman, R., Appleton, I. (2004) Inflammation and autoimmunity as a central theme in neurodegenerative disorders: fact or fiction? *Curr. Opin. Investig. Drugs*, **5**, 706-13.
- [11] Conti, S., Costa, B., Colleoni, M., Parolaro, D., Giagnoni, G. (2002) Anti-inflammatory action of endocannabinoid palmitoylethanolamide and the synthetic cannabinoid nabilone in a model of acute inflammation in the rat. *Br. J. Pharmacol.*, **135**, 181-7.
- [12] Dickason-Chesterfield, A.K., Kidd, S.R., Moore, S.A., Schaus, J.M., Liu, B., Nomikos, G.G., Felder, C.C. (2006) Pharmacological characterization of endocannabinoid transport and Fatty Acid amide hydrolase inhibitors. *Cell Mol. Neurobiol.*, **26**, 405-21.

- [13] Ehrhart, J., Obregon, D., Mori, T., Hou, H., Sun, N., Bai, Y., Klein, T., Fernandez, F., Tan, J., Shytle, R. (2005) Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. *J. Neuroinflamm.*, **2**, 29.
- [14] Eikelenboom, P., Veerhuis, R., Scheper, W., Rozeumuller, A.J., van Gool, W.A., Hoozemans, J.J. (2006) The significance of neuroinflammation in understanding Alzheimer's disease. *J. Neural. Trans.*, **113**, 1685-95.
- [15] Eljaschewitsch, E., Witting, A., Mawrin, C., Lee, T., Schmidt, P., Wolf, S., Hoertnagl, H., Raine, C., Schneider-Stock, R., Nitsch, R., Ullrich, O. (2006) The endocannabinoid anandamide protects neurons during CNS inflammation by induction of MKP-1 in microglial cells. *Neuron*, **49**, 67.
- [16] Facchinetti, F., Del Giudice, E., Furegato, S., Passarotto, M., Leon, A. (2003) Cannabinoids ablate release of TNFalpha in rat microglial cells stimulated with lipopolysaccharide. *Glia*, **41**, 161-8.
- [17] Felder, C.C., Joyce, K.E., Briley, E.M., Mansouri, J., Mackie, K., Blond, O., Lai, Y., Ma, A.L., Mitchell, R.L. (1995) Comparison of the pharmacology and signal transduction of the human cannabinoid CB1 and CB2 receptors. *Mol. Pharmacol.*, **48**, 443-50.
- [18] Fernandez-Lopez, D., Martinez-Orgado, J., Nunez, E., Romero, J., Lorenzo, P., Moro, M.A., Lizasoain, I. (2006) Characterization of the neuroprotective effect of the cannabinoid agonist WIN-55212 in an *in vitro* model of hypoxic-ischemic brain damage in newborn rats. *Pediatr. Res.*, **60**, 169-73.
- [19] Fernandez-Ruiz, J., Romero, J., Velasco, G., Tolon, R.M., Ramos, J.A., Guzman, M. (2006) Cannabinoid CB(2) receptor: a new target for controlling neural cell survival? *Trends Pharmacol. Sci.*, **30**, 30.
- [20] Franklin, A., Parmentier-Batteur, S., Walter, L., Greenberg, D.A., Stella, N. (2003) Palmitoylethanolamide increases after focal cerebral ischemia and potentiates microglial cell motility. *J. Neurosci.*, **23**, 7767-75.
- [21] Franklin, A., Stella, N. (2003) Arachidonylcyclopropylamide increases microglial cell migration through cannabinoid CB2 and abnormal-cannabinoid-sensitive receptors. *Eur. J. Pharmacol.*, **474**, 195-8.
- [22] Gardner, B., Zu, L.X., Sharma, S., Liu, Q., Makriyannis, A., Tashkin, D.P., Dubinett, S.M. (2002) Autocrine and paracrine regulation of lymphocyte CB2 receptor expression by TGF-beta. *Biochem. Biophys. Res. Commun.*, **290**, 91-6.
- [23] Glass, M., Northup, J.K. (1999) Agonist selective regulation of G proteins by cannabinoid CB(1) and CB(2) receptors. *Mol. Pharmacol.*, **56**, 1362-9.
- [24] Gong, J.P., Onaivi, E.S., Ishiguro, H., Liu, Q.R., Tagliaferro, P.A., Brusco, A., Uhl, G.R. (2006) Cannabinoid CB2 receptors: immunohistochemical localization in rat brain. *Brain Res.*, **1071**, 10-23.
- [25] Hanus, L., Breuer, A., Tchilibon, S., Shiloah, S., Goldenberg, D., Horowitz, M., Pertwee, R.G., Ross, R.A., Mechoulam, R., Fride, E. (1999) HU-308: a specific agonist for CB(2), a peripheral cannabinoid receptor. *Proc. Natl. Acad. Sci. USA*, **96**, 14228-33.
- [26] Hevner, R.F., Hodge, R.D., Daza, R.A., Englund, C. (2006) Transcription factors in glutamatergic neurogenesis: conserved programs in neocortex, cerebellum, and adult hippocampus. *Neurosci. Res.*, **55**, 223-33.
- [27] Honore, P., Mantyh, P.W. (2000) Bone cancer pain: from mechanism to model to therapy. *Pain Med.*, **1**, 303-9.
- [28] Huffman, J.W. (2005) CB2 receptor ligands. *Mini Rev. Med. Chem.*, **5**, 641-9.
- [29] Jin, K., Wang, X., Xie, L., Mao, X.O., Zhu, W., Wang, Y., Shen, J., Mao, Y., Banwait, S., Greenberg, D.A. (2006) Evidence for stroke-induced neurogenesis in the human brain. *Proc. Natl. Acad. Sci. USA*, **103**, 13198-202.
- [30] Jin, K., Xie, L., Kim, S.H., Parmentier-Batteur, S., Sun, Y., Mao, X.O., Childs, J., Greenberg, D.A. (2004) Defective adult neurogenesis in CB1 cannabinoid receptor knockout mice. *Mol. Pharmacol.*, **66**, 204-8.
- [31] Kishimoto, S., Muramatsu, M., Gokoh, M., Oka, S., Waku, K., Sugiura, T. (2005) Endogenous cannabinoid receptor ligand induces the migration of human natural killer cells. *J. Biochem. (Tokyo)*, **137**, 217-23.
- [32] Klegeris, A., Bissonnette, C., McGeer, P. (2003) Reduction of human monocyte cell neurotoxicity and cytokine secretion by ligands of the cannabinoid-type CB2 receptor. *Br. J. Pharmacol.*, **139**, 775-86.
- [33] La Rana, G., Russo, R., Campolongo, P., Bortolato, M., Mangieri, R.A., Cuomo, V., Iacono, A., Raso, G.M., Meli, R., Piomelli, D., Calignano, A. (2006) Modulation of neuropathic and inflammatory pain by the endocannabinoid transport inhibitor AM404 [N-(4-hydroxyphenyl)-eicos-5,8,11,14-tetraenamide]. *J. Pharmacol. Exp. Ther.*, **317**, 1365-71.
- [34] Landsman, R.S., Makriyannis, A., Deng, H., Consroe, P., Roeske, W.R., Yamamura, H.I. (1998) AM630 is an inverse agonist at the human cannabinoid CB1 receptor. *Life Sci.*, **62**, PL109-13.
- [35] Maresz, K., Carrier, E., Ponomarev, E., Hillard, C., Dittel, B. (2005) Modulation of the cannabinoid CB2 receptor in microglial cells in response to inflammatory stimuli. *J. Neurochem.*, **95**, 437-45.
- [36] Marsicano, G., Goodenough, S., Monory, K., Hermann, H., Eder, M., Cannich, A., Azad, S.C., Cascio, M.G., Gutierrez, S.O., van der Stelt, M., Lopez-Rodriguez, M.L., Casanova, E., Schutz, G., Ziegler, S., Berger, W., Di Marzo, V., Behl, C., Lutz, B. (2003) CB1 cannabinoid receptors and on-demand defense against excitotoxicity. *Science*, **302**, 84-8.
- [37] Marsicano, G., Moosmann, B., Hermann, H., Lutz, B., Behl, C. (2002) Neuroprotective properties of cannabinoids against oxidative stress: role of the cannabinoid receptor CB1. *J. Neurochem.*, **80**, 448-56.
- [38] Massa, F., Monory, K. (2006) Endocannabinoids and the gastrointestinal tract. *J. Endocrinol. Invest.*, **29**, 47-57.
- [39] McKallip, R.J., Lombard, C., Fisher, M., Martin, B.R., Ryu, S., Grant, S., Nagarkatti, P.S., Nagarkatti, M. (2002) Targeting CB2 cannabinoid receptors as a novel therapy to treat malignant lymphoblastic disease. *Blood*, **100**, 627-34.
- [40] McKallip, R.J., Lombard, C., Martin, B.R., Nagarkatti, M., Nagarkatti, P.S. (2002) Delta(9)-tetrahydrocannabinol-induced apoptosis in the thymus and spleen as a mechanism of immunosuppression *in vitro* and *in vivo*. *J. Pharmacol. Exp. Ther.*, **302**, 451-65.
- [41] Mechoulam, R., Lichtman, A.H. (2003) Neuroscience. Stout guards of the central nervous system. *Science*, **302**, 65-7.
- [42] Mestre, L., Correa, F., Docagne, F., Clemente, D., Ortega-Gutierrez, S., Arevalo-Martin, A., Molina-Holgado, E., Borrell, J., Guaza, C. (2006) Cannabinoid system and neuroinflammation: therapeutic perspectives in multiple sclerosis. *Rev. Neurol.*, **43**, 541-8.
- [43] Molina-Holgado, F., Pinteaux, E., Heenan, L., Moore, J.D., Rothwell, N.J., Gibson, R.M. (2005) Neuroprotective effects of the synthetic cannabinoid HU-210 in primary cortical neurons are mediated by phosphatidylinositol 3-kinase/AKT signalling. *Mol. Cell Neurosci.*, **28**, 189-94.
- [44] Mukhopadhyay, S., Das, S., Williams, E.A., Moore, D., Jones, J.D., Zahm, D.S., Ndegele, M.M., Lechner, A.J., Howlett, A.C. (2006) Lipopolysaccharide and cyclic AMP regulation of CB(2) cannabinoid receptor levels in rat brain and mouse RAW 264.7 macrophages. *J. Neuroimmunol.*, **181**, 82-92.
- [45] Munro, S., Thomas, K.L., Abu-Shaar, M. (1993) Molecular characterization of a peripheral receptor for cannabinoids. *Nature*, **365**, 61-5.
- [46] Murineddu, G., Ruii, S., Loriga, G., Manca, I., Lazzari, P., Reali, R., Pani, L., Toma, L., Pinna, G.A. (2005) Tricyclic pyrazoles. 3. Synthesis, biological evaluation, and molecular modeling of analogues of the cannabinoid antagonist 8-chloro-1-(2',4'-dichlorophenyl)-N-piperidin-1-yl-1,4,5,6-tetrahydrobenzo [6,7]cyclohepta[1,2-c]pyrazole-3-carbo-xamide. *J. Med. Chem.*, **48**, 7351-62.
- [47] Nilsson, O., Fowler, C.J., Jacobsson, S.O. (2006) The cannabinoid agonist WIN 55,212-2 inhibits TNF-alpha-induced neutrophil transmigration across ECV304 cells. *Eur. J. Pharmacol.*, **547**, 165-73.
- [48] Nunez, E., Benito, C., Pazos, M.R., Barbachano, A., Fajardo, O., Gonzalez, S., Tolon, R.M., Romero, J. (2004) Cannabinoid CB2 receptors are expressed by perivascular microglial cells in the human brain: an immunohistochemical study. *Synapse*, **53**, 208-13.
- [49] Ofek, O., Karsak, M., Leclerc, N., Fogel, M., Frenkel, B., Wright, K., Tam, J., Attar-Namdar, M., Kram, V., Shohami, E., Mechoulam, R., Zimmer, A., Bab, I. (2006) Peripheral cannabinoid receptor, CB2, regulates bone mass. *Proc. Natl. Acad. Sci. USA*, **103**, 696-701.
- [50] Onaivi, E.S., Ishiguro, H., Gong, J.P., Patel, S., Perchuk, A., Meozzi, P.A., Myers, L., Mora, Z., Tagliaferro, P., Gardner, E., Brusco, A., Akinshola, B.E., Liu, Q.R., Hope, B., Iwasaki, S., Arinami, T., Teasensitz, L., Uhl, G.R. (2006) Discovery of the presence and functional expression of cannabinoid CB2 receptors in brain. *Ann. N. Y. Acad. Sci.*, **1074**, 514-36.
- [51] Onaivi, E.S., Ishiguro, H., Sejal, P., Meozzi, P.A., Myers, L., Tagliaferro, P., Hope, B., Leonard, C.M., Uhl, G.R., Brusco, A., Gardner, E. (2006) Methods to study the behavioral effects and expression of CB2 cannabinoid receptor and its gene transcripts in the chronic mild stress model of depression. *Methods Mol. Med.*, **123**, 291-8.
- [52] Ortega-Gutierrez, S., Molina-Holgado, E., Guaza, C. (2005) Effect of anandamide uptake inhibition in the production of nitric oxide and in the release of cytokines in astrocyte cultures. *Glia*, **52**, 163-8.
- [53] Pacher, P., Batkai, S., Kunos, G. (2006) The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacol. Rev.*, **58**, 389-462.
- [54] Palazuelos, J., Aguado, T., Egia, A., Mechoulam, R., Guzman, M., Galve-Roperh, I. (2006) Non-psychoactive CB2 cannabinoid agonists stimulate neural progenitor proliferation. *FASEB J.*, **20**, 2405-7.
- [55] Panikashvili, D., Shein, N.A., Mechoulam, R., Trembovler, V., Kohen, R., Alexandrovich, A., Shohami, E. (2006) The endocannabinoid 2-AG protects the blood-brain barrier after closed head injury and inhibits mRNA expression of proinflammatory cytokines. *Neurobiol. Dis.*, **22**, 257-64.
- [56] Puffenberger, R.A., Boothe, A.C., Cabral, G.A. (2000) Cannabinoids inhibit LPS-inducible cytokine mRNA expression in rat microglial cells. *Glia*, **29**, 58-69.
- [57] Raduner, S., Majewska, A., Chen, J.Z., Xie, X.Q., Hamon, J., Faller, B., Altmann, K.H., Gertsch, J. (2006) Alkylamides from Echinacea are a new class of cannabinomimetics. Cannabinoid type 2 receptor-dependent and -independent immunomodulatory effects. *J. Biol. Chem.*, **281**, 14192-206.
- [58] Rahman, R.M.A., Nair, S.M., Appleton, I. (2005) Current and future pharmacological interventions for the acute treatment of ischaemic stroke. *Curr. Anaesth. Crit. Care*, **16**, 99-109.
- [59] Ramirez, B.G., Blazquez, C., Gomez del Pulgar, T., Guzman, M., de Ceballos, M.L. (2005) Prevention of Alzheimer's disease pathology by cannabinoids: neuroprotection mediated by blockade of microglial activation. *J. Neurosci.*, **25**, 1904-13.
- [60] Ronco, A.M., Llanos, M., Tamayo, D., Hirsch, S. (2006) Anandamide inhibits endothelin-1 production by human cultured endothelial cells: a new vascular action of this endocannabinoid. *Pharmacology*, **79**, 12-16.
- [61] Rosi, S., Pert, C.B., Ruff, M.R., McGann-Gramling, K., Wenk, G.L. (2005) Chemokine receptor 5 antagonist D-Ala-peptide T-amide reduces microglia and astrocyte activation within the hippocampus in a neuroinflammatory rat model of Alzheimer's disease. *Neuroscience*, **134**, 671-6.
- [62] Rosi, S., Ramirez-Amaya, V., Vazdarjanova, A., Worley, P.F., Barnes, C.A., Wenk, G.L. (2005) Neuroinflammation alters the hippocampal pattern of behaviorally induced Arc expression. *J. Neurosci.*, **25**, 723-31.
- [63] Ryu, J.K., Choi, H.B., McLarnon, J.G. (2006) Combined minocycline plus pyruvate treatment enhances effects of each agent to inhibit inflammation, oxidative damage, and neuronal loss in an excitotoxic animal model of Huntington's disease. *Neuroscience*, **141**, 1835-48.
- [64] Schwartz, M., Butovsky, O., Bruck, W., Hanisch, U.K. (2006) Microglial phenotype: is the commitment reversible? *Trends Neurosci.*, **29**, 68-74.
- [65] Schwartz, M., Shaked, I., Fisher, J., Mizrahi, T., Schori, H. (2003) Protective autoimmunity against the enemy within: fighting glutamate toxicity. *Trends Neurosci.*, **26**, 297-302.

- [66] Sheng, W.S., Hu, S., Min, X., Cabral, G.A., Lokensgard, J.R., Peterson, P.K. (2005) Synthetic cannabinoid WIN55,212-2 inhibits generation of inflammatory mediators by IL-1 β -stimulated human astrocytes. *Glia*, **49**, 211-9.
- [67] Shrikant, P., Lee, S.J., Kalvakolanu, I., Ransohoff, R.M., Benveniste, E.N. (1996) Stimulus-specific inhibition of intracellular adhesion molecule-1 gene expression by TGF- β . *J. Immunol.*, **157**, 892-900.
- [68] Skaper, S.D., Buriani, A., Dal Toso, R., Petrelli, L., Romanello, S., Facci, L., Leon, A. (1996) The ALIAmide palmitoylethanolamide and cannabinoids, but not anandamide, are protective in a delayed postglutamate paradigm of excitotoxic death in cerebellar granule neurons. *Proc. Natl. Acad. Sci. USA*, **93**, 3984-9.
- [69] Smith, P.F. (2004) GW-1000. GW Pharmaceuticals. *Curr. Opin. Investig. Drugs*, **5**, 748-54.
- [70] Smith, P.F. Will medicinal cannabinoids prove to be useful clinically? *Curr. Drug Ther.*, (In Press).
- [71] Stefano, G.B., Esch, T., Cadet, P., Zhu, W., Mantione, K., Benson, H. (2003) Endocannabinoids as autoregulatory signaling molecules: coupling to nitric oxide and a possible association with the relaxation response. *Med. Sci. Monit.*, **9**, RA63-75.
- [72] Steffens, S., Veillard, N.R., Arnaud, C., Pelli, G., Burger, F., Staub, C., Karsak, M., Zimmer, A., Frossard, J.L., Mach, F. (2005) Low dose oral cannabinoid therapy reduces progression of atherosclerosis in mice. *Nature*, **434**, 782-6.
- [73] Teixeira-Clerc, F., Julien, B., Grenard, P., Tran Van Nhieu, J., Deveaux, V., Li, L., Serriere-Lanneau, V., Ledent, C., Mallat, A., Lotersztajn, S. (2006) CB1 cannabinoid receptor antagonism: a new strategy for the treatment of liver fibrosis. *Nat. Med.*, **12**, 671-6.
- [74] Valenzano, K.J., Tafesse, L., Lee, G., Harrison, J.E., Boulet, J.M., Gottshall, S.L., Mark, L., Pearson, M.S., Miller, W., Shan, S., Rabadi, L., Rotshteyn, Y., Chaffer, S.M., Turchin, P.I., Elsemore, D.A., Toth, M., Koetzner, L., Whiteside, G.T. (2005) Pharmacological and pharmacokinetic characterization of the cannabinoid receptor 2 agonist, GW405833, utilizing rodent models of acute and chronic pain, anxiety, ataxia and catalepsy. *Neuropharmacology*, **48**, 658-72.
- [75] Van Sickle, M.D., Duncan, M., Kingsley, P.J., Mouihate, A., Urbani, P., Mackie, K., Stella, N., Makriyannis, A., Piomelli, D., Davison, J.S., Marnett, L.J., Di Marzo, V., Pittman, Q.J., Patel, K.D., Sharkey, K.A. (2005) Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science*, **310**, 329-32.
- [76] Waksman, Y., Olson, J.M., Carlisle, S.J., Cabral, G.A. (1999) The central cannabinoid receptor (CB1) mediates inhibition of nitric oxide production by rat microglial cells. *J. Pharmacol. Exp. Ther.*, **288**, 1357-66.
- [77] Walter, L., Franklin, A., Witting, A., Wade, C., Xie, Y., Kunos, G., Mackie, K., Stella, N. (2003) Nonpsychotropic cannabinoid receptors regulate microglial cell migration. *J. Neurosci.*, **23**, 1398-405.
- [78] Wei, B.Q., Mikkelsen, T.S., McKinney, M.K., Lander, E.S., Cravatt, B.F. (2006) A second fatty acid amide hydrolase with variable distribution among placental mammals. *J. Biol. Chem.*, **281**, 36569-78.
- [79] Yao, B.B., Mukherjee, S., Fan, Y., Garrison, T.R., Daza, A.V., Grayson, G.K., Hooker, B.A., Dart, M.J., Sullivan, J.P., Meyer, M.D. (2006) *In vitro* pharmacological characterization of AM1241: a protean agonist at the cannabinoid CB2 receptor? *Br. J. Pharmacol.*, **149**, 145-54.
- [80] Zhang, M., Martin, B.R., Adler, M.W., Razdan, R.K., Jallo, J.I., Tuma, R.F. (2007) Cannabinoid CB(2) receptor activation decreases cerebral infarction in a mouse focal ischemia/reperfusion model. *J. Cereb. Blood Flow Metab.*, **24**, 24.
- [81] Zipp, F., Aktas, O. The brain as a target of inflammation: common pathways link inflammatory and neurodegenerative diseases. *Trends Neurosci.*, **29**, 518-27.
- [82] Ziring, D., Wei, B., Velazquez, P., Schrage, M., Buckley, N.E., Braun, J. (2006) Formation of B and T cell subsets require the cannabinoid receptor CB2. *Immunogenetics*, **58**, 714-25.

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