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MINIREVIEW

Ligand- and Heterodimer-Directed Signaling of the CB_1 Cannabinoid Receptor

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ABSTRACT

Seven-transmembrane G protein-coupled receptors (GPCRs) represent the single largest family of cell surface receptors. Signaling through these receptors is controlled by changes in the conformation of the receptor from inactive to active conformations, which in turn lead to the activation of multiple downstream signaling pathways. To facilitate greater diversity in signaling responses, many of these receptors are capable of adopting several distinct active conformations, in which each couples preferentially to its own set of downstream signaling partners. Because these unique signaling responses result from specific receptor active conformations, GPCR signaling may be directed toward these selective responses through either strength-of-signal effects resulting from partial agonism or through biased agonism and functional selectivity, resulting from the selective stabilization of one active conformation over the others. This review uses the CB_1 cannabinoid receptor as a specific example to highlight the contribution of two important aspects of GPCR function—orthosteric ligand binding and receptor heterodimerization—toward directed GPCR signaling.

Seven-transmembrane G protein-coupled receptors (GPCRs) are the single largest family of receptors localized to the cell surface and represent the most common target for currently available therapeutics (Jacoby et al., 2006). These receptors are typically defined by both their seven-membrane-spanning regions and their ability to couple with, and signal through, heterotrimeric guanine nucleotide binding proteins (G proteins). By tradition, GPCRs were believed to exist as monomers at the cell membrane, in which agonist binding would lead to the recruitment of a specific GDP-bound G protein, followed by the exchange of GTP for GDP, subsequently allowing the activated G protein to then act on its downstream effectors and produce a biological response (Gether, 2000). However, as our understanding of GPCR function has evolved, it has become apparent that such a model is far too simple to fully explain the complex pharmacology of these important signal transduction proteins.

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Like other proteins, GPCRs may adopt many different structural conformations. Most importantly, these receptors switch between active conformations, capable of activating G proteins, and an inactive conformation that does not activate G proteins (Kenakin, 2001). Signaling through the GPCR is, therefore, determined by the relative stability of the active conformation compared with the inactive conformation of the receptor. In the absence of ligand, receptors typically adopt the inactive state. However, many GPCRs show an appreciable proportion of the active state, even in the absence of ligand, resulting in ligand-independent constitutive activity (Costa and Herz, 1989; Chidiac et al., 1994; Bond and Ijzerman, 2006). In this model of GPCR activation, agonist binding simply stabilizes the active conformation of the receptor and, thus, facilitates an increase in the proportion of receptors in the active state compared with those in the inactive state. An inverse agonist is thus defined as a ligand that stabilizes the inactive conformation of the receptor, reducing constitutive receptor signaling, whereas a neutral antagonist binds with no preference for the active or inactive conforma-

ABBREVIATIONS: GPCR, G protein-coupled receptor; 2-AG, 2-arachydonylglycerol; AEA, *N*-arachidonoylethanolamine or anandamide; β_2AR, β_2 adrenergic receptor; CNS, central nervous system; G protein, guanine nucleotide binding protein; MAEA, (*R*)-methanandamide; THC, (−)-*trans*-Δ^9-tetrahydrocannabinol; WIN, WIN 55,212-2; ERK, extracellular signal-regulated kinase; ICI 118,551, (±)-1-[2,3-(dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol.

tions of the receptor, effectively trapping it at whatever level of activity existed in the absence of ligand (Kenakin, 2001).

Adding to the complexity of GPCR signaling, it is now clear that GPCRs are capable of adopting more than one active conformation, in which each active state is capable of stimulating an overlapping yet distinct group of downstream effectors (Berg et al., 1998; Kenakin, 2001). This may allow signaling through the receptor to be directed through one pathway over the others through either strength-of-signal or biased agonism effects (Kenakin, 1995a,b). Strength-of-signal effects occur when a receptor is more strongly coupled to one of its signaling pathways than the others, and as a result, partial agonists not capable of fully activating the receptor may still completely activate the more strongly coupled signaling response while only partially activating the weakly coupled response (Fig. 1, A and B). The result of this is an agonist that will produce preferential signaling through pathways more strongly coupled to the receptor. In contrast, biased agonism occurs when agonist binding preferentially stabilizes one active signaling state over another (Fig. 1C) (Kenakin, 1995b; Berg et al., 1998). Similar to the strength-of-signal effects, this also results in preferential activation of one signaling pathway over another; however, unlike the strength-of-signal effects, biased agonism may selectively activate any signaling pathways downstream of a given receptor, not only the most strongly coupled one. In recent years, the term "biased agonism" has become synonymous with additional terms to indicate selective activation of GPCR signaling pathways, including stimulus trafficking and, the one that has become most common, functional selectivity (Kenakin, 2001; Bosier et al., 2008a).

Directed signaling and functional selectivity is most easily detected in GPCRs that pleiotropically couple to more than one Gα subtype (G_s, $G_{i/o}$, $G_{q/11}$, etc.), different active conformations of the receptor having differing abilities to couple with, and activate, each Gα subtype (Bosier and Hermans, 2007). However, functionally selective active conformations may also influence G protein-independent signaling pathways (Drake et al., 2008) or even alter patterns of desensitization and internalization of activated receptors. This is the case for the μ-opioid receptor, in which some active conformations lead to receptor desensitization and internalization, whereas others do not (Keith et al., 1996; Blake et al., 1997).

Given that these different GPCR active states are the result of distinct conformations of the receptor, any interacting molecule with the ability to stabilize one particular active conformation over the others may also direct receptor signaling through that specific active state and, in turn, toward its

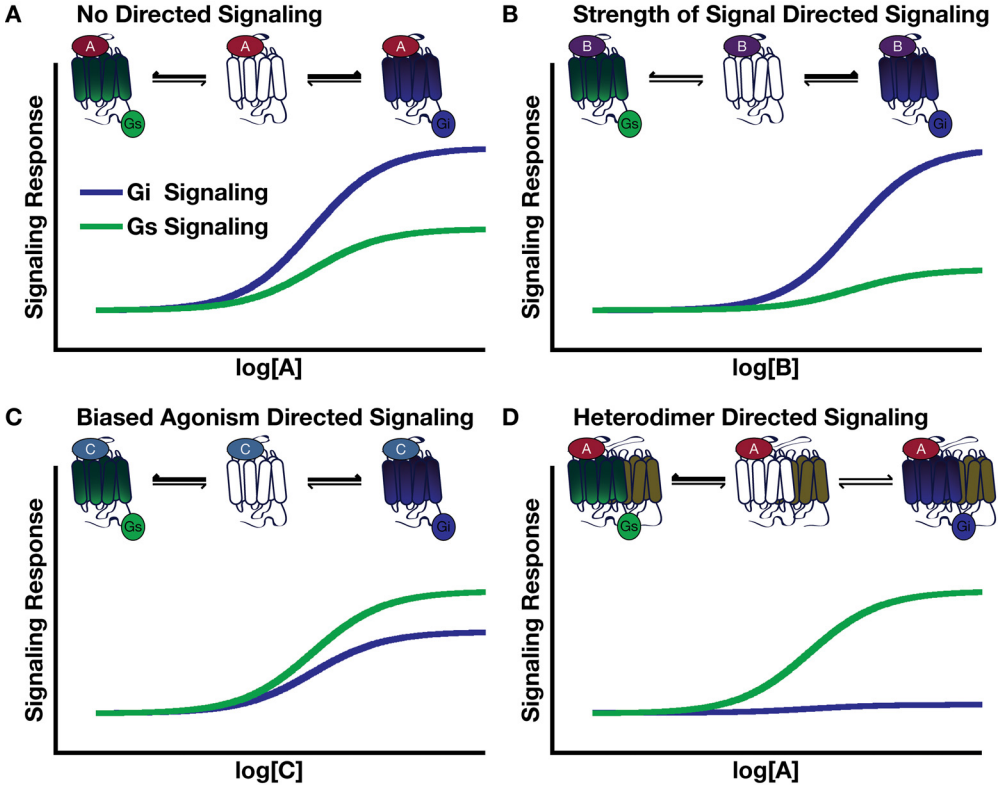

Fig. 1. Model of GPCR conformations and the resulting concentration-response curves for ligand- and heterodimer- directed signaling of a model GPCR. A, a model GPCR activated by a full agonist, ligand A, favors the G_i-coupled active state (blue) over the G_s-coupled active state (green). The resulting concentration-response shows a G_i response of greater efficacy than that of the G_s response. B, ligand B directs signaling toward G_i via strength-of-signal effects. Ligand B is a partial agonist with less efficacy than ligand A for the model receptor. As a result, ligand B activates the poorly coupled G_s pathway to a lesser extent than does ligand A, yet ligand B is still fully capable of activating the strongly coupled G_i pathway of the receptor. Instead, if ligands A and B have similar affinities for the receptor, the reduced efficacy of ligand B compared with A will result in the reduced potency of B to activate the strongly coupled G_i pathway and, in turn, a rightward shift in the concentration-response curve. C, biased agonism or true functional selectivity of the model GPCR is produced by the binding of ligand C. In this case, the ligand preferentially stabilizes the G_s-coupled active state of the receptor over the G_i-coupled state, resulting in increased G_s signaling efficacy compared with ligand A but decreased G_i signaling efficacy. D, heterodimer-directed signaling of the model GPCR favoring G_s signaling. When the model GPCR heterodimerizes with another GPCR (brown receptor), the G_s-coupled state is stabilized over the G_i-coupled state through allosteric effects. As a result, when acting on the heterodimer, ligand A no longer activates the G_i pathway but still activates the G_s pathway.

unique set of signaling pathways. In a model of GPCR activation that includes multiple active receptor conformations, different orthosteric agonist ligands may selectively bind to and stabilize specific active conformations of the receptor, resulting in ligand-directed functional selectivity (Kenakin, 1995b). In some cases, such directed selectivity can be so extreme that a ligand that acts as an inverse agonist in one pathway can be an agonist for another. For example, the β_2-adrenergic receptor (β_2AR) ligand ICI 118,551 is an inverse agonist for the β_2AR/G_s-coupled cAMP response while concurrently acting as an agonist to a β_2AR/G protein-independent, β-arrestin-mediated ERK mitogen-activated protein kinase signaling pathway (Azzi et al., 2003; Baker et al., 2003).

In addition to orthosteric ligand binding, allosteric effects on GPCRs may also affect receptor conformation and generate functional selectivity, either through ligand binding to allosteric sites, or through protein-protein interactions with the receptor (Bosier and Hermans, 2007; Leach et al., 2007; Piñeyro, 2009). Although allosteric ligands have been shown to produce functional selectivity in GPCR signaling for a few receptors (Maillet et al., 2007; Valant et al., 2008), the majority of work in this area has focused on how the protein-protein interactions of GPCRs may result in functional selectivity. Given that many of the downstream effectors activated by GPCRs, including adenylyl cyclase and K^+ and Ca^{2+} channels, form stable complexes with the receptor at the cell surface, it might be expected that direct receptor-effector physical interactions are capable of producing functionally selective signaling responses (Piñeyro, 2009). Recent observations have also demonstrated that Gα subunits may themselves alter the conformation of the receptor to affect ligand affinity and signaling (Yan et al., 2008).

In addition to the effects of G proteins and downstream effectors, when considering receptor-protein interactions, a significant amount of attention has been directed toward examining the influence of GPCR heterodimerization on the functional selectivity of these receptors. The first clear evidence that family A GPCRs form dimers or higher-order oligomers came with the demonstration of a β_2AR homodimer (Hebert et al., 1996; Angers et al., 2000). Since then, it has been found that most GPCRs form homodimers and heterodimers with other GPCRs (Pfleger and Eidne, 2005; Dalrymple et al., 2008; Panetta and Greenwood, 2008) and that these interactions influence many aspects of receptor function (Terrillon and Bouvier, 2004). One important aspect of GPCR function that may be affected by GPCR heterodimerization is the G protein-coupling preference of the receptors present in the dimer (George et al., 2000; Charles et al., 2003; Breit et al., 2004; Terrillon and Bouvier, 2004). As a result, a ligand that does not show functional selectivity in the absence of heterodimerization may produce a functionally selective response of the heterodimer because the second interacting receptor of the dimer stabilizes one active conformation over the others (Fig. 1D). Heterodimerization of these receptors, therefore, can be viewed as another means to direct functional selectivity in downstream signaling pathways that each receptor in the complex activates. The possibility of developing bivalent orthosteric ligands for heterodimers or bivalent orthosteric/allosteric modulators for GPCR homo- and heterodimers significantly increases drug development possibilities (Waldhoer et al., 2005; Valant et al., 2009). Whether such bitopic modulators might be designed to be functionally selective remains to be seen. This review compares ligand- and heterodimer-directed signaling of GPCRs, using the CB_1 cannabinoid receptor as a specific example.

The CB_1 Cannabinoid Receptor

First recognized for its ability to bind to and produce the psychotropic effects of the active agent of the plant *Cannabis sativa* (Matsuda et al., 1990), the CB_1 receptor has since become of significant physiological, pharmacological, and clinical interest. CB_1 is widely expressed both in the central nervous system (CNS) and in the periphery and is one of the central components of the endocannabinoid system composed of the cannabinoid receptors CB_1 and CB_2; the endocannabinoid ligands, including *N*-arachidonoylethanolamine or anandamide (AEA) and 2-arachydonylglycerol (2-AG); and the metabolic enzymes responsible for the production and breakdown of these ligands (Rodríguez de Fonseca et al., 2005). The endocannabinoid system, in particular CB_1, has generated considerable research interest as a potential therapeutic target in the treatment of a plethora of conditions, including pain, metabolic syndrome, neurological and psychiatric disorders, and addiction, among others (Piomelli et al., 2000; Croxford, 2003; Vinod and Hungund, 2006; Hosking and Zajicek, 2008; Pertwee, 2008).

CB_1 provides a key example of both ligand- and heterodimer-directed GPCR functional selectivity. Stimulation of CB_1 leads primarily to the activation of $G_{i/o}$ proteins to inhibit adenylyl cyclase, activate mitogen-activated protein kinases, inhibit voltage-dependent Ca^{2+} channels, and activate inwardly rectifying K^+ channels (Demuth and Molleman, 2006). However, activation of CB_1 also results in signaling through G_s proteins to activate adenylyl cyclase (Glass and Felder, 1997; Maneuf and Brotchie, 1997), as well as signaling through $G_{q/11}$ proteins to increase intracellular Ca^{2+} concentration (Lauckner et al., 2005; McIntosh et al., 2007). In addition to the fact that CB_1 couples with all three major Gα subtypes, ligands acting at this receptor are quite diverse in both their chemical structures and pharmacological effects (Pertwee, 2006), making CB_1 ideal for the study of ligand-directed functional selectivity. Finally, because CB_1 has been shown to form heterodimers with several other receptors, including the D_2 dopamine receptor, μ, κ, and δ opioid receptors, the orexin-1 receptor, the A_{2A} adenosine receptor, and the β_2AR (Kearn et al., 2005; Mackie, 2005; Ellis et al., 2006; Rios et al., 2006; Carriba et al., 2007; Hudson and Kelly, 2008), CB_1 is also a good candidate for examining heterodimer-directed GPCR functional selectivity.

Ligand-Directed Signaling of CB_1

Ligands with affinity for the CB_1 receptor are both structurally and pharmacologically diverse (Fig. 2). CB_1 agonists can be broadly divided into four structurally distinct classes: 1) the classic[1] cannabinoids, including natural compounds from the plant *C. sativa* such as (−)-*trans*-Δ^9-tetrahydrocannabinol (THC), and its related synthetic derivatives (e.g., the highly potent HU-210); 2) the nonclassic[2] cannabinoids, which are similar to the classic cannabinoids except that they

[1] Also known as "classical."
[2] Also known as "nonclassical."

lack the dihydropyran ring (e.g., CP 55,940); 3) the eicosanoids, which are derivatives of arachidonic acid and include the endocannabinoids AEA and 2-AG; and 4) the structurally distinct aminoalkylindoles, including WIN 55212-2 (WIN) and its related compounds (Howlett et al., 2002; Pertwee, 2006). The most common class of CB_1 antagonists is the diarylpyrazoles, compounds that are structurally dissimilar from the four classes of CB_1 agonists. These compounds are typically inverse agonists at CB_1 and are defined by the prototypical compound SR 141716 (Howlett et al., 2002). However, there are also examples of CB_1 antagonists with structures similar to those of the cannabinoid agonists. For example, the compound O-2050, which is a neutral antagonist at CB_1, is structurally similar to the classic cannabinoids (Gardner and Mallet, 2006).

Given the vast structural variation of the cannabinoid ligands, it is not surprising that they might possess significant selectivity in their ability to modulate the various CB_1 signaling pathways. This extends even to their actions on the classic pertussis toxin-sensitive $G_{i/o}$ signaling pathway of CB_1. In a study directly comparing the levels G_i and G_o activation by various CB_1 ligands, only the classic cannabinoid HU-210 produced maximal activation of both G protein subtypes (Glass and Northup, 1999). The aminoalkylindole WIN and the endocannabinoid AEA produced maximal G_i responses but submaximal G_o responses, whereas THC produced submaximal responses for both G_i and G_o. These findings demonstrate clear ligand-directed signaling for WIN and AEA in favor of CB_1-G_i activation. Because CB_1 is more strongly coupled to G_i than it is to G_o (Glass and Northup,

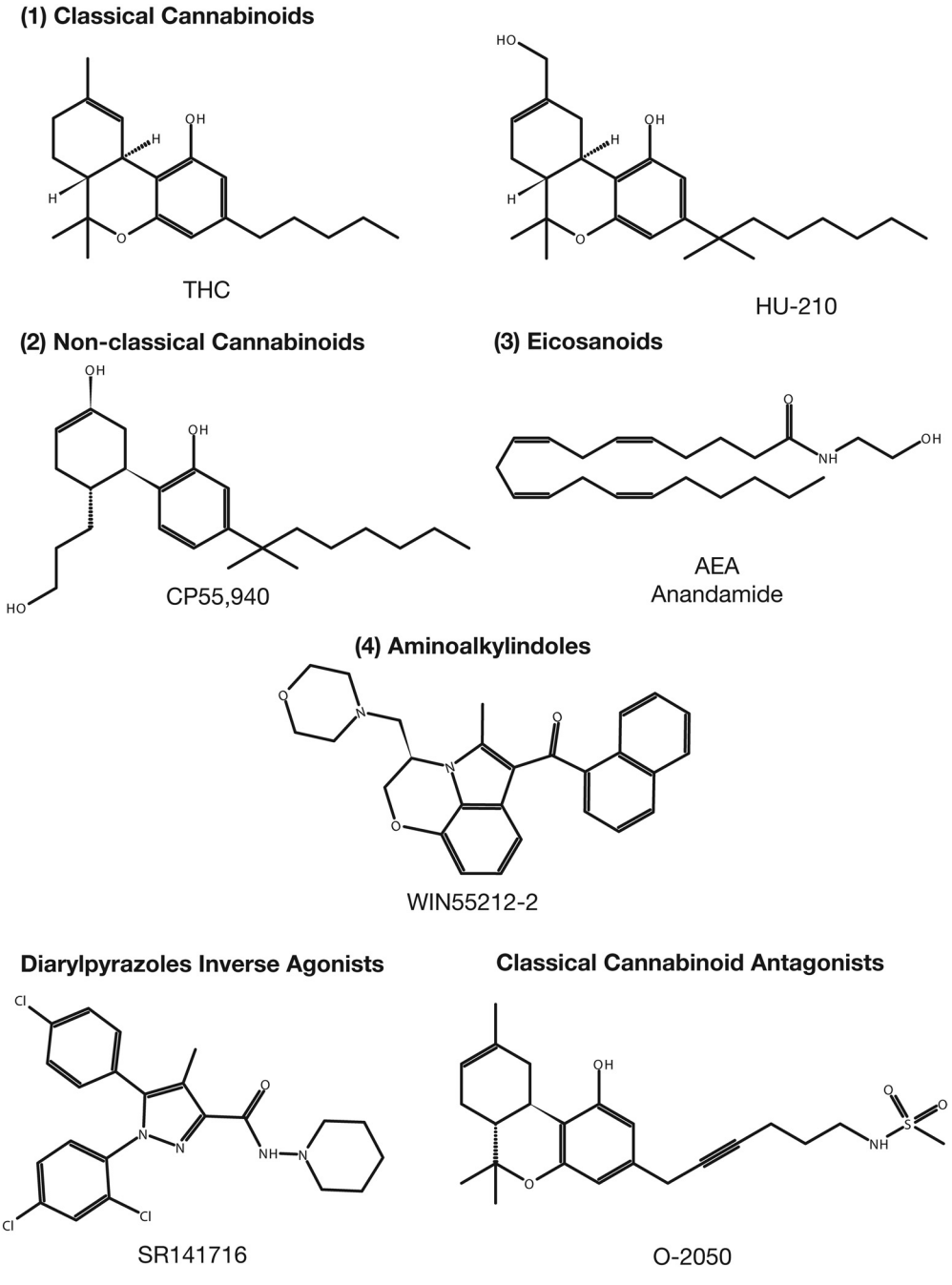

Fig. 2. Chemical structures of representative cannabinoid ligands.

1999), this ligand-directed signaling may be due to either strength-of-signal or biased agonism effects of these ligands. Subsequent work examining the specific G_i subtypes activated by cannabinoid ligands has now shown that WIN activates all of the G_{i1}, G_{i2}, and G_{i3} subtypes as an agonist, whereas other ligands, including the classic cannabinoid desacetyl-levonantradol and the endocannabinoid (*R*)-methanandamide (MAEA), activate only some G_i isoforms as an agonist while acting as inverse agonists at others (Mukhopadhyay and Howlett, 2005). Such observations clearly indicate that within CB_1-G_i signaling, there is true functional selectivity in the activation of G_i subtypes, because these results cannot be explained by strength-of-signal ligand-directed effects. In this study, the CB_1 ligand SR141716 acted as an inverse agonist for all three subtypes of G_i. In addition, lending further support for the concept of true CB_1-G_i functional selectivity, a separate study comparing the nonclassic cannabinoid CP 55,940 with the aminoalkylindole WIN clearly demonstrated that each ligand stabilized a qualitatively different conformation of the CB_1 receptor, and these conformations had differing affinities for G_{i1} (Georgieva et al., 2008).

In addition to evidence directly showing $G_{i/o}$ functional selectivity of CB_1 at the level of G protein activation, several reports have indirectly shown $G_{i/o}$ ligand-directed selectivity in signaling pathways downstream of CB_1. The classic cannabinoid HU-210 and the nonclassic cannabinoid CP 55,940 have reciprocal effects on tyrosine hydroxylase expression, as well as having opposite effects on ERK versus c-Jun NH_2-terminal kinase phosphorylation in neuroblastoma N1E-115 cells, with HU-210 being more effective in activating ERK and CP 55,940 being more effective in activating c-Jun NH_2-terminal kinase (Bosier et al., 2007; Bosier et al., 2008b). Because these reciprocal responses to the two ligands were at least partially sensitive to pertussis toxin, it suggests that they are the result of CB_1-$G_{i/o}$ subtype functional selectivity.

Under some circumstances, CB_1 may also signal through G_s to increase cAMP levels, in addition to its more traditional $G_{i/o}$-mediated pathway, extending the possibility of greater ligand-directed signaling and functional selectivity for this receptor (Maneuf and Brotchie, 1997; Felder et al., 1998; Calandra et al., 1999). A careful examination of the effect of various cannabinoid ligands on CB_1-G_s signaling showed clear ligand-directed signaling (Bonhaus et al., 1998). In particular, although WIN and HU-210 had comparable efficacies for the $G_{i/o}$ and G_s signaling pathways, CP 55,940 and AEA both had a significant preference for the CB_1-G_i pathway. Further support for CP 55,940 being poorly linked to CB_1-G_s signaling came from the observation that a CB_1 mutant that constitutively activated G_s did not exhibit increased binding to this ligand (Abadji et al., 1999). The ligand SR141716, which is an inverse agonist for the classic $G_{i/o}$ signaling pathway, seems to act as an inverse agonist for the G_s pathway as well, because in all cases, the CB_1-G_s response was blocked by SR141716, and this compound was also capable of attenuating the signaling of a CB_1 mutant constitutively active for G_s signaling (Maneuf and Brotchie, 1997; Bonhaus et al., 1998; Abadji et al., 1999; Calandra et al., 1999). However, because the CB_1 receptor is not as strongly coupled to G_s as it is to G_i (Felder et al., 1998), these ligand-directed G_s responses may not be true biased agonism functional selectivity but instead may be the result of strength-of-signal effects resulting from HU-210 and WIN being stronger agonists than CP 55,940 and AEA.

The more recent observation that CB_1 also signals through $G_{q/11}$ has added an additional level of complexity to the ligand-directed signaling of this receptor. In both a heterologous expression system using rat CB_1 and a human ocular cell line endogenously expressing CB_1, it was found that only the aminoalkylindole WIN was capable of activating CB_1-$G_{q/11}$ signaling to increase intracellular Ca^{2+} levels, whereas all other CB_1 agonists tested, including HU-210, THC, CP55,940, and MAEA, did not facilitate CB_1-$G_{q/11}$ signaling (Lauckner et al., 2005; McIntosh et al., 2007). The CB_1 inverse agonist SR141716 blocked the WIN response, indicating that it still acted as an antagonist, and possibly as an inverse agonist, for CB_1-$G_{q/11}$ signaling.

Taken together, it is clear that complex ligand-directed signaling events can be detected for CB_1 (Table 1). This includes the true functional selectivity observed within CB_1-$G_{i/o}$ and $G_{q/11}$ signaling and CB_1-G_s-directed signaling, which may result from either ligand strength-of-signal effects or functional selectivity. Among the cannabinoid ligands, the aminoalkylindole WIN seems to be the least restrictive in its

TABLE 1
Summary of the ligand-directed signaling of CB_1

Ligand	G_i	G_o	G_s	$G_{q/11}$	References
Classic					
HU-210	++	++	++	N.E.	Bonhaus et al., 1998; Glass and Northup, 1999; Lauckner et al., 2005
THC	+	+	+	N.E.	
Nonclassic					
CP 55,940	++[a]	×	+	N.E.	Bonhaus et al., 1998; Glass and Northup, 1999; Lauckner et al., 2005; McIntosh et al., 2007; Georgieva et al., 2008
Eicosanoid					
AEA	++[a]	+	+	N.E.[b]	Bonhaus et al., 1998; Glass and Northup, 1999; Mukhopadhyay et al., 2005; McIntosh et al., 2007
Aminoalkylindole					
WIN	++	+	++	+	Bonhaus et al., 1998; Glass and Northup, 1999; Lauckner et al., 2005; Mukhopadhyay et al., 2005; McIntosh et al., 2007
Diarylpyrazole					
SR 141716	−	×	−	N.E.[c]	Abadji et al., 1999; Lauckner et al., 2005; Mukhopadhyay et al., 2005

+, agonist effect; −, inverse agonist effect; N.E., no agonist or inverse effect (compounds probably act as neutral antagonists); ×, has not been directly tested for G_o-specific coupling.
[a] Acts as full G_i agonist but has reduced affinity or efficacy to specific G_i subtypes.
[b] Data obtained with MAEA instead of AEA.
[c] SR 141716 acted as an antagonist to the $G_{q/11}$ pathway but was not tested as an inverse agonist.

ability to activate all of the various CB_1-G protein subtype pathways, acting as an agonist for each. In contrast, the diarylpyrazole ligands, including SR141716, act as inverse agonists or antagonists for every pathway. HU-210 also showed less selectivity than the other ligands, activating every pathway except $G_{q/11}$, whereas the nonclassic cannabinoid CP55,940 and the eicosanoids AEA and MAEA, possessed significantly greater selectivity in their abilities to activate all CB_1 signaling pathways examined.

Heterodimer-Directed CB_1 Signaling

Like many other GPCRs, CB_1 physically and functionally interacts with other GPCRs as dimers or higher-order oligomers. To date, CB_1 has been found to form homodimers as well as heterodimers with the D_2 dopamine receptor, the μ-, κ-, and δ-opioid receptors, the orexin-1 receptor, the A_{2A} adenosine receptor, and the β_2AR (Wager-Miller et al., 2002; Kearn et al., 2005; Ellis et al., 2006; Rios et al., 2006; Carriba et al., 2007). These interactions have been demonstrated to influence several different aspects of CB_1 function, including the specific signaling pathways that are activated by cannabinoid agonists.

The most thoroughly studied CB_1 heterodimer with regard to its effect on CB_1 signaling is the CB_1/D_2 heterodimer. Even before a physical interaction between these two receptors had been demonstrated, it was observed that costimulation of CB_1 and D_2 in striatal neurons led to an accumulation of cAMP, whereas stimulation of either receptor alone led to an inhibition of cAMP (Glass and Felder, 1997). This response was suggested to be the result of CB_1 switching from G_i to G_s signaling if CB_1 was coactivated with the D_2 receptor. Thereafter, it was found that coexpression of the D_2 receptor alone was sufficient to switch CB_1 toward increased G_s coupling, even in the absence of a D_2 agonist (Jarrahian et al., 2004). Finally, with the demonstration that CB_1 does in fact form a heterodimer with D_2 by coimmunoprecipitation (Kearn et al., 2005), it can be suggested that the CB_1/D_2 interaction stabilizes a CB_1 active state with increased coupling to G_s. Because the initial study found that costimulation of the D_2 receptor was required for the CB_1 switch to G_s signaling (Glass and Felder, 1997), it is possible that it is only the active state of D_2 that facilities this switch. If this is the case, CB_1-G_s switching in the absence of D_2 agonist can be explained by the fact that D_2 is a constitutively active receptor in some systems (Hall and Strange, 1997).

Similar to the effect of the CB_1/D_2 heterodimer on CB_1-G_s signaling, A_{2A} adenosine receptor coactivation was required for effective CB_1-G_i signaling, as measured by inhibition of forskolin-mediated cAMP accumulation, in the context of a CB_1/A_{2A} heterodimer (Carriba et al., 2007). When CB_1 and A_{2A} were coexpressed, CB_1 only signaled through G_i when A_{2A} was coactivated (Carriba et al., 2007), even though CB_1 effectively coupled to G_i when expressed alone (Felder et al., 1995). These findings indicate that the physical interaction between CB_1 and A_{2A} in the absence of an A_{2A} agonist results in a conformation of CB_1 that does not allow for CB_1-G_i signaling to occur. In contrast, the opposite effect seems to occur in the CB_1/μ-opioid heterodimer. In this case, activation of either receptor alone within the heterodimer led to effective G_i signaling, but coactivation of both receptors resulted in inhibition of their G_i-mediated responses (Rios et al., 2006). Thus, it seems that only when the μ-opioid receptor is in the active state is this receptor able to inhibit CB_1 signaling through G_i. It has not yet been determined for either the CB_1/A_{2A} and CB_1/μ-opioid receptor heterodimers whether there is an effect on either CB_1-G_s- or CB_1-$G_{q/11}$-coupled signaling (Rios et al., 2006; Carriba et al., 2007), representing an important aspect of CB_1 pharmacology that deserves future attention.

Heterodimerization of CB_1 with β_2AR also results in directed CB_1 signaling, which may be relevant to many of the tissues that coexpress these two receptors, including the brain, cardiovascular system, bone, eye, and reproductive tract (Jampel et al., 1987; Wanaka et al., 1989; Tsou et al., 1998; Stamer et al., 2001; Wang et al., 2004; Pacher and Haskó, 2008). Specifically, when CB_1 and β_2AR are coexpressed, the G protein-coupling preference for CB_1 switches away from G_s and toward G_i (Hudson and Kelly, 2008). This was evident by an increase in CB_1-G_i-mediated pERK signaling and a decrease in CB_1-G_s-mediated phosphorylation of cAMP response element binding protein signaling when β_2AR was present. Unlike the effects seen with the D_2 dopamine and A_{2A} adenosine receptors, this effect seems to be independent of the activation state of β_2AR, because neither β_2AR agonist nor antagonist significantly altered either CB_1 response.

In considering the overall influence of the various CB_1 heterodimers on CB_1-directed signaling and functional selectivity (Table 2), it is clear that heterodimer-directed signaling has the potential to be much more complex than ligand-directed signaling, because it depends not only on which GPCRs are present in the complex but also on the (possibly multiple) active states of each receptor. Further complicating the issue is the recent demonstration, using sequential resonance energy transfer, that CB_1 forms a hetero-oligomer with the D_2 receptor and the A_{2A} receptor (Carriba et al., 2008). This $CB_1/D_2/A_{2A}$ complex probably results in even more complex CB_1 functional selectivity and directed signaling in cells that coexpress all three of these receptors.

Allosteric Ligands of CB_1 and Functional Selectivity

Despite the fact that allosteric ligands by definition alter receptor conformation through binding at sites distinct from the orthosteric ligand binding site, only recently has it been directly shown that these ligands produce functionally selec-

TABLE 2
Summary of heterodimer-directed CB_1 signaling

Heterodimer	$G_{i/o}$	G_s	References
CB_1			
Expressed alone	++	++	Felder et al., 1995; Hudson and Kelly, 2008
CB_1/D_2			
Activated D_2	++	+++	Glass and Felder, 1997; Jarrahian et al., 2004; Kearn et al., 2005
Basal D_2	++	++	
CB_1/A_{2A}			
Activated A_{2A}	++	N.D.	Carriba et al., 2007
Basal A_{2A}	–	N.D.	
CB_1/μ-opioid			
Activated μ-opioid	+	N.D.	Rios et al., 2006
Basal μ-opioid	++	N.D.	
CB_1/β_2AR			
Active or basal β_2AR	+++	+	Hudson and Kelly, 2008

+, CB_1 in heterodimer signals through this pathway; –, CB_1 in heterodimer does not signal through this pathway; N.D., not determined.

tive GPCR signaling responses (Leach et al., 2007; Maillet et al., 2007; Valant et al., 2008). CB_1 possesses at least one allosteric site, binding at which is typically associated with enhanced orthosteric agonist affinity yet decreased agonist efficacy (Price et al., 2005; Horswill et al., 2007; Ross, 2007). This contradictory effect suggests that allosteric ligands that bind at this site stabilize a CB_1 conformation with high agonist affinity but which is functionally less active (Ross, 2007). It is interesting that there does seem to be a ligand-specific component to the action of these allosteric modulators, because although they tend to enhance agonist binding, they inhibit the binding of the inverse agonist SR141716 (Price et al., 2005). This result may be explained if the receptor conformation stabilized by the allosteric ligand has a lower affinity for SR141716 but higher affinity for the CB_1 agonists. Although these results do not directly demonstrate CB_1 functional selectivity resulting from the binding of allosteric modulators, the fact that these modulators do seem to alter the conformation of CB_1 in such a way as to affect signaling efficacy suggests that such functionally selective CB_1 allosteric ligands may exist.

Conclusions

The ultimate goal in the development of any new therapeutic agent is to identify a drug that produces the desired effect with minimal side effects. To this end, the concept of directed signaling and functional selectivity has generated significant interest as a means to develop compounds that can selectively activate or block receptor-signaling pathways that lead only to the desired therapeutic effect. This is of particular importance for the CB_1 receptor as a potential druggable target for the treatment of pain, psychiatric, and neurodegenerative disorders given that many existing cannabinoid agonists for CB_1 are of little value therapeutically because of their unwanted psychotropic side effects (Croxford, 2003; Vinod and Hungund, 2006; Bambico and Gobbi, 2008; Bilsland and Greensmith, 2008; Hosking and Zajicek, 2008; Pazos et al., 2008; Pertwee, 2008). Several approaches have been undertaken to develop cannabinoid compounds that can produce analgesia with reduced psychotropic side effects. These include the development of CNS-excluded CB_1 ligands or targeting the nonpsychotropic CB_2 cannabinoid receptor (Agarwal et al., 2007; Hosking and Zajicek, 2008; Pertwee, 2008). However, because much of the therapeutic potential of cannabinoids lie in CNS-located CB_1 receptors (Croxford, 2003; Pertwee, 2008), neither of these strategies are likely to provide a complete solution. Instead, a combination of these approaches together with an approach that takes advantage of ligand- or heterodimer-directed CB_1 signaling may be the key to unlocking the full therapeutic potential of the cannabinoids.

Even with cannabinoid ligands already available, there is some promise to the idea of developing CB_1 agonists that produce only a desired therapeutic effect without the unwanted side effects. For example, it has been suggested that the reciprocal regulation of tyrosine hydroxylase, an enzyme involved in the synthesis of dopamine, by cannabinoid ligands HU-210 and CP 55,940 may present a means to develop cannabinoids with a reduced risk of addiction (Bosier and Hermans, 2007). This is still speculative, however, given that it is unclear which specific CB_1-signaling pathways should be activated to produce the desired therapeutic effects versus which should be excluded to reduce the unwanted side effects. Only with the development of suitable functionally selective CB_1 ligands will these questions truly be answered in vivo. Unfortunately, there is still the possibility that even a fully selective ligand activating only one receptor pathway may not be able to separate desired from unwanted pharmacological effects if both effects are mediated by the same CB_1-signaling pathway through actions on different cells, regions, or tissues.

To resolve such problems, heterodimer-directed signaling may prove to be a useful solution. Heterodimer-directed signaling is more complex than the ligand-directed selectivity because it is influenced not only by the receptors present in the dimer but also by the active state of each receptor. However, it also presents a greater challenge for drug development because controlling which receptors heterodimerize in vivo will be difficult. Instead, the answer probably will be to generate ligands that selectively activate one specific GPCR heterodimer over others. Such heterodimer-selective ligands have long been proposed as a means to generate more functionally and regionally selective GPCR responses (Dalrymple et al., 2008; Panetta and Greenwood, 2008), because these ligands would only have actions in regions and tissues in which the targeted heterodimer is expressed. Recent work has begun to show promise in the development of such ligands for several GPCR heterodimer pairs, but it remains to be seen whether these ligands will be useful clinically (Daniels et al., 2005; Xie et al., 2005; Breit et al., 2006). The prospect of such ligands for CB_1, which are capable of activating specific CB_1 signaling pathways in specific cells and tissues, may be the final step in the development of psychotropically inactive, clinically useful CB_1 cannabinoid agonists.

Although much has been learned about the selectivity of CB_1 with respect to its pleiotropic coupling to various G protein subtypes, additional forms of CB_1 functional selectivity still need to be examined. For example, neither ligand-directed selectivity in receptor desensitization and internalization, as has been shown with the μ-opioid receptor (Keith et al., 1996; Blake et al., 1997), nor ligand-directed selectivity in G protein-independent signaling pathways, as has been demonstrated with β-arrestin-biased ligands for the β_2AR (Drake et al., 2008), has been examined for CB_1. A more complete understanding of the ways in which these different forms of CB_1 functional selectivity are affected by cannabinoid ligands and heterodimer pairs is critical to the future development of effective therapeutics targeting the CB_1 receptor.

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