REVIEW

Signal transduction of the CB_1 cannabinoid receptor

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Abstract

The CB_1 cannabinoid receptor (CB_1R) is the major cannabinoid receptor in neuronal cells and the brain, but it also occurs in endocrine cells and other peripheral tissues. CB_1R is a member of the superfamily of G-protein-coupled receptors (GPCRs), which are characterized by seven transmembrane helices. The major mediators of CB_1R are the G proteins of the $G_{i/o}$ family, which inhibit adenylyl cyclases in most tissues and cells, and regulate ion channels, including calcium and potassium ion channels. Regulation of ion channels is an important component of neurotransmission modulation by endogenous cannabinoid compounds released in response to depolarization and Ca^{2+}-mobilizing hormones. However, evidence exists that CB_1Rs can also stimulate adenylyl cyclase via G_s, induce receptor-mediated Ca^{2+} fluxes and stimulate phospholipases in some experimental models. Stimulation of CB_1R also leads to phosphorylation and activation of mitogen-activated protein kinases (MAPK), such as p42/p44 MAPK, p38 MAPK and c-Jun N-terminal kinase, which can regulate nuclear transcription factors. Activated and phosphorylated CB_1Rs also associate with β-arrestin molecules, which can induce the formation of signalling complexes and participate in the regulation of GPCR signalling. Recent data also suggest that CB_1Rs can form homo- and heterodimers/oligomers, and the altered pharmacological properties of these receptor complexes may explain the pharmacological differences observed in various tissues.

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Introduction

Marijuana or cannabis is a very popular recreational drug due to its ability to alter sensory perception and cause euphoria. However, it has also been recognized thousands of years ago that extracts of *Cannabis sativa* can exert medicinal effects (Pacher *et al.* 2006). The correct chemical structure of the main psychoactive ingredient of marijuana, Δ^9-tetrahydrocannabinol (THC), was identified by Gaoni (1964). Although the lipophylic nature of cannabinoids slowed down the progress of pharmacological identification of its biological target, high-affinity-binding sites for cannabinoids in brain membranes were first reported in 1988 (Devane *et al.* 1988). Molecular biological studies have identified two major cannabinoid receptors, the CB_1 receptor (CB_1R) and CB_2 receptor (CB_2R; Matsuda *et al.* 1990, Munro *et al.* 1993). Anandamide and 2-arachidonylglycerol (2-AG) have been identified as the major endocannabinoids, but other endocannabinoid mediators, including 2-AG ether (noladin ether), *O*-arachidonoyl ethanolamine (virodhamine) and endogenous analogues of anandamide (eicosatrienoylethanolamide and docosatetraenoylethanolamide), were also identified (Freund *et al.* 2003, Pacher *et al.* 2006). Recent studies have also suggested that in addition to CB_1R and CB_2R, cannabinoid ligands can exert effects on other receptors, such as GPR55 and transient receptor potential vanilloid type 1 (Pacher *et al.* 2006). In the past decade, the endocannabinoid system has been implicated in a number of physiological functions in the central and peripheral nervous systems and in peripheral organs. Drugs acting on the endocannabinoid system have therapeutic potential in a number of pathologic conditions, including obesity and metabolic syndrome, mood and anxiety disorders, movement disorders, neuropathic pain, multiple sclerosis and spinal cord injury, as well as in atherosclerosis, myocardial infarction, stroke, hypertension, cancer, glaucoma and osteoporosis (Pacher *et al.* 2006, Table 1).

Rimonabant, an antagonist of CB_1R, has already been introduced to the market to treat obesity and nicotine addiction (Bellocchio *et al.* 2006). Although the central side effects seriously limit the widespread use of CB_1R antagonists (Steinberg & Cannon 2007),

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Table 1 Affinities of the main cannabinoid ligands to CB_1R

	K_i or K_d[a] values	
	Human	Rat
WIN55 212-2	16·7[a]	2·4[a]
SR141716A	2·9[a]	1[a]
CP 55 940	2·5[a]	0·98[a]
THC	25·1	42·6
AEA	239	88
2-AG	3423	1181
HU-210	0·25	0·34

[a]K_i or K_d values are based on a previous meta-analysis of CB_1R ligand affinities (McPartland *et al.* 2007).

the therapeutical potential of drugs acting on CB_1R is still very high (Kunos *et al.* 2008). The aim of this review is to discuss the intracellular mechanisms of the action of CB_1R. After summarizing the available data about the G-protein activation and signal transduction of CB_1R, this review also analyses recent data about dimerization or oligomerization of these receptors, and the tonic/basal activity of the receptor, which may contribute to its importance as a therapeutic target in metabolic diseases (Kunos *et al.* 2008).

Classical signal transduction pathways

Inhibition of cAMP production

Inhibition of adenylyl cyclase activity was the first characterized cannabinoid agonist-stimulated CB_1R signal transduction pathway (Howlett & Fleming 1984, Howlett 1985, Howlett *et al.* 1986; Fig. 1A). The effect was blocked by pertussis toxin, indicating that it was mediated through $G_{i/o}$ proteins. CB_1R-mediated inhibition of adenylyl cyclase activity was reported in neural and peripheral tissues, as well as in cells overexpressing CB_1R as reviewed previously (Howlett 2005). Coexpression of isoforms 1, 3, 5, 6 or 8 of adenylyl cyclase resulted in CB_1R-mediated inhibition of cAMP accumulation, suggesting that the activated $G_{i/o}$ proteins regulate these isoforms of the enzyme (Rhee *et al.* 1998). In recent years, numerous pharmacological studies have indicated that G-protein-coupled receptors (GPCRs) can adopt multiple conformations, leading to different signalling events. These different conformations can be stabilized by different ligands, causing ligand-biased signal transduction. In this concept, agonist and antagonist properties of a ligand can only be interpreted when a particular signal transduction pathway is defined, since the same ligand can behave as an agonist in one pathway, or antagonist or inverse agonist in another (Kenakin 2007). In the case of CB_1R, similar biased agonism was also observed when activation of different $G_{i/o}$ subtypes were measured (Houston & Howlett 1998, Glass & Northup 1999, Mukhopadhyay *et al.* 2002). In the membranes of Sf9 cells, the relative activation of G_i and G_o was dependent on agonist (Glass & Northup 1999). In N18TG2 cells, WIN55 212-2 behaved as full agonist for G_{i1}, G_{i2} and G_{i3}, while desacetyllevonantradol is agonist at G_{i1} and G_{i2} and inverse agonist at G_{i3}. Methanandamide, a more stable analogue of anandamide, acts as agonist at G_{i3} and inverse agonist at G_{i1} and G_{i2}. SR141716 was inverse agonist at all three G proteins tested by coimmunoprecipitation (Mukhopadhyay & Howlett 2005). In another study, it was demonstrated that WIN55 212-2 activated different $G_{i/o}$ subtypes with different efficacies, suggesting that ligand-biased agonism is a more complicated issue as even a single ligand can activate different pathways depending on ligand concentration (Prather *et al.* 2000). Another complicating factor is that CB_1R may have different affinity states, which may represent different conformations (Houston & Howlett 1998).

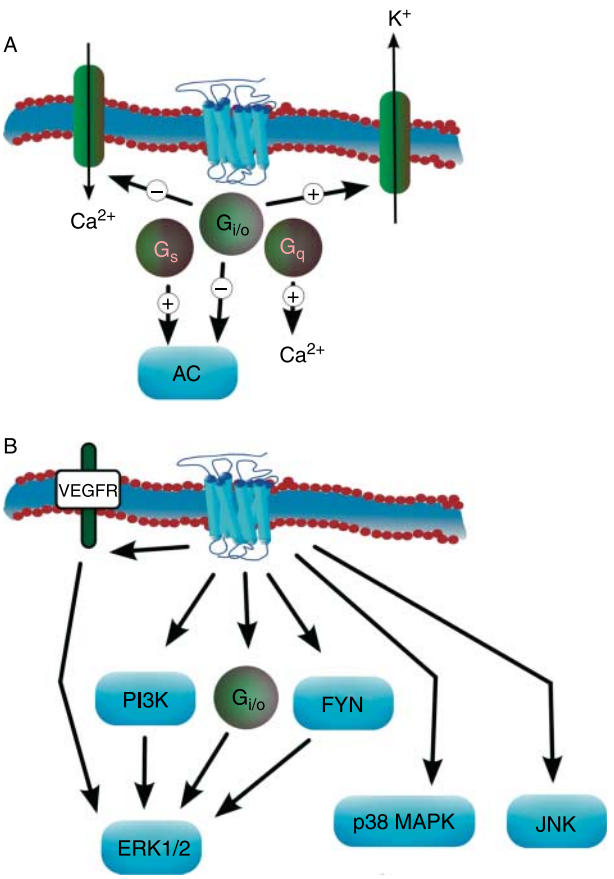

Figure 1 Main signal transduction pathways of CB_1R activaton. G-protein activation and modulation of ion channels (A), and activation of different MAPK pathways (B).

In agreement with the concept of multiple receptor states, CB_1R coupled with CP 55 940 or WIN 55 212-2 adopted different conformations as detected with plasmon-waveguide resonance spectroscopy (Georgieva *et al.* 2008). In addition, relative signalling efficacies of CB_1R ligands may be also different in different brain regions, and the same ligand could activate $G_{i/o}$ subtypes with different potencies in a number of brain regions (Sim *et al.* 1996, Breivogel *et al.* 1997, 2004, Breivogel & Childers 2000). These later results may be explained by different profiles of G proteins, or possibly with diverse interactions (e.g. dimerization) with other GPCRs (see below).

Stimulation of cAMP production

It has been reported that in pertussis-pretreated cells, CB_1R stimulation leads to adenylyl cyclase activation suggesting that in certain circumstances, CB_1R can couple to G_s proteins (Glass & Felder 1997, Abadji *et al.* 1999, Calandra *et al.* 1999, Kearn *et al.* 2005; Fig. 1A). However, others could not immunoprecipitate G proteins, other than $G_{i/o}$, with CB_1R (Mukhopadhyay *et al.* 2000). Coexpression of isoforms 2, 4 or 7 of adenylyl cyclase also resulted in CB_1R-mediated stimulation of cAMP formation, but this effect was probably mediated by Gβγ dimers released from G_i proteins (Rhee *et al.* 1998).

Activation of $G_{q/11}$ proteins

Ca^{2+} signalling after CB_1R stimulation has also been reported (Fig. 1A), however, the mechanism of this response is not clear. CB_1R stimulation leads to an increase in Ca^{2+} levels in N18TG and NG108-15 cells, but not in C9 cells (Sugiura *et al.* 1996). It has also been reported that this response was pertussis toxin-sensitive in NG108-15 cells, suggesting the role of $G_{i/o}$ proteins, which can regulate β2 isoform of phospholipase C (PLC) via Gβγ subunits, in this response (Sugiura *et al.* 1996, 1997). In contrast, in HEK cells expressing CB_1R, only WIN55 212-2 caused Ca^{2+} signal generation, which occurred through activation of G_q proteins (Lauckner *et al.* 2005). Ca^{2+} signalling after stimulation of CB_1R and CB_2R has also been reported in insulinoma cells, and this response was G_q/PLC dependent and induced by arachidonoyl-chloro-ethanolamide and JWH133, a CB_2R agonist (De Petrocellis *et al.* 2007). Anandamide also induced Ca^{2+} signal CB_1R dependently. These cell-type-specific differences in the mechanism of CB_1R-mediated Ca^{2+} signal generation may point to the different G-protein subunit composition of various cell types, or may be caused by different dimerization/interaction of CB_1R with other receptors in these cells.

In contrast to CB_1R and CB_2R, GPR55, which has been suggested to be another cannabinoid receptor, seems to be coupled mainly to Ca^{2+} signalling (Baker *et al.* 2006, Ross 2009). It has been reported that GPR55 induces Ca^{2+} signal generation, but it is a lysophosphatidylinositol receptor (Oka *et al.* 2007). Another report showed, using GTPγS binding in HEK293 cells, that GPR55 was activated by CP 55 940, O-1602, THC, palmitoylethanolamide, anandamide, 2-AG, cannabinoidal (abnormal-CBD) and virodhamine, and was coupled to G_{13} protein, without activation of Ca^{2+} signal generation (Ryberg *et al.* 2007). In this report, WIN55 212-2 neither coupled nor activated GPR55, similar to findings of Johns *et al.* (2007). In contrast, in another report, GPR55 expressed in HEK293 cells, and its stimulation by THC, anandamide, methanandamide and JWH015, but not by 2-AG, palmitoylethanolamide, CP 55 940, virodhamine, and abnormal-CBD lead to G_q activation and Ca^{2+} signal generation (Lauckner *et al.* 2008). In endothelial cells, GPR55 was also coupled to Ca^{2+} signal, but it was dependent on CB_1R activity (Waldeck-Weiermair *et al.* 2008). Since GPR55 and CB_1R may have overlapping ligand specificity and, more importantly, both are inhibited by rimonabant (SR141716A), it has to be emphasized that calcium signals observed in natural tissues and inhibited by rimonabant (SR141716A) can also be mediated by GPR55. On the other hand, GPR55 may turn out not to be a cannabinoid receptor after all, since inverse agonists AM251 and rimonabant induce β-arrestin coupling to the receptor, suggesting that they activate it, while classical cannabinoids have no such or a weak effect (Yin *et al.* 2009).

Modulation of ion channels

Stimulation of CB_1Rs leads to activation of G-protein-coupled inwardly-rectifying potassium channels (GIRKs) through pertussis-sensitive $G_{i/o}$ proteins (Henry & Chavkin 1995, Mackie *et al.* 1995), and this activation can be induced by different CB_1R agonists. In *Xenopus laevis* oocytes, GIRK1 and GIRK4 activation was reported, and this response was also detected in neuronal cells (Henry & Chavkin 1995, McAllister *et al.* 1999, Guo & Ikeda 2004, Azad *et al.* 2008).

CB_1Rs can also influence the function of voltage-gated calcium channels. Inhibition of L-type calcium channels by cannabinoid stimulation was detected in cerebral vessels (Gebremedhin *et al.* 1999), in retinal bipolar cells (Straiker *et al.* 1999) and in neonatal rat solitary tract cells (Endoh 2006), whereas activation of these channels was reported in N18TG2 cells (Rubovitch *et al.* 2002). Inhibition of N-type calcium channels was detected in a number of experiments (Caulfield & Brown 1992, Mackie & Hille 1992, Felder *et al.* 1993, Mackie *et al.* 1993, Pan *et al.* 1996,

Brown *et al.* 2004, Azad *et al.* 2008), and this effect may have a role in the presynaptic inhibition and retrograde signalling induced by cannabinoids (Freund *et al.* 2003, Howlett 2005). P/Q-type calcium channels are also negatively modulated by CB_1R (Mackie *et al.* 1995, Hampson *et al.* 1998, Ho *et al.* 2000, Brown *et al.* 2004, Fisyunov *et al.* 2006).

β-Arrestin binding to CB_1R

β-Arrestins were initially identified as proteins that play a role in the desensitization of GPCRs. Following receptor activation and subsequent phosphorylation by GPCR kinases (GRKs), β-arrestins bind to receptors and initialize their internalization. In addition to their role in regulation of GPCR internalization, β-arrestins can also serve as scaffolds for signalling complexes. The mechanism of β-arrestin binding to various GPCRs can be different. Relatively few data are available about the β-arrestin-binding properties of cannabinoid receptors. However, reports have indicated that β-arrestins play a role in desensitization of CB_1Rs (Jin *et al.* 1999, Kouznetsova *et al.* 2002, Breivogel *et al.* 2008, Daigle *et al.* 2008*b*). It has been shown that expression of β-arrestin 2 and GRK3 in *Xenopus* oocyte accelerates desensitization of CB_1Rs (Jin *et al.* 1999), and expression of dominant negative GRKs and β-arrestins interferes with CB_1R desensitization in hippocampal neurons (Kouznetsova *et al.* 2002). Residues S426 and S430 were identified as responsible for β-arrestin-dependent desensitization of CB_1Rs; however, mutations of these amino acids did not interfere with internalization of the receptor in AtT20 and HEK293 cells at least at early time points (Jin *et al.* 1999, Daigle *et al.* 2008*a*). Both wild-type and S426A/S430A mutant CB_1Rs recruit β-arrestins in HEK293 cells following stimulation with CP 55 940 (Daigle *et al.* 2008*a*). In contrast, serine and threonine amino acids in the extreme carboxyl-terminal of CB_1Rs are involved both in internalization and β-arrestin binding (Daigle *et al.* 2008*b*). Thus, it seems that different residues are responsible for β-arrestin-mediated desensitization and internalization; however, it cannot be ruled out that β-arrestin-independent CB_1R internalization mechanisms are responsible for these different structural requirements. Receptor densities of CB_1Rs in brain regions were not affected in β-arrestin knockout mice, and their desensitization was affected only when receptors were stimulated with THC (Breivogel *et al.* 2008), suggesting that control of receptor desensitization by β-arrestin may be dependent on agonist type. In conclusion, the data available today show that β-arrestins are involved in the desensitization of CB_1R, although whether it has role in the internalization or G-protein-independent signalling remains to be elucidated.

Activation of mitogen-activated protein kinase pathways

Mitogen-activated protein kinase (MAPK) pathways are often activated after stimulation of GPCRs. GPCRs can regulate cell proliferation, cell differentiation, cell movement and cell death through MAPK. MAPK-signalling cascades are organized hierarchically, as MAPKs are activated by MAPK kinases (MAPKKs), which are activated by MAPKK kinases (MAPKKKs). MAPKKKs are activated by small GTPases or other protein kinases. MAPK cascades include pathways leading to activation ERK1/2, c-Jun N-terminal kinase (JNK), p38 MAPK or ERK5 proteins.

Stimulation of CB_1R *in vitro* and *in vivo* leads to activation of ERK1/2 kinases in a variety of cell types (Howlett 2005). CB_1R-mediated activation of ERK1/2 proteins can involve a number of mechanisms including activation of $G_{i/o}$ proteins (Howlett 2005), phosphatidylinositol 3-kinase (PI3K; Galve-Roperh *et al.* 2002), transactivation of VEGF receptors (Korzh *et al.* 2008), through inhibition of adenylyl cyclase and protein kinase A (Davis *et al.* 2003) and the Src tyrosine kinase FYN (Derkinderen *et al.* 2003). Similar to G-protein activation, ERK1/2 stimulation also seems to be dependent on the particular cell type and different agonists can activate ERK1/2 through different pathways. CB_1R stimulation is also followed by p38 MAPK and JNK activation (Liu *et al.* 2000, Rueda *et al.* 2000, Paradisi *et al.* 2008; Fig. 1B). It has been reported that CB_1R activation can stimulate ERK1/2, p38 MAPK and JNK in endothelial cells (Liu *et al.* 2000).

In rat hippocampal slices, cannabinoids were able to activate p38 MAPK, but not JNK (Derkinderen *et al.* 2001). In cultured cortical neurons, JNK activation was observed when stimulated with THC (Downer *et al.* 2003), but no ERK1/2, p38 MAPK and JNK activation was observed after stimulation with another agonist, HU-210 (Molina-Holgado *et al.* 2005). These differences may reflect the dependence of these pathways on the maturation of neural cells (Downer *et al.* 2007). In Neuro2a cells, HU-210 activated only ERK1/2, but not JNK or p38 MAPK pathways (Graham *et al.* 2006), although in another study JNK activation through Src kinase was reported (He *et al.* 2005, 2006). In Chinese Hamster Ovary (CHO) cells, both JNK and p38 MAPK were activated after stimulation with THC. JNK activation was dependent on $G_{i/o}$ proteins, PI3K and Ras, and involved platelet-derived growth factor (PDGF) receptor transactivation. On the other hand, p38 MAPK was not dependent on PDGF receptor activation (Rueda *et al.* 2000).

CB1R stimulation, in addition to direct modulation of MAPK pathways, may also regulate these pathways indirectly by modulating MAPK activation induced by

other GPCRs (Ellis *et al.* 2006, Canals & Milligan 2008), or insulin and growth factor receptors (Bouaboula *et al.* 1997, Rajesh *et al.* 2008).

Other aspects of CB_1R signalling

Homo- and heterodimerization of CB_1R

Soon after identification of the β-adrenergic receptor, the physical interaction and cooperativity between receptor molecules were suggested (Limbird & Lefkowitz 1976). Since then, a large amount of data has accumulated suggesting that GPCRs function in dimers or in higher order oligomers (Bulenger *et al.* 2005, Waldhoer *et al.* 2005, Milligan *et al.* 2006, Milligan & Smith 2007, Szidonya *et al.* 2008). Strong evidences suggest that coexpression of two receptors leads to functional consequences; however, the exact nature of these interactions is difficult to determine using currently available techniques (Szidonya *et al.* 2008). It is usually accepted that there are direct interactions between GPCRs, however, the majority of data was obtained with biophysical, biochemical and structural methods that have been criticized by recent publications that challenge the concept. Despite these concerns, GPCR dimerization is a useful paradigm to explain the altered functional properties of these receptors in the presence of other GPCRs. Since dimerization can influence the signal transduction of GPCRs, it must be taken into consideration when the function of a GPCR is analyzed. In recent years, evidence has been presented that supports homo- or heterodimerization of CB_1Rs. The first observation of the homodimerization of the CB_1Rs was presented by Wager-Miller *et al.* (2002) using western blotting. These authors showed high molecular weight bands of CB_1Rs, and suggested that these bands correspond to dimerized receptors (Wager-Miller *et al.* 2002). Another paper reported that CB_1R and D2 dopamine receptors can form heterodimers using coimmunoprecipitation, and suggested that this dimerization tends to be dependent on the activity of CB_1R (Kearn *et al.* 2005). However, activity-dependent localization of CB_1R in different membrane compartments has also been reported (Sarnataro *et al.* 2006), which may affect the interpretation of the above-mentioned coimmunoprecipitation data. Although western blot and coimmunoprecipitation have been widely used for detection of GPCR dimers, these techniques may have several drawbacks including difficulties with solubilization of membranes, formation of GPCR aggregates, inappropriate selection of detergents, remaining membrane patches in supernatant and effects of the receptor glycosylation (Szidonya *et al.* 2008). Because of these and other difficulties, the data obtained with western blotting cannot be accepted as conclusive evidence for CB_1R homodimerization, and data obtained with other methods are necessary to verify these findings. Using resonance energy transfer methods, heterodimerization of CB_1R with D_2 and A_{2A} receptors (Carriba *et al.* 2008), opiate receptors (Rios *et al.* 2006) has been observed in transfected cells, and the functional role of these heterodimerizations has also been suggested (Hojo *et al.* 2008, Marcellino *et al.* 2008). Although bioluminescence resonance energy transfer (BRET) has also been widely used in dimerization studies, it needs careful experimental design and appropriate controls to conclude on specific interaction between two receptors (Marullo & Bouvier 2007). The pure BRET ratio cannot be interpreted as indicator of the proximity of two receptors and specificity of the interaction as it depends on many conditions including expression levels, expression ratios and the orientation of fluorophores. For example, the BRET ratio can be greatly influenced by simply changing the donor–acceptor ratio.

Another approach to study the interaction between GPCRs is detection of the functional changes caused by coexpression of receptors. When CB_1R and μ-opioid receptors (μ-OR) were coexpressed, it has been shown that stimulation of one receptor can attenuate the signal transduction of the other receptor (Rios *et al.* 2006). Similarly, it has been reported that basal activity of CB_1R also attenuates signalling of μ-OR (Canals & Milligan 2008). Although these interactions were suggested to be caused by dimerization, other alternative mechanisms may explain these findings, such as sequestration of G proteins (Bouaboula *et al.* 1997, Vasques & Lewis 1999, Nie & Lewis 2001, Chillakuri *et al.* 2007). Reciprocal inhibition between $GABA_B$ and CB_1R has been reported in hippocampal membranes (Cinar *et al.* 2008). Another interesting interaction between $G_{i/o}$-coupled CB_1R and D2-dopamine receptors has been reported (Kearn *et al.* 2005). Activation of both receptors decreased the forskolin-induced adenylyl cyclase activity; however, when the receptors were coexpressed, CB_1R stimulation reversed the inhibition caused by D2 stimulation and led to an increase in cAMP levels. These data have been interpreted as a consequence of receptor heterodimerization; however, if we take into consideration that CB_1R can couple to G_s proteins when $G_{i/o}$ proteins are inhibited, the sequestration of $G_{i/o}$ proteins cannot be ruled out as an alternative explanation for this finding. Another interesting functional interaction between CB_1R and orexin-1 receptor was reported by Hilairet *et al.* (2003). They showed that CB_1R coexpression increased the potency of orexin A to induce MAPK activation by orexin-1 receptor and this was blocked by CB_1R antagonist. However, since orexin-1 is a $G_{q/11}$-coupled receptor, transactivation of CB_1Rs by endocannabinoids

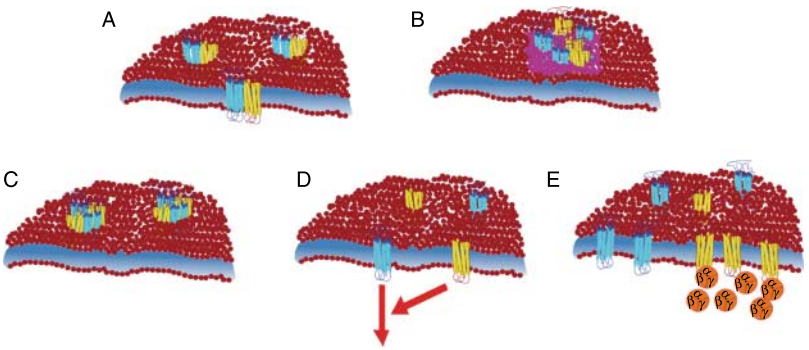

Figure 2 Possible mechanisms of interactions between GPCRs. Dimerization (A), clustering of GPCRs in specific membrane subdomains (B), higher order GPCR oligomers (C), interaction between GPCRs in signal transduction pathways (D) and sequestration of G-proteins (E).

and CB_1R-mediated MAPK activation in this set-up cannot be ruled out. When the G_q-coupled AT_1R was coexpressed with CB_1R in CHO cells, AT_1R stimulation with angiotensin II lead to a diacylglycerol lipase (DAGL)-mediated transactivation of CB_1Rs (Turu *et al.* 2007). This effect was mediated by the formation of endocannabinoids in CHO cells, and similar CB_1R transactivation was observed with all other tested G_q-coupled GPCRs (Turu *et al.* 2009). Since $G_{q/11}$ activation can lead to generation of 2-AG, the above-mentioned interaction between orexin-1 receptor and CB_1R may also occur with this mechanism.

Perhaps the most convincing evidence for dimerization of GPCRs came from studies with $GABA_B$ receptors, because it has been shown convincingly that $GABA_{B1}$ and $GABA_{B2}$ work as a functional dimers in which $GABA_{B1}$ binds the agonists and $GABA_{B2}$ is responsible for efficient trafficking of the dimer and for coupling to the G proteins (Galvez *et al.* 2001). One of the consequences of dimerization could be coupled trafficking of GPCRs to and from the plasma membrane. Such coupled trafficking of CB_1Rs has been recently demonstrated by Ellis *et al.* (2006), who showed that orexin-1 receptor distribution in the cells was markedly changed after coexpression of CB_1R. It changed its localization from plasma membrane to intracellular vesicles, which is similar to the cellular distribution of CB_1Rs. Both CB_1R and orexin-1 antagonists externalized both receptors to the plasma membrane, and this observation is difficult to interpret differently than a direct interaction (dimerization or oligomerization) between receptors. Interestingly, on the other hand, the other previously suggested dimer pair of the CB_1R, the μ-OR, failed to traffic together with CB_1R in the same experimental setting (Ellis *et al.* 2006).

In conclusion, although the dimerization of GPCRs is an emerging important paradigm, it requires careful examination. In order to conclude the physiological relevance of the dimerization of GPCRs, coherent data obtained with biochemical, biophysical and pharmacological approaches should be assembled. The available data show clearly that coexpression of CB_1R alters the function and signal transduction of other GPCRs, and vice versa. However, the data obtained with different methods are either lacking or contradictory, suggesting that firm interpretation of the available data as a consequence of dimerization or other type of interactions needs further investigations (Fig. 2).

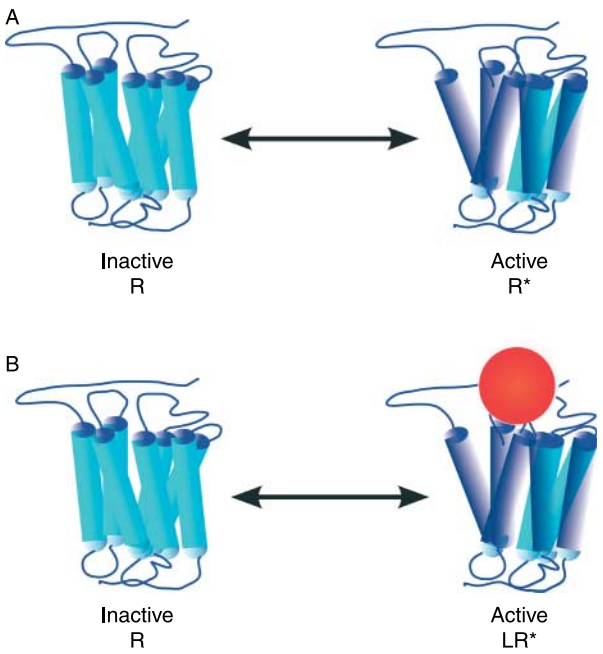

Figure 3 Two possible explanations of CB_1R's basal activity: constitutive activity, when some receptors are present in active state even in the absence of any ligand (top); or endogenously produced agonists cause basal activity (bottom).

Constitutive versus basal activity

Although a number of GPCRs show basal activity even in the absence of agonists, especially when overexpressed, the role of the constitutive activity in physiological conditions (when the receptor is not overexpressed) is known only in some cases (Seifert & Wenzel-Seifert 2002). The best example is the MC_4 melanocortin receptor and its inverse agonist, the agouti-related peptide (Adan & Kas 2003), which has an extensively studied role in regulation of weight balance.

CB_1R also shows a high degree of basal activity, both in expression systems and in native tissues and, as such, it is usually referred as a constitutively active receptor (Bouaboula *et al.* 1997, Rinaldi-Carmona *et al.* 1998, Pertwee 2005). However, in the case of receptors expressed in native tissues, the endogenous ligands released by the tissues may represent an alternative explanation to the high level of basal activity (Seifert & Wenzel-Seifert 2002). In many tissues, 2-AG, an endocannabinoid, is present in concentrations close to the K_d of CB_1R (Sugiura *et al.* 2006). Moreover, DAGLs, the enzymes responsible for 2-AG generation, are present in most tissues, suggesting that endocannabinoid production could be a common property of different cell types (Bisogno *et al.* 2003). Despite these data, it has been widely accepted that the CB_1R is constitutively active even in the absence of endocannabinoids, and there is some evidence that points to this conception, as reviewed recently (Pertwee 2005). Some other data suggest that the constitutive activity is not present in all CB_1R expressing cells, or it can be inhibited by Ca^{2+} chelation (Katona *et al.* 1999, Hoffman & Lupica 2000, Wilson & Nicoll 2001, Chevaleyre & Castillo 2003, Savinainen *et al.* 2003, Breivogel *et al.* 2004, Hentges *et al.* 2005, Zhu & Lovinger 2005, Neu *et al.* 2006) or by inhibition of DAGL (Turu *et al.* 2007). There is also an observation that SR141716A, the CB_1R-inverse agonist, can inhibit other receptors as well (Savinainen *et al.* 2003, Lauckner *et al.* 2008), and this can complicate the interpretation of its inverse agonist effects.

A pure neutral antagonist would help to decide at which degree is CB_1R constitutively active. Indeed, there are some neutral CB_1R antagonists in the literature (Pertwee 2005). Neutral antagonists have no effect on the constitutive CB_1R activity, although they should inhibit it if endocannabinoids were the cause of basal activity. Three antagonists, NESS 0327, O-2654 and O-2050, do not show inverse agonist effects, although they do antagonize the agonists (Ruiu *et al.* 2003, Pertwee 2005, Canals & Milligan 2008). Similarly, cannabinol, another cannabis derivate, can antagonize the binding of CP 55 940, although it shows no inverse agonism (MacLennan *et al.* 1998). These observations argue against the role of endocannabinoids in the basal activity of CB_1R.

Although these data would show that CB_1R is constitutively active, the way how the neutral antagonist properties of these compounds were defined makes this issue a bit complicated. These compounds were named neutral antagonists because they do not change the basal activity of the CB_1R in some experimental conditions. However, they can only be accepted as neutral antagonists if we accept that CB_1R is constitutively active. If we would presume that the basal activity was caused by endocannabinoids present in the tissues, these compounds should be referred to as partial agonists, causing a similar degree of activation as endogenous cannabinoids. In concert with this, although the neutral antagonist effect of the compound O-2050 has not been described in publication, but is taken as such ligand (Martin *et al.* 2002, Pertwee 2005, Gardner & Mallet 2006, Canals & Milligan 2008); in the patent description, it has a similar partial agonist effect as THC, the first known cannabinoid agonist, when measured with [^{35}S]-GTPγS binding on membranes (Martin *et al.* 2005). So these compounds are taken as neutral antagonists based on the observation that in certain experiments, they do not change the activity of CB_1R. Since we assume that CB_1R is constitutively active when we define these antagonists as neutral ones, results obtained with these ligands cannot be used as evidence of constitutive activity. To decide whether these compounds are really neutral antagonists, we need further experiments, maybe with mutated receptors that have altered basal activity.

In conclusion, although the CB_1R is usually referred as a constitutively active receptor, the significant role of endocannabinoids in high basal activity cannot be ruled out (Fig. 3).

Declaration of interest

I declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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