The Development of Cannabinoid CBII Receptor Agonists for the Treatment of Central Neuropathies

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Abstract: Two cannabinoids receptors have been characterised in mammals; cannabinoid receptor type 1 (CBI) which is ubiquitous in the central nervous system (CNS), and cannabinoid receptor type 2 (CBII) that is expressed mainly in immune cells. Cannabinoids have been used in the treatment of nausea and emesis, anorexia and cachexia, tremor and pain associated with multiple sclerosis. These treatments are limited by the psychoactive side-effects of CBI activation. Recently CBII has been described within the CNS, both in microglia and neuronal progenitor cells (NPCs), but with few exceptions, not by neurons within the CNS. This has suggested that CBII agonists could have potential to treat various conditions without psycho-activity. This article reviews the potential for CBII agonists as treatments for neurological conditions, with a focus on microglia and NPCs as drug targets. We first discuss the role of microglia in the healthy brain, and then the role of microglia in chronic neuroinflammatory disorders, including Alzheimer's disease and Parkinson's disease, as well as in neuroinflammation following acute brain injury such as stroke and global hypoxia. As activation of CBII receptor on microglia results in suppression of the proliferation and activation of microglia, there is potential for the anti-inflammatory properties of CBII agonist to treat neuropathologies that involve heightened microglia activity. In addition, activating CBII receptors may result in an increase in proliferation and affect migration of NPCs. Therefore, it is possible that CBII agonists may assist in the treatment of neuropathologies by increasing neurogenesis. In the second part of the article, we review the state of development of CBII selective drugs with an emphasis on critical aspects of CBII agonist structural activity relationship (SAR).

Keywords: Cannabinoid, CBII receptor, microglia, neurodegeneration, neuroinflammation.

INTRODUCTION

Cannabinoids are a group of compounds that bind to the G protein coupled receptors cannabinoid receptor I (CBI) and cannabinoid receptor II (CBII) [1-3]. Cannabinoids have been used medicinally in the treatment of nausea and emesis, anorexia and cachexia, tremor and pain associated with multiple sclerosis (MS) [4]. Until recently, the CBI receptor was thought to be the only cannabinoid receptor expressed in the brain, and because CBI was found ubiquitously throughout the brain, it was widely agreed that CBI was responsible for the analgesic, neuroprotective and psycho-active effects of cannabinoids [5]. The CBII receptor had been detected in immune cells, but not reliably in any cells in the central nervous system (CNS) [1].

Recently, the CBII receptor has been detected in microglial cells in the brain following injury [6-8]. *In vitro* studies have demonstrated that microglia proliferate and migrate in response to cell damage, and then release inflammatory cytokines that cause further immune cell migration and activation [9]. This results in inflammation which leads to further damage in the surrounding penumbra [10].

Microglia-dependent inflammation has been shown to cause neuronal damage in acute brain injuries, such as

ischemic stroke and global hypoxia [11], as well as in chronic neurological disorders such as: Alzheimer's disease (AD) [12-15], Huntington's disease (HD) [16], Parkinson's disease (PD) [17], multiple sclerosis (MS) [18], major depression and schizophrenia [19], prion disease [20], and Down's syndrome (DS) [21].

Immune-suppressants including cyclosporin-A, methylprednisolone and tumor necrosis factor β (TNF-β) inhibitors have been shown to temporarily reduce neuronal damage during neuroinflammation by suppressing microglia activation [22]. However, negative side effects include neurological disruption, nephrotoxicity, arterial hypertension, reductions in clotting and pathogen susceptibility, which makes these drugs unsuitable as treatments for chronic or acute brain injury [22, 23]. Recently, evidence has accumulated that CBII receptor agonists may be potential alternatives to current immune-suppressants. Activation of the CBII receptor appears to block activation of microglial cells but has little effect on the normal functioning of neurons within the CNS [24].

In addition, CBII agonists may have effects on neurogenesis. Neurogenesis in the adult brain is upregulated following a neuropathological event [25]. Newly formed neurons have been shown to migrate to the site of neurological damage, some of which may become part of the surrounding circuitry [26]. CBII receptors have also been found on the surface of neuronal progenitor cells (NPCs), and CBII expression is similarly upregulated following neuropathologi-

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cal events [27]. The CBII receptor may have roles in modulating adult neurogenesis [8].

There is potential for the development of CBII agonists in the treatment of stroke patients, and chronic neurodegenerative disease sufferers. This review will address the role of the CBII in the CNS, specifically focusing on microglial function and pathology, modulation of microglia by the CBII receptor, and the role of CBII in modulating neurogenesis in the adult CNS.

CBII AND NEUROINFLAMMATION

Microglia and the Central Nervous System

Microglia are small glial cells in the CNS that are ubiquitous and abundant, making up 5-15% of all cells within the CNS [9]. Although still debated, it is commonly viewed that they are derived from mesodermal precursor cells of hematopoietic lineage, and that they enter the CNS during embryonic and early postnatal phases of development [28-30]. The primary role of microglia appears to be to modulate and participate in CNS immune responses [31]. Microglia can sense and respond to changes in the CNS micro-environment using fine processes that extend into the surrounding tissue [32, 33]. These processes are continuously retracting and expanding, sensing the micro-environment [32, 34]. The processes have swellings at the distal ends, suggesting that microglia may be constantly clearing debris in the cerebral environment [34]. When microglia recognize foreign particles, such as viral proteins or lipopolysaccharide (LPS) they become activated. Activation can also occur by signaling from interferon gamma (INF-γ) or proinflammatory cytokines, or changes in the extra-cellular environment as a result of cellular damage [13, 35-40].

Once activated, microglia modulate the CNS immune response via secretion of cytokines. These cytokines can be anti-inflammatory such as TGF-β1 and IL-10, which signal to reduce the activity of immune cells (including microglia) [2, 36, 41], or proinflammatory, such as TNF-β and IL-1B. These induce expression of adhesive molecules on both endothelial and systemic leukocyte cells, which allows these leukocytes to attach and migrate in the CNS [2, 36, 39, 41]. In addition, these cytokines also cause the activation, differentiation and proliferation of endemic and invading leukocytes [2, 36, 41]. This process results in inflammation which can be neuropathological.

Following activation, microglia increase expression of the major histocompatibility complex (MHCII), and can then act as antigen presenting cells to the infiltrating type one T helper cells (Th1) [33, 42-44]. This causes the Th1 cell to produce both INF-γ, which in turn causes the further activation and proliferation of microglia, and IL-2 which induces the activation, proliferation and differentiation of invading leukocytes [33].

Once activated, microglia also produce chemokines including IP-10, MIP-1, MIP-2, CCL19, MCP-1, and MCP-2 [45, 46], which induce the migration of leukocytes and other microglia, in a concentration dependent manner, to the site of damage/infection [45, 46]. This migration may also be mediated by endocannabinoids produced by microglia, astrocytes and neurons in the damage area [46-48].

The role of microglia in the CNS is a complicated one. The exact function and interaction is still being investigated; however, it is clear that microglia are crucial in initiating and propagating the CNS immune response to infection and tissue damage.

Neuroinflammation and Neuropathology

Under normal conditions microglia have important roles in removal of debris, small immune responses and neuronal remodeling, within the CNS [28, 30, 49]. However, microglia are also sensitive to changes in the microenvironment, and can quickly mediate a pathological inflammatory response when confronted with an activator stimuli, such as LPS or INF-γ [28]. During microglia-induced inflammation, the CNS microenvironment can become neurotoxic and result in extensive tissue damage. Several mechanisms involved in microglial neurotoxicity were investigated in a study by Bal-price and Brown [11]. In this study, co-cultures of microglia and neurons were exposed to microglia activating agents LPS and INF-γ, and levels of nitric oxide (NO) released and neuron respiration were measured. Upon activation the microglia produced large amounts of NO. Previous studies had shown that moderate levels of NO has antimicrobial and anti-tumor effects, as well as acting as a vasodilator and a neuromodulator, suggesting a possible biological function of microglial NO production [50]. However, this NO production can cause pathological levels of NO resulting in 45%-95% of co-cultured neurons died. Neuronal death was almost completely prevented when cultures were pretreated with an inducible nitric oxide synthase (iNOS) inhibitor. Without pretreatment, the levels of neuronal respiration dropped noticeably, due to NO reversibly inhibiting cytochrome-oxidase, causing depolarization of the neuron and subsequent glutamate release via GluT transport protein [51-53]. These high levels of glutamate cause excitotoxicity via the N-methyl-D-aspartate (NMDA) receptor, and is probably the reason for the neuronal death [52].

Activation of the NMDA receptor may also result in the activation of neuronal NOS enzymes (nNOS) in a calcium dependent manner, generating even more NO within the cell, possibly adding to the pathological effects of NO produced by activated microglia [11]. However, the role of nNOS in neurodegeneration is still unclear, with some studies showing a neuroprotective role [50]. Other sources of NO include surrounding astrocytes, NO production, via the iNOS enzyme, is induced by IL-1B and TNF-β produced by microglia during inflammation [53-55]. From this it is evident that microglia activation results in the production of NO though several mechanisms, and that this has a role in neurotoxicity.

In addition to being a neuromodulator, NO is also a reactive oxygen species (ROS), and can take part in redox reactions [56]. Furthermore, NO also causes the production of other members of the ROS family by a number of different mechanisms. NO inhibition of cytochrome-oxidase, affects the electron transport chain, causing electrons within the chain to react with other molecules in the mitochondria leading to the production of superoxide, which in turn can react with H_2O to form the ROS H_2O_2. NO can then react with H_2O_2 to produce peroxynitrite. These ROS can react with cellular components, severely disrupting cellular function

causing mutation, mitochondria breakdown leading to apoptosis, membrane disruption leading to depolarization, and protein disruption which can lead to necrosis [11]. To summarize this section, the NO produced by microglia can cause necrosis and apoptosis of neurons via both ROS related damage, as well as by NMDA mediated excitotoxicity.

Microglia and Neurodegenerative Diseases

Microglia have been implicated in having some pathological involvement in many neurological pathologies, including: AD [12-15], HD [16], PD [17], MS [30], major depression and schizophrenia [19], prion disease [20], and DS [21]. This section will address each pathology and evidence for microglia involvement.

Microglia and Alzheimer's Disease

Alzheimer's disease (AD) is a disease characterized by β-amyloid plaques and neurofibrillary tangles in the brain, cognitive decline and memory deficits [57]. The plaques are believed to be formed by the abnormal processing of a 42 amino acid peptide (β-amyloid peptide) which precipitates in the extra-cellular space, setting off several responses that lead to neuronal death [5, 58]. It has been shown that AD β-amyloid plaques are surrounded by activated microglia, and that microglia activation occurs before the onset of neuronal death [57, 59]. *In vivo* it has also been shown that by inhibiting microglia activity, with either minocycline or cannabinoids, during a β-amyloid peptide induced neurodegeneration model for AD, the neurodegeneration normally observed following β-amyloid treatment is significantly reduced [5, 13, 57, 60]. It can therefore be inferred that microglial activation has an important role in the neurodegeneration that characterizes AD.

Microglia and Huntington's Disease

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease, characterized by loss of spiny projection neurons in the striatum and loss of pyramidal neurons in most areas of the cortex [61]. This is related to an abnormal Huntington protein, which aggregates in the cytoplasm and nucleus of the affected cell [61]. The exact relationship between HD and microglia is not fully understood, and debate continues as to whether microglia are hyperactive or hypoactive in the HD brain. Ma *et al*. [62] used a mouse model of HD (R6/2) to determine that levels of microglia decline over time in a healthy brain, and that this decrease appears to be accelerated in the HD mice. This suggests that the hypoactivity of microglia could lead to a buildup of cellular debris, and a reduction in neuronal support and remodeling, resulting in the neurodegeneration observed in HD [62]. However, in contrast there are several studies that suggest that microglia are hyperactive and more prevalent in HD. For example, Sapp *et al*. [16] investigated human brains postmortem for activated microglia. They observed not only heightened levels of activated microglia, but the number of activated microglia was positively related to the severity of HD. A further study by Pavese *et al*. [63] used the radioactive ligand [11C](R)-PK11195, which binds to a microglia specific peripheral benzodiazepine binding site. Visualizing the ligand using positron emission tomography (PET) revealed that activated microglia are more prevalent in the HD patients compared with healthy controls, and that microglia activation is positively related to HD severity. The increased activity of microglia in human HD observed by Pavese *et al*. [63] and Sapp *et al*. [16] suggests that the microglia could be a contributing factor to the neurodegeneration observed in the HD brain, though the direction of causation remains to be determined.

The studies carried out by Sapp *et al*. [16] and Pavese *et al*. [63] demonstrate that the results obtained by Ma *et al*. [62] may be incorrect. Further evidence that supports the results of Sapp *et al*. [16] and Pavese *et al*. [63] are the many studies of other neurodegenerative diseases such as AD and PD, that have revealed hyperactivity of microglia in the affected areas within the human brain (see above). The likely reason for Ma *et al*. [62] differing results, is the limitation in the animal model that they used. As the R6/2 model does not model the microglial or other neuroinflammatory responses found in the human condition, this model should be used with caution as a general model of HD and should be avoided when studying neuroinflammatory aspects of the disease.

Microglia and Parkinson's Disease

Parkinson's disease (PD) is characterized by loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) [64]. This results in a loss of motor reward signaling in the basal ganglia. The clinical symptoms of this are stiffness, tremors and postural instability [64]. Although the exact etiology of PD is unknown, probable contributing factors include toxins, genetic factors, and trauma [65]. There is mounting evidence that activated microglia have a role in neurodegeneration in PD [66, 67]. An increased prevalence of MHCII positive microglia were observed in human PD SNpc [68, 69]. Furthermore, the relationship between the severity of PD and microglia activation was found to be strongly co-related [68, 69]. A study by Yasuda *et al*. [67] demonstrated that using 1-methyl-4phenyl-1,2,3,6-tetrahydro-pyridine induce PD-like symptoms in mice resulted in only transient symptoms. However, the same toxin in a primate model resulted in long term and degenerative PD symptoms. Yasuda *et al*. [67] noted that the microglia in the mouse model, did not persistently express MHCII, suggesting that continual expression of MHCII is required for degenerative PD. This demonstrates that microglial phenotype has an impact on the severity of the neurodegeneration. Supporting work by Wu *et al*. [66] showed that PD symptoms were less severe in the 1-methyl-4phenyl-1,2,3,6-tetrahydro-pyridine mouse model of PD, when the mice were pretreated with the microglia inhibitor minocycline. From these studies, it seems clear that microglia have a role in neurodegeneration in various animal models, and that microglia may have a similar role in neurodegeneration in PD.

Microglia and Multiple Sclerosis

Multiple sclerosis (MS) is a demyelinating disease, which causes debilitating symptoms which initially include extreme fatigue, weakness and numbness; then, as the disease progresses the symptoms become more severe including ataxia, spasms, and cognitive decline [45]. It is probable that the development of MS requires both genetic predisposition

and exposure to an antigen from a bacterial, viral, or environmental source, that will activate the immune system [70, 71]. The antigen will be structurally similar to a component of myelin, and this would cause the immune system to become sensitive to the presence of myelin, responding to it as a potential pathogen. This will result in the immune system attacking the myelin sheath that surrounds neuronal processes [70].

A considerable number of studies have now been carried out that suggest a microglial role in the progression of MS [18]. For example, Banati *et al.* [72] used a radiolabelled ligand and PET to visualize activated microglia in MS sufferers. This revealed that activated microglia were present in increased numbers in patients with MS, particularly in the spinal region of the CNS and that microglia activation was positively correlated with MS severity. In addition, Felts *et al.* [18] demonstrated that demyelination of spinal axons can occur in rats by consistent activation of microglia by LPS injection. Furthermore, work published by Zabad *et al.* [73] trialled the use of the microglial activation suppressant minocycline, in the treatment of MS in a clinical trial. During the trial, the development of gadolinium-enhancing lesions in the spinal tissues, were observed using MRI. The results of the trial showed that minocycline reduced the progression of these lesions and improved the prognosis of the MS sufferer. In addition, preliminary studies have shown that inhibition of microglial activity reduces disease progression in animal models of MS [30, 45, 74]. In conclusion, there appears to be evidence for microglia activity playing an integral part in the development of MS.

Microglia in Schizophrenia and Major Depression

Schizophrenia and major depression have only recently been linked to microglia activation. A recent study by Steiner *et al.* [19] investigated postmortem microglial numbers and activation, in both suicidal and schizophrenic patients. They found a significantly higher number of activated microglia in suicidal and schizophrenic dorsolateral prefrontal cortexes and hippocampi, compared to healthy controls. However, the direction of cause and effect underlying this correlation has yet to be elucidated. It is possible that increased microglia activation was due to the stress prior to suicide, or that microglia activation has an effect on the catecholaminergic neurotransmission and serotonin hormones within the brain. The latter seems plausible, considering that previous studies have shown two cytokines produced by microglia, IL-1B and IL-2, are implicated in catecholaminergic neurotransmission modulation. In addition, IL-2 has been implicated in clinical trial as a contributing factor to low levels of serotonin resulting in depression [75-77]. The relationship between microglia and depression, schizophrenia and suicide needs further research, and could possibly lead to microglia suppression drugs as a new treatment for these disorders.

Microglia and Prion Disease

Prion disease is a rare neurodegenerative disease, symptoms of which include rapid cognitive disruption, memory loss, personality changes, hallucinations, and speech impediment. Motor disruption also occurs including ataxia, irregular gait, rigid posture and seizures [78, 79]. The cause

of the neurodegeneration is linked to the presence of an aberrant prion protein (PrP^{sc}) [80]. The normal prion protein (PrP^{c}) is a copper binding protein and is likely to have a role as an antioxidant. PrP^{c} is present in many cells but in particularly in high concentration at neuronal synapses [20]. PrP^{sc} is resistant to degradation by proteases, which causes it to accumulate and aggregate into plaques and fibrils [81]. PrP^{sc} plaques have been shown to induce a considerable microglia response in animal models [78, 79].

Giese *et al.* [79] has also demonstrated that in mice infected with the prion disease scrapie, significant neuronal apoptosis only occurs following the microglial response. In addition, Brown *et al.* [78] demonstrated that microglia have a clear role in neuronal death in prion disease. Using an *in vitro* model, they showed that neuronal death only occurred in the presence of both a PrP^{sc} analogue and microglia and that this could be inhibited by strong enzymatic antioxidants. This suggests that NO released by microglia upon activation, and other oxidants induced by NO, are one of the causes of neuronal death in prion disease, clearly implicating a role for microglia in prion disease neurodegeneration.

Microglia and Down's Syndrome

Down's syndrome (DS) is a rare chromosomal disorder that causes a variety of symptoms [82], including neurological symptoms such as cognitive impairment and the early development of AD after the age of 40 [83]. Although microglia appear to have a role in the accumulation of β-amyloid in AD, it appears that in DS microglia activate before the development of β-amyloid plaques and may cause an upregulation in the amyloid protein [84]. Wierzba-Bobrowicz *et al.* [21] investigated microglial prevalence during the development of fetuses with DS, and observed a greater number of microglia in fetuses with DS, compared with healthy fetuses. This suggests that microglia may be a contributing factor in not only the development of AD in later life, but also in the early cognitive impairment of people with DS.

A study by Goldgaber *et al.* [85] investigated interleukin-1 and its effects on β-amyloid synthesis in endothelial cells, and observed an increase in β-amyloid precursor expression upon exposure to interleukin-1. This was later repeated in neurons by various research groups, with similar results [86, 87]. As interleukin-1 is produced by activated microglia, this process could be the mechanism by which the increased number of microglia in people with DS can lead to early onset AD. Furthermore, as β-amyloid protein induces microglia activation, this results in the production of increased levels of interleukin-1 by the activated microglia, which in turn causes endothelia cells and neurons to produce even more β-amyloid. Hence, there is potential for an ongoing increase in the microglia response, and progressively worsening pathology [86, 87].

To summarise this section, studies on microglia in both the early development of DS, and on AD development in the later stages of DS, have suggested that there are greater numbers of activated microglia in the brains of people with DS than in healthy brains. It is unclear why there is an initial increase in microglia prevalence in DS sufferers, but it is clear that microglia are a contributing factor of both cogni-

tive impairment and the development of AD in DS sufferers [82, 86, 87].

Microglial Involvement in Ischemic Stroke and Global Hypoxia

Ischemic stroke results in various cognitive and behavioral deficits, the precise nature of the deficits depending on location of the stroke. These deficits include losses in cognitive function, motor function, and personality changes [37, 88, 89]. Without blood supply, toxins and CO_2 build up in the tissues surrounding the occlusion, as tissues are starved of oxygen, glucose and other nutrients [89-91]. This leads to disruptions in protein synthesis and osmotic balance, resulting in release of excitotoxic levels of glutamate [90, 91]. This results in necrosis and apoptosis of neurons around the affected vessel, referred to as the infarct core [91, 92]. The tissue that surrounds the infarct core is referred to as the penumbra, and undergoes necrosis and apoptosis of neurons for days and weeks after the initial insult [92-95].

Microglia have been implicated in this delayed neurodegeneration within the penumbra [60, 92-95]. A study by Fan *et al.* [96] used a rat model of cerebral ischemia to investigate the effect of the inhibition of microglia by minocycline on penumbral damage, and found that microglia inhibition significantly reduced that size of the infarction, suggesting that microglia activation is critically involved in penumbral tissue damage. Microglial induced damage is probably due to iNOS expression which results in pathological levels of NO. During hypoxia the expression of iNOS is upregulated by several mechanisms; one major contributor is the small peptide hypoxia inducible factor-1α (HIF-1 α) [51, 97, 98]. Under normal conditions, oxygen reacts with the present HIF-1 α causing the HIF-1 α to become hydroxylated and acetylated, allowing HIF-1 α to bind to protein complexes that assist in degrading the HIF-1 α protein. The hydroxylation also prevents the binding of HIF-1α to DNA binding complex, preventing the transcription of HIF-1 α mediated genes, such as genes associated with the production of iNOS. During hypoxia oxygen is not present in high enough concentrations to effectively inhibit the binding of HIF-1 α to the DNA binding complex, allowing the expression of the HIF-1 α mediated genes [97, 98].

Global cerebral hypoxia can be caused by low levels of systemic oxygen, or by the blocking of carotid arteries [54, 99], and involves the same mechanisms of cellular damage as ischemic stroke. In humans global hypoxia can often occur during birth complications, and has been linked with attention deficient disorders [100]. Global hypoxia has been induced in animal models to model neuroinflammation, particularly in ischemic stroke induced inflammation [54, 91, 99]. Crucially, studies of ischemic stroke and global hypoxia demonstrate a clear link between microglia activity and the severity of the neuropathological insult.

CBII AND MICROGLIA

Neuroprotective Effects of CBII

Cannabinoids modulate nearly every aspect of the peripheral immune response, including antibody production by B-cells, T-cell proliferation, and macrophage phagocytosis [101-104]. The CBII receptor was first discovered in peripheral immune cells, and was proposed as the receptor responsible for the immuno-modulation properties of cannabinoids [102, 105]. This was confirmed in a study by Buckley *et al.* [106] used CBII knock-out mice. Specifically, the immune suppression caused by the cannabinoid Δ9-tetrahydrocannabinol (Δ9-THC, Fig. **1**) did not occur in the CBII knock-out mice.

Cannabinoid receptors are G protein-coupled receptors. Activation of the Gi/o by the CBII receptor results in the inhibition of adenylate-cyclase and the activation of mitogen activated protein kinase (MAPK), this results in the buildup of AMP and the phosphorylation of various secondary messengers which illicit the cellular responses observed [1, 107, 108]. Recently, the CBII receptor has been found to be expressed in certain cells within the CNS, including microglia [84, 109]. This was not entirely unexpected, as microglia are the resident immune cells of the CNS and derive from the same cellular lineage as peripheral immune cells [28-30].

Recent work has shown that microglia activation caused by various treatments can be suppressed by CBII activation [3-5, 110-116]. As CBII activation may suppress microglia proliferation and activation, CBII selective agonists may have potential therapeutic applications. For example, a study by Kelgeris *et al.* [117] demonstrated that activation of microglia by LPS could be inhibited by CBII activation. The authors of this study also showed that when the supernatant from the microglia cultures was administered to cultured neurons it was neurotoxic, but not following CB2 activation. In addition, a study by Fernandez-Lopez *et al.* [118] used *in vitro* brain slices to demonstrate a neuroprotective effect by CBII activation during oxygen and glucose deprivation. Finally, *in vivo* studies by Zhang *et al.* [24] have shown a neuroprotective effect from suppressing microglia activation in an ischemia model in rats, specifically showing that the size of the infarction caused by middle cerebral artery occlusion was significantly reduced in animals treated with CBII selective agonists. To conclude this section, it is likely that the microglia suppression caused by CBII agonists could be beneficial in any inflammation induced neurodegeneration or injury, including AD, HD, PD, MS, major depressive disorder, schizophrenia, prion disease, DS, global hypoxia and ischemic stroke.

CBII AND NEUROGENESIS

Sites of Neurogenesis

Neurogenesis is the generation of new functional neurons. It is now accepted that neurogenesis in the brain occurs throughout adult life [119-122]. The two confirmed regions of the brain that neurogenesis occurs are the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus within the hippocampus [119, 123, 124]. In the SVZ and SGZ, new neurons are derived from neural stem cells and possibly astrocytes and radial glia [119, 123]. Neurons produced in the SGZ migrate to the granular layer of the dentate gyrus, where they become granule cells and interneurons forming new synapses [121, 123]. Neurons produced in the SVZ migrate along the rostral migratory stream, integrating into the existing circuitry of the olfactory bulb [119]. Although, it is clear that neurons are produced in the SVZ

and SGZ, other possible sites including the neocortex, striatum, amygdala and substantia nigra, are debated as sites of adult neurogenesis [125].

Hippocampal Neurogenesis

Although thymine analogue staining has clearly demonstrated that neurons are produced in the SGZ and develop into mature neurons within the dentate gyrus granular layer [121, 126], it is unclear whether these new neurons are crucial to cognitive function [127]. Many of the newly formed neurons die within 1-3 weeks of migration [126]. However, a study by Sisti *et al.* [128] has shown that giving rats repeated learning and memory challenges increases the survival rate of the newly formed neurons. In addition, work by van Praag *et al.* [129] demonstrated that exercise in mice induces increased neurogenesis, which leads to improved performance in spatial learning and memory tasks. Further evidence for the importance of neurogenesis includes work by Bruel-Jungerman *et al.* [130] which showed an anti-mitotic agent impaired learning and memory in rats. In contrast, a study by Meshi *et al.* [127] demonstrated that learning and memory appeared to be unaffected by the inhibition of neurogenesis. These discrepancies could be due to the differences in the methods used to inhibit neurogenesis. Meshi *et al.* [127] used irradiation to specifically target the hippocampus, whereas Breul-Jungerman *et al.* [130] administered a systemic antimitotic agent. The latter could have dramatic effects on many physiological functions and make interpretation of the results difficult, whilst the former induces neuroinflammation which would have negative effects on many neurological processes. The overall body of evidence is in contrast to the work done by Meshi *et al.* [127], and suggests that adult neurogenesis is a highly regulated and crucial process, involved in memory [123, 131-133].

Sub-Ventricular Neurogenesis

Neurons produced in the SVZ migrate along the rostral migratory stream to the olfactory bulb (OB), where most become GABA producing granule cells [134]. Like new neurons produced in the SGZ, neurons produced in the SVZ often do not survive more than 28 days after migration. Survival of the newly formed neurons appears to depend on olfactory experience [121, 124]. Alonso *et al.* [135] investigated the effects of olfactory experience on NPCs survival in the mouse olfactory bulb, they found that challenging the study animal to discriminate between odors resulted in an increase in NPC survival. Similarly, Gheusi *et al.* [136] demonstrated that knockout mice lacking an adhesion protein required for rostral migratory stream migration had no loss in olfactory sensitivity. However, the ability to distinguish between two similar olfactory cues was impaired in the knockout mice. These studies suggest that SVZ neurogenesis is required for olfactory learning [124, 137].

The Link Between Neuroinflammation, Neurodegeneration and Neurogenesis

Neurogenesis in both the SGV and the SVZ is upregulated in response to both global hypoxia and ischemic stroke [121, 138, 139]. New neurons migrate to the infarcted region along blood vessels that are generated after CNS injury [26,

140]. Some studies have shown that implanted NPCs migrate towards the site of the infarction [26, 46-48]. A study by Belmadani *et al.* [141] showed that NPC migration and survival, is partially regulated by chemokines such as MCP-1 and IL-6. These chemokines are produced by microglia activated by ischemia. Another mechanism that guides the migration of NPCs was investigated by Wang *et al.* [142] who found that the interneurons that surround an infarction produce neurotrophins that guide NPCs to the site of neuronal damage.

The direction of change in levels of neurogenesis during neurodegeneration is debated. One theory proposes that neurogenesis is upregulated in a healing response to replace the neurons that die during the degeneration [121, 140]. Evidence for this has been provided by a study by Jin *et al.* [143] who investigated immature neurons in the brains of people who died with AD, compared with healthy controls. In this study, immunohistochemistry was use to identify immature and migrating neurons, and it was found that AD patients had increased levels of immature and migrating neurons compared to healthy controls. Similar results were found in a study by Curtis *et al.* [144] for people with HD. Using post mortem brains of people with HD, Curtis *et al.* [144] used proliferating cell nuclear antigen (PCNA) imaging to characterize the generation of new neuronal cells in the brain, and found that neurogenesis was higher in HD patients than health controls. The question of how neuronal damage can lead to an upregulation of neurogenesis was investigated by Zhu *et al.* [25], who used an ischemia rat model, that showed a seven fold upregulation of neurogenesis in the hippocampus, following ischemia. Zhu *et al.* [25] also found that neurogenesis correlated with an increased expression of iNOS within the hippocampus, and that administering an iNOS inhibitor after the ischemia could inhibit the increase in neurogenesis in the hippocampus. As iNOS is expressed in microglia during activation, and activated microglia are important players during ischemia, it is possible that neuronal damage leads to proliferation and activation of microglia, which then leads to NO generation from iNOS, which in turn induces neurogenesis, [28, 50, 92, 97, 132, 145].

An alternative theory proposes that neurogenesis is down-regulated during neuroinflammation, and that this adds to neurodegeneration. This was suggested by Ekdahl *et al.* [146] as the cause of the neurodegeneration suffered by recipients of radiation treatment. Specifically, the radiation is thought to induce a neuroinflammatory response, which in turn inhibits neurogenesis. Similarly, neuroinflammation induced by LPS causes a down-regulation of neurogenesis in the rat SGZ, and this is partially alleviated by the anti-inflammatory compound minocycline. This inhibition of neurogenesis is thought to be caused by increased production of interleukin-6 by microglia. A similar study by Monje *et al.* [147] used both irradiation and LPS to induce neuroinflammation. The combined treatments reduced neurogenesis in the SGZ, whereas inhibiting inflammation with indomethacin restored neurogenesis to normal levels. These studies conflict with the many human studies of neurodegenerative and neuroinflammatory conditions which show that neuroinflammation is associated with increased neurogenesis [132, 145]. These discrepancies are probably due to differ-

ences in the methods used to induce neuroinflammation. Furthermore, it is possible that intensity, duration, and location, of neuroinflammation could result in differing effects on neurogenesis.

The results of studies reviewed here, especially the human studies, provide strong evidence that neurogenesis is upregulated following insult; either by an acute ischemia or a more chronic pathology, and that new neurons migrate to the site(s) of damage. It is possible that some of these new neurons become functioning neurons. However, it remains unconfirmed whether these new neurons improve cognitive function [132, 145].

CBII Expression and Activation and Neurogenesis

Until recently, little was known about the role of the CBII receptor and neurogenesis. However, recent work by Palazuelos *et al.* [8] using RTPCR and immunohistochemistry has demonstrated that the CBII receptor is expressed by NPCs present in the dentate gyrus and has a role in regulating neurogenesis. Using neurosphere cell cultures Palazuelos *et al.* [8] showed that administering the CBII agonist HU-308 increased NPC proliferation. However, this proliferation was not seen in cultures of neurospheres that did not express the CBII receptor. Palazuelos *et al.* [8] also showed that CBII activation also induced migration of the neurosphere cells suggesting that endocannabinoids may be involved in signaling both the increase of neurogenesis and chemotaxic migration of NPCs.

Endocannabinoids are produced by both neurons and microglia [148, 149]. Neurons secrete endocannabinoids in response to NMDA activation, which can be caused by glutamate release due to microglial iNOS dependent production of NO [27, 148, 149]. Carrier *et al.* [148] also demonstrated that activated microglia produce endocannabinoids in culture. Conceivably endocannabinoid production by microglia

could act as a negative feedback signal to control microglial proliferation, and also as a signal to other cells such as NPCs [3, 14, 46, 115, 148-151].

To summarize, microglia that have been activated during a neuropathological insult may induce endocannabinoid production in neurons via an iNOS-NMDA mediated mechanism, and produce endocannabinoids themselves [51, 97, 148, 152, 153]. These endocannabinoids signal various types of cells to proliferate, including NPCs [27, 131, 138, 154]. Microglia and other surrounding cells then signal to NPCs to migrate towards the site of damage via cannabinoids, chemokines, neurotrophins and cytokines, [26, 46, 47, 155]. Here it is possible that the NPCs differentiate into functional neurons, replacing the neurons that had been lost during the neuropathological insult [155].

THE DEVELOPMENT OF CBII-SELECTIVE AGONISTS

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

Computer Models and CBII Ligand SAR

WIN55,212,2 (**3**) is a moderately CBII-selective agonist for human cannabinoid receptors. This property of WIN55,212,2 (**3**) has led to considerable research into CBII ligand selectivity. This is discussed in relation to the development of CBII-selective agonists further below. Cannabinoid receptors belong to the α-rhodopsin family of GPCRs,
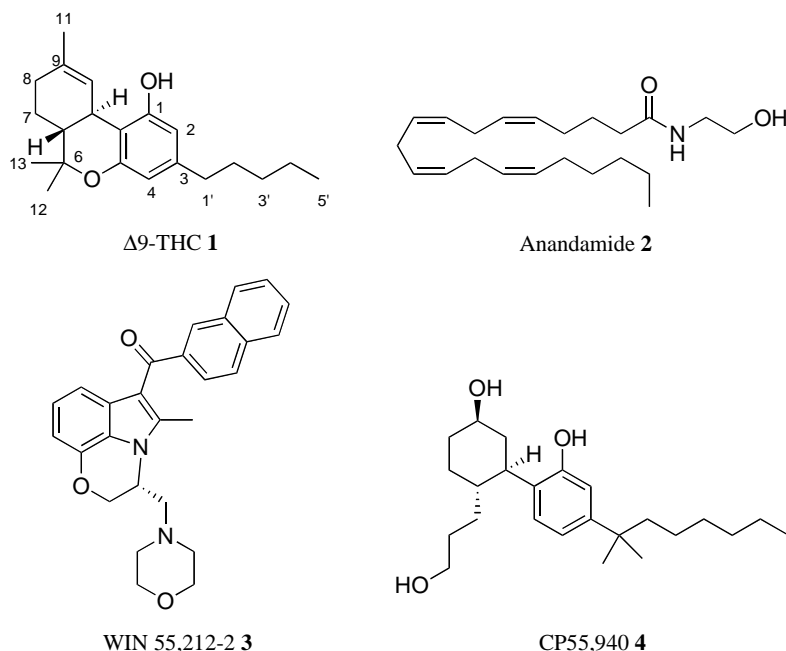

Fig. (1). Non-selective CBI/CBII receptor agonists.

and have the same basic structure [156]. All current understanding of the three-dimensional structure of mammalian GPCRs comes from X-ray crystallographic analysis of bovine rhodopsin [157], although the structure of the β_2 adrenergic receptor has just been published [158]. All GPCR models are based upon bovine rhodopsin. However, cannabinoid receptors share only 20-21% sequence identity with bovine rhodopsin. Despite this, modelling has contributed a great deal to current understanding of cannabinoid ligand-receptor interactions [159-165], including CBII [166-168], and have helped explain why some ligands are selective for one receptor over another [160, 166, 167, 169].

Automated docking to computer models is problematic for highly flexible ligands such as anandamid (**2**), but has been more successful with more rigid cannabinoids such as **3**. Both CBI and CBII have a central ligand binding pocket that is surrounded by the seven transmembrane helices. Several subdomains in CBI and CBII are important for ligand binding, with key residues in each of these subdomains spanning several of the transmembrane helices. Anandamide (**2**) consists of a long hydrophobic alkyl tail, and an ethanolamide head group. The lipophilic alkyl chain folds into a hydrophobic pocket involving transmembrane helix 2 (henceforth "TM2"), TM3, TM6, and TM7. By contrast, the alkyl tail of classical and bicyclic cannabinoids folds into a hydrophobic subdomain involving TM3, TM5, TM6, and TM7. Criticaly, hydrophobic interactions for **3** involve another (overlapping) subdomain spanning TM3, TM4, TM5, and TM6.

Many residues have been identified by mutation and binding studies as critical for cannabinoid SAR. However, we discuss only a small selection here (for reviews see [161, 170]. Mutation and binding studies have revealed that residue K3.28 in particular is critical for hydrogen bonding to anandamide (**2**), and to classical cannabinoids and their analogues, which share with endocannabinoids a lipophilic group along with groups able to form hydrogen bonds. Δ9-THC (**1**) is a fused ring compound that consists of a dihydropyran ring fused with a cyclohexene and a phenol ring. A lipophilic alkyl tail is attached to the phenol ring (position 3). Pfizer developed non-classical cannabinoids such as CP55,940 (**4**) by removing the middle dihydropyran ring of the classical cannabinoids, thus producing bicyclic analogues. These compounds were modified by the substitution of hydroxyl groups, designated the southern aliphatic hydroxyl (SAH) and the northern aliphatic hydroxyl (NAH). The most potent analogues contain the SAH. Up to a point, increasing the length of the alkyl tail increases potency, with a chain consisting of 7 carbons considered optimal [171].

CP55,940 (**4**) is considerably more potent than Δ9-THC (**1**) as an agonist at both CBI and CBII. When **4** enters the receptor binding site, the alkyl tail folds into the lipophilic binding subdomain and hydrogen bonds are formed at each of the three hydroxyl groups [159, 164, 165], with residue K3.28 considered critical in all current models. For instance, hypothesis postulates that the SAH interacts with K3.28, the phenolic hydroxyl with D6.58, and the NAH hydroxyl with K258 (E258 in the human receptor), which is located on the second extracellular loop (ECL2) [159].

Several rules have emerged from the study of classical cannabinoids and their analogues. First, increasing the length of the alkyl side chain increases receptor affinity up to a point. Second, hydrogen bonds are a critical feature of CBI agonism, particularly via residue K3.28. Loss of the phenolic hydroxyl group leads to a loss of CBI activity, but not activity at CBII. Third, configuration of the hydroxyl groups constrains the orientation of lipophilic groups with respect to hydrophobic binding pockets, and thereby modulates CBI activity. These three points correspond to the three-point interaction model for the activity of Δ9-THC (**1**). Interestingly, this predates the development of receptor models [172].
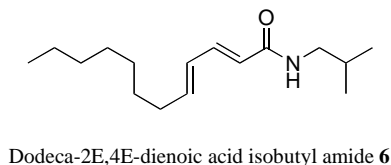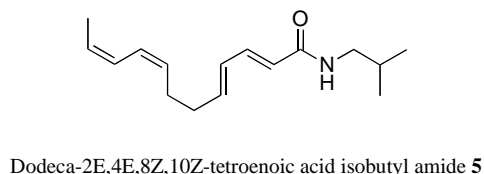
WIN55,212-2 (**3**) also binds into the transmembrane helix-bound hydrophobic pocket, but there are key differences in the way that it binds to CBI. Current evidence suggests that aromatic stacking in an aromatic subdomain is a critical feature of **3** binding to both CBI and CBII [159, 167, 169, 170, 173, 174]. In contrast to endocannabinoids, classical, and non-classical cannabinoids, **3** does not form hydrogen bonds with K3.28, and mutations at K3.28 do not cause a loss of activity for **3** at CBI [175]. However, replacing aromatic residues in the aromatic subdomain with alanine reduces binding [176]. These properties of **3** are starting points for the development of CBII selective agonists.

Huffman *et al.* [177] proposed that the naphthalene ring of **3** is an overlapping moiety with cyclohexene group in Δ9-THC (**1**), with similar π−π interactions during receptor binding. Loss of the phenolic hydroxyl group from Δ9-THC (**1**) results in a loss of activity at CBI but not CBII. Therefore, loss of hydrogen bonding at K3.28 and increased reliance on aromatic stacking has been a key strategy in developing CBII-selective agonists. Consistent with this, anandamide (**2**) may have WIN55,212-2 (**3**) like binding into the aromatic subdomain in CBII. Mutational studies have shown that hydrogen bonds at K3.28 are not important for anandamide (**2**) (or **4**) binding to CBII [175], and residues important for WIN55,212-2 (**3**) binding to CBII are also identified as important for anandamide (**2**) [170]. For instance, **2** may form hydrogen bonds with Y5.39 in CBII [178], and Xie *et al.* [168] postulated that this residue forms part of a distinct subdomain; an amphipathic cavity that consists of a hydrophobic and hydrophilic centre surrounded by aromatic residues. Raduner *et al.* [179] have modelled the binding of *N*-alkyl amides (**5**, **6**, Fig. **2**) to CBII using this theory. These compounds show CBII selective binding, and differ from endocannabinoids chiefly in that they lack a hydroxyl at the amide headgroup. Raduner *et al.* [179] proposed that the alkyl tails fold into the hydrophobic cleft across TM3,5,6,7 surrounded by aromatic residues and framed by the polar residues homologous to the CBI binding site for **3**. Y5.39 is thought to form a hydrogen bond with the amide oxygen and π-π interactions with the alkyl side chain. Loss of the hydroxyl head-group leads to decreased CBI activity due loss of hydrogen bonding to K3.28.

CBII-selective agonists such as JWH-015 (**7**) and AM1241 (**8**) [180], GW405833 (**9**) [181], and **3** have the potential to form hydrogen bonds with Y5.39. However, aromatic stacking rather than hydrogen bonding appears to be the key interaction at Y5.39 for binding CBII agonist

(excepting the *N*-alkyl amides, **5** and **6,** discussed above) [166, 167, 169], and the carbonyl oxygen in **3** and its CBII-selective analogues does not appear to form hydrogen bonds at Y5.39. However, Huffman *et al.* [177] have argued that the phenolic hydroxyl in Δ9-THC (**1**) is an overlapping moiety with the carbonyl oxygen in **3**, suggesting that the carbonyl oxygen of **3** forms hydrogen bonds with K3.28 in CBI. However, mutations at K3.28 do not reduce **3** binding to CBI. In addition, despite the importance of the carbonyl group of analogues of **3** for CBII-selectivity, modelling analysis suggests that it is not required for interaction with the aromatic subdomain [173].

Dodeca-2E,4E,8Z,10Z-tetroenoic acid isobutyl amide **5**

Dodeca-2E,4E-dienoic acid isobutyl amide **6**

Fig. (2). *N*-alkyl amides derived from *Echinacea* spp. With selective agonist activity at the CBII receptor.

One polar residue, S3.31 (G3.31 in CBI) appears to be a critical site for the formation of hydrogen bonds with CBII agonists. Chin *et al.* [182] postulated that S3.31 forms hydrogen bonds with the carbonyl oxygen of **3**. By contrast, Tuccinardi *et al.* [167] argued that the oxygen in the morpholine ring of **3** forms a hydrogen bond with S3.31. However, JWH-015 (**7**), an analogue of **3** without a morpholine ring [183], is more selective for CBII than is **3**. Therefore, the morpholine ring may be important for **3** binding to CBI but not to CBII. Tuccinardi *et al.* [167] concluded that CBII selectivity is chiefly determined by interactions with S3.31 and F5.46 (cognate residues are G3.31 and V5.46 in CBI), but through different chemical interactions. In contrast to S3.31, F5.46 appears to increase CBII activity by enhancing aromatic stacking [184].

G3.31S mutation in CBI enhances **3** binding to CBI, and S3.31G mutation in CBII severely reduces agonist activity. Tuccinardi *et al.* [167] speculated that CBII selectivity could be increased for ligands that form aromatic π-stacking interactions with F5.46 in CBII, and form a hydrogen bond with S3.31. Mutation of S3.31G results in a loss of affinity to CBII for non-selective cannabinoids such as Δ9-THC (**1**), CP55,940 (**4**), and anandamide (**2**) [175], and on the balance of evidence, it seems that S3.31 in CBII replaces K3.28 in CBI as the critical hydrogen bonding site. A similar change may occur from K3.28 to S3.31 (and Y5.39) for **2**.

Hydrogen bonding to K3.28 in CBI is a critical feature of CBII selective agonists, because CBII selectivity depends on maintaining affinity for CBII whilst losing affinity for CBI. Therefore, a key strategy for the development of CBII-selective agonist is to target moieties that form hydrogen

bonds with K3.28. For instance, JWH-133 (**10**) is a THC-analogue that acts as a CBII agonist with 200-fold selectivity for human CBII over rat CBI, and lacks a phenolic hydroxyl [170, 180]. Similarly, HU-308 (**11**) is a greater than 440-fold selective bicyclic CBII-agonist with a single hydroxyl group but that otherwise lacks hydrogen bond donors [185]. Conceivably the hydroxyl group of HU-380 binds to S3.31 in CBII, with the majority of interactions with the receptor being π-stacking interactions in the aromatic subdomain.

A limitation of computer modelling is that models are based on the structure of inactive bovine rhodopsin [186]. GPCRs, including cannabinoid receptors, undergo a conformational change upon bind by an agonist [187]. Therefore, current cannabinoid receptor models are only moderately useful for understanding binding of receptor agonists [169, 188]. However, the current consensus is that TM6 moves up and away from the receptor core, rotating in the counter clockwise direction, and that there is a small rotation of TM3, also in the counter clockwise direction [186, 189-191]. Ebersole *et al.* [192] postulated that the indole ring of 5-HT interacts with the aromatic subdomain of the 5-HT$_{2a}$ receptor, and thereby induces a change to the activated state. Given the importance of aromatic π-stacking for CBII agonism, aromatic stacking might be more readily induced to cause a change to the active conformation in CBII than in CBI. Consistent with this, Shim and Howlett [169] have recently suggested that aromatic stacking is important for both cannabinoid receptor ligand binding, and for the induction of conformational changes.

**CBII-Selective Agonists: State of the Play**

Compounds under investigation for CBII-selective agonism include indoles, classical cannabinoid analogues, cannabimimetic amides, pyrazoles, and a number of compounds that do not fall into any traditional class of cannabinoids. Each of these are discussed in turn below.

Indoles

The selectivity of WIN55,212-2 (**3**) for CBII led to the development of **7**, which is more selective for CBII than **3** [193] (Fig. **3**). This compound does not have a morpholine ring and has a shortened alkyl tail. JWH-046 is an example of a moderately CBII-selective (21-fold) derivative of **7** [172, 194]. At the same time that these series were being developed, the Merck Frosst group developed several indoles related to GW405833 (**9**), that had up to 146-fold selectivity for CBII [195]. AM1241 (**8**) is another (patented) analogue of **3** that is reportedly a selective agonist for CBII [196]. This compound has been described in [197] as possessing nearly 100-fold selectivity for CBII over CB1. In contrast, in [182] it has been described as having 538-fold selectivity. A similar compound, AM1221, is also selective for CBII (187-fold) [198].

Huffman & Padgett [199] have discussed various compounds that have been developed working from the hypothesis that cannabinoid indoles bind to CBI and CBII chiefly through aromatic stacking. In addition, Barth *et al.* [200] have described patented and CBII-selective indoles, and more recently, Pagé *et al.* [201] have reported the

development of CBII agonists that bind to CBII with greater than 350-fold selectivity for CBII.

JWH-015 **7**   AM1241 **8**

GW405833 **9**
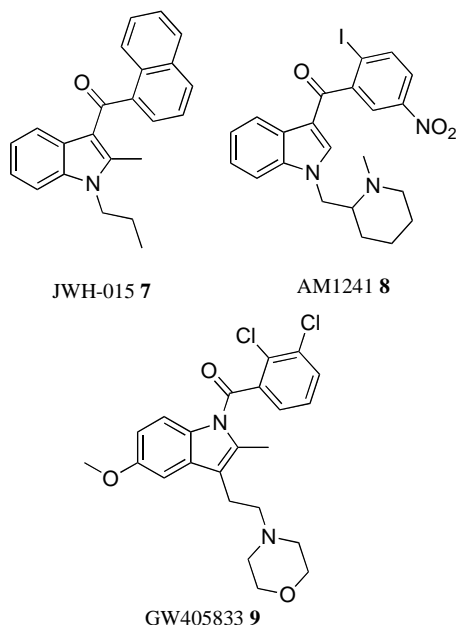
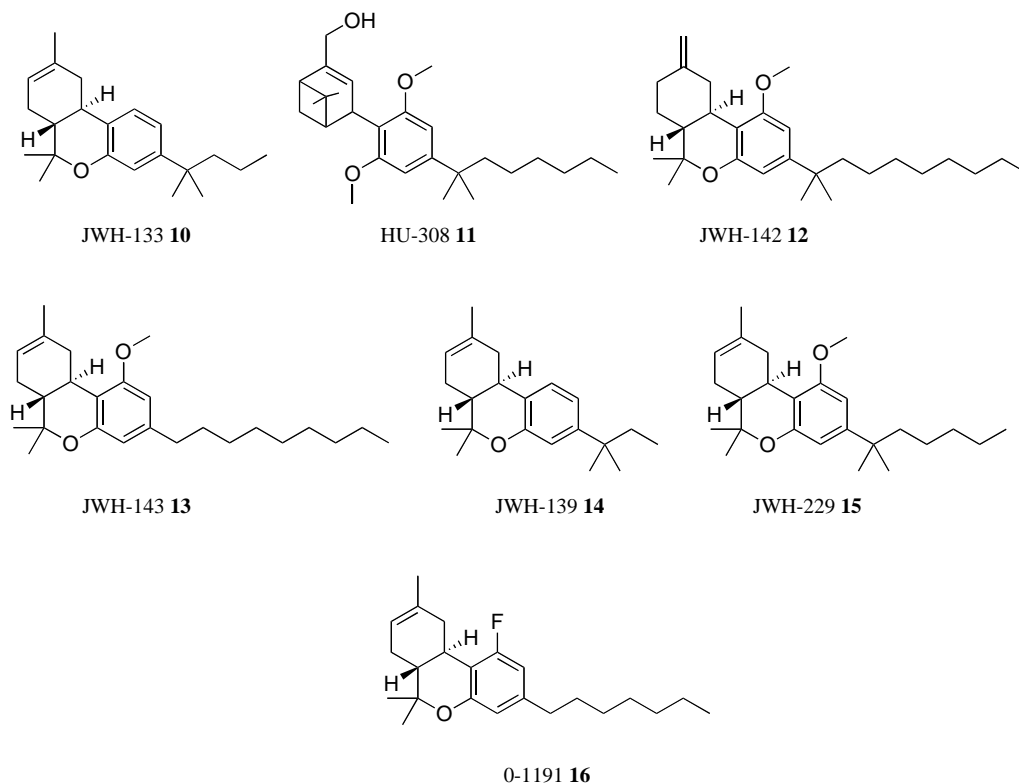**Fig. (3).** WIN55,212-2 analogues with selective agonist activity at the CBII receptor.

Classical Cannabinoids and Analogues

Derivatives of THC (**1**) (e.g., JWH-142, **12** and JWH-143, **13**) are moderately selective for CBII [202-205]. This

discovery led to the development of analogues with increase CBII-selectivity, such as JWH-139 (**14**) [180, 199, 203]. Related series of analogues [199, 206] are discussed by Huffman [180]. Notably, analogues, such as JWH-229 (**15**) are highly selective for mouse CBII over human CBI.

Compounds with very shortened alkyl tails, such as analogues related to **10**, have significant selectivity for CBII. JWH-133 (**10**) itself has nearly 200-fold selectivity for CBII [203]. Partly, this is because **10** lacks a C1 phenolic hydroxyl, and therefore probably does not bind to K3.28 in CBI. In general, replacing the phenolic hydroxyl with a methoxy group or a fluorine atom increases CBII-selectivity. For example, JWH-142 (L-759,656, **12**) and JWH-143 (L-759,633, **13**) both have C1 methoxy substituents and are potent CBII agonists, with 414-fold and over 163-fold selectivity over CBI respectively. Also, O-1191 (**16**) has a fluorine atom substituent, and has with 35-fold selectivity for CBII (Fig. **4**).

Adam *et al.* [207] describe a pyridone-based bicyclic cannabinoid that is moderately CBII-selective, and GlaxoSmithKlein have reported pyridine derivatives with more than 100-fold selectivity [207]. Shionogi & Co have also described pyridine analogues with CBII-selective agonism [208]. Astra Zeneca have developed a series of alkoxyaryl-benzimidazolecarboxamides, several of which have CBII-selectivity approaching 1000-fold, and show agonist activity [201]. Manera *et al.* [209, 210] have also reported that several 1,8-naphthyridin-4(1H)-on-3-carboxamide and quinolin-4(1H)-on-3-carboxamide derivatives bind selectively to CBII.

JWH-133 **10**   HU-308 **11**   JWH-142 **12**

JWH-143 **13**   JWH-139 **14**   JWH-229 **15**

O-1191 **16**

Fig. (4). Δ9-THC and Δ8-THC analogues with selective agonist activity at the CBII receptor.

HU-308 (**11**) is a potent CBII agonist, covered under patent for various clinical and experimental uses [211]., and has more than 440-fold selectivity for rat CBII [185]. HU-308 (**11**) is a bicyclic resorcinol derivative. Compounds in this series have a bicyclic ring system ortho to the phenolic hydroxyl that corresponds to the cyclohexene ring of **1**. HU-308 (**11**) also has a methoxy substituent replacing the hydroxyl group at C1, and another methoxy substuent in a meta position, as well as a hydroxymethyl substituent on the aliphatic ring, and a 7 carbon alkyl tail.

Replacing the dihydropyran ring in THC analogues with phenanthridine produces tricyclic cannabinoids termed octahydrophenanthridines with selectivity for CBII [212]. Replacing the C-3 alkyl side chain of classical cannabinoids with a cyclised side chain produces a series of compounds with considerable selectivity for CBII [213]. Lastly, heterocyclic cannabinoids with CBII selectivity have been patented by Kai *et al.* [214] and by Hanasaki *et al.* [215].

Cannabimimetic Amides

Jürg Gertsch and colleagues [216] described the development of cannabimimetic N-alkyl amides (**5**, **6**) with selectivity for CBII (30 to 50-fold) [216] that are natural constituents of *Echinacea angustifolia* and *E. purpura*. Gertsch *et al.* [216] have also published preliminary results showing that some N-benzyl amides have moderate affinity for CBII. Inaba *et al.* [217] have also reported that several N-substituted aromatic amides, cinnamides, and heterocyclic amides have high affinity binding to CBII.

Pyrazoles

SR141716 (**17**) and SR144528 (**18**) are tricyclic pyrazoles that are highly selective inverse agonists at CBI and CBII respectively Fig. (**5**). Huffman [180] has discussed the development of bridged pyrazoles that are highly selective for CBII. However, it was not reported whether any of the series have agonist activity.
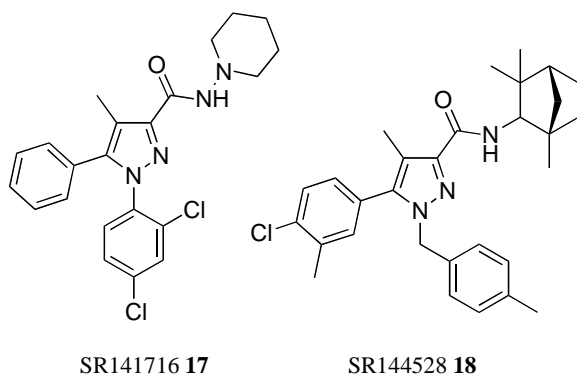
SR141716 **17**   SR144528 **18**

Fig. (5). Selective cannabinoid receptor antagonists.

Mussinu *et al.* [218] have described 1,4-dihydroindeno[1,2-c]pyrazol-based compounds which bind to both CBI and CBII, and Murineddu *et al.* [219] have developed analogues that are selective CBII. One compound has agonist activity *in vitro* and is 6029-fold selective for mouse CBII, over mouse CBI.

Miscellaneous CBII Agonists

Adam *et al.* [207] have described monocyclic CBII agonists, including a tetrazine derivative with 90-fold CBII-selectivity, and a 2-imino-1,3-thiazolidine derivative with more than 500-fold CBII-selectivity. Gonsiorek *et al.* [220] have described Sch35966 (**19**), a benzoquinolinone derivative with more than 450-fold selectivity for CBII over CBI Fig. (**6**). They reported that the compound is a more potent agonist at CBII than **4** for both rodent and primate receptors. Schering Corporation have also patented several series of piperidine [221], indanesulfonamide [222], N-(α-methyl-benzyl, and heteroarene [223] derivatives [224] with CBII-selective activity.
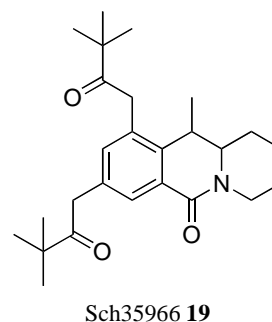
Sch35966 **19**

Fig. (6). Sch35966, a potent and selective CBII agonist in both rodents and primates.

DISCUSSION: POTENTIAL THERAPEUTIC USES OF CBII AGONISTS IN BRAIN PATHOLOGIES

CBII and Neuroinflammation

Microglia are crucial for the normal functioning of the healthy brain by responding to small responses to changes in the CNS micro-environment, clearing debris, and carrying out other metabolic functions. Microglia can also, induce neuroinflammation by initiating immune cell migration and proliferation through the production of cytokines, growth factors, and chemokines. Microglia also produce NO via iNOS and this is implicated in inhibiting mitochondrial function in the surrounding neurons, which causes neuronal depolarization and the release of excitotoxic levels of glutamate. This cascade of events has been implicated in many pathologies including acute injuries caused by hypoxia and ischemic stroke, as well as by chronic neurological dysfunctions such as AD, HD, PD, MS, major depression, schizophrenia, prion disease, and DS. Upon activation microglia increase expression of the CBII receptor, and various lines of evidence have suggested that activation of this receptor results in inhibition of microglial proliferation and activation, and prevents production of proinflammatory factors and NO. This results in reduced inflammation and neuronal damage. Therefore there is potential for CBII modulators as treatments for a variety of neuropathologies.

CBII and Neurogenesis

NPCs produced in both the dentate gyrus SGZ and the SVZ have been shown to express the CBII receptor. Under normal conditions newly formed neurons migrate to either the olfactory bulb or the SGZ of the dentate gyrus. During

neuropathological insult proliferation of NPCs is up to seven fold greater than those in healthy brains. NPCs also migrate to sites of neurological damage. Both proliferation and chemotaxic signals may involve activation of the CBII receptor by endocannabinoids in combination with the actions of chemokines, cytokines, and neurotrophic factors produced by interneurons and microglia. It is possible that once the NPCs reach the site of neuronal damage they differentiate into functional neurons, reducing the cognitive impact of the neurological damage. Therefore, CBII agonists may have the potential to treat neuropathologies not only by preventing damage caused by neuroinflammation, but perhaps also by stimulating the regeneration of function through neurogenesis.

Outstanding Questions with Respect to CBII Function in the Pathological CNS

Elucidating the role of the CBII receptor in the CNS has been challenging. Discrepancies between studies have occurred due to the complexity of the system, differences between *in vivo* and *in vitro* results, and the differences in animal models and the human system. This is particularly evident in the animal models of neurodegenerative conditions and the role microglia have in these conditions, as well as the role CBII have in these microglia. Many studies on CBII agonist's role in reducing neuroinflammation have administered agonists before or directly after initiating neuroinflammation [30, 116]. Furthermore, these studies often only observed the adverse effects of the neuroinflammation for a short period.

Before CBII agonist can been seen as having potential medical importance, several interactions need to be investigated. First, the window of effectiveness is largely undetermined, i.e., for how long after a neuroinflammatory insult application of a CBII agonist may be effective as a treatment. This is a particularly important consideration for treatment of acute injuries, for example human stroke, where preadministration of a CBII agonist is impossible, and diagnosis and treatment may not occur for several hours after the stroke. The long term effects of CBII on neuroinflammation induced neuronal death also requires investigation. It is currently unknown where administering of a CBII agonist merely delays the damage that occurs during neuroinflammation.

The involvement of the CBII receptor in neurogenesis is a very new field. Future studies could fruitfully focus on how CBII induced neurogenesis either delays neurodegeneration or helps cognitive recovery from a neuropathology. The difficulty lies in distinguishing the neuroprotective/anti-inflammatory properties of CBII agonists and the possible positive effects that CBII agonists have on neurogenesis. For this reason animal models which involve long term neuroinflammation cannot be used in investigation of neurogenesis and CBII.

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