

MINIREVIEW

CB₂ Cannabinoid Receptors as a Therapeutic Target—What Does the Future Hold?

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ABSTRACT

The past decades have seen an exponential rise in our understanding of the endocannabinoid system, comprising CB₁ and CB₂ cannabinoid receptors, endogenous cannabinoids (endocannabinoids), and the enzymes that synthesize and degrade endocannabinoids. The primary focus of this review is the CB₂ receptor. CB₂ receptors have been the subject of considerable attention, primarily due to their promising therapeutic potential for treating various pathologies while avoiding the adverse psychotropic effects that can accompany CB₁ receptor-based therapies. With the appreciation that CB₂-selective ligands show marked functional selectivity,

there is a renewed opportunity to explore this promising area of research from both a mechanistic as well as a therapeutic perspective. In this review, we summarize our present knowledge of CB₂ receptor signaling, localization, and regulation. We discuss the availability of genetic tools (and their limitations) to study CB₂ receptors and also provide an update on preclinical data on CB₂ agonists in pain models. Finally, we suggest possible reasons for the failure of CB₂ ligands in clinical pain trials and offer possible ways to move the field forward in a way that can help reconcile the inconsistencies between preclinical and clinical data.

Introduction

The endocannabinoid system consists of endogenous cannabinoids (endocannabinoids), cannabinoid receptors (primarily CB₁ and CB₂), and the enzymes that synthesize and degrade endocannabinoids. A complete [receptor(s), enzymes, and endocannabinoids] endocannabinoid system appears to be present in all vertebrates (Elphick and Egertová, 2005). Δ^9 -Tetrahydrocannabinol (Δ^9 -THC), the primary psychoactive component of cannabis, produces many of its psychoactive effects by engaging CB₁ cannabinoid receptors. In addition to its psychoactivity, cannabis has been shown (or suggested) to be efficacious for multiple therapeutic indications and maladies (Grotenhermen and Müller-Vahl, 2012; Borgelt et al., 2013). Most of these appear to be mediated by Δ^9 -THC's activation of either CB₁ or CB₂ receptors, though cannabidiol can be an important factor in the therapeutic efficacy of *Cannabis*-based medicines (Campos et al., 2012). These potential therapeutic effects of Δ^9 -THC have motivated a great deal of drug development over the past 40 years. Most of these efforts have taken the form of targeted manipulation of endocannabinoid

engagement with cannabinoid receptors or inhibition of the enzymes that degrade endocannabinoids.

A major limitation for the therapeutic development of compounds that directly activate CB₁ receptors is unwanted psychotropic effects (Volkow et al., 2014). These CB₁-mediated psychotropic actions produce both practical and administrative hurdles that have severely curtailed the development of direct-acting CB₁ agonists. In contrast, activation of CB₂ receptors does not appear to produce these psychotropic effects (Deng et al., 2014). Thus, the observation that CB₂ receptor activation produces desirable actions in a range of preclinical models (Leleu-Chavain et al., 2012; Han et al., 2013) attracted considerable interest and generated much activity in both academic and commercial laboratories. For example, agonists targeting CB₂ receptors have been proposed as therapies for the treatment or management of a range of painful conditions, including acute pain, chronic inflammatory pain, and neuropathic pain (Ehrhart et al., 2005). They may also be helpful in treating diseases that have a neuroinflammatory or neurodegenerative component, such as multiple sclerosis (Pertwee, 2007; Dittel, 2008), amyotrophic lateral sclerosis (Kim et al., 2006; Shoemaker et al., 2007), Huntington's disease (Sagredo et al., 2012), and stroke (Zhang et al., 2007; Pacher and Hasko, 2008). CB₂ agonists have also been proposed as therapeutics in peripheral

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ABBREVIATIONS: AM1241, 2-iodo-5-nitrophenyl-(1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl)methanone; CB, cannabinoid receptor; CHO, Chinese hamster ovary; CP55940, (-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)hexanol; ERK, extracellular signal-regulated kinase; GPCR, G protein-coupled receptor; GW842166X, 2-(2,4-dichloroanilino)-*N*-(tetrahydropyran-4-ylmethyl)-4-(trifluoromethyl)pyrimidine-5-carboxamide; MAPK, mitogen-activated protein kinase; Δ^9 -THC, Δ^9 -tetrahydrocannabinol; WIN55212-2, (*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*cd*]-1,4-benzoxin-6-yl]-1-naphthalenylmethanone mesylate.

disorders that involve inflammation, including atherosclerosis (Mach et al., 2008), inflammatory bowel diseases (Izzo and Camilleri, 2008; Wright et al., 2008), ischemia/reperfusion injury (Bátkai et al., 2007), renal fibrosis (Barutta et al., 2011), and liver cirrhosis (Mallat et al., 2007; Izzo and Camilleri, 2008; Lotersztajn et al., 2008). Both epidemiologic and preclinical data suggest that activation of CB₂ receptors may be protective in osteoporosis (Ofek et al., 2006). Finally, CB₂ agonists have shown efficacy in preclinical cancer models (Guzman, 2003; Izzo and Camilleri, 2008; Wright et al., 2008). However, despite very favorable efficacy in a range of preclinical models, CB₂ agonists have fared poorly in the clinic. In this review, we summarize our current state of knowledge of CB₂ receptor signaling, review preclinical and clinical studies using CB₂ agonists, discuss the mismatch between preclinical and clinical results, and suggest possible ways forward. As mentioned above, CB₂ agonists may be beneficial for a variety of ailments. However, this minireview focuses primarily on CB₂ agonists for treating chronic pain. Nonetheless, many of the concepts discussed apply to the use of CB₂ agonists for other therapeutic indications.

CB₂ Receptors

Like CB₁ receptors, CB₂ receptors are class A serpentine receptors that couple primarily to G_{i/o} proteins to modulate an array of signaling pathways: adenylyl cyclase, mitogen-activated protein kinase [MAPK (p44/42 and p38)], c-Jun N-terminal kinase, Akt kinase/protein kinase B, phosphoinositide 3-kinase/Akt nuclear factor κ -light-chain-enhancer of activated B cells, nuclear factor of activated T cells, cAMP response element-binding protein/activating transcription factor, Janus kinase/signal transducer and activator of transcription, sphingomyelinase, and caspase, as well as some potassium and calcium ion channels (Bouaboula et al., 1996; Pertwee, 1997; McAllister et al., 1999; Sugiura et al., 2000; Howlett et al., 2002; Molina-Holgado et al., 2002; Ehrhart et al., 2005; Herrera et al., 2005, 2006; Pertwee et al., 2010; Atwood et al., 2012a) (Fig. 1). Despite activation of a wide range of signaling pathways by CB₂ receptors, characterization of CB₂ receptor ligands has primarily focused on modulation of adenylyl cyclase and extracellular signal-regulated kinases 1/2 (ERK1/2), while other pathways, such as those involving arrestin, Akt, ceramide, and ion channel modulation, and the physiologic processes they mediate, are much less well studied.

Adenylyl Cyclase

CB₂ receptor-mediated pertussis toxin-sensitive G_{i/o} protein stimulation leads to inhibition of adenylyl cyclase and decreased cAMP levels (Felder et al., 1995; Slipetz et al., 1995; Mukherjee et al., 2004). However, expression levels and the environment of expression influence strongly influence the coupling of CB₂ to adenylyl cyclase inhibition. For example, stimulation of CB₂ receptors on human lymphocytes that endogenously express CB₂ receptors poorly inhibits forskolin-stimulated adenylyl cyclase compared with CB₂-transfected human embryonic kidney or Chinese hamster ovary (CHO) cells (Bouaboula et al., 1996; Pertwee, 1997, 1999; Schatz et al., 1997; Herring et al., 1998; Gardner et al., 2002; Massi et al., 2003). Similarly, activation of CB₂ receptors in mouse spleen cells that endogenously express CB₂ receptors does not inhibit forskolin-stimulated adenylyl cyclase at physiologically relevant agonist concentrations

(Kaminski, 1993; Kaminski et al., 1994). However, activation of CB₂ receptors in the BV2 microglial cell line inhibits adenylyl cyclase (Franklin et al., 2003). A particularly interesting example is the natural product 4'-*O*-methylhonokiol, which shows inverse agonism for cAMP production and agonism for release of intracellular calcium (Schuehly et al., 2011).

MAPK

MAPKs are enzymes involved in a wide variety of vital signaling cascades in many cellular responses, including cell proliferation, migration, transformation, and cell death. Bouaboula et al. (1996) were the first to report the time- and dose-dependent activation of ERK1/2 by CB₂ agonists in CHO cells transfected with CB₂ receptors. They found this activation to be pertussis toxin-sensitive, indicating involvement of G_{i/o} protein, but adenylyl cyclase-independent. They further showed that activation of this signaling cascade results in phosphorylation of transcription factor Krox-24, thus indicating potential control of gene transcription by CB₂ receptors. Unlike inhibition of adenylyl cyclase, ERK1/2 activation is routinely observed in both recombinant as well as nonrecombinant cells/systems (Beltramo, 2009). For example, robust ERK1/2 activation was also reported in immune cells as well as microglia and macrophages, thus confirming the likely physiologic relevance of this pathway (Beltramo, 2009; Merighi et al., 2012).

p38 MAPK activation by a nonselective CB₂ receptor agonist (Δ^9 -THC) was found to have a proapoptotic effect in the Jurkat human leukemia cell line (Herrera et al., 2005) and cytotoxicity in J774-1 macrophages (Yamaori et al., 2013). This effect was exclusively mediated by CB₂ receptors (Herrera et al., 2005; Yamaori et al., 2013; Kauppinen et al., 2014). Interestingly, Yamaori et al. (2013), in the same cells, also found a c-Jun N-terminal kinase-mediated cytoprotective effect mediated by Δ^9 -THC activation of CB₂ receptors. Thus, the same CB₂ receptor ligand can activate different MAPKs with varied responses and outcomes (Lopez-Illasaca, 1998).

Ion Channels

Although initial experiments failed to detect functional coupling of CB₂ receptors to G protein-gated inwardly rectifying potassium channels and calcium channels (Felder et al., 1995; Pertwee, 1997), other reports suggest that CB₂ receptors can modulate the activity of these channels (Ho et al., 1999; McAllister et al., 1999; Atwood et al., 2012b). Atwood et al. (2012b) showed CB₂ receptor-mediated inhibition of voltage-gated calcium channels in AtT20 cells. CP55940 [(-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol effectively inhibited voltage-gated calcium channels, while WIN55212-2 [(*R*)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-*de*]-1,4-benzoxin-6-yl]-1-naphthalenylmethanone mesylate] was inactive on its own and antagonized CP55940 inhibition. Thus, the reason some of the earlier studies failed to find ion channel modulation can likely be attributed to the functional selectivity of the ligands used in the earliest studies (see below) (Atwood et al., 2012b).

Internalization

Most G protein-coupled receptors (GPCRs) undergo some degree of internalization following agonist binding. Internalization

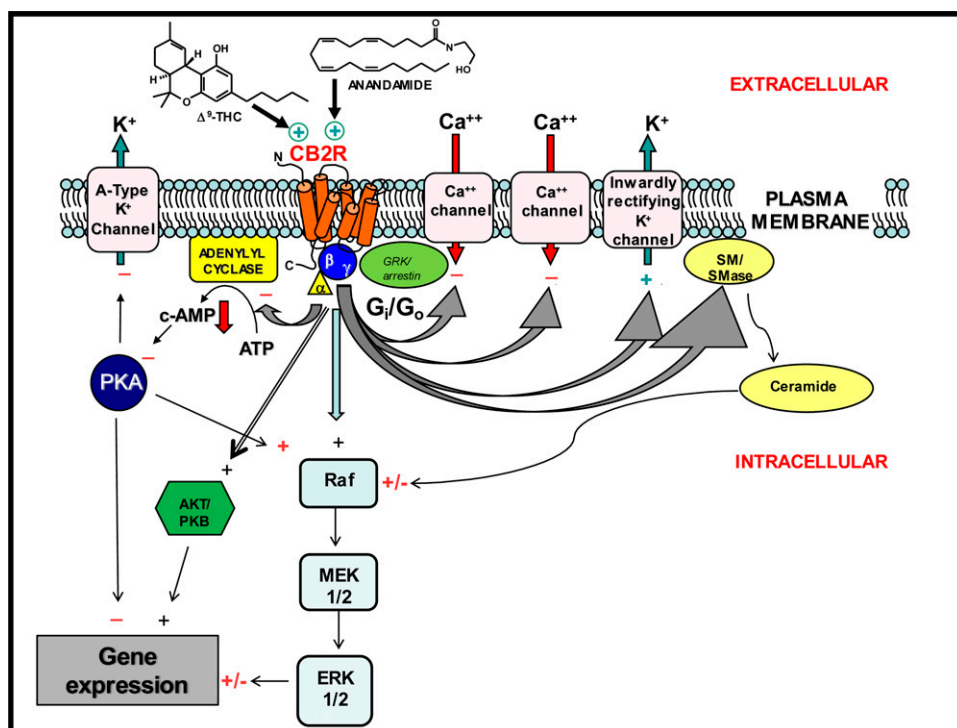


Fig. 1. Activation of CB₂ receptors by natural or synthetic ligands favors a range of receptor conformations that can variably affect different signaling pathways in the following ways: 1) inhibition of adenylyl cyclase, decreased cAMP production, and less activation of cAMP-dependent protein kinase (PKA), culminating in inhibition of A-type potassium channels and inhibition of some gene expression; 2) activation of Akt/protein kinase B, stimulating cell survival, migration, and growth; 3) activation of the MAPK cascade, favoring cell survival and modulating gene expression; 4) inhibition of specific calcium channels and enhanced opening of G protein-gated inwardly rectifying potassium (GIRK) channels; 5) stimulation of de novo synthesis of ceramide and inhibition of the MAPK cascade, promoting apoptosis; 6) recruitment of β-arrestin to the activated CB₂ receptor, resulting in desensitization and/or internalization of the receptor and potential activation of arrestin-specific signaling; and 7) decreased PKA activity, which increases Raf-1 to stimulate the MAPK cascade, positively regulating the expression of many genes. + indicates activation of pathway by CB₂ receptor agonists; - indicates inhibition/downregulation of pathway by CB₂ receptor agonists; +/- indicates a variable outcome.

can play a role in downregulation of the GPCR's ability to signal at the membrane (Ferguson, 2001). Additionally, internalized GPCRs can engage novel signaling pathways inaccessible to GPCRs residing on the surface membrane (Miller and Lefkowitz, 2001). Thus, internalization of a GPCR in response to a ligand can be considered a form of signaling. CB₂ receptors exhibit variable internalization in response to an agonist, with some agonists promoting marked internalization and others being inactive (Grimsey et al., 2011; Atwood et al., 2012b; Petrov et al., 2013).

β-Arrestin Signaling

β-Arrestins are multifunctional proteins that downregulate G protein signaling through direct interactions with GPCRs (Ferguson et al., 1996), as well as serving as scaffolds to recruit other signaling complexes to GPCRs (Miller and Lefkowitz, 2001). β-Arrestin activation is conventionally measured by enzyme complementation, bioluminescence resonance energy transfer, or optical imaging of the recruitment of fluorescently labeled arrestin molecules to the plasma membrane. Two studies have systematically evaluated arrestin recruitment by CB₂ receptors and found that the limited number of CB₂ ligands tested recruited arrestin to the plasma membrane (McGuinness et al., 2009; Atwood et al., 2012b).

CB₂ Receptor Dimerization

Although class A GPCRs can signal as monomers (Milligan, 2013), much GPCR signaling appears to involve homo- or heterodimerization of GPCRs (Milligan, 2013). Heterodimerization can greatly enrich the range of intracellular responses elicited by a ligand (Rozenfeld et al., 2012) and alter the pharmacology of receptor ligands (Rozenfeld and Devi, 2007). The capacity of

CB₂ receptors to dimerize and the potential implications of CB₂ heterodimerization have not been deeply explored (Callén et al., 2012), but are likely to contribute to the heterogeneity of CB₂ receptor signaling reported in various preparations. A particularly fascinating possibility is that dimerization of CB₁ and CB₂ receptors might produce novel receptor pharmacology as well as introduce a confound in interpreting the results of experiments using CB₁/CB₂ antagonists or deletion of one of the CB₁ or CB₂ receptor genes.

Functional Selectivity of CB₂ Signaling

Functional selectivity is the phenomenon in which different agonists activate distinct (or overlapping) intracellular signaling pathways (Kenakin, 2011) and is a concept that has important implications for drug development (Kenakin and Miller, 2010; Violin et al., 2014). Functional selectivity, also known as biased agonism or stimulus trafficking, is often noted as different agonists activating signaling pathways with different rank order potencies (Kenakin et al., 2012). A balanced agonist activates all pathways similarly, whereas a biased agonist shows bias toward a subset of pathways. In the most extreme example of functional selectivity, an agonist may maximally activate some signaling pathways and not others.

Theoretically, functional selectivity offers the opportunity to "fine-tune" receptor stimulation. Functional selectivity could facilitate the discovery of agonists that stimulate signaling pathways to elicit desirable therapeutic benefits while avoiding activation of signaling pathways that may lead to undesirable side effects. However, if endogenous ligands are present at significant levels and are balanced agonists, which appears to be the case for endocannabinoids (particularly for 2-arachidonoylglycerol), functionally selective ligands may

actually antagonize some signaling pathways activated by endocannabinoids, which could be detrimental.

Interestingly, CB₂ ligands show significant functional selectivity. For example, Shoemaker et al. (2005) found that endocannabinoids activated distinct signaling pathways with varied rank order potencies in CHO cells transfected with CB₂ receptors. The endocannabinoid 2-arachidonoylglycerol was most potent in activating the ERK1/2-MAPK pathway, but higher concentrations were needed to inhibit adenylyl cyclase and induce calcium transients. On the other hand, noladin ether displayed higher potency in inhibiting adenylyl cyclase as compared with activating ERK1/2 and calcium transients. An even more extreme example of functional selectivity occurs for several commonly used CB₂ agonists. Certain CB₂ agonists (e.g., the aminoalkylindoles) inhibit adenylyl cyclase and activate ERK1/2, but fail to induce CB₂ internalization or inhibition of voltage-sensitive calcium channels (Atwood et al., 2012b). This striking functional selectivity of CB₂ receptor agonists must be considered during the therapeutic development of CB₂ agonists and enriches the possibilities for developing drugs targeting CB₂ receptors (Atwood et al., 2012b; Han et al., 2013). Arrestin-biased signaling has been found to produce useful therapeutic effects for other GPCRs (Wisler et al., 2007). To date, no strongly β -arrestin-biased CB₂ agonists have been described, so it will be interesting to screen the rich repertoire of CB₂ ligands synthesized to determine if arrestin-biased signaling exists for CB₂ ligands and if arrestin-biased signaling is necessary or dispensable for CB₂ actions in preclinical models (e.g., analgesia and anti-inflammatory).

Regulation of CB₂ Receptor Expression and CB₂ Receptor Localization

An interesting biologic property of CB₂ receptors is their high inducibility, with CB₂ mRNA levels often increasing as much as 100-fold following nerve injury or during inflammation (Maresz et al., 2005; Hsieh et al., 2011). If these increases in mRNA are followed by a corresponding increase in functional receptor protein, and activation of the receptor is therapeutically beneficial, then theoretically this leads to a therapeutically desirable situation in which agonists will stimulate CB₂ receptors primarily where their activation will be beneficial. In healthy organisms, CB₂ receptors are most abundant in cells of macrophage lineage, though they are also found in other immune cells (Galiègue et al., 1995). The extent of their expression in healthy central nervous system tissue is quite controversial. This topic has been recently reviewed. Caveats of the existing literature and suggestions for resolving the controversy can be found there (Atwood and Mackie, 2010). The bottom line is that immunocytochemical studies purporting to show CB₂ expression are many, but are often flawed, and the inclusion of concurrent and careful controls is mandatory before accepting any claim of CB₂ expression in a particular tissue. These conditions have been met for many immune cells, and possibly in neurons following pathologic insult, but remain to be established for most other tissues. In this regard, carefully conducted pharmacology has much to offer in discussions on CB₂ receptor localization as the key question is often whether CB₂ receptors are functionally involved in a response. The anatomic demonstration of CB₂ receptors in this style of experiment is a secondary concern.

CB₂ Ligands

A full consideration of the range of CB₂ receptor ligands that have been synthesized and characterized is beyond the scope of this minireview. Several recent reviews have comprehensively considered this topic (Han et al., 2013, 2014) and can be consulted by the interested reader. An interesting development in the identification of naturally occurring ligands for CB₂ is the existence of a number of abundant phytochemicals that engage CB₂ receptors. Perhaps the best example of this is β -caryophyllene (Gertsch et al., 2008), which offers a starting point for novel compounds that influence endocannabinoid signaling (Chicca et al., 2014). A key concept to keep in mind when evaluating experiments conducted with CB₂ ligands is that many of the commonly used CB₂ ligands are only relatively selective with regard to CB₁. This is because most of the commonly encountered CB₂ ligands were evolved from molecules that have appreciable affinity for CB₁ receptors. Therefore, the concentrations of CB₂-preferring agonists that are commonly encountered in the literature (low micromolar) can result in significant occupancy of CB₁ receptors, with subsequent signaling (Murataeva et al., 2012). Similarly, CB₂-preferring antagonists at micromolar concentrations can substantially antagonize CB₁-mediated responses (Murataeva et al., 2012). Thus, when interpreting the results of experiments conducted solely using a pharmacological approach, careful attention needs to be paid to the controls and a healthy skepticism maintained.

Genetic Tools To Study CB₂ Receptor Signaling

Because of the typically low specificity of cannabinoid ligand pharmacology, the complementary use of mice lacking CB₂ receptors is desirable to implicate CB₂ receptors in a specific response. There are two CB₂ receptor knockout lines in wide use. The first line was developed by Nancy Buckley and Andreas Zimmer (Buckley et al., 2000). This mouse was made by replacing the sequence coding for the receptor from the middle of its third intracellular loop through its stop codon, corresponding to amino acids 217–347, with a phosphoglycerate kinase–neomycin resistance cassette. Although this receptor is nonfunctional when tested for classical CB₂ activity (Buckley et al., 2000), these mice make mRNA for the proximal part of the receptor (Liu et al., 2009), so there is the concern that this mRNA might be translated. This translated mRNA for the partial receptor could interact with other GPCRs to form partial dimers. Interestingly, if the protein corresponding to the truncated CB₂ receptor is heterologously expressed in HEK293 cells, it is trafficked to a sub-plasma membrane compartment (B. Atwood and K. Mackie, unpublished data). In addition, background strain can strongly affect the immune phenotype of this mouse, necessitating caution in using this line to determine the utility of therapeutic manipulations of the endocannabinoid system in immune disorders, and presumably chronic pain, which often has a substantial immune component (Sisay et al., 2013). A second CB₂ knockout line in common use is one that was made by Deltagen (San Mateo, CA) and is available from the Jackson Laboratory (Bar Harbor, ME) through the Knockout Mouse Project Repository (Davis, CA) (Wotherspoon et al., 2005). This mouse was constructed by deleting the sequence corresponding to amino acids 26–140, which comprises a portion of the amino terminus and the first

three transmembrane domains (Yao and Mackie, 2009). Although the deletion is more proximal in this mouse, the presence of mRNA for the residual receptor still remains to be carefully examined. Thus, interpretation of experiments using either line of CB₂ receptor knockout mice must be interpreted with the caveats that variable portions of the CB₂ receptor may be present. Recently, the generation and characterization of a CB₂ conditional knockout was reported. In this mouse an internal ribosomal entry site followed by the gene for green fluorescent protein was inserted downstream of the CB₂ coding sequence, which facilitates localization of CB₂ expression (Vazquez et al., 2014). An additional genetic resource that would be very helpful for the field is a knockin mouse expressing human CB₂ in the mouse CB₂ locus.

Preclinical Studies of CB₂ Agonists in Pain

The initial studies by Ibrahim and colleagues (Malan et al., 2001; Ibrahim et al., 2003) showing that the CB₂ agonist AM1241 [2-iodo-5-nitrophenyl-(1-(1-methylpiperidin-2-ylmethyl)-1*H*-indol-3-yl)methanone] reversed tactile and thermal hyperalgesia in neuropathic rats and mice, independent of CB₁ receptors (that is, the effect persisted in CB₁ knockouts), stimulated a great deal of excitement. These findings offered the possibility of producing significant analgesia through the endogenous cannabinoid system in the absence of CB₁-mediated psychoactivity. Subsequent work by a number of groups has confirmed and extended these studies with a variety of structurally distinct CB₂ agonists (Murineddu et al., 2013). Generally, the field has moved away from AM1241, as AM1241 appears to engage a unique endogenous opioid-mediated component for its analgesia (Ibrahim et al., 2005; Whiteside et al., 2005); its enantiomers have different actions and its efficacy is species-dependent (Bingham et al., 2007); and it is a low-efficacy agonist, as it exhibits protean agonism (Yao et al., 2006).

Significant issues in developing GPCR agonists for therapy include the potential for tolerance (i.e., the need for more drug to achieve the desired effect) or physical dependence (i.e., physical signs and symptoms upon cessation of drug administration) (Williams et al., 2013). When given in preclinical neuropathic pain models, tolerance develops quickly to efficacious doses of CB₁ agonists (Deng et al., 2014). Similarly, physical dependence can be demonstrated following repeated administration of low doses of a CB₁ agonist (Deng et al., 2014). In comparison with CB₁-mediated analgesia, tolerance to CB₂-mediated analgesia in neuropathic pain models does not appear to develop, at least over the course of ~7 days of treatment (Deng et al., 2014). This lack of tolerance to the analgesic effects of a CB₂ agonist raises the question of whether the immune modulation by CB₂ agonists will also be persistent, and if so, whether this will be detrimental. Importantly, abrupt antagonism of CB₂ receptors after chronic dosing with an effective dose of a CB₂ agonist does not appear to elicit signs of physical or autonomic withdrawal (Deng et al., 2014). Finally, although CB₂ agonists do not appear to be rewarding by themselves (Ignatowska-Jankowska et al., 2013), rats will only self-administer CB₂ agonists and will only show preference for the environment in which they received a CB₂ agonist if they are experiencing a painful stimulus (e.g., neuropathy) (Gutierrez et al., 2011).

Reconciling Preclinical and Clinical Studies

Conventional drug development relies heavily on preclinical models to evaluate efficacy of compounds toward potential targets. The high failure rate of agents in clinical trials has prompted a re-evaluation of the predictive reliability of this approach (Paul et al., 2010; Baxter et al., 2013). There are many potential reasons why an agent that appears effective in preclinical models fails in the clinic. These include 1) irreproducible preclinical studies, 2) irrelevance of the preclinical model being evaluated to the clinical condition being treated, 3) an inability in humans to reach tissue concentrations that were necessary for efficacy in animal models because of dose-limiting side effects, 4) species differences in how a ligand engages and activates its targets, 5) off-target negative effects that were not detectable in animal toxicology tests, and 6) lack of selectivity of the ligands used to validate the target in preclinical models. Which of these might explain why CB₂ agonists have failed in the preclinical-to-clinical transition? As numerous groups have demonstrated efficacy of CB₂ agonists in diverse preclinical models of pain, and CB₂ agonists as used in clinical trials appear to have minimal side effects, reasons 1, 3, and 5 are unlikely to explain the failure of CB₂ agonists in clinical trials, so we will focus on possibilities 2, 4, and 6. In the case of possibility 6, many CB₂-“selective” agonists still have significant affinity for CB₁. Because CB₁ receptors are highly abundant, low occupancy still results in the activation of a significant number of CB₁ receptors, potentially producing CB₁-mediated effects, including significant analgesia. Analgesia produced by these agonists’ activating CB₁ receptors will be absent in mice lacking functional CB₂ receptors (but see above for the caveats in using existing CB₂ knockout lines).

The outcome of clinical trials in pain is strongly affected by the type of pain being treated, study design, patient population, and many related factors (Gewandter et al., 2014). Whereas preclinical studies are typically conducted in a genetically and environmentally (usually unenriched) uniform population of often young (and male) rodents, human populations enrolled in clinical pain trials tend to be older and of both sexes and are invariably more heterogeneous. In addition, practical concerns on adequate subject enrollment and the ease of evaluating subject response often result in clinical pain trials being performed on populations whose disease may be quite distinct from those represented in the preclinical models. For example, third molar extraction and osteoarthritis patients are often examined in early-phase analgesic trials, and commonly used preclinical pain models do not faithfully recapitulate these conditions. Indeed, this appears to be the case in that reported trials in which CB₂ agonists have failed include third molar extraction (GW842166X [2-(2,4-dichloroanilino)-*N*-(tetrahydropyran-4-ylmethyl)-4-(trifluoromethyl)pyrimidine-5-carboxamide]; GlaxoSmithKline, Brentford, Middlesex, UK) (Ostenfeld et al., 2011), topical capsaicin-induced pain (PRS-211,375 [cannabinor]; Pharmos, Iselin, NJ) (Roche and Finn, 2010), and osteoarthritis (LY2828360; Eli Lilly, Indianapolis, IN) (Pereira et al., 2013). An additional complicating issue is that early-stage clinical trials are often not reported or are only reported in abstract form. The paucity of details and lack of peer review can make it very difficult to determine why a clinical trial has been considered to “fail,” as the decision not to pursue a clinical target may be a commercially driven rather than an efficacy-driven decision (Hay et al., 2014).

Species differences in receptors and signaling pathways are additional potential confounds in moving from exploratory and preclinical studies to human therapies. Differences in sequence between receptors from different species can lead to different pharmacologies. Thus, a ligand for a rodent receptor may be inactive or have different signaling properties than the human receptor. An example of this is the H₃ histamine receptor, for which ligands switch from agonism to antagonism depending on the species of receptor (Ireland-Denny et al., 2001; Yao et al., 2003). Thus, whereas in vitro pharmacology can be done in cell lines expressing receptors of the correct species (or possibly in induced pluripotent stem cells), pre-clinical testing is often done in rodents. Although mice expressing the human version of the appropriate receptor can be generated and used for testing (e.g., Jun et al., 2014), this is often not done. Furthermore, even if a “humanized” mouse is used, coupling between the receptor and downstream signaling pathways may be species-dependent. This concern is especially significant in situations in which there are substantial species differences in non-ligand-binding portions of a receptor, for example, in domains that may be important for signal transduction or receptor regulation, such is the case for the highly divergent carboxy termini of the CB₂ receptor (Brown et al., 2002). The development and characterization of a mouse expressing humanized CB₂ receptors will be very useful to address these and related questions.

Concluding Comments

The development of the CB₂ receptor as a therapeutic target has gained significant momentum over the past decade due to the identification of CB₂-specific synthetic and natural product ligands, a better understanding of the range of physiologic processes mediated by CB₂ receptors, the regulation of CB₂ receptors, and promising preclinical studies. However, the publicly available clinical data have thus far been disheartening. One reason for this may be discrepancies in pain mechanisms between the preclinical models, in which CB₂ agents show efficacy, and the patients enrolled in clinical trials. Thus, efforts to examine the clinical efficacy of CB₂ agonists in (neuro)inflammatory conditions and neuropathic pain syndromes (e.g., chemotherapy or diabetic) may be more productive. A second potential reason for the lack of translation is that CB₂ agonists show very strong functional selectivity, and this functional selectivity may significantly affect agonist efficacy across species and types of pain. With the availability of increasingly precise and selective pharmacological, genetic, preclinical, and clinical tools and a more complete understanding of the importance of CB₂ agonist functional selectivity, CB₂ receptors still appear to be promising targets for drug development, both for chronic pain and other indications.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Dhopeswarkar, Mackie.

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