

Review Article

The Central Role of Glia in Pathological Pain and the Potential of Targeting the Cannabinoid 2 Receptor for Pain Relief

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Under normal conditions, acute pain processing consists of well-characterized neuronal signaling events. When dysfunctional pain signaling occurs, pathological pain ensues. Glial activation and their released factors participate in the mediation of pathological pain. The use of cannabinoid compounds for pain relief is currently an area of great interest for both basic scientists and physicians. These compounds, bind mainly either the cannabinoid receptor subtype 1 (CB₁R) or cannabinoid receptor subtype 2 (CB₂R) and are able to modulate pain. Although cannabinoids were initially only thought to modulate pain via neuronal mechanisms within the central nervous system, strong evidence now supports that CB₂R cannabinoid compounds are capable of modulating glia, (e.g. astrocytes and microglia) for pain relief. However, the mechanisms underlying cannabinoid receptor-mediated pain relief remain largely unknown. An emerging body of evidence supports that CB₂R agonist compounds may prove to be powerful novel therapeutic candidates for the treatment of chronic pain.

1. Introduction

Chronic pathological pain is one of the most common reasons to seek medical attention and is a worldwide epidemic [1]. Chronic pain becomes pathological as a consequence of abnormal pain signaling and is often manifested in numerous diseases, such as diabetes, arthritis, amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), and cancer [2–6]. Glial cells, which include oligodendrocytes, astrocytes, and microglia, have been found to play key roles when chronic pain becomes pathological. Given less is known about the involvement of oligodendrocytes, this paper will focus primarily on astrocytes and microglial cells in chronic pain processing.

Cannabinoid compounds are emerging as novel therapeutic targets for the treatment of chronic neuropathic pain [7]. These compounds, with subsequent CB₁ and CB₂ receptor (CB₁R and CB₂R, resp.) activation, are able to modulate pain through a number of mechanisms including microglial mechanisms [8]. This paper will first discuss how normal pain becomes pathological and the role of activated glia in mediating such pain. These sections will be followed

by addressing cannabinoid-mediated modulation of glial proinflammatory factors, which are known to produce chronic neuropathic pain in animal models. An emphasis will be made on the CB₂R. Given that this paper focuses on the action of the CB₂R, a discussion is included on the current states of clinical trials examining the potential efficacy of CB₂R agonists as pain therapeutics.

2. Normal versus Pathological Pain

2.1. Acute Pain Signaling. Acute pain processing is distinct from the etiology underlying chronic pathological pain. Distinguishing the cellular responses and underlying signaling cascades that are unique to pathological pain may prove critical in understanding why many neuronally targeted treatments do not prove to be effective in relieving chronic pathological pain in the clinical setting. In acute pain, such as that caused by high intensity stimuli from mechanical stimulation (e.g., pinprick), unmyelinated C and lightly myelinated A δ nociceptive nerve fiber terminals in the body depolarize and transduce this information into action

potentials that travel through the peripheral axon to the dorsal root ganglia (DRG). The centrally projecting terminals of these nociceptors predominantly enter the spinal cord dorsal horn to reach the superficial (laminae I-II) and deeper lamina IV-V and synapse onto second order pain projection neurons located in lamina I, IV, and V [9–11]. The classical neurotransmitter primarily responsible for synaptic communication between nociceptors and pain projection neurons is the excitatory amino acid glutamate. Glutamate then binds and activates the ionotropic α -amino-3-hydroxyl-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate receptors as well as metabotropic glutamate receptors (mGluR 1, 3, 5, and 7) [12]. Additionally, a number of nociceptive-related neuropeptides acting in the spinal cord dorsal horn have been identified to play key roles in pain neurotransmission. For example, the classic neuropeptide, substance P, is released from primary nociceptive afferents [13]. Substance P then binds and activates its receptor, neurokinin 1 (NK1), which is present in high concentrations on dorsal horn lamina I neurons. Both substance P and its NK1 receptor are widely known to play a significant role in nociceptive processing [14]. These spinal cord nociceptive neurotransmitters, along with their receptors, are critical for activating second-order neurons, which communicate to supraspinal pain-processing centers and elicit reflexive and protective responses to avoid potential or further tissue damage.

2.2. Central Sensitization. However, under some circumstances, incoming nociceptive signaling is prolonged leading to clinical manifestations of pathological neuronal signaling. Examples of such pathological states are hyperalgesia, which is decreased threshold to nociceptive stimuli, and dynamic tactile allodynia, which is increased sensitivity to nonnociceptive light touch. Both pain states often occur in regions beyond the tissue-injured site. The underlying neurobiological events initiated by prolonged nociceptive signaling include increased synaptic function triggered within the central nervous system. Specifically, these events are known to occur within the dorsal horn of the spinal cord and culminate in a process termed *spinal sensitization* of pain projection neurons [15, 16]. Once triggered, this central sensitization is sustained despite the termination of noxious input. Experimentally, continued activity is substantially extended following the end of the stimulus application [17, 18]. These seminal early studies suggested that pain may be experienced even in the absence of peripheral noxious stimuli.

Pathological pain results from inflammation and/or trauma to peripheral nerve(s), tissue(s), or the central nervous system (CNS) and may arise as a complication to numerous medical conditions. Various animal models have been developed to induce conditions similar to those observed clinically. Neuropathic pain is commonly studied in models of peripheral nerve injury/inflammation. Models of diabetic neuropathy, chemotherapy-induced pain, post-surgical pain, and osteoarthritis pain are well-established examples, and reports of these are cited throughout this

paper. Although distinct in disease etiology, peripheral neuropathies share in the manifestation of pathological pain. This pathological processing is initially triggered by incoming noxious signals from nociceptors leading to central sensitization. One classically known mechanism for spinal sensitization involves excitation of pain projection neurons in the superficial laminae of the spinal cord dorsal horn as well as wide dynamic range neurons (WDR) located in deeper lamina IV and V that process the rapid and intense nerve depolarizations. Following prolonged and significant depolarization by the actions of glutamate and substance P, spinal pain projection neurons become sensitized, leading to the activation of N-Methyl-D-Aspartic Acid (NMDA) receptors that are normally inactive due to a Mg^{2+} plug within the cation channel. Prolonged depolarization induces Mg^{2+} release followed by enhanced influx of Ca^{2+} [19, 20]. A cascade of intracellular events occurs, which ultimately leads to postsynaptic enhancement of AMPA and mGlu receptor action, thereby increasing synaptic efficacy [21].

2.3. Sensory Changes in Pathological Pain. Increasing synaptic efficacy exerts profound changes in dorsal horn sensory processing [16, 22]. Indeed, enhanced synaptic efficacy, initiated by low intensity mechanosensitive $A\beta$ fibers, occurs at synapses on pain projection neurons in the dorsal horn [23], creating the perceptual equivalent of a noxious stimulus. Activated low-intensity $A\beta$ fibers, that carry nonpainful information such as light touch, are now capable of activating high intensity nociceptive neurons resulting in the clinical phenomenon known as allodynia. That is, nonpainful light touch is coded as painful, leading to a pain sensation that occurs in the absence of noxious input. Despite the fact that the stimulus is initiated in the periphery, its manifestation is a consequence of central changes like sensitization in the spinal cord [15]. Both allodynia and hyperalgesia are a hallmark of pathological pain [15, 16].

3. The Role of Glia in Pathological Pain

3.1. Glial Activation. While it is clear that neuronal processes are critical for spinal sensitization leading to pathological pain signaling, nonneuronal glial mechanisms are also important [24]. Under persistent pathological conditions, the availability of neuropeptides, such as substance P and amino acid neurotransmitters like glutamate is increased and able to bind their receptors not only on neurons, but also on astrocytes as well as parenchymal and perivascular microglia. Glial “activation” ensues and sets in motion a cascade of excitatory signaling events [25].

At the onset and during pathological pain conditions, multiple signaling cascades within glia are triggered including the activation of p38 mitogen-activated protein kinase (p-p38MAPK) and the c-Jun N-terminal kinase (JNK) pathways via phosphorylation events. Consequently, downstream cascades are initiated, including NF- κB activation, a cytokine nuclear transcription factor, and lead to the subsequent production of proinflammatory cytokines such

as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α), as well as chemokines (chemoattractant cytokines) [25–30]. It is important to note that multiple signaling pathways can activate NF- κ B leading to altered gene expression. For example, glially released TNF- α , when bound to its receptor, leads to phosphorylation of p38MAPK (p-p38MAPK) and NF- κ B activation. Alternatively, IL-1 β , when bound to its receptor, can directly activate NF- κ B [31, 32]. In the spinal cord, IL-1 β and TNF- α can further directly excite neurons because neurons express receptors for these cytokines. Indirect neuronal stimulation occurs by cytokine-induced release of additional excitatory mediators such as prostaglandins and nitric oxide (NO). It has been reported that spinal p38MAPK, JNK, and the extracellular signal-regulated kinase, ERK1/2, also referred to as MAPK3/1, are critical mediators of pathological pain in animal models [33–37]. For example, sciatic nerve ligation (SNL), a well-characterized rodent model of peripheral nerve injury, leads to increased p38MAPK in spinal astrocytes and microglia, and upon spinal pharmacological blockade with the p-p38MAPK inhibitor SB203580, p38MAPK activation with associated neuropathic pain is diminished [34]. Although very little is known about oligodendrocyte signaling in chronic pain, emerging evidence suggests that these cells are not merely passive observers to chronic pain, but rather these cells may also upregulate (phosphorylated) p-AKT, a factor that has been found to mediate apoptosis, cell migration, and motility in the dorsal horn of the spinal cord. Phosphorylated-AKT may be critical to the previously discussed spinal cord neuronal sensitization process [38].

It is notable that activated microglia respond to and produce inducible nitric oxide synthase (iNOS), and likewise activated astrocytes release NO [39, 40]. The production of NO by both neurons and glia is characteristic of neuroinflammation [41–44]. Thus, upon spinal glial activation from NO (among several other activating factors), intracellular signaling cascades lead to increases in cytokines and diffusible factors that *further* activate neighboring neurons and glia. That is, IL-1 β and TNF- α lead to a feed-forward loop of further JNK and MAPK signaling, NF- κ B activation, and increased NO, cytokine, and chemokine production, which all contribute to ongoing pathological pain.

While microglia and immune-like astrocytes respond to spinal IL-1 β and TNF- α resulting in pathological pain, increased peripheral immune cell (neutrophils, lymphocytes, monocytes, macrophages) migration to critical regions of nociceptive processing, such as the DRG and the spinal cord dorsal horn also occurs in response to cytokines [39, 45–47]. The specific underlying mechanisms are poorly understood. What is known, however, is that cellular enrichment at these critically important anatomical sites takes place via increased immune cell actin remodeling and proliferation in response to chemotactic signaling [46, 48, 49].

3.2. Glial Morphology and Activation Markers. Activated glial cells typically undergo changes in morphology, proliferation, and migration, termed gliosis. For example, astrocytes upregulate vimentin and glial fibrillary acidic

protein (GFAP) and become highly arborized with thickened processes [50]. These changes in morphology and increased GFAP expression are often considered a sign of spinal cord pathogenesis during the expression of neuropathic pain in animal models and are thought to be indicative of CNS inflammatory processes [26, 50, 51]. A report examining cellular enrichment of the spinal cord in a peripheral nerve injury rat model of pain identified that microglial cells are more proliferative and undergo more clustering than astrocytes [52]. Microglia, when activated, typically upregulate the cellular makers, ionized calcium binding adaptor molecule-1 (Iba-1), and CD11b/c, also known as OX42 [52–57]. However, the upregulation of these proteins is not always indicative of proinflammatory phenotypic processes of glial cells. For example, activated microglia can additionally express ED2, a classic anti-inflammatory marker, suggesting that activated microglia are not solely engaged in proinflammatory processes [58, 59].

Although these cellular changes have been widely documented in animal models of chronic pain, less is known about whether glial activation always reflects inflammation and whether it contributes to chronic pain in humans. What is known is that gliosis occurs within the spinal cord of patients with neuroimmune diseases such as ALS, MS, and spodylotic myopathy [60, 61]. It is noteworthy that these patients often report chronic pain symptoms [4, 6]. Furthermore, postmortem tissue analysis from these patients often reveals gliosis concomitant with the disease, and as such, these glial changes may contribute to chronic pain in these patients. However, the role of these cellular markers in animal models of chronic pain are not fully understood, as reports show a disconnection between glial marker upregulation, proinflammatory signaling markers, and behavior associated with pain. For example, while fluorocitrate attenuated upregulation of GFAP in mice with the chronic constriction injury of the sciatic nerve (CCI), another commonly used model of chronic neuropathic pain, chronic pain symptoms remained unchanged [55]. Additionally, in separate studies utilizing a paw incision model of postsurgical pain, chronic morphine administered subcutaneously delayed the normal resolution of allodynia and hyperalgesia, which was observed with saline-injected controls. Tissues from the corresponding groups in this study were analyzed for GFAP, Iba-1, p-ERK, and p-p38MAPK, with saline-injected animals showing clear behavioral resolution, which was absent in the morphine-treated groups. Strikingly, no differences in GFAP or Iba-1 immunoreactivity were observed between saline- or chronic morphine-treated groups. However, p-ERK and p-38MAPK were increased in the chronic morphine-treated groups, corresponding to their behavioral profile [62]. Conversely, perivascular microglia have been shown to remain in an activated state as assessed by immunohistochemical detection of ED2 during the presence of pain reversal [63]. From these studies, and as noted previously, the presence or absence of glial activation, per se, is too simplistic to fully understand a glial role in chronic pain. It is possible that microglia can remain activated while producing and releasing anti-inflammatory factors that ultimately lead to pain suppression [64, 65].

3.3. Downstream Glial Signaling of Cytokines. Both IL-1 β and TNF- α induce chemotactic activity on CNS microglia and astrocytes. Indeed, once activated, microglia and astrocytes are well known to undergo migration and proliferation in the spinal cord under conditions of chronic pain [52]. Recently, it has been shown that an increase in glial cell numbers occurs within the ipsilateral dorsal horn of the spinal cord following unilateral peripheral nerve injury [66].

During pathological pain states, peripheral immune cells additionally migrate to critical CNS pain processing sites. However, the contribution of peripheral versus CNS immune cell actions with subsequent cytokine signaling to neuropathic pain is not fully understood. Rat spinal cord meninges contain peripheral immunocompetent cells such as macrophages, and following *in vitro* stimulation of isolated meninges with the administration of the HIV-1 envelope glycoprotein gp120, IL-1 β , and TNF- α were released [67]. These data suggest that meningeal cells, characterized to include peripheral immune cells like macrophages, contribute to ongoing spinal cord glial activation via proinflammatory cytokine actions. Given these compelling data, we explored the possibility that anatomically intact meninges contain macrophages that express IL-1 β . Here, we utilized immunofluorescent histochemical procedures followed by detection with confocal microscopy and demonstrated that IL-1 β is indeed present within the meningeal layers surrounding the spinal cord of neuropathic rats (Figures 1(a), 1(b), and 1(c)). Histologically, these data confirm prior reports showing, via *in situ* hybridization, that IL-1 β mRNA was colabeled with Iba-1 [68], indicating infiltrating monocytes/macrophages. Within deeper dorsal horn laminae, IL-1 β is colabeled with Iba-1 that identifies microglia (Figures 1(d), 1(e), and 1(f)). While we found some colabeling of IL-1 β with GFAP, no co-labeling with NF-H (data not shown) within the dorsal horn of the spinal cord was observed. Given the evidence that immune cell and glial-derived IL-1 β (as well as other cytokines discussed, above) has a critical role in animal models of pathological pain, targeting neurons alone is now thought to be an incomplete approach. Immune and glial cells within the CNS may serve as novel targets to modulate enduring pathological pain.

3.4. Glia in DRG. Glial satellite cells in the DRG are also important in mediating pathological pain in addition to spinal cord glial cytokine actions. Satellite glia completely surround DRG neurons and together form a functional unit [69]. Glial satellite cells become activated and contribute to pathological pain in response to peripheral injury by several possible mechanisms [69–73]. For example, glial satellite cells generate cytokines, including IL-1 β and TNF- α , which have been characterized to activate peripheral immune cells [71, 73–75]. DRG invasion by peripheral immune cells [76–79] occurs as a consequence of peripheral nerve injury [29, 80, 81]. Neuroimmune activity is a potential mechanism because DRG neurons have receptors for these cytokines and, when stimulated, lead to the production of the chemokine monocyte chemoattractant protein-1 (MCP-1), which induces peripheral immune cell migration to the

DRG [49, 81]. In addition, neuroactive IL-1 β and other immune signals released from satellite glia act in a paracrine fashion to stimulate neighboring sensory ganglia and their axons, creating allodynia [69, 73, 82–84]. Indeed, stimulating sensory neurons in the DRG with IL-1 β leads to further axonal release of substance P [85] within the dorsal horn of the spinal cord. IL-1 β acts in a p-p38MAPK-dependent manner in the DRG [82], and increased p-p38MAPK expression is well characterized in the DRG following peripheral nerve injury that produces pathological pain [82, 86, 87]. Here we show an example of DRG IL-1 β in close proximity with sensory neurons. IL-1 β is colabeled with GFAP-positive satellite cells within a DRG from an animal with ongoing CCI-induced neuropathy which is shown (Figures 1(g), 1(h), and 1(i)). The actions of glially released cytokines such as IL-1 β on nearby neuronal processing in both spinal cord and DRG indicate that not only neuronal, but also glial systems are altered during conditions that lead to and promote chronic pain. These data strongly suggests that in order to efficiently control chronic or pathological pain associated with numerous disease states, including diabetic neuropathy and cancer, promising therapeutics will need to address this underlying glial contribution.

3.5. Modulating Glial Activation for Pain Relief. Several compounds specifically targeting glial activation have been developed with the potential for the treatment of pain. While a full discussion of such compounds is beyond the scope of this paper (for review, see [88]), one example drug is discussed here to underscore the supposition that altering glial activation states is a highly promising approach to control pathological pain. An example of a compound that targets microglial activation is minocycline, a well-characterized microglial inhibitor [89]. In numerous animal models, minocycline robustly produces antiallodynia and hyperalgesia [55, 89–91]. However, globally disrupting the function of microglia as well as peripheral immune cells may produce unintended side effects, such as increased susceptibility to CNS infection [92]. An alternative approach using cannabinoid-related compounds appears to be very promising for clinical pain relief. Cannabinoids may act in an anti-inflammatory manner, and these anti-inflammatory actions may have a glial role [7, 93]. Intriguingly, the cannabinoid receptor subtype 2, CB₂R, has been identified primarily on microglia [94]. Published reports strongly suggest that activation of this receptor subtype leads to pain control [95, 96]. In the remainder of this paper we will provide a brief overview of cannabinoids, specifically discussing published data in support of cannabinoid-related compounds for pain control with a glial-centric view.

4. The Endocannabinoid System

4.1. Components of the Endocannabinoid System. The endogenous cannabinoid (endocannabinoid) system is comprised of multiple components, including receptors, ligands, and degradative enzymes. Each will be discussed in turn, below. Within the past 6 years, an explosion of reports has

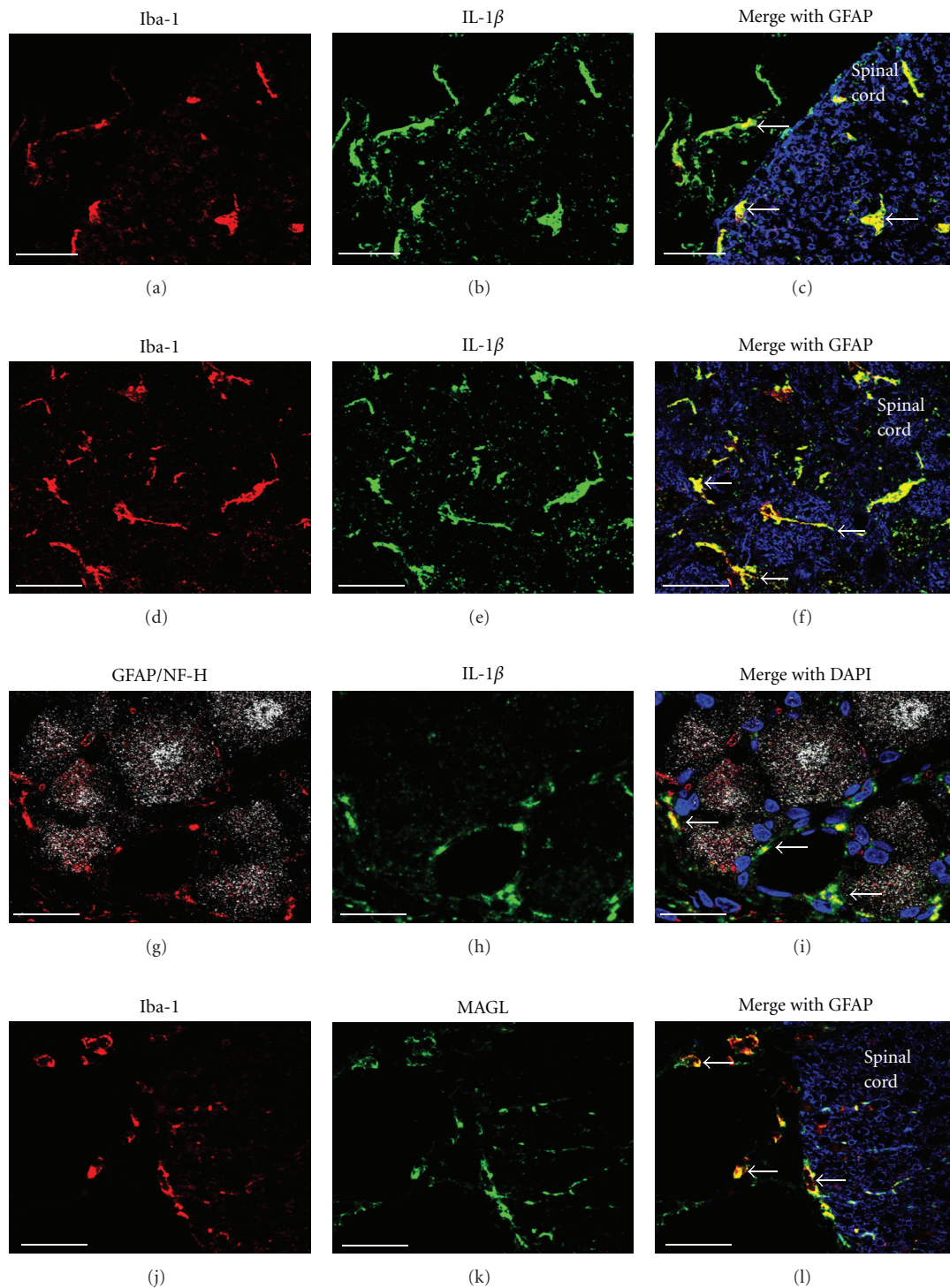


FIGURE 1: Qualitative confocal images of cellular immunostaining. (a) Immunostaining of Iba-1 (red) for infiltrating macrophages and microglia in the meninges and superficial white matter of the spinal cord in a rat with ongoing neuropathy. (b) Immunostaining with IL-1 β (green). (c) Arrows indicate yellow colabeling of IL-1 β - and Iba-1-positive cells and not with GFAP (blue). (d) Immunostaining of Iba-1 (red) in the deeper laminae of the spinal cord dorsal horn in a rat with ongoing neuropathy. (e) Immunostaining with IL-1 β (green). (f) Arrows indicate yellow colabeling of IL-1 β - and Iba-1-positive cells and not with GFAP (blue). (g) DRG immunostaining of GFAP positive satellite cells (red) and neurons stained for neurofilament-heavy (NF-H, white) from a rat with ongoing neuropathy. (h) DRG immunostaining for IL-1 β (green). (i) Arrows indicate yellow DRG IL-1 β and GFAP colabeling with DAPI nuclear labeling (blue). (j) Immunostaining of Iba-1 (red) in meninges and superficial laminae of the dorsal horn spinal cord in a rat with ongoing neuropathy. (k) Immunostaining of MAGL (green). (l) Arrows indicate yellow colabeling of MAGL and Iba-1-positive cells, and not with GFAP (blue). Scale bars for all images indicate 20 μ m.

occurred on the endocannabinoid system and its potential role in modulating numerous disease processes, including those associated with pathological pain conditions. This is due, in part, following the identification of cells that express cannabinoid receptors and subsequent signaling mechanisms. In general, endocannabinoid signaling was thought to involve only neurons [97–99]. Glia in the CNS had no role. However, immune cells, including microglia are now known to be involved in endocannabinoid signaling cascades (discussed further, below). While the underlying mechanisms involved in mediating the therapeutic effects of the endocannabinoid system are still a mystery, new breakthroughs have elucidated the bioavailability of endocannabinoids and cannabinoid receptor action with regard to the mediation of pain processing.

The two widely acknowledged cannabinoid receptors are the CB₁R and the CB₂R. Both have shown great potential for the development of therapeutics targeted at pain control. The putative cannabinoid receptor subtype of the “orphan” receptor, GPR55 [100], remains controversial as several reports indicate opposite pharmacological profiles [101–104]. Research targeting this receptor with cannabinoid ligands has just begun to gain momentum [105–107]. However, there are reports that at least five distinct cannabinoid receptors have been identified [8]. The most well-characterized cannabinoid receptor, the CB₁R, is primarily found on neurons within the heart, small intestine, urinary bladder and vas deferens in the periphery and, within the CNS, has the highest concentrations in the cerebellum, hippocampus, basal ganglia and cerebral cortex [108–110]. However, the CB₂R has a distinctly different distribution and is primarily found on immune cells [111–113]. Current evidence demonstrates that the endocannabinoid system may have potential as a target for pain control, and thus the remainder of this paper will focus on the endocannabinoid system relative to pain therapeutics.

4.2. Classical Cannabinoid Receptor Signaling. Both the CB₁R and CB₂R belong to the G-protein coupled receptor (GPCR) superfamily and couple to the inhibitory G_{i/o} and G_i, respectively. Activation of either receptor leads to p42/44 MAPK signaling and inhibits adenylate cyclase, limiting the ATP production of cyclic AMP (cAMP) and leading to lessened activity of protein kinase A (PKA) [108, 114, 115]. CB₁R activation, but not CB₂R activation, can modulate ionic Ca²⁺ and K⁺ channels, which is blocked with pertussis toxin, indicating that the CB₁R G_{i/o} proteins are directly responsible for modulation of these ion channels [108, 116–118]. Evidence exists that CB₁R activation can activate p38MAPK *in vitro* [119, 120]. However, this is a paradoxical finding, because activation of p38MAPK can lead to increased pain signaling, which opposes the therapeutic efficacy of CB₁R agonists for pain control. A mechanism for these findings has not been elucidated, but may include or be wholly dependent on noncannabinoid receptor signaling cascades. No similar *in vivo* report exists detailing p38MAPK activation from CB₁R activation.

Although a few of the above mentioned signaling properties of the CB₁R have proven to be sufficient in leading to pain control, the practical implications of CB₁R agonists in a clinical setting are limited. The CB₁R was first discovered as the receptor for the major psychoactive ingredient in *Cannabis sativa*, Δ⁹-tetrahydrocannabinol (THC) which was first isolated in 1965 [121–123]. The attractiveness for clinical application of compounds selectively acting on the CB₁R is limited by the development of tolerance [124] and its psychotropic effects [7, 125], which include cognitive impairment [126], catalepsy [127–129], hypothermia [127–129], and negative impacts on learning and memory [130, 131]. This is in contrast to the effects of cannabidiol, another active compound of marijuana [132]. Cannabidiol does not produce unwanted CNS side effects by itself, but it is not widely thought to act robustly at either the CB₁R or the CB₂R due to low binding affinities observed *in vitro* [132]. Despite low CB₁R and CB₂R binding properties that cannabidiol possesses, it remains as a promising therapeutic for chronic pain treatment based on its anti-inflammatory actions.

In vivo, cannabidiol within the CNS may still produce CB₂R activation resulting in anti-inflammatory properties. It was recently demonstrated in a mouse model of diabetic neuropathy that intranasal administration of cannabidiol produces anti-inflammatory actions via downregulation of p-p38MAPK in spinal glia [133]. In this animal model, spinal glia are characterized to become activated and contribute to neuropathic conditions resulting in mechanical sensitivity and thermal hyperalgesia through the activation of proinflammatory signaling cascades like p38MAPK [134–136]. Cannabidiol is sufficient to produce neuropathic pain relief that is dependent on CB₂R activation [133]. These data demonstrate that there is a critical link between cannabidiol's therapeutic action, which includes a CB₂R role, to spinal glial activation and pain control.

4.3. Cannabinoid 2 Receptors. The CB₂R is predominantly found on immune cells, such as lymphocytes, neutrophils, and macrophages, with the highest peripheral concentrations in the spleen, lymph nodes, and testes [94, 111–113]. Within the CNS, the CB₂R is found primarily on microglia, and some neurons specifically within the hippocampus, cortex, and substantia nigra [110, 137, 138]. However, the spinal cord and DRG distribution of the CB₂R is an area of much debate, as the current literature frequently reports conflicting data. One report suggests that the CB₂R is expressed only on neurons [139], while a separate report reveals CB₂R expression on microglia, and to a lesser extent on neurons [96], and yet another report demonstrates CB₂R on microglia with no neuronal expression [138]. The discrepancy between studies identifying the CB₂R expression on specific cell types may be partially due to the recent discovery of two separate isoforms of the CB₂R. The most prevalent isoform of the CB₂R within the periphery, termed “CB2B”, is found extensively within the spleen, and to a lesser degree, in the liver, intestines, and leukocytes. The shorter isoform, “CB2A”, is predominately found within the brain, testes, and to a lesser degree in the spleen, kidney, muscle, and leukocytes [140].

As most commercially available antibodies for the CB₂R utilize CB₂R isolated from spleen, it is possible that the CB₂B isoform, found in much greater abundance within the spleen than the CB₂A isoform, is the isoform recognized by most commercially available antibodies for immunohistochemistry. For example, Wotherspoon and colleagues, utilizing immunohistochemistry and CB₂R null mice, showed that CB₂R expression was induced by nerve ligation and was localized to the spinal cord superficial lamina ipsilateral to the nerve damage, while null CB₂R mice revealed no upregulation [139]. The authors suggest that CB₂R was expressed on sensory afferent terminals because colocalization with growth-associated protein-43 and the neuropeptide galanin, was observed. However, Romero-Sandoval and colleagues demonstrated, also through immunohistochemistry, that the CB₂R was primarily found on parenchymal and perivascular microglial cells. The authors additionally noted very limited and sparse staining in neurons [63, 96]. Lastly, in a study using *in situ* hybridization (ISH), CB₂R mRNA was present on immunohistochemically identified microglia [138].

Based on the above-noted discrepancies of the cellular localization of the CB₂R in the spinal cord, one possible consideration should be the animal model that is utilized. It has been demonstrated that the CB₂R may not be upregulated in the dorsal horn of the spinal cord in inflammatory pain models, but rather in chronic neuropathic pain models [138]. This suggests that the degree of CB₂R upregulation in chronic pain is heavily dependent on the model. The type of the injury induced in a specific model may dictate the overall receptor upregulation and the cellular colocalization of the CB₂R. The CB₂R isoform distribution within the spinal cord and DRG under basal and chronic inflammatory pain conditions has not been systematically examined. Given these potential confounds, identifying the cellular distribution of the CB₂R within the spinal cord remains elusive.

4.4. Bioavailability of Endocannabinoids. The endogenous cannabinoid system is also comprised of a number of endogenous ligands for the CB₁R and CB₂R, which includes anandamide (AEA), 2-arachidonyl glycerol (2-AG), as well as degradative enzymes [141, 142]. The endocannabinoids AEA and 2-AG are produced and released from neurons and microglia [94], which are controlled by enzymatic hydrolysis of fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively [141, 143]. The enzyme MAGL has been identified on presynaptic axon terminals in brain, suggesting that it can terminate 2-AG activity in presynaptic nerve terminals [142, 144] of centrally projecting afferent nociceptors in the spinal cord dorsal horn. Interestingly, it has been found that microglia release 2-AG, and functional MAGL has been described in primary microglial cell cultures [145]. Recently, a novel isoform of MAGL has been described in BV-2 microglial cell cultures, although it is uncertain if this isoform occurs within microglial cells *in vivo* [146]. To date, MAGL cellular coexpression utilizing immunohistological techniques has not been performed on pain-relevant spinal cord dorsal horn regions or spinal

cord tissue. We show here, utilizing confocal microscopy, that within the meningeal layer surrounding the spinal cord taken from behaviorally verified neuropathic rats, MAGL is colabeled with Iba-1-positive infiltrating monocytes. In superficial laminae, MAGL is colabeled with either resident microglia or infiltrating monocytes/macrophages (Figures 1(j), 1(k), and 1(l)). We additionally observed in the deeper dorsal horn laminae, that MAGL is colabeled with Iba-1-positive microglia and morphologically identifiable neuronal cell bodies (data not shown).

4.5. The Implications of the Endocannabinoid System in Pain Modulation. Following peripheral nerve or tissue injury, increased expression of endocannabinoids, CB₂R, FAAH, and MAGL occurs in DRG and spinal cord [94, 109, 147]. For example, AEA and 2-AG are upregulated in DRG following L5 spinal nerve ligation (SNL), a well-described animal model that leads to neuropathic pain [109]. Additionally, in a paw incision model of pain, 2-AG, widely characterized to produce analgesia, was found to be upregulated in the ipsilateral lumbar spinal cord on days 3 and 9 and in the contralateral lumbar spinal cord on days 1 and 9 after surgery [63]. These data suggest that endocannabinoid compounds may act to counterbalance the cytokine actions known to mediate neuropathic pain.

Recent studies show that exogenous application of the endocannabinoids, AEA and 2-AG, leads to pain control. Exogenous AEA administered spinally reverses carageenan-induced nociception [148], and exogenous 2-AG injected into the hindpaw blocks nociceptive responses due to formalin injection [149, 150]. Paradoxically, administration of exogenous AEA to the hindpaw or high intrathecal doses produce nociceptive behavior and, in both cases, is mediated by the ionotropic transient receptor potential cation channel, superfamily V subtype 1 (TRPV-1) [148, 151]. Several reports detail that the actions of AEA may be mediated by TRPV-1, and as such, caution must be taken when assigning endocannabinoid actions to only the CB₁R or the CB₂R.

Manipulating the enzymes responsible for the bioavailability of AEA or 2-AG is additionally effective for pain control. Altering endocannabinoid levels by inhibiting the actions of MAGL and/or FAAH increases available endogenous AEA and 2-AG and results in therapeutic actions. Following localized administration of MAGL inhibitors (JZL184, URB602), into rat hindpaws increases local levels of 2-AG, with simultaneous attenuation of formalin-induced pain in rats [149, 150]. Additionally, systemic administration of FAAH inhibitors (PF-3845, URB597), MAGL inhibitors (JZL184, URB602) or the dual FAAH/MAGL inhibitor, JZL195, increases CNS levels of AEA and 2-AG, with attenuation of CCI-induced pain in mice [152, 153]. Specifically, the pharmacological FAAH inhibitor, PF-3845, decreased allodynia and hyperalgesia in CCI-induced neuropathic mice without the development of tolerance [129]. Numerous studies have demonstrated that MAGL inhibitors increase 2-AG accumulation [129, 152, 154]. However, recent studies indicate that following challenge with CB₁R agonists, increased 2-AG availability leads to CB₁R downregulation,

desensitization, and lessened CB₁R effects [127, 129]. These data suggest that significantly increasing the levels of 2-AG may not be a clinically viable approach for treating chronic pain conditions.

Although pain behavior is suppressed following exogenous administration of 2-AG and AEA or by increased levels of endocannabinoids via enzyme inhibitors, the exact mechanisms of these cannabinoids underlying the modulation of inflammation and pain are not well understood. Studies are currently underway by several groups to elucidate the mechanisms whereby the endocannabinoid system is able to lead to pain control [63, 129, 152, 153]. Alkatis and colleagues, utilizing dual CB₁R and CB₂R antagonists, AM281 and AM630, respectively, recently found that the endocannabinoid system plays critical roles in the resolution of allodynia from surgical hindpaw incision, an animal model of postsurgical pain [63]. In addition, blocking the activation of both the CB₁R and the CB₂R resulted in increased p-p38MAPK levels in this model of postsurgical pain, suggesting that constitutive endocannabinoid actions play a role in modulating p-p38MAPK. These findings support that the endocannabinoid system alters factors which are critical mediators of inflammatory processes underlying pain responses in a wide range of medical conditions where chronic pain is a component. Although speculative, CB₂R actions during chronic pain may be primed for enhanced activity to ultimately produce pain modulation following CB₂R stimulation, as downregulation and desensitization previously noted to occur with the CB₁R have not been observed with the CB₂R [7, 95, 155]. Numerous synthetic CB₂R agonists are currently being explored as potential therapeutic interventions for the treatment of chronic pain.

5. Well-Characterized CB₂R Synthetic Compounds

Growing evidence that CB₂R agonism appears to lack the adverse CNS effects that activated CB₁R exerts has fueled the development of clinically viable CB₂R agonists. Therefore, a strong research effort in pursuit of the development and characterization of synthetic CB₂R agonists with modified chemical structures to facilitate selective binding to the CB₂R over the CB₁R is ongoing. The remainder of this paper will address the current evidence of synthetic CB₂R selective compounds for the treatment of different animal models of pathological pain. This is not an exhaustive review of all studies, but rather an overview. Additional reviews detailing the chemistry, bioavailability, efficacy, and kinetics of specific drug compounds are available elsewhere [7, 156, 157].

Synthetic agonists selective for CB₂R have been shown to produce anti-inflammatory effects with modulation of signaling cascades favorable for controlling chronic pain. Caution must be used in assuming that specific anti-inflammatory effects seen with a particular CB₂R agonist will additionally be seen with all other CB₂R agonists, as the binding site for different compounds may not be the same. This factor may further influence the cellular signaling pathways that occur, downstream of cannabinoid receptor

binding, and the compound's ultimate intracellular fate, such as degradation by MAGL as opposed to FAAH. Therefore, each selected CB₂R agonist and its observed actions are presented in a table (Table 1). The most recent findings for each compound are summarized. JWH-015 is a CB₂R selective agonist from the aminoalkylindole classification of CB₂R agonists with a 27-fold affinity for the CB₂R over the CB₁R [158, 159]. Collectively, the aminoalkylindoles represent the most studied group of synthetic CB₂R agonists. Romero-Sandoval and colleagues have recently used a well-characterized *in vitro* model of inflammation to examine the anti-inflammatory actions of JWH-015. Lipopolysaccharide (LPS), an outer cell-wall particle of Gram-negative bacteria which strongly activates innate immune cells, was given to macrophages in cell culture. It was demonstrated that incubation with JWH-015 leads to a decrease in phosphorylated extracellular signal-regulated kinase-1 (P-ERK) that is mediated by mitogen-activated kinase phosphatase (MKP) 1 and 3 [93]. MKP-3 is a selective negative modulator of the ERK-2 signaling pathway through negative feedback loop mechanisms, while in the same *in vitro* studies, neither JNK nor p38MAPK signaling was affected [160]. CB₂R agonist treatment failed to suppress LPS-stimulated increases in p-ERK-2 in the presence of MKP-3 inhibitors, supporting the possibility that CB₂R agonists exert antiinflammatory actions via MKP-3 signaling [93]. These data support that CB₂R activation, by binding highly selective synthetic agonists, may control proinflammatory processes.

Other CB₂R agonists that produce therapeutic effects to control chronic pain are described below. The compound AM1241, a CB₂R selective agonist also from the aminoalkylindole class, has a 36-fold affinity for the CB₂R > CB₁R [157, 161, 162]. Despite the fact that it has been described as a protean agonist because it exerts different inverse agonist properties [162], it is widely characterized as an effective compound for pain suppression. For example, AM1241 has been found to be effective in treating experimental models of bone cancer pain. Acute and sustained intraperitoneal (i.p.) administration of AM1241 to mice decreased pain symptoms and additionally lessened the amount of bone loss during bone cancer-induced neuropathic pain. The authors suggest that these observations were mediated via the CB₂R, as acute behavioral effects observed were not present with the addition of SR144528, a CB₂R antagonist [163]. In a separate study using two models of bone cancer pain, systemic administration of AM1241 was efficacious in reducing pain symptoms and was reliant on *spinal* CB₂R. Interestingly, the authors concluded that the actions of opioid receptors were necessary to achieve these analgesic effects, as the administration of naloxone, a short-acting opioid antagonist in a replicate experiment, blocked the development of AM1241-mediated analgesia [164]. The involvement of endogenous opioids was further supported in mediating CB₂R analgesia by an earlier study using non-neuropathic naïve rats [165]. However, Rahn and colleagues have recently demonstrated that the anti-nociceptive effects of systemic AM1241 in naïve rats are not dependent on the actions of opioid receptors or downstream effects [166]. In this study, the reported dose of AM1241 utilized by Ibrahim

TABLE 1: CB₂R agonist effects from animal and clinical studies. For the compound/produced by column, * indicates compounds that are also examined in clinical trials. For the CB₂R > CB₁R selective column, human binding (h), rat binding (r). For *in vivo* models of pain, the abbreviations are as follows: local inflammatory pain models of lipopolysaccharide (LPS), complete Freund's adjuvant (CFA). The systemic visceral model of pain: intraperitoneal acetic acid (i.p. acetic acid). Neuropathic pain models: chronic constriction injury (CCI), sciatic nerve ligation (SNL), intraperitoneal chemotherapy-induced pain (i.p. paxlitaxol), bone cancer-induced pain (bone cancer), osteoarthritis induction via knee joint synovium injection (knee joint osteoarthritis). Other models of pain utilized are noted as: postoperative model of surgical pain (PSP) and intrathecal administration of the HIV-1 envelope glycoprotein, gp120 (i.t. gp120).

Compound/produced by	Classification	CB ₂ R > CB ₁ R selective	CB ₂ R compound route of administration	<i>In Vivo</i> models of pain	Tested efficacy	Glial effects	References
A-796260 Abbott Laboratories	Alkylindole	h = 206-fold r = 26-fold	i.p.	CFA, CCI, PSP, Knee joint osteoarthritis	Mechanical allodynia, Grip force	Unknown	[182–184]
A-836339 Abbott Laboratories	Aminothiazole	h = 421-fold r = 189-fold	i.p., plantar hindpaw, intra-DRG, i.t.	CFA, CCI, PSP	Thermal hyperalgesia, mechanical allodynia	Unknown	[167, 182–184]
AM1241 Cayman Chemicals, Sigma-Aldrich, Enzo Life Sciences, A. Makryannis (Northeastern University)	Amino-alkylindole	h = 125-fold r = 36-fold protean agonist	i.p., i.t., intra-DRG, plantar hindpaw	Bone cancer, SNL, CCI	Mechanical allodynia, thermal hyperalgesia	↓ astrocyte activation	[157, 161–163, 167–169]
AM1710 A. Makryannis (Northeastern University)	Cannabylactone	r = 57-fold	i.t., i.p.	i.p. paxlitaxol, CCI, i.t. gp120	Mechanical allodynia, thermal hyperalgesia	Prevented macrophages/microglial mediated induction of i.t. gp120	[157, 168, 169, 176, 177, 179]
AM1714 A. Makryannis (Northeastern University)	Cannabylactone	r = 490-fold	i.p.	i.p. paxlitaxol	Mechanical allodynia, thermal hyperalgesia	Unknown	[157, 176, 178]
*Cannabinor Pharmos Scientific	Bicyclic	h ~ 80–90-fold	i.p., oral, s.c.	Hindpaw carrageenan, i.p. acetic acid, CCI	Thermal hyperalgesia, mechanical allodynia, cold allodynia	Unknown	[156, 189, 190]
CBS0550 S. Saito (Taisho Pharmaceutical Co.)	Imine Derivative	h = 1000-fold	oral	Hindpaw yeast cell wall	Mechanical hyperalgesia	Unknown	[186]
*GRC10693 Glenmark Pharmaceutical Limited	Unknown	Species unknown >4700-fold	oral	CFA, CCI, SNL	Unknown	Unknown	Glenmark Pharmaceuticals website
GW405833 Sigma-Adrich, Tocris Bioscience, Santa Cruz Biotechnology Inc, Enzo Life Sciences	Amino-alkylindole	h = 1200-fold r = 78-fold	i.p., chronic i.p.	SNL, CCI, Knee joint osteoarthritis	Mechanical allodynia	↓ astrocyte activation, ↓ microglial activation	[170, 171, 173]

TABLE 1: Continued.

Compound/produced by	Classification	CB ₂ R > CB ₁ R selective	CB ₂ R compound route of administration	<i>In Vivo</i> models of pain	Tested efficacy	Glial effects	References
*GW842166X Glaxo-Smith-Klein	Pyrimidine Ester	h ~ 500-fold	Oral	CFA	Paw weight bearing	Unknown	[191]
JWH-015 Cayman Chemicals, Sigma-Aldrich, Tocris Bioscience	Amino-alkylindole	h = 27-fold	Plantar hindpaw, i.t.	CCI, PSP	Thermal hyperalgesia, mechanical allodynia, cold allodynia	↓ microglial activation, ↓ astrocyte activation i.t., prevented microglial activation (<i>in vitro</i>)	[93, 158, 159, 192]
MDA19 M. Naguib (University of Texas)	N-alkyl isatin acylhydrazone derivative	h ~ 14-fold r ~ 70 fold protean agonist	i.p.	SNL, i.p. paxlitaxol	Mechanical allodynia	Unknown	[181]
O-3223 A. Lichtman (Virginia Commonwealth University)	Ethyl Sulfonamide derivative of THC	h = 80-fold	i.p.	LPS, SNL	Edema, thermal hyperalgesia	Unknown	[185]

and colleagues, 0.1 mg/kg i.p., did not achieve reliable effects, and so higher doses of AM1241, up to 1 mg/kg, were evaluated. Additionally, in the SNL model of neuropathic pain, the effects of AM1241 following i.p. administration were not blocked by naloxone suggesting that AM1241 does not act via opioid receptors to exert analgesic effects [167] (Table 1). The discrepancy between these studies suggests that bone cancer pain may uniquely involve endorphin-endocannabinoid interactions while other discrete peripheral nerve lesions or naïve conditions may involve only the endocannabinoid system.

Spinal sensitization is a key component of chronic pathological pain. Thus, compounds developed for chronic pain control will require centrally mediated actions and may be insufficient if they do not cross the blood brain barrier because their actions will be sequestered to peripheral sites of pain processing. A growing body of evidence supports that spinal administration of AM1241 produces significant control over pathological pain in several models using peripheral manipulations. For example, intrathecal (perispinal, i.t.) AM1241 reverses allodynia induced by either SNL or intrapaw injection of complete Freund's adjuvant (CFA), a model of local inflammatory pain [167]. Additionally, i.t. AM1241 has been found to reverse CCI-induced allodynia [168] and leads to a corresponding decrease in spinal cord astrocyte activation of these previously neuropathic animals [169]. In separate studies that used SNL to induce peripheral neuropathy in rats, both astrocyte and microglial phenotypic markers of activation were decreased following either i.t. administration of JWH-015 [96] or i.p. administration of GW405833 [170], a partial CB₂R agonist. Taken together, these reports demonstrate that CB₂R agonists are able to alter spinal glial activation states and create *in vivo* anti-inflammatory effects suitable for pain control.

The ability for CB₂R agonists to be administered without the development of tolerance or reliance on μ -opioid actions within the spinal cord has been studied utilizing GW405833. This compound is also classified as an aminoalkylindole and is additionally known as L-768,242. Conflicting reports of GW405833's affinity for the CB₂R over the CB₁R exist [157]. However, it is generally accepted that at the human CB₂R the compound displays a 1,200-fold affinity for the CB₂R over the CB₁R, and at the rat CB₂R there is a 78-fold affinity for the CB₂R over the CB₁R [171]. Leichsenring and colleagues recently demonstrated that chronic repeated i.p. injection of GW405833 was able to provide sustained reversal from allodynia following SNL. That is, animals did not develop tolerance to this compound, which was in stark contrast to treatment with the mixed CB₁R/CB₂R agonist WIN55,212-22 [170]. Additionally, allodynia returned after intermittent treatment of GW405833. The authors also performed immunohistochemistry and, as previously noted, found diminished astrocyte and microglial activation. However, after cessation of GW405833 treatment, astrocyte and microglial activation returned, which occurred in parallel with the return of allodynia. In addition to the above mentioned benefits of CB₂R agonist actions, it has recently been reported that GW405833 can reverse CCI-induced increased helplessness responses, as assessed in the forced

swim test for rats, which is a model that may elucidate depression-like symptoms in animals [172]. Furthermore, GW405833 is efficacious in treating knee pain however, these studies indicate that GW405833 may have partial agonist actions at the TRPV-1 [173]. While endocannabinoids are capable of acting at the TRPV-1 receptor at high doses that subsequently lead to TRPV-1 desensitization [174, 175], the report by Schuleret and colleagues is the first electrophysiological demonstration of CB₂R agonist actions on neuronal TRPV-1 ion channels. Further research is needed to understand if the downstream signaling following GW405833 binding to neuronal TRPV-1 may enhance this CB₂R agonist compound's antinociceptive actions.

6. Newer CB₂R Agonist Compounds

Several newer classes of CB₂R agonists have been developed to examine therapeutic efficacy for chronic pain relief. AM1714 and AM1710 are members of the novel cannabillactone classification [157, 176]. AM1710's pharmacological profile has recently been characterized both *in vitro* and *in vivo* [169, 177]. AM1710 does not cross the blood brain barrier and is 57-fold more selective for the CB₂R over the CB₁R [177]. Systemic i.p. AM1710 in naïve rats was able to produce antinociceptive mechanical responses when a 100-fold dose range (from 0.1 mg/kg–10 mg/kg) was examined. At the 0.1 mg/kg dose, AM1710's effects were altered only by the administration of a CB₂R antagonist, but not the administration of a CB₁R antagonist. However, at the dose of 5 mg/kg, both CB₁R and CB₂R antagonists diminished AM1710's antinociceptive actions. The doses of either 0.1 mg/kg or 10 mg/kg AM1710 did not produce behaviors typically associated with CB₁R activation. This was in stark contrast to the observed CB₁R-induced effects from the mixed CB₁R/CB₂R agonist, WIN 55,212-2. Antinociceptive effects of 5 mg/kg AM1710 were observed for as long as 120 minutes after i.p. injection, while no effects at 0.1 mg/kg were observed at the same timepoint, showing a dose effect on the duration of AM1710 efficacy [177]. In separate studies, i.t. injection of AM1710 reverses CCI-induced allodynia for approximately 3 hours [168, 169]. Additionally i.t. pretreatment with AM1710 blocks the development of allodynia in a rat model of sterile spinal cord inflammation using i.t. administration of the HIV-1 envelope glycoprotein, gp120 [168]. Separately, Rahn and colleagues have shown that AM1714 is capable of reversing chemotherapy-induced pain [178] while AM1710 prevented pain in the same model [179]. NESS400, a novel CB₂R agonist, decreased spinal astrocyte and microglial activation and reversed signs of neuropathic pain behavior following i.p. administration [180]. MDA19 is also a novel CB₂R agonist with moderate selectivity for the CB₂R over the CB₁R (approximately 14-fold) and displays properties of a protean agonist *in vitro* [181], like AM1241. MDA19 was found to reverse both the spinal nerve ligation and chemotherapy-induced models of chronic pain (Table 1).

Abbott Laboratories has developed two novel compounds, A-796260 and A-836339, both of which are selective

for the CB₂R over the CB₁R [182–184]. A-796260, when given to rats i.p., was able to produce relief from local inflammatory pain, neuropathic pain, postoperative pain, and osteoarthritis pain. These effects were due only to the actions of the CB₂R, and not CB₁R or μ -opioid receptor actions, and without the development of CB₁R-mediated psychotropic effects. It was found *in vitro* that A-836339 could act as a CB₁R agonist, and studies *in vivo* revealed that high doses of A-836339 produced CB₁R-mediated psychotropic effects [183]. Further studies with A-836339 reveal that this compound was also efficacious in animal models of inflammatory, neuropathic, postoperative, and osteoarthritis pain, when administered locally to the hindpaw, intra-DRG, and intrathecally. As before, the actions of A-836339 at these sites were due primarily to the CB₂R, and not μ -opioid receptor agonism [167] (Table 1).

Several independent groups have developed and characterized additional promising CB₂R selective compounds. The Lichtman laboratory has recently synthesized an ethyl sulfonamide THC analog: O-3223. This compound is also a novel CB₂R agonist with a 79-fold affinity for the CB₂R over the CB₁R, and administration of this compound in naïve mice did not produce the psychotropic effects associated with CB₁R activation [185]. *In vivo* antinociceptive effects of this compound were determined to be reliant on CB₂R, but not CB₁R function. Pretreatment with i.p. O-3223 was efficacious in lowering the amount of edema in the paws of mice given the immune stimulant LPS, and i.p. O-3223 reversed hyperalgesia in a mouse model of sciatic nerve ligation [185]. CBS0550 is a novel CB₂R agonist with high selectivity for the CB₂R and, when given orally to rats, was efficacious in reversing yeast cell-wall-induced local inflammatory pain [186]. Taken together, these studies reflect just a sample of the efforts being made toward identifying optimal CB₂R compounds for pain therapeutics (Table 1).

7. Clinical Use of CB₂R Agonists

The current clinical trials using cannabinoid compounds for the treatment of chronic pain have examined mixed CB₁R/CB₂R agonists or CB₁R agonists. Sativex, Marinol/Dronabinol, and Nabilone, all containing THC derivatives, have reached late stage or regulatory approval in various countries [187, 188]. To date, only three CB₂R compounds have entered into clinical trials for human evaluation. First noted by Beltramo [156], the progress of CB₂R agonists in clinical trials has not been swift. Glenmark Pharmaceuticals reported in a press release (April 13th, 2009) that its CB₂R compound, GRC10693, successfully completed a phase I clinical trial, showing good tolerance and no serious adverse events in the 80 healthy patients enrolled. This safety profile of GRC10693 was observed with the highest dose of GRC10693 evaluated—1200 mg. Glenmark Pharmaceuticals states that GRC10693 shows a CB₂R selectivity of >4700-fold over the CB₁R. Additionally, peripheral and oral administration of GRC10693 showed efficacy in modulating the *in vivo* animal models of systemic

acetic acid-induced visceral pain and hindpaw carageenan-induced local inflammation, as well as CCI [189, 190]. However, the company has decided not to move forward with phase II clinical trials, as it is currently contemplating licensing the compound to other pharmaceutical companies (<http://www.glenmarkpharma.cz/clin2.php?lang=en>). Early clinical trials showed a safety and tolerability profile of Pharmos Scientific's Cannabinor CB₂R selective compound, but it lacked reliable analgesia. Cannabinor is no longer being developed as an i.v. therapeutic (<http://www.pharmoscorp.com/development/cannabinor.html>). Glaxo-Smith-Klein reports numerous phase I and II trials for its CB₂R agonist GW842166X. GW842166X was described as highly selective for the CB₂R over the CB₁R, with the ability to cross the blood brain barrier in animals. Additionally, this compound showed efficacy in the CFA inflammatory model of pain, without the development of tolerance [191] (Table 1). Interestingly, the only completed phase I clinical trial examined the distribution of radiolabeled GW842166X (specifically, [¹¹C]GW842166X) via positron emission tomography (PET) analysis (<http://clinicaltrials.gov/ct2/show/NCT00511524?term=GW842166X&rank=2>). The rationale was to identify whether this compound was able to cross the blood brain barrier in 6 healthy males. All other phase I studies of this compound were terminated prior to study completion. Glaxo-Smith-Klein reports a total of 3 phase II clinical studies, all aimed at oral dosing, with all reaching completion. The first phase II study examined molar tooth extraction with enrollment in European sites. The other two studies, also with European enrollment sites, examined GW842166X efficacy in osteoarthritis pain (Table 1). Reports from these studies have not been released, and all were completed by September 2009.

The outcomes from the above-noted early clinical trials, specifically those of Cannabinor from Pharmos Scientific, suggest that there may be intrinsic differences between the cellular mechanisms within the human patient that has suffered with pain for an indeterminate amount of time. Further, intrinsic physiological differences may also exist, even in a closely monitored animal model of pain. One potential explanation may lie within the previously described spinal cord mechanisms underlying the maintenance of chronic pain. The clinical studies described did not administer these CB₂R agonist compounds to the spinal cord. The restriction of these compounds to peripheral sites (i.e., poor blood brain barrier permeability) is desired to ensure that even minuscule CB₁R nonspecific binding within the CNS does not occur. This is thought to be an optimal approach to avoid off-target (i.e., CB₁R) psychotropic effects. However, it may be that the administration of these compounds to reach the spinal cord is necessary to produce enduring pain relief due to the potential spinal glial mechanisms underlying chronic pathological pain. Indeed, the argument can be made that these compounds, lipophilic in nature, do possess the ability to penetrate the blood brain barrier. Additionally, it may be that these compounds, acting as very weak CB₁R agonists within the CNS, at levels that do not produce psychotropic or motor side effects, are beneficial in producing pain relief.

8. Summary

CB₂R agonists are emerging as favorable therapeutics over CB₁R for the treatment of chronic pain, as these compounds produce relief from pain symptoms without the commonly reported CB₁R-related side-effects, like catalepsy and motor ataxia. CB₂R agonists may exert their actions independently from μ -opioid receptor actions, and no evidence currently exists related to the development of tolerance or addiction following CB₂R agonist administration. While CB₂R agonists appear to be highly promising as a new avenue for pain therapeutics, the actual direct CNS and DRG effects of CB₂R agonists on the endocannabinoid system are largely unknown. In addition, the CNS role in pain modulation of the endocannabinoid system is itself currently not fully understood and is an area of intense research. The findings discussed in this paper suggest that CB₂R ligands hold promise as future therapeutics to treat chronic pain problems. However, greater research efforts are required to yield new clinically useful CB₂R ligands, as the evidence and outcomes from clinical trials is limited regarding the efficacy of these compounds. Although speculative, spinal CB₂R activation in humans may be necessary to reverse ongoing chronic pathological pain. This approach would preferentially target activated glia which are critical modulators of chronic neuropathic pain. Targeting glial cells, including microglial cells, with CB₂R ligands may hold the key to unlocking an efficacious treatment for chronic pain patients.

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