Chapter 66

Molecular Pharmacology of CB1 and CB2 Cannabinoid Receptors

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Abbreviations

CB1 Cannabinoid type 1 receptor
CB2 Cannabinoid type 2 receptor
EC Extracellular
ECS Endocannabinoid system
GPCR G-protein-coupled receptor
^{3}H Tritium
MAP Mitogen-activated protein
PLC Phospholipase C
TMH Transmembrane helix
WT Wild type

INTRODUCTION

In the 1990s, a number of surprising discoveries laid a strong, new foundation for both *Cannabis* and cannabinoid research. The identification and early characterization of the cannabinoid receptors (Console-Bram, Marcu, & Abood, 2012; Matsuda, Lolait, Brownstein, Young, & Bonner, 1990; Pacher, 2006) literally provided substrate for the future of cannabinoid science. The CB1 receptor is now understood to be one of the most abundant proteins in the mammalian brain. The CB2 receptor—sharing significant genetic sequence (homology) with the CB1 receptor—has been found to be expressed predominately in immune tissue and cells of immune origin. Because of this characteristic distribution, cannabinoid receptors provide an attractive therapeutic target. The discovery and subsequent availability of various synthetic cannabinoid analogues have allowed researchers to begin characterizing the various physiological roles of these abundant G-protein-coupled receptors (GPCRs). The sum of all knowledge of how these cannabinoid receptors and their endogenous ligands work together to modulate mammalian biology and behavior is referred to as the endocannabinoid system (ECS).

INTRODUCTION TO CANNABINOID RECEPTOR RESEARCH

The investigation of novel compounds acting through CB1 and CB2 receptors has revealed at least five structurally distinct classes of cannabinoid ligands, each possessing their own unique binding properties (Hurst et al., 2002; Song & Bonner, 1996). Known cannabinoid classes include tricyclic cannabinoids (i.e., Δ^9-tetrahydrocannabinol (Δ^9-THC)), bicyclic cannabinoids (CP 55,940), aminoalkylindole or indole derivatives (WIN 55,212), fatty acid derivatives or eicosanoids (anandamide), and antagonist/inverse agonists (SR141716A).

Structure–activity relationship studies of ligands such as SR141716A have aided in the design of more high-affinity and selective ligands (Hanuš, 2009). Many of these cannabinoid compounds have demonstrated selective binding properties (see Figure 1 for cannabinoid ligand descriptions and a review of K_i values). For instance, CP 55,940, Δ^9-THC, and anandamide activate both CB1 and CB2 receptors with similar efficacies, whereas compounds like WIN 55,212 possess a significantly higher affinity for CB2 over CB1. The synthesis and availability of novel and potent cannabinoid ligands has driven significant research gains in recent years.

Recovering useful data on the transmembrane helical clusters that surround and comprise the ligand-binding pocket of a membrane-bound receptor is crucial to understanding how that protein carries out its functions. Investigation represents significant technical challenges. Crystallization techniques, often useful in revealing important structural information of proteins, failed to successfully crystallize the cannabinoid receptors. Whereas mutational analysis of transfected cell lines—cells forced to express the CB1 receptor—serve as a reliable tool for studying both the structure of the CB1 receptor and the pharmacology of cannabinergic ligands (Abood, 2009; Matsuda et al., 1990), the molecular structure of known cannabinoid receptors was largely determined through experiments combining site-directed mutagenesis with molecular modeling techniques. Cannabinoid receptors collected and processed from cell cultures have been used to study GPCR activity through various methods, including, but not limited to, GTPγS assays to determine receptor activity and tritiated (using [^{3}H]) cannabinoid assays to determine binding affinities and distribution (Figure 2). The techniques developed for delineation of the ECS have been among the most innovative in all of scientific enquiry.

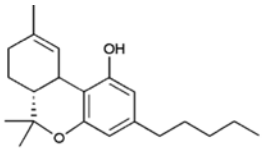
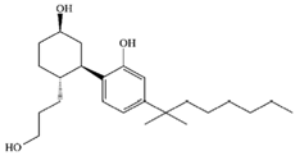
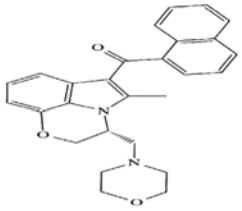
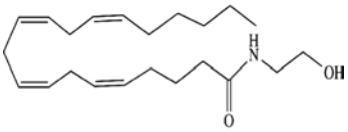
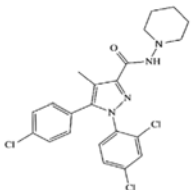
Structure	Description	Reported Binding Activity: K_i (nM)
	**THC** Botanical, Agonist	CB1: 5.05–80.3 CB2: 3.13–75.3
	**CP55,940** Synthetic, Agonist	CB1: 0.06–0.073 CB2: 0.17–0.52
	**WIN55,212** Synthetic, Indole or aminoalkylindole, Agonist	CB1: 1.89–124 CB2: 0.28–16.2
	**Anandamide** Endocannabinoid, Fatty acid derivative, Agonist	CB:1 61–543 CB2: 279–1940
	**SR141716A** Synthetic, Antagonist / Inverse agonist	CB1: 1.8–12.3 CB2: 5.14–13,2000

FIGURE 1 Structures and definitions of cannabinoid ligands. The structures of prototypical cannabinoid compounds from various structural classes, with binding activity values reported as a range of average K_i values for the displacement of a tritiated compound from CB1 and CB2 receptors (rat, human, or mouse) by a nonradioactive compound. In most cases, the tritiated compound used was [^{3}H]CP 55,940, but K_i values may also be derived from displacement of [^{3}H]SR141716A, *R*-(+)-[^{3}H]WIN55,212, or [^{3}H]HU-210. (For full references of reported values see Pertwee, 2014. All images were generated using ChemDraw by the author.)

OVERVIEW OF ENDOCANNABINOID SYSTEM MOLECULAR BIOLOGY

The CB1 receptor has a fundamental, perhaps unsung, role in the mammalian central nervous system (CNS). Dubbed "cannabinoid type 1," the CB1 is a member of the class A rhodopsin-like family of GPCRs found primarily in the CNS, where they are key to the regulation of neuronal activity. There is some evidence that the CB1 receptor is also expressed in peripheral tissues, albeit to a lesser extent, including the adrenal gland, bone marrow, heart, lung, and prostate (Howlett et al., 2002). The CB1 is a $G_{i/o}$-coupled GPCR that binds five structurally diverse classes of ligands; these include the previously mentioned endocannabinoids (typified by anandamide and 2-arachidonoylglycerol (2-AG)), the classical and nonclassical cannabinoids (typified by Δ^9-THC and CP 55,940, respectively), the indoles (typified by WIN 55,212-2), and the diarylpyrazole antagonists/inverse agonists (typified by SR141716A) (Picone, Fournier, & Makriyannis, 2002).

GPCRs are a superfamily of cell surface receptors with the principal role of transmitting information concerning the environment exterior to the cell to the inside of the cell. This transfer of information across the cellular membrane is relayed via heterotrimeric guanine nucleotide-binding regulatory proteins (i.e., G proteins) to other proteins (i.e., kinases). As cannabinoid receptors belong the "class A" family of GPCRs, they share a common membrane topology (Kristiansen, 2004). Class A receptors are generally characterized as having three distinct features: an extracellular N-terminus, an intracellular C-terminus, and seven transmembrane helices (TMHs), connected by loops (Ballesteros & Weinstein, 1995). Those regions crucial for ligand binding, receptor activation, and signal transduction lay within the bundles and stretches of amino acids throughout the seven TMHs (Abood, 2009).

Functional residues of the CB1 receptor can be divided into at least four groups by function; there are residues that affect ligand binding, receptor conformation, receptor activation, and G-protein

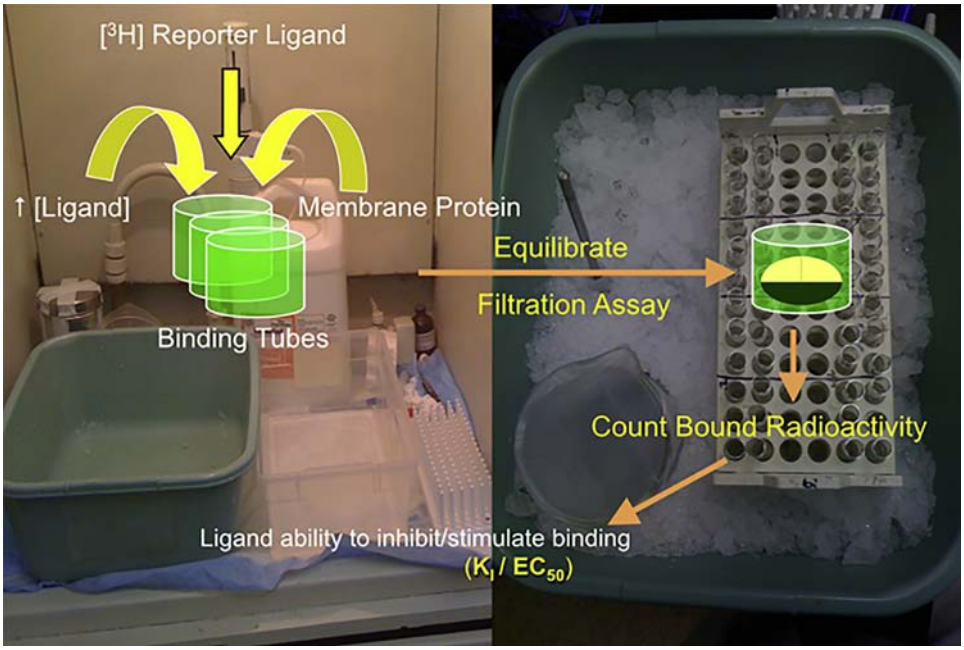


FIGURE 2 **Ligand binding experimental setup and work flow.** A reporter ligand, membrane protein, and salt solution are placed in siliconized tubes with increasing amounts of the reporter ligand. The assay is equilibrated and filtered. The radioactivity is collected on filter paper and counted via a scintillation counter. The count of bound radioactivity is used to generate binding and stimulation values. The background image on the left shows the setup for siliconizing glass tubes, the background image on the right shows the experimental setup prior to equilibration.

association. Alterations in binding affinities and conformations are usually associated with changes in basal and maximal stimulation. Mutations of residues that affect receptor confirmation may cause a failure of expression due to protein misfolding and/or a suppression of binding affinities and receptor activation. For instance, on the extracellular portion of TMHs 3–4–5–6, there is an aromatic cluster consisting of tryptophan, tyrosine, and phenylalanine residues. The residues F3.25, F3.36, W4.64, Y5.39, W5.43, and W6.48 constitute a "microdomain" of aromatic clusters that face the binding pocket (numbering is based on location and conservation of the residue across species Ballesteros & Weinstein, 1995). Study of this microdomain revealed that a tyrosine, Y5.39, is conserved between both CB1 and CB2 receptors. A mutation of this Y5.39 to another aromatic residue, phenylalanine (Y5.39F), resulted in only minor differences in affinity and transduction compared to the wild-type (WT) CB1 receptor. However, an isoleucine (Y5.39I) mutation results in a loss of ligand binding along with pronounced topological changes in TMHs 3–4–5. Such systematic analysis of aromatic microdomain demonstrates that the cannabinoids SR141716A and WIN 55,212 require aromatic stacking interactions of TMHs 3–4–5–6 to activate the CB1 receptor (McAllister et al., 2003). This study also revealed a selective binding region for aminoalkylindoles and diaryl pyrazole cannabinoids, but not for endogenous (anandamide) and bicyclic (CP 55,940) cannabinoids.

Aromatic residues F3.36 and W6.48 form a "toggle switch," and mutational analysis revealed that these residues are important for both binding and activation (McAllister et al., 2004). A mutation of F3.36 to alanine resulted in an increase in constitutive activity, confirmed by using the inverse agonist SR141716A to reduce basal levels of GTPγS binding. Mutation of W6.48 did not cause a rise in basal levels but, rather, an increase in ligand affinities. Together, the F3.36 and W6.48 residues form an interaction that exists in the inactive state of the CB1 receptor. As a ligand is recognized by the receptor, these "bridges" are broken and the protein conformation changes—operating as a toggle switch mechanism.

Other residues may have more dramatic effects on protein folding or conformation. For example, tryptophan 4.64 (W4.64) is conserved between CB1 and CB2 receptors and mutational analysis suggests this aromatic residue is important for ligand binding and signaling (McAllister et al., 2003; Rhee, 2002). Substituting W4.64 with the nonaromatic residues leucine (W4.64L) and alanine (W4.64A) resulted in drastic reductions—W4.64L and W4.64A mutants displayed a significant loss of ligand binding and signal transduction. The W4.64A mutant did not localize to the cell surface and this may be the result of protein misfolding. (In many GPCRs, W4.64 is known for its role in proper protein folding. See Wess, Nanavati, Vogel, & Maggio, 1993). As another example, mutations in the N-terminus of the CB1 second extracellular loop (EC-2) resulted in retention of the receptor in the endoplasmic reticulum (Ahn, Bertalovitz, Mierke, & Kendall, 2009).

In contrast, isoleucine 2.62 (I2.62) and aspartate 2.63 (D2.63) are located in a nonbinding region of the cannabinoid receptor, but may be an important structural requirement (Kapur, Samaniego, Thakur, Makriyannis, & Abood, 2008). A mutation of I2.62 to threonine (I2.62T)—and substituting D2.63 for the (likewise similarly charged) glutamate residue D2.63E—did not significantly alter either binding or activation compared to WT. However, substituting the neutrally charged asparagine (D2.63N) reduced the potency of CP 55,940, WIN 55,212, HU-210, and AM4056. The double mutation (of I2.62T–D2.63N) resulted in a synergistic increase in the EC_{50} required for activation. Accordingly, D2.63A is hypothesized to form a "salt bridge" with a lysine (K) residue in

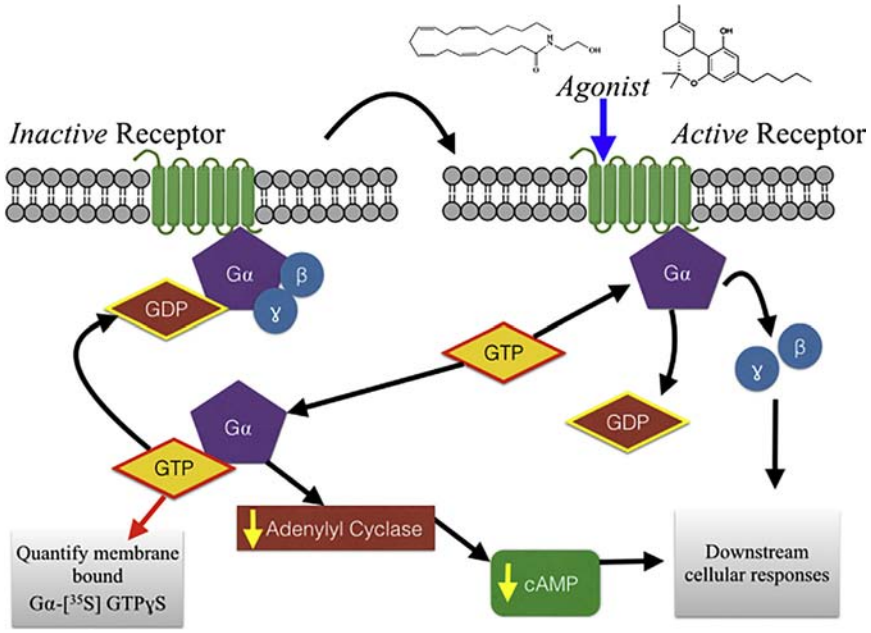


FIGURE 3 **Basic model of cannabinoid receptor signaling.** In this conceptual model, the inactive receptor sits in the plasma membrane with the G-protein complex. Upon the ligand binding, the active receptor undergoes a conformational change, which releases GDP for GTP, and the β and γ subunits separate to affect downstream cellular responses, such as increasing MAP kinase activity. The Gα–GTP complex can be used to measure receptor stimulation in bioassays using radiometric techniques, in this case adding Gα–[^{35}S]GTPγS, which irreversibly binds to Gα and can then be collected and measured.

the third EC loop (K373). Salt bridges between charged residues are the result of proximity and favorable electrostatic attractions and contribute to protein structure and the specificity of interaction of proteins with various biomolecules (Bosshard, Marti, & Jelesarov, 2004). Studies on GPCRs suggest these interactions are between charged residues from spatially distinct domains, i.e., a salt bridge between TMH2 and TMH7 modulates receptor function. Salt bridges are generally composed of negative charges from Asp, Glu, Tyr, Cys, and the C-terminal carboxylate group and of positive charges from His, Lys, Arg, and the N-terminal amino group (Kumar & Nussinov, 2002). In consideration of facts such as D2.63A–K373A producing dramatic reductions in receptor activation, salt-bridge interactions may be of particular importance for maintaining the structure of the CB1 receptor (see Figure 4 for schematic representation of important amino acids).

The CB1 and CB2 receptors share a significant degree of sequence homology, despite being predominately located in the CNS and immune system, respectively. The high degree of homology between the amino acid residue sequences from TMHs of various GPCRs has led to the identification of conserved residues shown to be crucial for receptor function (Kapur et al., 2008; Roche, Hoare, & Parker, 1992; Tao et al., 1999). For example, in the third TMH of both CB1 and CB2 receptors, there is a conserved lysine residue, Lys 192 (K3.28), which plays an important role in selective ligand binding.

In addition, charged interactions between amino acid residues from different TMH domains have been shown to be essential for either ligand binding or receptor function (Marcu et al., 2013; Sealfon et al., 1995; Xu et al., 1999; Zhou et al., 1994). Residues from the extracellular loops demonstrate a comparatively low sequence homology and were initially thought to connect the TMH domains rather than having a direct role in receptor functioning. However, more recent studies have demonstrated the critical role of the EC loops in ligand binding and receptor signaling (Marcu et al., 2013; Shore et al., 2014). Mutation studies have demonstrated that the first EC loop (EC-1) is important to the activation of the adenosine A_{2B} receptor (Peeters et al., 2011). The EC-2 loop has been shown to be important in ligand binding and activation at the V_{1a} vasopressin receptor (Conner et al., 2007), helix movement in rhodopsin (Ahuja et al., 2009), and the binding of allosteric modulators at the M_2 acetylcholine receptor (Avlani et al., 2007). Considerably less is known about the EC-3 loop. However, a key salt bridge between the EC-3 and the EC-2 loops has been observed to influence ligand binding and receptor activation at the β_2-adrenergic receptor (Bokoch et al., 2010).

While both the EC-1 and the EC-2 loops of the CB1 receptor have, thus far, been better characterized than its EC-3 loop (Ahn et al., 2009; Ahn, Mahmoud, & Kendall, 2012; Bertalovitz, Ahn, & Kendall, 2010; Murphy & Kendall, 2003), modeling studies suggest that EC-3 residue K373 may form a functionally important ionic interaction with a transmembrane residue, D2.63^{176}. Previous D2.63^{176} mutational studies demonstrated that the negative charge of D2.63^{176} is critical for agonist efficacy, but not for ligand binding at the CB1 receptor (Kapur et al., 2008). This functional requirement (of a negatively charged residue at 2.63^{176}) might be due to the residue's participation in an ionic interaction with K373—a relationship that is necessary for signal transduction. The efficacy of CP 55,940 and WIN 55,212-2 was markedly reduced by alanine-substitution mutations at D2.63 and K373, while the charge reversal mutation led to a partial rescue of WT levels of efficacy. Computational results indicate that the D2.63^{176}–K373 ionic interaction strongly influences the conformation of the EC-3 loop, providing a structure-based rationale for the importance of the EC-3 loop to signal transduction in CB1. This putative ionic

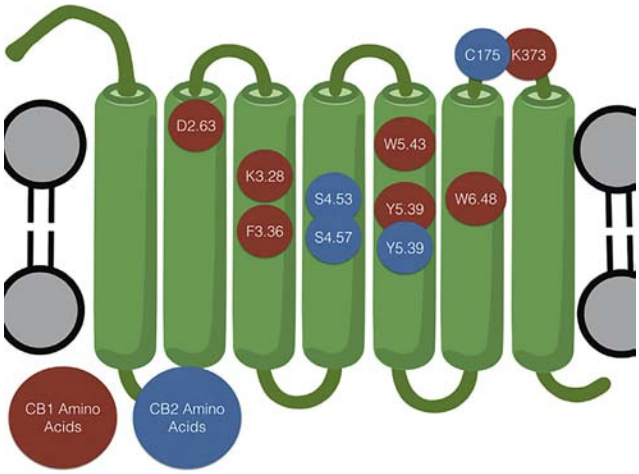


FIGURE 4 Important amino acids for CB1/CB2 receptors. The amino acid residues important for proper functioning of the CB1 and CB2 receptor are noted in red and blue spheres, respectively. The placement of the circles is a conceptual approximate location of the amino acids on this generic diagram of a seven-TMH GPCR.

interaction results in the EC-3 loop pulling over the top (EC side) of the receptor, resulting in an EC-3 loop conformation that may serve both protective and mechanistic roles (Figure 4).

Finally, EC loops may be important for allosteric interactions with cannabinoid receptors (Ahn et al., 2012). Stimulating allosteric sites on cannabinoid receptors can alter binding affinity and efficacy of orthosteric ligands. This offers a unique approach to cannabinoid receptor-based strategies, promising a more precise control of the effects of cannabinoid-based treatments by controlling the conformation of the receptor and the selectivity of the ligand it can interact with (Price, 2005; Shore et al., 2014).

OVERVIEW OF CANNABINOID SIGNALING IN CELLS

Cannabinoid receptors are members of the rhodopsin subfamily of GPCRs, meaning their signal transduction mechanisms are affected by G-protein coupling (Figure 3). As of this writing, cannabinoid receptor activation is known to involve regulation of adenylyl cyclase, alterations in mitogen-activated protein (MAP) kinases, modulation of ion channels, and modulation of intracellular Ca^{2+}. Cannabinoid signaling cascades are linked to processes of cell proliferation, cell differentiation, cell movement, and cell death. Understanding the molecular pathways involved in, and coupled to, cannabinoid receptor signaling is key to elucidating the mechanisms and control of mammalian physiology.

CB1 and CB2 receptors are coupled to $G_{i/o}$ proteins. Cannabinoid receptor signaling events are usually pertussis toxin sensitive, implicating the involvement of $G_{i/o}$-coupled proteins (Howlett, Qualy, & Khachatrian, 1986). Additional evidence from experiments that sequester $G_{i/o}$ proteins from being used by cannabinoid receptors has shown that CB1 can also associate with G_s. CB2 receptors are not known to associate with G_s (Calandra et al., 1999; Glass & Felder, 1997). In contrast, experiments have revealed CB1 receptor coupling with G_q proteins in HEK cells (Lauckner, Hille, & Mackie, 2005). Both CB1 and CB2 receptor stimulation can induce G_q-mediated Ca^{2+} release in insulinoma cells (De Petrocellis et al., 2007). The $G_{i/o}$ and G_s proteins can modulate cAMP and Ca^{2+} currents, while G_q is mainly involved with intracellular Ca^{2+} release. CB1 and CB2 receptors are known to associate with $G_{i/o}$ and G_q, but only the CB1 receptor is known to associate with G_s proteins.

Upon receptor stimulation—by synthetic, phytological, or endocannabinoid means—there is generally an inhibition of adenylyl cyclase and activation of the MAP kinase signaling pathway (Howlett, 1984; Howlett et al., 2002; Pertwee, 2005). The CB1 receptor when coupled to $G_{i/o}$ can also modulate A-type and inwardly rectifying potassium currents and cause an inhibition of N- and P/Q-type calcium currents.

The CB1 receptor-mediated inhibition of adenylyl cyclase was the first cannabinoid receptor mechanism to be characterized (Howlett, 1984). More than a decade later, it was discovered that CB2 receptors can also inhibit adenylyl cyclase activity (Bayewitch et al., 1995). The ability of cannabinoid receptors to interact with $G_{i/o}$ is exploited in the adenylyl cyclase assay (and the [^{35}S]GTPγS receptor stimulation assay). Functional inhibition of adenylyl cyclase, and thus cAMP has been demonstrated in many other experiments (Demuth & Molleman, 2006). This cannabinoid-mediated effect is sensitive to both pertussis toxin and CB1 antagonists such as SR141716A and leads to an attenuation of cAMP accumulation. Such alterations in cAMP concentrations can regulate protein kinase A (PKA) phosphorylation, resulting in significant changes in cellular activity (Childers & Deadwyler, 1996; Mu, Zhuang, Hampson, & Deadwyler, 2000). As one example, PKA activity can affect gene expression and regulate potassium channel activity (Hampson et al., 1995).

MAP kinase pathways are often activated by GPCRs. MAP kinase cascades can lead to the activation of extracellular signal-regulated kinase 1/2 (ERK1/2), c-Jun N-terminal kinase (JNK), p38 MAP kinase, and/or ERK5 proteins. Stimulation of CB1 and CB2 receptors, both in vivo and in vitro, has been shown to activate ERK1/2, phosphoinositide 3-kinase, and PKC through mechanisms that involve the activation of $G_{i/o}$ proteins and inhibition of adenylyl cyclase and alterations in the activity of protein kinases (Davis, Ronesi, & Lovinger, 2003). Via these mechanisms, cannabinoid receptor signaling can lead to alterations in the levels of p38 MAP kinase and JNK. The stimulation of various signaling molecules can also be dependent on the cell type or tissue.

Ion channels can also be modulated by CB1 and CB2 receptor stimulation (Atwood, Wager-Miller, Haskins, Straiker, & Mackie, 2012; McAllister, Griffin, Satin, & Abood, 1999). CB1 receptors can stimulate G-protein-coupled inwardly rectifying potassium channels, which are activated through $G_{i/o}$ proteins, and this stimulation may be induced by a variety of CB1 agonists (Turu & Hunyady, 2010). Experiments using *Xenopus* oocytes transfected with CB2 and G-protein-coupled inwardly rectifying potassium channels did not demonstrate CB2 modulation of ion channel function. However, CB2 cannabinoid receptors inhibit synaptic transmission when expressed in cultured autaptic neurons. CB1 receptors can also affect the function of L-type, N-type, and P/Q-type calcium channels (Mackie, Devane, & Hille, 1993; Mackie & Hille, 1992; Mackie, Lai, Westenbroek, & Mitchell, 1995).

Cannabinoids can modulate levels of intracellular calcium. Both 2-AG and WIN 55212-2 were able to increase intracellular Ca^{2+} in NG108-15 cells, a neuroblastoma–glioma hybrid cell line (Sugiura

et al., 1996). These effects were inhibited by pertussis toxin and SR141716A, again suggesting the effect is mediated by $G_{i/o}$ proteins via the CB1 receptor. A phospholipase C (PLC) inhibitor was also able to block the response, implicating the involvement of IP_3 in the release of Ca^{2+} (Sugiura et al., 1997). Additional evidence supports cannabinoid modulation of Ca^{2+} in endothelial, cerebellar granule, canine kidney, and smooth muscle cells (Demuth & Molleman, 2006). Anandamide has also been shown to modulate intracellular Ca^{2+} in endothelial cells by activation of PLC, the effect of which was blocked by the CB2 antagonist SR144528 but not the CB1 antagonist SR141716A (Zoratti, Kipmen-Korgun, Osibow, Malli, & Graier, 2003). Collectively, these data support a CB1- and CB2-receptor-mediated release of intracellular Ca^{2+} through $G_{i/o}$ proteins and increases in PLC activity.

The CB1 receptor-coupled G proteins are also associated with other GPCRs, and these G proteins have been demonstrated to affect important signaling molecules in osteoblasts. $G_{i/o}$ proteins may regulate osteoblast proliferation by interacting with the MAP kinase cascade and by affecting downstream ERK activation. Antiapoptotic signals can result from AKT activation, which can induce a caspase cascade under the regulation of G_i proteins. The effect on such downstream mediators has been explored in cells, with promising results in osteoclasts (Ross, 2005).

G_q may regulate both osteoblast proliferation and differentiation. In vitro assays have revealed that G_q signaling promotes osteoblast proliferation (Wu, Deng, Zhu, & Li, 2010). In an apparent contradiction, transgenic mice with a constitutively activated G_q in osteoblasts appear to have reduced numbers of osteoblasts and lower bone marrow density (Ogata, Kawaguchi, Chung, Roth, & Segre, 2007). Cultures from these G_q-transgenic mice show impaired osteoblast differentiation confirmed by a decrease in activity of the differentiation marker alkaline phosphatase. This study was conducted with FVB/N mice and MC3T3 cells—it will be interesting to see if future studies find a consistent G_q-associated inhibition of osteoblastic differentiation across different mouse backgrounds such as C57/BL6 or CD1 mouse strains.

G_s proteins have a demonstrated role in bone remodeling as well. Transgenic animals with an induced G_s deficiency exhibit significant skeletal malformations, which surely supports the role played by G_s proteins in endochondral bone development (Castrop et al., 2007). A G_s deficiency in chondrocytes is lethal in mice, as the animals displayed severe growth plate defects, while osteoblast-specific G_s deficiency in mice reduced bone turnover, thickened cortical bone, and produced a narrow bone marrow cavity (Sakamoto, Chen, Kobayashi, Kronenberg, & Weinstein, 2005; Sakamoto, Chen, Nakamura, et al., 2005). G_s promotes bone formation in vivo, and osteoblast differentiation and proliferation are promoted in vitro.

SUMMARY AND APPLICATIONS TO OTHER ADDICTIONS AND SUBSTANCE MISUSE

While this biochemical saga continues to be elucidated, at the same time thousands of research articles have reported that cannabinoid receptor stimulation clearly mitigates numerous pathologies in humans. Positive outcomes have been evidenced in pathologies including, but clearly not limited to, Alzheimer disease, cancer, obesity, and pain (Pacher, 2006; Pertwee, 2014; Pertwee, 2009; Russo, 2008a; Russo, 2011). Additionally, cannabinoid receptor activity may decrease the untoward effects of drug withdrawal in animals, enhance the pain-attenuating effects of morphine in humans, and have a prophylactic effect on developing opiate or other drug addiction-related disorders (Abrams, Couey, Shade, Kelly, & Benowitz, 2011; Bachhuber, Saloner, Cunningham, & Barry, 2014; Ledent et al., 1999). Unfortunately, many attempts at harnessing the therapeutic potential of the cannabinoid receptors have failed owing to overregulation and "unacceptable" subjective CNS-related side effects, such as euphoria and, with far less incidence, depression and suicidal fixation (Christopoulou & Kiortsis, 2011). Clearly, a better understanding of the cannabinoid receptor signal transduction mechanism(s), at a molecular level, would be useful in realizing the therapeutic potential of modulating the ECS.

Mutational analysis of the cannabinoid receptor will surely contribute to our base of knowledge, building an increasingly accurate molecular model of the CB1 receptor. Tailoring future cannabinoid ligands to the receptor will herald the rise of potentially useful synthetic/modified cannabinergic therapeutic drugs and will very probably serve as future treatment modalities for symptoms of drug withdrawal and addiction. One tantalizing possibility, on the forefront of pharmacogenomics research, is to create compounds that may activate cannabinoid receptors when there is a loss of endogenous ligand activity or ligand recognition due to a mutation of those genes encoding the cannabinoid receptors.

Many studies have found associations with ECS polymorphisms in human diseases such as depression, drug addiction, and abnormal mental health (Hillard, Weinlander, & Stuhr, 2012). Hypothetically, a patient might possess a polymorphism (or acquire a mutation) that hinders efficient interactions between cannabinoid receptors and endocannabinoids. In this scenario, a drug or treatment might be chosen based on the patient's genotype. Creation of a different class of bespoke, neo-cannabinoids might be able to replace lost endogenous compounds or perhaps increase the efficiency of existing endogenous receptor–ligand interactions. Such a personalized approach would require either selecting a compound that has structurally distinct binding requirements from extant endogenous compounds (i.e., an indole such as WIN 55,212) or a compound that would enhance baseline endocannabinoid activity at the target. A "clinical endocannabinoid deficiency syndrome"—resulting from defects in the ECS—has already been proposed to underlie certain pathologies, including otherwise treatment-resistant conditions, and cannabinoid receptor polymorphisms are already associated with several diseases (Russo, 2008b). To date, a mutation has yet to be identified in the human cannabinoid receptor that results in conclusive alteration of ligand–receptor interactions. The future of *Cannabis*- and cannabinoid-based clinical trials could be enhanced by developing methods for screening patient polymorphisms or mutations in those genes associated with the ECS.

DEFINITION OF TERMS

Cannabinoids These are a group of closely related compounds, similar to Δ^9-THC or other compounds found in plants.

CB1 receptor This is a GPCR densely located in the brain and nervous tissue.

CB2 receptor This is a GPCR densely located in immune tissue and also found in the brain.

Endocannabinoid system This is a mammalian biological system consisting of receptors (i.e., CB1, CB2), endogenously produced compounds (i.e., anandamide, 2-AG), and proteins responsible for the synthesis, breakdown, and transport of endogenous cannabinoids.

Mutational analysis This is an experimental process of mutating amino acids of a protein and investigating the mutant protein's function.

SUMMARY POINTS

- The cannabinoid receptors are highly expressed in nearly all mammalian tissues.
- The various classes of cannabinoid receptors share a significant amount of sequence homology.
- The CB1 and CB2 receptors have distinct expression profiles; CB1 is localized in neuronal tissue, while CB2 is found on cells of immune origin.
- There exist at least five distinct classes of cannabinoid ligands.
- Genomics studies have revealed strong associations between substance abuse and cannabinoid receptor polymorphisms.
- Developing modern therapeutic cannabinergic agents will depend on how well the ECS is understood.
- Cannabinoid receptor mutational analysis has provided insight into how these receptors can influence drug addiction-related disorders and disease progression.

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