Cannabinoid receptors: nomenclature and pharmacological principles

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Abstract

The CB₁ and CB₂ cannabinoid receptors are members of the G protein-coupled receptor (GPCR) family that are pharmacologically well defined. However, the discovery of additional sites of action for endocannabinoids as well as synthetic cannabinoid compounds suggests the existence of additional cannabinoid receptors. Here we review this evidence, as well as the current nomenclature for classifying a target as a cannabinoid receptor. Basic pharmacological definitions, principles and experimental conditions are discussed in order to place in context the mechanisms underlying cannabinoid receptor activation. Constitutive (agonist-independent) activity is observed with the overexpression of many GPCRs, including cannabinoid receptors. Allosteric modulators can alter the pharmacological responses of cannabinoid receptors. The complex molecular architecture of each of the cannabinoid receptors allows for a single receptor to recognize multiple classes of compounds and produce an array of distinct downstream effects. Natural polymorphisms and alternative splice variants may also contribute to their pharmacological diversity. As our knowledge of the distinct differences grows, we may be able to target select receptor conformations and their corresponding pharmacological responses. Importantly, the basic biology of the endocannabinoid system will continue to be revealed by ongoing investigations.

Highlights

► New information on cannabinoid receptor nomenclature and pharmacological principles. ► The potential classification of GPR18 and GPR55 as cannabinoid receptor subtypes. ► Distinction among agonists, antagonists, inverse agonists and allosteric modulators. ► Practical information on binding and signal transduction for cannabinoid receptors. ► Relevance of CB1 and CB2 mutations, splice variants and polymorphisms to physiology.

Abbreviations

abn-CBD, Abnormal cannabidiol; ACEA, Arachidonyl-2′-chloremethylamide; AEA, N-arachidonoyl ethanolamide, a.k.a. anandamide; 2-AG, 2-arachidonoyl glycerol; CB₁,
1. Introduction

The first cannabinoid receptor, designated CB₁, was discovered (Devane et al., 1988) and subsequently cloned (Matsuda et al., 1990) on the basis of its responsiveness to (−)-Δ⁹-tetrahydrocannabinol (Δ⁹-THC) and related synthetic analogs. Δ⁹-THC is the primary psychoactive constituent in marijuana (a.k.a. cannabis), hence the name “cannabinoid” receptor. It is worth noting that Cannabis sativa produces over 80 cannabinoïds, including Δ⁹-THC (Ahmed et al., 2008, Elsohly and Slade, 2005, Radwan et al., 2008 and Turner et al., 1980). CB₁ is a member of the G-protein coupled receptor (GPCR) family. The discovery of an endogenous ligand that activated the CB₁ receptor led to subsequent breakthroughs elucidating an “endocannabinoid” system (Di Marzo et al., 1998). This arachidonic acid moiety, N-arachidonylethanolamide (AEA) was named “anandamide” for the Sanskrit word for “bliss” (Devane et al., 1992). A second cannabinoid receptor was isolated by a PCR-based strategy designed to isolate GPCRs in differentiated myeloid cells (Munro et al., 1993). The CB₂ receptor has 44% amino acid
identity with CB₁, and a distinct yet similar binding profile, and thus represents a receptor subtype. The nomenclature for cannabinoid receptors has recently been reported by a subcommittee of the International Union of Basic and Clinical Pharmacology (IUPHAR) (Pertwee et al., 2010); a brief summary is presented here. A synopsis of basic pharmacological definitions and principles is also discussed which may be useful when considering new developments in cannabinoid receptor pharmacology.

In the two decades following the discovery of the first cannabinoid receptor, a range of pharmacological and genetic tools have been developed, and used to delineate "cannabinoid receptor"-mediated activity by a variety of compounds. Basically, five distinct classes of cannabinoid compounds have been identified (Fig. 1): the classical cannabinoids (e.g., Δ⁹-THC, 11-hydroxy-Δ⁸-THC-dimethylheptyl (HU210)); non-classical cannabinoids (e.g., CP-55,940); indoles (e.g., WIN 55,212), eicosanoids (e.g., the endogenous ligands; e.g., AEA, 2-arachidonylglycerol) and antagonist/inverse agonists (e.g., SR141716A and AM251 for CB₁, SR145528 and AM630 for CB₂) (Devane et al., 1992, Eissenstat et al., 1995, Howlett, 1995, Mechoulam et al., 1995, Rinaldi-Carmona et al., 1994, Rinaldi-Carmona et al., 1998 and Xie et al., 1996). In general the agonists show little selectivity between the CB₁ and CB₂ receptors, while the antagonist compounds are highly selective (> 1000 fold selective for CB₁ vs. CB₂ and vice versa with nanomolar affinity at the relevant receptor). The selectivity of these antagonists allows the discrimination of CB₁- vs CB₂-mediated effects in vitro and in vivo. Despite the existence of numerous non-selective agonists, there are some that exhibit selectivity for CB₁ vs CB₂ receptors. One example is arachidonyl-2′-chlorethylamide (ACEA) (Hillard et al., 1999b), which is highly selective for CB₁ (nanomolar affinity at CB₁ and > 1000 fold selectivity for CB₁ vs. CB₂). HU-308, a Δ⁹-THC analog, is a highly selective CB₂ agonist with nanomolar affinity at CB₂ and > 1000 fold selectivity for CB₂ vs. CB₁ (Hanus et al., 1999). Several other compounds show > 100 fold selectivity and are generally classified as selective agonists (please see (Pertwee et al., 2010) for more examples). However, these compounds are used at micromolar concentrations in vitro, and therefore may be acting at both receptors. Thus additional controls should be performed to ensure the site of action of these compounds. Fortunately, in addition to the selective CB₁ and CB₂ antagonists that can be used to block agonist effects, there are also genetic tools available to the research community. CB₁ knockout mice have been generated in several laboratories; with both global (Ledent et al., 1999, Marsicano et al., 2002 and Zimmer et al., 1999) and tissue-specific inactivation of CB₁, including select CNS neuronal populations (Marsicano et al., 2003), spinal cord specific nociceptors (Agarwal et al., 2007) dorsal horn inhibitory interneurons (Pernia-Andrade et al., 2009), and liver specific (hepatocytes) (Osei-Hyiaman et al., 2008). CB₂ knockout mice (global inactivation) have also been generated (Buckley et al., 2000).
Since the discovery of AEA, several other arachidonic acid derivatives have been identified that interact with the CB₁ and/or CB₂ cannabinoid receptors and are also considered endocannabinoids. In 1995, Mechoulam and Frde (Mechoulam and Frde, 1995) isolated 2-arachidonoylglycerol (2-AG) from canine intestines and demonstrated binding to both CB₁ and CB₂ receptors. Initially both AEA and 2-AG were thought to bind with similar affinities to CB₁ and CB₂ (Pertwee, 1999). However, another set of investigators found that 2-AG was more potent than AEA at eliciting increases in intracellular calcium (Sugiura et al., 2000). Furthermore, this transient effect was inhibited by CB₂ not CB₁ antagonists. It is now generally accepted that 2-AG acts as a full agonist, whereas anandamide is a partial agonist, at both CB₁ and CB₂ receptors (Gonsiorek et al., 2000 and Sugiura, 2009). Recently these endocannabinoids have demonstrated a differential role in memory and anxiety (Busquets-Garcia et al., 2011). Although both appear to be involved in anxiolytic responses, only AEA was reported to modulate memory consolidation. Anxiolytic responses evoked by 2-AG were mediated by CB₂ whereas CB₁ receptors mediated AEA anxiolytic effects. Additionally, 2-AG antinociception has been reported to be mediated both CB₁ and CB₂ receptors (Guindon et al., 2011) whereas, AEA mediated antinociception is largely via CB₁ (Naidu et al., 2009). Collectively these findings indicate that the biological effects of 2-AG and AEA are differentially modulated by the ECS, perhaps reflective of their differing cannabinoid receptor potencies and/or a consequence of their regional levels at a particular instance due to the surrounding milieu.

Homo-γ-linolenylethanolamide and docosatetraenylethanolamide, isolated from brain (Hanus et al., 1993), compete for binding at CB₁ receptors; although these lipids have not been very well studied. Virodhamine, arachidonic acid and ethanolamine joined by an ester linkage, has also been isolated and shown to act as a partial agonist at the CB₁ receptor and a full agonist at the CB₂ receptor (Porter et al., 2002). However, in another investigation virodhamine was found to behave as a CB₁ receptor antagonist/inverse agonist (Steffens et al., 2005). N-arachidonyl-dopamine (NADA), is primarily a vanilloid receptor agonist, but has some activity at CB₁ receptors as well (Huang et al., 2002).

Another class of lipids has also been identified which has an effect on 2-AG mediated
In this report, 2-linoleoyl-glycerol and 2-palmitoyl-glycerol were isolated along with 2-AG from spleen, brain and gut. Although neither bind nor activate CB$_1$ or CB$_2$ cannabinoid receptors, they significantly potentiate the apparent binding of 2-AG and its apparent capacity to inhibit adenylyl cyclase. Together these esters also significantly potentiate the behavioral effects produced by 2-AG. The inactivation of 2-AG in neuronal cells is inhibited by 2-linoleoyl-glycerol, but not 2-palmitoyl-glycerol. This enhancement of the biological activity of 2-AG by related, endogenous 2-acyl-glycerols, which alone show no significant activity in any of the tests employed, was termed an "entourage effect".

Additionally, palmitoylethanolamide (PEA) has been suggested as a possible endogenous ligand at the CB$_2$ receptor (Facci et al., 1995). Subsequent studies showed no affinity for PEA at the CB$_2$ receptor (Griffin et al., 2000, Lambert et al., 1999 and Showalter et al., 1996), and suggest that another GPCR may be responsible for PEA's actions (Franklin et al., 2003). A metabolite of AEA, N-arachidonyl glycine (NAGly), present in bovine and rat brain as well as other tissues (Bradshaw et al., 2009), has been shown to suppress tonic inflammatory pain (Huang et al., 2001). This arachidonic acid-glycine conjugate has poor affinity for CB$_1$ and CB$_2$ (Sheskin et al., 1997).

The actions of endocannabinoids are not restricted to the CB$_1$ and CB$_2$ receptors. Additional GPCRs as well as ion channels, ion channel receptors (i.e., transient receptor potential cation channel; TRP) and nuclear receptors (peroxisome proliferator-activated receptor; PPAR) have also been identified as sites of endocannabinoid interaction. Activation of transient receptor potential cation channel vanilloid (TRPV) receptors was demonstrated with both AEA (Zygmunt et al., 1999) and NADA (Huang et al., 2002). Activation via AEA was reported to induce vasodilation of isolated vascular preparations as a consequence of calcitonin gene-related peptide (CGRP) (Zygmunt et al., 1999), whereas NADA activation of rat dorsal root ganglion and hippocampal TRPV receptors resulted in the release of substance P and CGRP (Hejazi et al., 2006 and Huang et al., 2002). Evidence for phytocannabinoid interaction with TRP channels has also been demonstrated (De Petrocellis et al., 2011). NADA and AEA have also been shown to modulate calcium channels (Romano and Lograno, 2006, Ross et al., 2009 and White et al., 2001). The channels targeted by synthetic cannabinoids have recently been extensively reviewed (Pertwee et al., 2010).

Both 2-AG and AEA have been shown to mediate activities of PPARs (Lenman and Fowler, 2007 and Rockwell et al., 2006). Furthermore, findings from a recent study suggest that 2-AG activation of CB$_1$ receptors enables cross-talk between PPARy and nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (Du et al., 2011). Δ$_9$-THC, AEA and NAGly have all been reported to potentiate the function of glycine receptors via allosteric interaction (Hejazi et al., 2006 and Yevenes and Zeilhofer, 2011). In addition NAGly is a reversible, non-competitive inhibitor of glycine transport via GLYT2a (Wiles et al., 2006). Interaction between cannabinoids, both endogenous and synthetic, and toll-like receptors (TLRs) has been the focus of much research, and has recently been reviewed (Downer, 2011). Indeed the relevance of the endocannabinoid system in physiology is further complicated by demonstrations of endocannabinoid interaction with GABAergic/glutamatergic, biogenic amine, and opioid neurotransmission (Lopez-Moreno et al., 2008).

In addition to endocannabinoid activation of receptors other than CB$_1$ and CB$_2$, a synthetic cannabinoid compound also demonstrated interaction with non-cannabinoid receptors. In particular, WIN 55,212 as well as AEA elicited guanosine [gamma-thio] triphosphate (GTP$_\gamma$S) activity in brain membranes from CB$_1$ receptor knockout mice (Breivogel et al., 2001). These effects were not sensitive to inhibition by SR141716A. This same phenomenon has also been demonstrated in a second strain of CB$_1$ receptor knockout mice (Monory et al., 2002). The identity of this receptor remains unknown. That AEA produces the full range of behavioral effects (antinociception, catalepsy and
impaired locomotor activity) in CB1 receptor knockout mice (Di Marzo et al., 2000) may be related to this receptor or may be due to AEA’s ability to act at TRP channels (Zygmunt et al., 1999). Another putative cannabinoid receptor subtype that is responsive to WIN 55,212 and CP 55,940 and blocked by capsazepine has been found in the hippocampus (Hajos et al., 2001). These receptors are found on excitatory (pyramidal) axon terminals and have been shown to suppress glutamate release in CB1 receptor knockout animals.

Cannabinoids including AEA elicit cardiovascular effects via peripherally located CB1 receptors (Ishac et al., 1996, Jarai et al., 1999 and Wagner et al., 1999). Abnormal cannabidiol (abn-CBD), a neurobehaviorally inactive synthetic cannabinoid, AEA and a stable analog of AEA (methanandamide) caused hypotension and mesenteric vasodilation in wild-type mice as well as in mice lacking CB1 receptors or both CB1 and CB2 receptors (Jarai et al., 1999). As a consequence of these findings, in addition to the lack of abn-CBD binding to CB1 and CB2 receptors observed in this study, the existence of an endothelial “abn-CBD receptor” has been suggested. In contrast to the studies described above with AEA-stimulated GTPγS activity, the cardiovascular and endothelial effects mediated by the “abn-CBD receptor” were SR141716A-sensitive. These effects were not due to activation of TRPV receptors as the TRPV receptor antagonist capsazepine did not inhibit these endothelium-dependent cardiovascular effects (Zygmunt et al., 1999). The cannabidiol analog and selective inhibitor of the “abn-CBD receptor”, O-1918, inhibits the vasorelaxant effects of abn-CBD and AEA (Offertaler et al., 2003). Furthermore, a lack of abn-CBD binding to CB1 and CB2 was corroborated in this study, and the authors reported that O-1918 did not bind to either of these cannabinoid receptors. The putative “abn-CBD receptor” has also been characterized in immortalized and primary microglia (Franklin and Stella, 2003, Kreutz et al., 2009 and Walter et al., 2003). These studies provide evidence that the “abn-CBD receptor” is involved in microglial migration.

It is well accepted that cannabinoids play a role in immune function. The exact nature of this involvement has not been resolved. As previously mentioned, NAGly suppresses inflammatory pain independent of CB1 and CB2 (Huang et al., 2001). In 2006 a group of investigators suggested that NAGly is the endogenous ligand for GPR18 (Kohno et al., 2006). In a recent report, NAGly was found to induce apoptosis of pro-inflammatory macrophages, further supporting the role of NAGly in inflammation (Takenouchi et al., 2012). Attenuation of apoptosis following knock-down of GPR18 expression by siRNA supports a role for GPR18 in immune function. The finding that abn-CBD, AEA and NAGly act as full agonists at GPR18 suggests that the “abn-CBD receptor” is in fact the GPR18 receptor (McHugh et al., 2010 and McHugh et al., 2011).

The endocannabinoids 2-AG and AEA have also been reported to bind to the lysophosphatidylinositol (LPI) — sensitive receptor, GPR55 (Ryberg et al., 2007). The findings that Δ9-THC, CBD and the synthetic cannabinoid CP-55940 also bind to GPR55 led this group to postulate that GPR55 is a novel cannabinoid receptor. However, GPR55 exhibits only 10–15% homology to cloned CB1 and CB2 receptors (Baker et al., 2006). More importantly, the reported pharmacology of GPR55 is conflicting; studies from different laboratories have found widely discrepant results as summarized in several recent reviews (Henstridge et al., 2011, Pertwee et al., 2010, Ross, 2009 and Sharir and Abood, 2010).

A large body of evidence is emerging which indicates that the resulting effects of endo-, phyto-, and synthetic cannabinoid interactions cannot be definitively explained based on the two-cannabinoid receptor theory. Activation of previously orphaned G-protein receptors, GPR18 and GPR55, by endo-, phyto-, and synthetic cannabinoids suggests that these receptors may have a role in the wide ranging neuro-modulatory effects of the endocannabinoid system (reviewed in Pertwee et al., 2010 and Stella, 2010).

2. Cannabinoid receptor nomenclature

Defining a cannabinoid receptor has become more complex. The IUPHAR committee on
Receptor Nomenclature and Drug Classification, Subcommittee on Cannabinoid Receptors, has proposed a set of criteria for classifying a target as a cannabinoid receptor (Pertwee et al., 2010). This nomenclature committee consists of a number of scientists who are actively involved in research on cannabinoids, and who review on a regular basis, new targets and new nomenclature for the cannabinoid receptors. The current criteria are as follows:

1. It should be activated at its orthosteric site and with significant potency by an established CB₁/CB₂ receptor ligand.

2. It should be activated by at least one established endogenous CB₁/CB₂ receptor agonist at ‘physiologically relevant’ concentrations.

3. If it is a GPCR, it should display significant amino acid sequence similarity with the CB₁ or the CB₂ receptor and hence be a member of the α group of Class A rhodopsin-type GPCRs.

4. It should not be a ‘well-established’ non-CB₁, non-CB₂ receptor or channel, especially if there is already strong evidence that (i) this is activated endogenously by a non-CB₁, non-CB₂ receptor ligand with appropriate potency and relative intrinsic activity and (ii) this is not activated endogenously by any endocannabinoid with appropriate potency and relative intrinsic activity.

5. It should be expressed by mammalian cells that are known to be exposed to concentrations of endogenously released endocannabinoid molecules capable of eliciting a response.

These IUPHAR criteria have been partially met with respect to GPR18 and GPR55. AEA, an undisputed endocannabinoid and ∆⁹-THC have been reported to act as full agonists at GPR18 (McHugh et al., 2011). Interestingly, NAGly, an AEA metabolite, was described by these investigators as more potent than AEA at GPR18. The site of GPR18 activation, orthosteric or allosteric, by ∆⁹-THC, AEA and NAGly has not been elucidated. Findings of AEA mediated activation at GPR55 remain controversial. Previous studies from our lab (Kapur et al., 2009) indicated that GPR55 was not activated by AEA, whereas Ryberg et al. (2007) reported that AEA was equipotent at GPR55, CB₁ and CB₂. Again, the location of activation, orthosteric vs. allosteric at these receptors by this endocannabinoid is of yet unknown. GPR18 and GPR55 share little (less than 15%) homology with CB₁ and CB₂ (Pertwee et al., 2010). Nucleotide sequence homology between CB₁ and CB₂ is reported to be 44%; 68% within the residues of the transmembrane domain (Munro et al., 1993). Moreover, in opposition to the third IUPHAR criteria, CB₁ and CB₂ belong to the Class A rhodopsin α-group, whereas both GPR18 and GPR55 are members of the Class A rhodopsin δ-group of GPCRs (Fredriksson et al., 2003).

Since AEA also binds to TRPV channels, additional studies are needed to ensure that AEA has a greater potency and affinity at cannabinoid receptors as opposed to TRP receptors. The localization of receptors, along with endogenous cannabinoids (virodamine and AEA, as well as AEA’s metabolite NAGly), and synthetic/degradative endocannabinoid enzymes, within the same peripheral and/or brain tissue lends support for GPR18 and GPR55 as cannabinoid receptors with respect to IUPHAR criteria number 5 (Bradshaw et al., 2009, Di Marzo et al., 1994, Howlett et al., 2002, Porter et al., 2002 and Stella, 2010).

Further research is required to fully characterize GPR18 and GPR55 prior to definitive classification as cannabinoid receptors. Such studies should include: competitive binding experiments of labeled agonists in transfected and non-transfected cell lines, displacement binding assays, modeling of binding pockets, point mutations of binding pocket domain(s), and development of knock-out mice. Data from these experiments, along with the development of high potency synthetic agonists, and antagonists will provide the necessary insight whether these two GPCRs should join the ranks of cannabinoid receptors.
3. Basic pharmacological definitions, principles and experimental considerations

Consequent to the interaction of endocannabinoids with a multitude of endogenous receptor systems, classification of orphaned GPCRs as cannabinoid receptors should proceed prudently. The breadth of knowledge gained from cannabinoid research brings to light the relevance of ligand concentration with respect to conclusions regarding cannabinoid involvement in biological events. A suggested “rule of thumb” in determining cannabinoid mediated effects is that cannabinoid compounds generally ligate their receptor(s) in the nanomolar range (Stella, 2010). Hence, the use of concentrations greater than 1 µM may produce off-target effects. For example, cannabinoid agonists were demonstrated to elicit increases in intracellular calcium and arachidonic acid release in both transfected and non-transfected CHO cells at a concentration of 10µM (10 to 100 fold greater than the K_i at the CB_1 receptor) (Felder et al., 1992). The increases in calcium and arachidonic acid release were not observed at concentrations close to the agonist's K_i values. Consequently, cannabinoids at these high concentrations elicited receptor and non-receptor mediated effects. In the field of cannabinoid pharmacology, the nature of the cannabinoid compound-receptor interaction has been upstaged by the biological effect that it imparts. In the quest for answers regarding the purpose of endocannabinoids, it is useful to review the theory of drug–receptor interaction.

The field of pharmacology has its roots in the desire to protect mankind from ailments. Chemicals were introduced into the body as a means of alleviating symptoms. Due to the intrinsic curiosity of man, the science of pharmacology expanded to include how the chemical interacts with biological systems to produce its effects (pharmacodynamics), and how the drug is handled by the body (pharmacokinetics). For a comprehensive review of basic pharmacology the reader is referred to Katzung (Katzung et al., 2009). Conceptually the receptor, site of drug interaction with the body, was borne from experiments of Ehrlich and Langley in the nineteenth and twentieth centuries. In the most classical sense the receptor was considered a membrane bound protein. With the identification of receptors, searches for the endogenous compound(s) which interact with the receptor began. Hence binding of a drug/endogenous compound with the receptor elicits a biological effect. Traditionally, when an endogenous compound binds to a receptor causing activation of the receptor to yield a biological response, the compound is referred to as an agonist. Conversely, if a compound binds to a receptor, at the same site as the agonist, and inhibits the biological response it is an antagonist. Chemicals like therapeutic compounds are designed to either mimic or inhibit this biological response to avoid illness or improve symptomatology. The interaction of receptor and agonist/antagonist was presumed reversible and competitive with the biological effect being proportional to the number of receptors occupied. The agonist was thought to shift the conformation of the receptor to an active state whereas the antagonist permitted the receptor to remain in an inactive state. As our knowledge of receptors and biological effectors became more expansive, the basic agonist/antagonist definition also expanded. For example, a new class of compounds for GPCRs, allosteric modulators has been identified. A compound can now be regarded as an agonist whether it binds to the same (orthosteric) or distinct (allosteric) site as the endogenous compound to elicit its effect (Fig. 2). Recently, allosteric modulators have been identified for cannabinoid receptors (Iliff et al., 2011).
Fig. 2.
Schematic of G protein coupled receptor (GPCR). Panel A. Depiction of inactive receptor (left) and agonist-activated receptor (right). In the inactive state, the G protein–GDP bound subunit complex is bound to the receptor protein (left); whereas upon binding of agonist at the orthosteric site, the receptor is activated and the G protein subunits βγ dissociate from the GTP bound α subunit (right). Panel B illustrates binding of either competitive antagonist or negative allosteric modulator at orthosteric and allosteric sites respectively. Note that the receptor is not activated by either of these ligands. Panel C. Binding of a positive allosteric modulator, at an allosteric site. Note that this ligand does not activate the receptor in and of itself. Panel D. Constitutive activity is demonstrated on the left. The receptor is in an activated state, bound to the α subunit of the GDP–G protein complex, in the absence of agonist. Presumably in this state the receptor conformation is different from the agonist-receptor conformation, indicated by a difference in receptor shape and shading. Binding of an inverse agonist, at the orthosteric site of a constitutively active receptor causes a “deactivation” of constitutive activity, a presumed conformational change, and re-coupling of the G-protein subunits with the receptor. Note that the position of the G protein complex is shown bound to the receptor in a slightly different location to illustrate a different G protein-receptor conformational state, accommodating decreased basal activity consequent to inverse agonist binding.

Agonist binding to the orthosteric site initiates a conformational change concomitant with the dissociation of the G-protein from the receptor and exchange of the bound GDP for GTP (Kenakin, 2001). In the absence of bound endogenous agonist, GPCRs exist in an inactive conformation while receptors are coupled to G-proteins, bound by GDP. Orthosteric binding of receptor molecules is modeled as a competitive saturable process. However, interactions at an allosteric site may not be competitive. The receptor may undergo covalent modification such that the conformational state induced by the allosteric agonist shifts the equilibrium of receptor state. This alteration in receptor state may either enhance (positive allostery) or attenuate (negative allostery) the ability of the receptor to couple to its G-protein(s) thereby affecting the continuation/magnitude of the biological response. Wang et al., (2009) provide a comprehensive discussion of allostery (Wang et al., 2009). Accurate identification and characterization of novel drugs as allosteric modulators (positive versus negative) is imperative in drug
development (Conn et al., 2009).

The ability of an agonist to interact with its receptor to produce a certain level of response is related to the compound's intrinsic activity or efficacy. Some agonists yield a reduced level of response. These are known as partial agonists (Fig. 3). The maximal effect of a partial agonist is independent of the number of receptors occupied and receptor affinity. Rather the ability of the partial agonist to induce G-protein receptor coupling (intrinsic activity/efficacy) is reduced, resulting in a submaximal effect. In the presence of a full agonist, partial agonists can appear to be competitive antagonists as they compete with the agonist for the orthosteric site on the receptor. The response of a full agonist will be augmented in the presence of low concentrations of a partial agonist. However, as the concentration of partial agonist increases the effect of the full agonist is attenuated. Thus the partial agonist appears as if it is a competitive antagonist with respect to the biologic response being measured. If the compound is a neutral (silent) competitive antagonist, having no intrinsic activity/efficacy of its own, the response of the agonist in the presence of competitive antagonist will be attenuated. Antagonism of an agonist-receptor response can be either described as competitive or non-competitive inhibition. Similar to agonist binding, antagonists can either bind to the orthosteric site OR allosteric site. Orthosteric antagonist binding results in either blockade or diminution of endogenous agonist response whereas binding of an antagonist to an allosteric site results in an attenuated endogenous agonist response. Competitive antagonism presumes that competitive antagonist binding occurs at the receptor site where the endogenous agonist binds, the orthosteric site (Fig. 4). As the name implies, competition exists between agonist and antagonist for the same site on the receptor. In the presence of this type of antagonist, a greater concentration of agonist will be required to elicit the same magnitude of response observed with agonist alone, resulting in an increased EC\textsubscript{50} value. Unlike competitive antagonism, non-competitive antagonism renders the receptor unavailable for agonist binding. A modification of the receptor is induced, be it a covalent alteration or a conformational alteration, the orthosteric site is not available to the endogenous agonist. However, the effects of the non-competitive antagonist are insurmountable. Despite increasing concentrations of agonist, the maximal agonist induced response will not be attained. The EC\textsubscript{50} of the agonist often is not different in the presence of non-competitive antagonist. This scenario can be mimicked by negative allosteric modulation. Whether a compound non-competitively antagonizes a receptor mediated response via binding at the orthosteric, or to an allosteric site, the antagonism produced yields a receptor unrecognizable to the endogenous agonist, and/or an alteration in the agonist mediated response.

Fig. 3.
Concentration–response analysis with agonists illustrating full and partial agonists. Shown are a full agonist WIN 55,212-2 (●), a potent, partial agonist CP 55,940 (▲), and a less potent partial agonist anandamide (O). (Data adapted from McAllister et al., 1999).
An additional type of antagonism, uncompetitive antagonism, has been described with respect to NMDA receptor activity (Lipton, 2004). Uncompetitive antagonists require receptor activation by an agonist at the orthosteric site prior to being capable of binding to an allosteric site. Inhibition of the agonist mediated biologic response by uncompetitive antagonists is greater in the presence of high levels of agonist in comparison to lower levels of agonists. Thus far uncompetitive antagonism has not been described for cannabinoid receptors, although it poses an interesting pursuit.

The finding that antagonists exhibited "negative intrinsic activity" was first observed decades ago in a receptor recombinant system (Costa and Herz, 1989). This negative intrinsic activity, the ability to yield an opposite effect as compared to an agonist's "positive" intrinsic activity, has been coined inverse agonism. The concept of inverse agonism is extensively reviewed (Greasley and Clapham, 2006 and Kenakin, 2004). Compounds which exhibit negative intrinsic activity, i.e., inhibitors of basal G protein activity, are called inverse agonists (Fig. 5). With in vitro systems, inverse agonists often exhibit competitive antagonism, also referred to as functional antagonism.

To accommodate the observation of "negative intrinsic activity", the ternary complex model of receptors, a model accommodating GPCRs, was extended to include an additional receptor state. In this state receptors spontaneously become activated thereby...
promoting uncoupling of the G-protein receptor complex (Kenakin, 2001). This spontaneous receptor activity is described as constitutive activity. A priori, constitutive activity assumes receptor activity in the absence of endogenous compound. Constitutive activity is described in detail in section four of this manuscript.

As discussed previously, allosterism, either positive or negative, affects the agonist mediated biologic response. Therefore, exploitation of allosterism can aid in the development of novel therapeutic compounds or perhaps aide in enhancing the biologic effect of an existing therapeutic agent with low intrinsic activity. Additionally, if a therapeutic agent exhibits off-target effects at a particular dose, an allosteric modulator could be used such that a lower concentration of the therapeutic compound could be used to achieve the same response without the unwanted off-target effects. Allosteric modulators could also be a valuable research tool to obtain more reliable results from binding assays investigating orthosteric sites and constitutive activity. For example, it has been proposed that an allosteric modulator could stabilize the ligand–receptor interaction during filtration (Hulme and Trevethick, 2010). An allosteric modulator could reduce unwanted receptor–ligand dissociation during the wash steps, thereby increasing reproducibility of binding parameters obtained from radioligand assays.

Radioligand and $[^{35}\text{S}]\text{GTP} \gamma \text{S}$ binding assays are tools which can allow the characterization of agonists, antagonists, and inverse agonists at GPCRs. There are some practical guidelines and specific considerations regarding assays for cannabinoid receptors. It is assumed that the reader has a basic knowledge of radioligand and $[^{35}\text{S}]\text{GTP} \gamma \text{S}$ techniques. An assortment of resources is available, which provides a thorough background of the theoretical and practical aspects of these assays (Boulton et al., 1986, Hulme, 1992, Hulme and Birdsell, 1992, Hulme and Trevethick, 2010, Kenakin, 1993, Limbird, 1986, Marangos et al., 1984, Williams and Timmermans, 1989 and Yamamura et al., 1990). The main goals of cannabinoid receptor radioligand binding and signal transduction studies are to obtain reliable affinities of ligands for the receptor of interest as well as to analyze the mechanism of action responsible for binding and/or stimulation.

The study of ligand–receptor interactions requires standardization of the assay if any meaningful comparisons are to be made between different laboratories. It is important to consider experimental conditions for these assays as slight changes in materials can lead to alterations in binding and stimulation values. There are conflicting reports as to what extent particular compounds are binding at putative cannabinoid receptors (e.g., at GPR55) and this may be due to labs using a preferred buffer recipe, out of custom or efficiency (reviewed in (Pertwee et al., 2010 and Sharir and Abood, 2010)). A far-reaching comparison of assay protocols between laboratories is the beyond the scope of this review, however to aid in the determination of reliable values from binding data practical aspects of basic ligand binding assays need to be addressed as they pertain to cannabinoid receptor analysis.

There are issues that are specific to cannabinoid receptors that should be considered when conducting ligand binding assays. A few factors that should be considered are the lipophilicity, aqueous solubility, purity, and the type (agonist vs. antagonist) of radioligand being used. Cannabinoids are generally non-polar compounds, having poor aqueous solubility and a tendency to stick to glass and plastic surfaces; this will lead to inaccurate estimates of ligand affinity. Hence, when using cannabinoids in binding assays it is important to coat the test tubes with a silanizing agent, such as AquaSil™.

Cannabinoid receptor antagonists are ideal compounds to use for radioligand assays. As mentioned above, the antagonists display high selectivity and affinity for CB₁ or CB₂. While an agonist may label only a portion of the total receptor population (high affinity state), antagonists generally recognize all available receptors. Using a compound with high affinity and selectivity is ideal because lower concentrations of ligand can be used, which can result in lower levels of nonspecific binding. Nonspecific binding includes
binding to other receptors and to glass filter fibers. Choosing the appropriate filters and pretreatment can also reduce nonspecific binding, i.e., soaking filters with polyethyleneimine grants a positive surface charge, which reduces nonspecific binding of cationic but not anionic (i.e., GTPγS) fragments.

Another consideration for increasing the reliability of binding data is quality control of the radioligand. In general, based on the data sheet from the manufacturer or vial label, the assumption is made that the radioligand is chemically pure. Unfortunately, the effective radioligand concentration may become inaccurate for numerous reasons. Improper handling and storage conditions contribute to degradation and thus alterations in specific activity, which subsequently affect the $K_D$. Ligands may also degrade significantly even when storage conditions are optimal. Contamination with binding and non-binding impurities can also lead to over- and under-estimations of binding parameters, which may cause inaccurate interpretations due to ambiguous data (Sum et al., 2001). These binding and non-binding contaminants contribute to non-specific binding (Hulme and Trevethick, 2010).

Radioligand supply companies (e.g., product sheet for $[^3H]$-SR141716A from Perkin Elmer) recommend that laboratories verify the purity of the radioligand with sensitive analytical techniques such as High-Performance Liquid Chromatography. Analytical chemistry can become costly and time consuming; a simple check for analyzing the quality of a radioligand is to compare Bmax and $K_D$ values in a standard membrane preparation. If radioligands from different batches have similar purities, then the binding values should not be significantly different.

4. Constitutive activity

Constitutive (agonist-independent) activity is observed with the over-expression of many GPCRs (Lefkowitz et al., 1993). Experimental evidence for constitutively active CB$_1$ receptors was first noted when SR141716A, initially described as a CB$_1$ antagonist, was found to have inverse agonist properties with respect to stimulation of mitogen-activated protein kinase (MAPK) activity (Bouaboula et al., 1997). Cannabinoid agonists activated mitogen-activated protein kinase (MAPK) activity in transfected CHO cells expressing CB$_1$ (Bouaboula et al., 1995). However, basal levels of MAPK activity were higher in CB$_1$-transfected cells as compared to untransfected cells, suggesting the presence of autoactivated CB$_1$ receptors. SR141716A not only antagonized the agonist effect on MAPK, but also reduced basal MAPK activity in CB$_1$-transfected but not untransfected cells. Similarly, basal cAMP levels were reduced and SR141716A raised basal cAMP levels in transfected cells. The $EC_{50}$ for SR141716A was similar to its $IC_{50}$, suggesting that these effects are a result of direct binding to unoccupied (pre-coupled) CB$_1$ receptors and not due to the presence of endogenous ligands in the cultures. A significantly higher $EC_{50}$ would be predicted if endogenous agonists were competing with SR141716A. Subsequent studies extended these findings to CB$_1$ receptor-activated GTPγS binding (Landsman et al., 1997) and inhibition of calcium conductance (Pan et al., 1998). Additionally, CB$_1$ receptors can sequester G proteins, making them unavailable to couple to other receptors (Vasquez and Lewis, 1999). SR141716A is also an inverse agonist when CB$_1$ receptors are co-expressed with G-protein coupled potassium channels in Xenopus oocytes (McAllister et al., 1999). A study in primary cultures of rat cerebellar granule neurons presented evidence for inverse agonism by SR141716A on nitric oxide synthase activity (Hillard et al., 1999a). Evidence for inverse agonism was also reported in the guinea pig small intestine (Coutts et al., 2000).

Constitutive receptor activity, a priori, occurs in the absence of endogenous ligands as previously mentioned. In a recent review the authors caution identification of cannabinoid receptor activation as constitutive activity unless endogenous ligands are known not to be present (Howlett et al., 2011).

Mutations (either naturally occurring or engineered) can also give rise to constitutively active GPCRs. Mutations that result in constitutive activity may provide clues to the key
amino acids involved in receptor activation. Generally, constitutively active receptors are also constitutively phosphorylated and desensitized, providing support for a model where a single active state conformation is the target for phosphorylation, internalization and desensitization (Leurs et al., 1998). However, a study on the angiotensin II receptor and a series of studies on the CB₁ receptor suggest that GPCRs may possess several transition states, each associated with conformationally distinguishable states of receptor activation and regulation (Houston and Howlett, 1998, Hsieh et al., 1999, Jin et al., 1999, Roche et al., 1999 and Thomas et al., 2000).

Nie and Lewis (2001) found that the C-terminal domain contributes to constitutive activity of CB₁. Truncation of the distal C-terminal tail of the CB₁ receptor (at residue 417 in rat CB₁) enhanced both the constitutive activity and the ability of the receptor to sequester G-proteins. Conversely, mutation of a highly conserved aspartate residue in TMH2, D2.50 (164 in rat CB₁) abolished G-protein sequestration and constitutive receptor activity without disrupting agonist-stimulated activity at Ca²⁺ channels. They concluded that the distal C-terminal tail acts to constrain the receptor from activating G-proteins, whereas the aspartate (D2.50) in the second transmembrane domain stabilizes the receptor in both the inactive RG(GDP) state and the active R*G(GTP) state.

An interaction between F3.36/W6.48 has also been proposed to be a key to the maintenance of the CB₁ inactive state (Singh et al., 2002). Previous modeling studies had suggested that an F3.36/W6.48 interaction requires a F3.36 trans χ1/W6.48 g + χ1 rotameric state. SR141716A stabilizes this F3.36/W6.48 aromatic stacking interaction, while WIN 55,212-2 favors an F3.36 g + χ1/W6.48 trans χ1 state (Singh et al., 2002). McAllister et al. explored this hypothesis in a mutation study of mouse CB₁ (McAllister et al., 2004). An F3.36(201)A mutation showed statistically significant increases in ligand-independent stimulation of GTPγS binding versus wild type CB₁. Basal levels for the W6.48(357)A mutant were not statistically different from wild type CB₁. F3.36(201)A demonstrated a limited activation profile in the presence of multiple agonists. In contrast, enhanced agonist activation was produced by W6.48(357)A. These results suggest that a F3.36(201)/W6.48(357)-specific contact is an important constraint for the CB₁-inactive state that may need to break during activation. Modeling studies suggested that the F3.36(201)/W6.48(357) contact can exist in the inactive state of CB₁ and be broken in the activated state via a χ1 rotamer switch (F3.36(201) trans, W6.48(357) g +) → (F3.36(201) g+, W6.48(357) trans) as previously proposed. The F3.36(201)/W6.48(357) interaction therefore may represent a “toggle switch” for activation of CB₁. Similar results were reported with mutation of F3.36(200) in the human CB₁ receptor (Shen et al., 2006).

Constitutive activity has also been shown with the CB₂ receptor (Bouaboula et al., 1999c). CB₂ receptors expressed in CHO cells also sequester Gᵢ proteins; the CB₂ inverse agonist SR144528 inhibits basal G-protein activity as well as switching off MAPK activation from receptor tyrosine kinases and the GPCR lysophosphatidic acid (LPA) receptor (Bouaboula et al., 1999a). When expressed in heterologous systems, CB₂ receptors are constitutively phosphorylated and internalized (Bouaboula et al., 1999b). Autophosphorylation as well as agonist-induced phosphorylation occur on S352 and involve a G-protein coupled receptor kinase (GRK) (Bouaboula et al., 1999c). In transfected HEK293 cells, mutations of CB₂ at H316Y, which corresponds to a single nucleotide polymorphism, caused higher constitutive activity than the CB₂ wild-type receptor (Carrasquer et al., 2010). These data suggest that CB₂ polymorphic receptors may contribute to the etiology of certain diseases.

5. Gene structure, polymorphisms and species diversity

The roles of specific amino acids within cannabinoid receptors have been studied in detail and researchers have identified many requirements essential for a compound to bind and/or activate these receptors. There is mounting evidence that different types of cannabinoids may require different amino acids for binding and activation (reviewed in
Abood, 2005). There are a number of published examples described below demonstrating a potentially vast amount of CB₁ and CB₂ gene divergence in human populations, which can arise from splice variations, polymorphisms, and somatic mutations.

The human CB₁ receptor has distinct splice variant forms. A PCR amplification product was isolated that lacked 167 base pairs of the coding region of the human CB₁ receptor (Shire et al., 1995). This alternative splice form (CB1a) is unusual in that it is generated from the mRNA encoding CB₁, and not from a separate exon (ibid.). When expressed, the CB1a clone would translate to a receptor truncated by 61 amino acid residues with 28 amino acid residues different at the NH2-terminal. A second splice variant of the coding region has been reported in which a 99 base portion of the coding exon is spliced out of the human mRNA leading to an in-frame deletion of 33 amino acids (Ryberg et al., 2005). This hCB1b cDNA was isolated while cloning the previously reported splice variant. Both the CB1a and CB1b variants show altered ligand binding and GTPγS activity compared with CB₁ when the cDNAs are expressed in HEK293 cells (Ryberg et al., 2005). Of the six endocannabinoids tested, only 2-AG showed significant affinity for hCB1b; furthermore, 2-AG acted as an inverse agonist at both variants. Anandamide was able to activate the variants at concentrations > 10 µM. However, Δ⁹-THC, CP55940, WIN55,212, HU210 and SR141716 exhibited good affinity and GTPγS activity with the variants. CB1a and CB1b expression has been detected in many tissues by RT-PCR (Ryberg et al., 2005 and Shire et al., 1995). It will be important to confirm that the CB1a and CB1b receptor proteins are indeed expressed as splice variants often arise due to incomplete splicing during library construction and RT-PCR techniques. The construction of antibodies selective to CB₁ or CB1a/CB1b peptides would be useful to detect these proteins. Neither splice variant is present in rat or mouse, because the splice consensus sequence is absent in these genes (Bonner, 1996).

Previous studies have suggested the presence of three exons upstream of the coding region of the CB₁ receptor (Bonner, 1996). The genomic structure of the human CB₁ receptor has been reported (Zhang et al., 2004). In this study, three exons upstream of the coding exon were identified (a total of 4 exons), with a variation in the first exon. Five distinct variant exonic structures were demonstrated.

The CB₁ receptors are highly conserved among vertebrate species and have also been found in some invertebrates (Elphick and Egertova, 2001, McPartland and Glass, 2003 and Murphy et al., 2001). Shortly after the cloning of the rat cannabinoid receptor, isolation of a human CB₁ receptor cDNA was reported (Gerard et al., 1991). The human CB₁ receptor has one less amino acid in the N-terminus as compared to the other mammalian species (472 amino acids vs. 473 amino acids). The rat and human receptors are highly conserved, 93% identity at the nucleic acid level and 97% at the amino acid level. Similarly, the mouse and rat clones have 95% nucleic acid identity (100% amino acid identity) and the mouse and human clones have 90% nucleic acid identity (97% amino acid identity) (Abood et al., 1997, Chakrabarti et al., 1995 and Ho and Zhao, 1996).

A molecular phylogenetic analysis which included the CB₁ receptor showed that the sequence diversity in 62 mammalian species varied from 0.41 to 27% (Murphy et al., 2001). In addition to mammals, the CB₁ receptor has been isolated from birds (Soderstrom et al., 2000), fish (Yamaguchi et al., 1996), amphibia (Cottone et al., 2003 and Soderstrom et al., 2000), and an invertebrate, Ciona intestinalis (Elphick et al., 2003). This deuterostomian invertebrate CB receptor contains 28% amino acid identity with CB₁, and 24% with CB₂ (Elphick et al., 2003). Since a CB receptor ortholog has not been found in Drosophila melanogaster or Caenorhabditis elegans, it has been suggested that the ancestor of vertebrate CB₁ and CB₂ receptors originated in a deuterostomian invertebrate (Elphick et al., 2003).

Several human CB₁ receptor polymorphisms have also been identified. The initial
Polymorphism found was a restriction fragment length polymorphism (RFLP) in the intron preceding the coding exon of the receptor (Caenazzo et al., 1991). The CB₁ receptor gene is intronless in its coding region, but possesses an intron 5' to the coding exon with three putative upstream exons (Bonner, 1996 and Zhang et al., 2004).

A positive association between a microsatellite polymorphism ((AAT)$_n$) in the CB₁ gene and IV drug abuse has been described (Comings et al., 1997). This polymorphism has subsequently been localized 3' to the coding exon of the CB₁ receptor (Zhang et al., 2004). Although there are differences between populations, the CB₁ (AAT)$_n$ polymorphism has also been associated with schizophrenia (Ujike et al., 2002) as well as with depression in Parkinson's disease (Barrero et al., 2005), providing genetic evidence for a role of the cannabinoid system in these disorders.

The first polymorphism in the coding exon described was a silent mutation in T453 (G to A), a conserved amino acid present in the C terminal region of the CB₁ and CB₂ receptors, that was a common polymorphism in the German population (Gadzicki et al., 1999). While this mutation is silent, analysis of several human sequences present in the database reveals that CB1K5 (accession #AF107262), a full length sequence, contains 5 nucleotide changes, three of which result in amino acid differences. Coincidentally, two amino acid differences are in the third transmembrane domain, F200L and I216V. The third variant is in the fourth transmembrane domain, V246A. A report by the group that submitted the sequence to the database revealed that this was a somatic mutation in an epilepsy patient; i.e., DNA obtained from their blood was unaltered, but DNA from the hippocampus showed the mutation (Kathmann et al., 2000). The presence of a somatic mutation rather than a polymorphism is generally indicative of the disease process in cancers (e.g. mutant p53 or APC expression in tumors but not normal tissues (Baker et al., 1989 and Lamlum et al., 2000)). CB₁ receptor polymorphisms may affect responsiveness to cannabinoids.

Zhang et al. (2004) studied several polymorphisms in control and drug-abusing individuals from European, African and Japanese ethnicities and found association with a 5'“TAG” haplotype that was highly associated with substance abuse in all three populations. Analysis of mRNA levels from post-mortem brain samples of individuals with the TAG haplotype showed reduced expression for individuals expressing this allele. In sum, the genomic studies implicate the CB₁ receptor in drug addiction and disease.

Polymorphisms in the CB₂ receptor have been identified as well (Karsak et al., 2005 and Sipe et al., 2005). Polymorphisms of the human CB₂ gene are linked to osteoporosis in several studies (Karsak et al., 2005, Karsak et al., 2009 and Yamada et al., 2007). Karsak et al. (2005) examined CB₁ and CB₂ receptor DNA in a sample of French post-menopausal patients and female controls. The authors report that certain changes in CB₂ receptor, but not the CB₁ receptor, were strongly associated with osteoporosis. A second study replicated these findings in a group of pre and post menopausal Japanese women (Yamada et al., 2007). In contrast, a recent study has found only nominally significant correlations with CB₂ polymorphisms and osteoporosis in a Chinese population; the role of the CNR2 gene in the etiology of Chinese osteoporosis thus requires further study in larger samples (Huang et al., 2009).

A recent study examined the role of CB₂ DNA or genes on hand bone strength (Karsak et al., 2009). The authors analyzed radiographic images and DNA samples from a Chevashian population, an ethnically homogeneous population of people of Bulgaric ancestry that live along the Volga River. Several SNPs (small nucleotide polymorphisms) were significantly associated with certain bone phenotypes as previously reported (Karsak et al., 2005). Two of the associated SNPs were in adjacent nucleotides (“double SNP” rs2502992–rs2501432) within the coding region of CB₂ and result in a non-conservative missense variant (Gln63Arg). This variant is probably functionally relevant as demonstrated by a differentially endocannabinoid-induced inhibition of T lymphocyte proliferation (Sipe et al., 2005). A less functional form of the CB₂ receptor appears to lead
to weak hand bone strength and is associated with osteoporosis.

In addition to the human CB$_2$ receptor, clones have been isolated from mouse (Shire et al., 1996 and Valk et al., 1997), rat (Griffin et al., 2000) (Brown et al., 2002 and Liu et al., 2009), dog (Ndong et al., 2011), the puffer fish Fugu rubripes (Elphick, 2002) as well as zebrafish (McPartland et al., 2007). There is also information in the GenBank database on additional species. The CB$_2$ receptor shows less homology between species than does CB$_1$; for instance the human and mouse CB$_2$ receptors share 82% amino acid identity (Shire et al., 1996), and the mouse and rat 93% amino acid identity. The human, rat and mouse sequences diverge at the C-terminus; the mouse sequence is 13 amino acids shorter, whereas the rat clone is 50 amino acids longer than the human CB$_2$ (Brown et al., 2002).

The first evidence for alternative splice forms of CB$_2$ was in the C-terminus of the rat CB$_2$ receptor. (Brown et al., 2002 and Griffin et al., 2000). That this may give rise to rat-specific pharmacology of the CB$_2$ receptor was suggested by differences in ligand recognition with a number of compounds at the rat CB$_2$ receptor compared to the human CB$_2$ receptor in transfected cells (Griffin et al., 2000). The clone described in these studies was amplified from genomic DNA rat CB$_2$; however this isoform has subsequently been shown to be the major splice form of rat CB$_2$ (Liu et al., 2009). Now, variants of the human and mouse CB$_2$ receptors have been reported as well (ibid).

In summary, from what we know so far, the diversity in the regulatory regions of the CB$_1$ and CB$_2$ genes may provide extensive flexibility in gene regulation of these receptors in health and disease. Cannabinoid receptors may be an excellent candidate for developing personalized medicine, as health professionals may be able to screen the gene encoding a cannabinoid receptor (i.e., CNR1 or CNR2) of a patient, and determine which class of cannabinoids to administer. For instance, a patient could possibly possess a polymorphism or acquire a mutation, which prevents efficient interactions at cannabinoid receptors by endocannabinoids. In this scenario, a drug is chosen based on the patient's genotype. A different class of cannabinoids may be able to replace the endogenous compounds; this personalized approach would require selecting a compound that has structurally distinct binding requirements from endogenous compounds (i.e., an indole such as WIN 55,212). A 'clinical endocannabinoid deficiency syndrome' resulting from defects in the endocannabinoid system (i.e. receptor mutations, alterations in endocannabinoid production), has already been proposed to underlie certain diseases including treatment resistant conditions (Russo, 2008). To date a mutation is yet to be identified in the human cannabinoid receptor that results in conclusive alteration of ligand–receptor interactions; however, molecular biologists have discovered amino acids residues important for selective ligand recognition and maintaining receptor–ligand interactions in vitro (Kapur et al., 2008 and Song and Bonner, 1996). The efficacy of future cannabis-based clinical trials could be enhanced by developing patient screening methods for polymorphisms or mutations in genes associated with the endocannabinoid system.

6. Conclusions

There is a complex molecular architecture of the cannabinoid receptors. This arrangement allows for a single receptor to recognize multiple classes of compounds and produce an array of distinct downstream effects. Classification of other GPCRs, such as GPR18 and GPR55, as bona fide cannabinoid receptors awaits further studies. Allosteric modulators can alter the pharmacological responses of cannabinoid receptors. Natural polymorphisms and alternative splice variants may also contribute to the pharmacological diversity of the cannabinoid receptors. As our knowledge of the distinct differences grows, we may be able to target select receptor conformations and their corresponding pharmacological responses. Importantly, the basic biology of the endocannabinoid system will continue to be revealed by ongoing investigations.
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Species differences in cannabinoid receptor 2 (CNR2 gene): identification of novel human and rodent CB2 isoforms, differential tissue expression and regulation by cannabinoid receptor ligands  
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Cannabinoid receptors can activate and inhibit G protein-coupled inwardly rectifying potassium channels in a Xenopus oocyte expression system  
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Structural mimicry in class A G protein-coupled receptor rotamer toggle switches: the importance of the F3.36(201)/W6.48(357) interaction in cannabinoid CB1 receptor activation  
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Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide

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