REVIEW

Signal transduction of the CB$_1$ cannabinoid receptor

Gábor Turu[1] and **László Hunyady**[1,2]

[1]Department of Physiology, Faculty of Medicine, Semmelweis University, PO Box 259, H-1444 Budapest, Hungary

[2]Laboratory of Neurobiochemistry and Molecular Physiology, Semmelweis University and Hungarian Academy of Sciences, Budapest, Hungary

(Correspondence should be addressed to L Hunyady at Department of Physiology, Faculty of Medicine, Semmelweis University; Email: hunyady@eok.sote.hu)

Abstract

The CB$_1$ cannabinoid receptor (CB$_1$R) is the major cannabinoid receptor in neuronal cells and the brain, but it also occurs in endocrine cells and other peripheral tissues. CB$_1$R is a member of the superfamily of G-protein-coupled receptors (GPCRs), which are characterized by seven transmembrane helices. The major mediators of CB$_1$R 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$_1$Rs can also stimulate adenylyl cyclase via G$_s$, induce receptor-mediated Ca^{2+} fluxes and stimulate phospholipases in some experimental models. Stimulation of CB$_1$R 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$_1$Rs 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$_1$Rs can form homo- and heterodimers/oligomers, and the altered pharmacological properties of these receptor complexes may explain the pharmacological differences observed in various tissues.

Journal of Molecular Endocrinology (2010) **44,** 75–85

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$_1$R) and CB$_2$ receptor (CB$_2$R; 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$_1$R and CB$_2$R, 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$_1$R, 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$_1$R antagonists (Steinberg & Cannon 2007),

Table 1 Affinities of the main cannabinoid ligands to CB$_1$R

	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$_1$R ligand affinities (McPartland *et al.* 2007).

the therapeutical potential of drugs acting on CB$_1$R is still very high (Kunos *et al.* 2008). The aim of this review is to discuss the intracellular mechanisms of the action of CB$_1$R. After summarizing the available data about the G-protein activation and signal transduction of CB$_1$R, 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$_1$R 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$_1$R-mediated inhibition of adenylyl cyclase activity was reported in neural and peripheral tissues, as well as in cells overexpressing CB$_1$R as reviewed previously (Howlett 2005). Coexpression of isoforms 1, 3, 5, 6 or 8 of adenylyl cyclase resulted in CB$_1$R-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$_1$R, 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$_1$R may have different affinity states, which may represent different conformations (Houston & Howlett 1998).
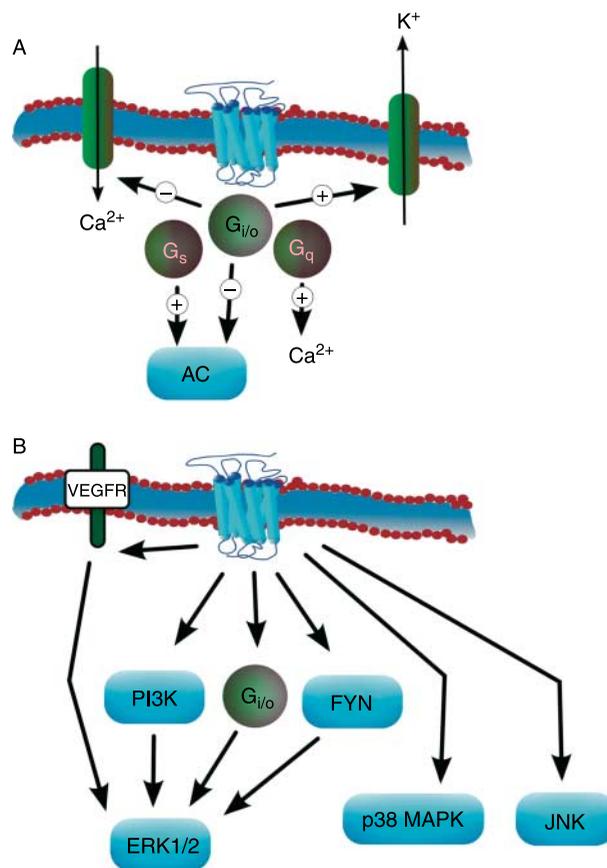
Figure 1 Main signal transduction pathways of CB$_1$R activaton. G-protein activation and modulation of ion channels (A), and activation of different MAPK pathways (B).

In agreement with the concept of multiple receptor states, CB$_1$R 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$_1$R 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$_1$R stimulation leads to adenylyl cyclase activation suggesting that in certain circumstances, CB$_1$R 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$_1$R (Mukhopadhyay *et al.* 2000). Coexpression of isoforms 2, 4 or 7 of adenylyl cyclase also resulted in CB$_1$R-mediated stimulation of cAMP formation, but this effect was probably mediated by G$\beta\gamma$ dimers released from G$_i$ proteins (Rhee *et al.* 1998).

Activation of G$_{q/11}$ proteins

Ca^{2+} signalling after CB$_1$R stimulation has also been reported (Fig. 1A), however, the mechanism of this response is not clear. CB$_1$R 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 G$\beta\gamma$ subunits, in this response (Sugiura *et al.* 1996, 1997). In contrast, in HEK cells expressing CB$_1$R, 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$_1$R and CB$_2$R 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$_2$R agonist (De Petrocellis *et al.* 2007). Anandamide also induced Ca^{2+} signal CB$_1$R dependently. These cell-type-specific differences in the mechanism of CB$_1$R-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$_1$R with other receptors in these cells.

In contrast to CB$_1$R and CB$_2$R, 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$_1$R activity (Waldeck-Weiermair *et al.* 2008). Since GPR55 and CB$_1$R 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$_1$Rs 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$_1$R 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$_1$Rs 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$_1$R (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$_1$R

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$_1$Rs (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$_1$Rs (Jin *et al.* 1999), and expression of dominant negative GRKs and β-arrestins interferes with CB$_1$R desensitization in hippocampal neurons (Kouznetsova *et al.* 2002). Residues S426 and S430 were identified as responsible for β-arrestin-dependent desensitization of CB$_1$Rs; 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$_1$Rs 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$_1$Rs 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$_1$R internalization mechanisms are responsible for these different structural requirements. Receptor densities of CB$_1$Rs 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$_1$R, 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$_1$R *in vitro* and *in vivo* leads to activation of ERK1/2 kinases in a variety of cell types (Howlett 2005). CB$_1$R-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$_1$R 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$_1$R 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$_1$R signalling

Homo- and heterodimerization of CB$_1$R

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$_1$Rs. The first observation of the homodimerization of the CB$_1$Rs was presented by Wager-Miller *et al.* (2002) using western blotting. These authors showed high molecular weight bands of CB$_1$Rs, and suggested that these bands correspond to dimerized receptors (Wager-Miller *et al.* 2002). Another paper reported that CB$_1$R and D2 dopamine receptors can form heterodimers using coimmunoprecipitation, and suggested that this dimerization tends to be dependent on the activity of CB$_1$R (Kearn *et al.* 2005). However, activity-dependent localization of CB$_1$R 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$_1$R homodimerization, and data obtained with other methods are necessary to verify these findings. Using resonance energy transfer methods, heterodimerization of CB$_1$R 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$_1$R 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$_1$R 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$_1$R has been reported in hippocampal membranes (Cinar *et al.* 2008). Another interesting interaction between G$_{i/o}$-coupled CB$_1$R 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$_1$R 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$_1$R 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$_1$R and orexin-1 receptor was reported by Hilairet *et al.* (2003). They showed that CB$_1$R coexpression increased the potency of orexin A to induce MAPK activation by orexin-1 receptor and this was blocked by CB$_1$R antagonist. However, since orexin-1 is a G$_{q/11}$-coupled receptor, transactivation of CB$_1$Rs by endocannabinoids
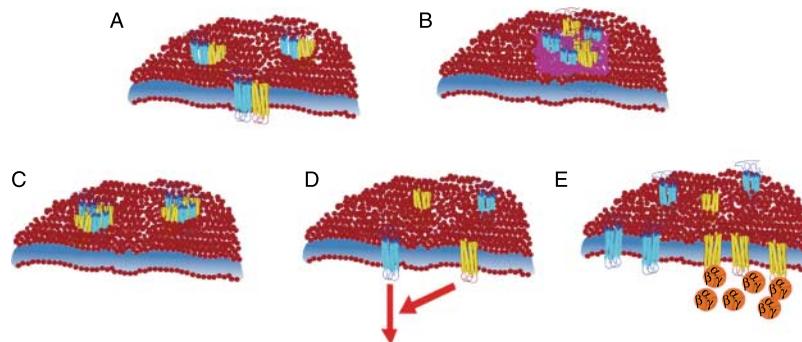
Figure 2 Possible mechanisms of interactions between GPCRs. Dimerization (A), clustering of GPCRs in specific membrane subdomains (B), higher order GPCR oligomers (C), interaction between GPCRs in signal transduction pathways (D) and sequestration of G-proteins (E).

and CB$_1$R-mediated MAPK activation in this set-up cannot be ruled out. When the G$_q$-coupled AT$_1$R was coexpressed with CB$_1$R in CHO cells, AT$_1$R stimulation with angiotensin II lead to a diacylglycerol lipase (DAGL)-mediated transactivation of CB$_1$Rs (Turu *et al.* 2007). This effect was mediated by the formation of endocannabinoids in CHO cells, and similar CB$_1$R 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$_1$R 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$_1$Rs 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$_1$R. It changed its localization from plasma membrane to intracellular vesicles, which is similar to the cellular distribution of CB$_1$Rs. Both CB$_1$R 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$_1$R, the μ-OR, failed to traffic together with CB$_1$R 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$_1$R 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).
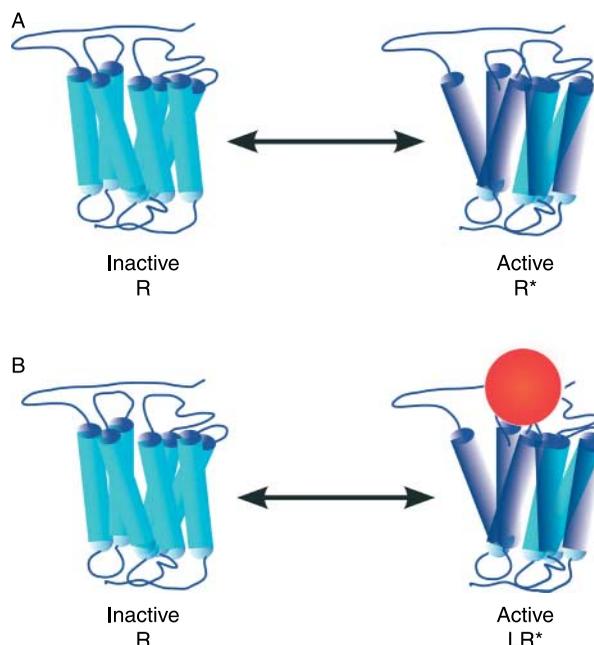
Figure 3 Two possible explanations of CB$_1$R's basal activity: constitutive activity, when some receptors are present in active state even in the absence of any ligand (top); or endogenously produced agonists cause basal activity (bottom).

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

Funding

References

Abadji V, Lucas-Lenard JM, Chin C & Kendall DA 1999 Involvement of the carboxyl terminus of the third intracellular loop of the cannabinoid CB1 receptor in constitutive activation of G$_s$. *Journal of Neurochemistry* **72** 2032–2038.

Adan RA & Kas MJ 2003 Inverse agonism gains weight. *Trends in Pharmacological Sciences* **24** 315–321.

Azad SC, Kurz J, Marsicano G, Lutz B, Zieglgansberger W & Rammes G 2008 Activation of CB1 specifically located on GABAergic interneurons inhibits LTD in the lateral amygdala. *Learning and Memory* **15** 143–152.

Baker D, Pryce G, Davies WL & Hiley CR 2006 *In silico* patent searching reveals a new cannabinoid receptor. *Trends in Pharmacological Sciences* **27** 1–4.

Bellocchio L, Mancini G, Vicennati V, Pasquali R & Pagotto U 2006 Cannabinoid receptors as therapeutic targets for obesity and metabolic diseases. *Current Opinion in Pharmacology* **6** 586–591.

Bisogno T, Howell F, Williams G, Minassi A, Cascio MG, Ligresti A, Matias I, Schiano-Moriello A, Paul P, Williams EJ *et al.* 2003 Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signalling in the brain. *Journal of Cell Biology* **163** 463–468.

Bouaboula M, Perrachon S, Milligan L, Canat X, Rinaldi-Carmona M, Portier M, Barth F, Calandra B, Pecceu F, Lupker J *et al.* 1997 A selective inverse agonist for central cannabinoid receptor inhibits mitogen-activated protein kinase activation stimulated by insulin or insulin-like growth factor 1. Evidence for a new model of receptor/ligand interactions. *Journal of Biological Chemistry* **272** 22330–22339.

Breivogel CS & Childers SR 2000 Cannabinoid agonist signal transduction in rat brain: comparison of cannabinoid agonists in receptor binding, G-protein activation, and adenylyl cyclase inhibition. *Journal of Pharmacology and Experimental Therapeutics* **295** 328–336.

Breivogel CS, Sim LJ & Childers SR 1997 Regional differences in cannabinoid receptor/G-protein coupling in rat brain. *Journal of Pharmacology and Experimental Therapeutics* **282** 1632–1642.

Breivogel CS, Walker JM, Huang SM, Roy MB & Childers SR 2004 Cannabinoid signalling in rat cerebellar granule cells: G-protein activation, inhibition of glutamate release and endogenous cannabinoids. *Neuropharmacology* **47** 81–91.

Breivogel CS, Lambert JM, Gerfin S, Huffman JW & Razdan RK 2008 Sensitivity to delta9-tetrahydrocannabinol is selectively enhanced in beta-arrestin2 $-/-$ mice. *Behavioural Pharmacology* **19** 298–307.

Brown SP, Safo PK & Regehr WG 2004 Endocannabinoids inhibit transmission at granule cell to Purkinje cell synapses by modulating three types of presynaptic calcium channels. *Journal of Neuroscience* **24** 5623–5631.

Bulenger S, Marullo S & Bouvier M 2005 Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. *Trends in Pharmacological Sciences* **26** 131–137.

Calandra B, Portier M, Kerneis A, Delpech M, Carillon C, Le Fur G, Ferrara P & Shire D 1999 Dual intracellular signalling pathways mediated by the human cannabinoid CB1 receptor. *European Journal of Pharmacology* **374** 445–455.

Canals M & Milligan G 2008 Constitutive activity of the cannabinoid CB1 receptor regulates the function of co-expressed μ opioid receptors. *Journal of Biological Chemistry* **283** 11424–11434.

Carriba P, Navarro G, Ciruela F, Ferre S, Casado V, Agnati L, Cortes A, Mallol J, Fuxe K, Canela EI *et al.* 2008 Detection of heteromerization of more than two proteins by sequential BRET–FRET. *Nature Methods* **5** 727–733.

Caulfield MP & Brown DA 1992 Cannabinoid receptor agonists inhibit Ca current in NG108-15 neuroblastoma cells via a pertussis toxin-sensitive mechanism. *British Journal of Pharmacology* **106** 231–232.

Chillakuri CR, Reinhart C & Michel H 2007 C-terminal truncated cannabinoid receptor 1 coexpressed with G protein trimer in Sf9 cells exists in a precoupled state and shows constitutive activity. *FEBS J* **274** 6106–6115.

Chevaleyre V & Castillo PE 2003 Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. *Neuron* **38** 461–472.

Cinar R, Freund TF, Katona I, Mackie K & Szucs M 2008 Reciprocal inhibition of G-protein signalling is induced by CB(1) cannabinoid and GABA(B) receptor interactions in rat hippocampal membranes. *Neurochemistry International* **52** 1402–1409.

Daigle TL, Kearn CS & Mackie K 2008*a* Rapid CB(1) cannabinoid receptor desensitization defines the time course of ERK1/2 MAP kinase signalling. *Neuropharmacology* **54** 36–44.

Daigle TL, Kwok ML & Mackie K 2008*b* Regulation of CB1 cannabinoid receptor internalization by a promiscuous phosphorylation-dependent mechanism. *Journal of Neurochemistry* **106** 70–82.

Davis MI, Ronesi J & Lovinger DM 2003 A predominant role for inhibition of the adenylate cyclase/protein kinase A pathway in ERK activation by cannabinoid receptor 1 in N1E–115 neuroblastoma cells. *Journal of Biological Chemistry* **278** 48973–48980.

Derkinderen P, Ledent C, Parmentier M & Girault JA 2001 Cannabinoids activate p38 mitogen-activated protein kinases through CB1 receptors in hippocampus. *Journal of Neurochemistry* **77** 957–960.

Derkinderen P, Valjent E, Toutant M, Corvol JC, Enslen H, Ledent C, Trzaskos J, Caboche J & Girault JA 2003 Regulation of extracellular signal-regulated kinase by cannabinoids in hippocampus. *Journal of Neuroscience* **23** 2371–2382.

Devane WA, Dysarz FA III, Johnson MR, Melvin LS & Howlett AC 1988 Determination and characterization of a cannabinoid receptor in rat brain. *Molecular Pharmacology* **34** 605–613.

Downer EJ, Fogarty MP & Campbell VA 2003 Tetrahydrocannabinol-induced neurotoxicity depends on CB1 receptor-mediated c-Jun N-terminal kinase activation in cultured cortical neurons. *British Journal of Pharmacology* **140** 547–557.

Downer EJ, Gowran A & Campbell VA 2007 A comparison of the apoptotic effect of $\Delta(9)$-tetrahydrocannabinol in the neonatal and adult rat cerebral cortex. *Brain Research* **1175** 39–47.

Ellis J, Pediani JD, Canals M, Milasta S & Milligan G 2006 Orexin-1 receptor-cannabinoid CB1 receptor heterodimerization results in both ligand-dependent and -independent coordinated alterations of receptor localization and function. *Journal of Biological Chemistry* **281** 38812–38824.

Endoh T 2006 Pharmacological characterization of inhibitory effects of postsynaptic opioid and cannabinoid receptors on calcium currents in neonatal rat nucleus tractus solitarius. *British Journal of Pharmacology* **147** 391–401.

Felder CC, Briley EM, Axelrod J, Simpson JT, Mackie K & Devane WA 1993 Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. *PNAS* **90** 7656–7660.

Fisyunov A, Tsintsadze V, Min R, Burnashev N & Lozovaya N 2006 Cannabinoids modulate the P-type high-voltage-activated calcium currents in purkinje neurons. *Journal of Neurophysiology* **96** 1267–1277.

Freund TF, Katona I & Piomelli D 2003 Role of endogenous cannabinoids in synaptic signalling. *Physiological Reviews* **83** 1017–1066.

Galve-Roperh I, Rueda D, Gomez DP, Velasco G & Guzman M 2002 Mechanism of extracellular signal-regulated kinase activation by the CB(1) cannabinoid receptor. *Molecular Pharmacology* **62** 1385–1392.

Galvez T, Duthey B, Kniazeff J, Blahos J, Rovelli G, Bettler B, Prezeau L & Pin JP 2001 Allosteric interactions between GB1 and GB2 subunits are required for optimal GABA(B) receptor function. *EMBO Journal* **20** 2152–2159.

Gaoni YMR 1964 Isolation, structure, and partial synthesis of an active constituent of hashish. *Journal of the American Chemical Society* **86** 1646–1647.

Gardner A & Mallet PE 2006 Suppression of feeding, drinking, and locomotion by a putative cannabinoid receptor 'silent antagonist'. *European Journal of Pharmacology* **530** 103–106.

Gebremedhin D, Lange AR, Campbell WB, Hillard CJ & Harder DR 1999 Cannabinoid CB1 receptor of cat cerebral arterial muscle functions to inhibit L-type Ca^{2+} channel current. *American Journal of Physiology* **276** H2085–H2093.

Georgieva T, Devanathan S, Stropova D, Park CK, Salamon Z, Tollin G, Hruby VJ, Roeske WR, Yamamura HI & Varga E 2008 Unique agonist-bound cannabinoid CB1 receptor conformations indicate agonist specificity in signalling. *European Journal of Pharmacology* **581** 19–29.

Glass M & Felder CC 1997 Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a G$_s$ linkage to the CB1 receptor. *Journal of Neuroscience* **17** 5327–5333.

Glass M & Northup JK 1999 Agonist selective regulation of G proteins by cannabinoid CB(1) and CB(2) receptors. *Molecular Pharmacology* **56** 1362–1369.

Graham ES, Ball N, Scotter EL, Narayan P, Dragunow M & Glass M 2006 Induction of Krox-24 by endogenous cannabinoid type 1 receptors in Neuro2A cells is mediated by the MEK–ERK MAPK pathway and is suppressed by the phosphatidylinositol 3-kinase pathway. *Journal of Biological Chemistry* **281** 29085–29095.

Guo J & Ikeda SR 2004 Endocannabinoids modulate N-type calcium channels and G-protein-coupled inwardly rectifying potassium channels via CB1 cannabinoid receptors heterologously expressed in mammalian neurons. *Molecular Pharmacology* **65** 665–674.

Hampson AJ, Bornheim LM, Scanziani M, Yost CS, Gray AT, Hansen BM, Leonoudakis DJ & Bickler PE 1998 Dual effects of anandamide on NMDA receptor-mediated responses and neurotransmission. *Journal of Neurochemistry* **70** 671–676.

He JC, Gomes I, Nguyen T, Jayaram G, Ram PT, Devi LA & Iyengar R 2005 The G alpha(o/i)-coupled cannabinoid receptor-mediated neurite outgrowth involves Rap regulation of Src and Stat3. *Journal of Biological Chemistry* **280** 33426–33434.

He JC, Neves SR, Jordan JD & Iyengar R 2006 Role of the G$_{o/i}$ signalling network in the regulation of neurite outgrowth. *Canadian Journal of Physiology and Pharmacology* **84** 687–694.

Henry DJ & Chavkin C 1995 Activation of inwardly rectifying potassium channels (GIRK1) by co-expressed rat brain cannabinoid receptors in *Xenopus* oocytes. *Neuroscience Letters* **186** 91–94.

Hentges ST, Low MJ & Williams JT 2005 Differential regulation of synaptic inputs by constitutively released endocannabinoids and exogenous cannabinoids. *Journal of Neuroscience* **25** 9746–9751.

Hilairet S, Bouaboula M, Carriere D, Le Fur G & Casellas P 2003 Hypersensitization of the orexin 1 receptor by the CB1 receptor: evidence for cross-talk blocked by the specific CB1 antagonist, SR141716. *Journal of Biological Chemistry* **278** 23731–23737.

Ho BY, Stadnicka A, Prather PL, Buckley AR, Current LL, Bosnjak ZJ & Kwok WM 2000 Cannabinoid CB1 receptor-mediated inhibition of prolactin release and signalling mechanisms in GH4C1 cells. *Endocrinology* **141** 1675–1685.

Hoffman AF & Lupica CR 2000 Mechanisms of cannabinoid inhibition of GABA(A) synaptic transmission in the hippocampus. *Journal of Neuroscience* **20** 2470–2479.

Hojo M, Sudo Y, Ando Y, Minami K, Takada M, Matsubara T, Kanaide M, Taniyama K, Sumikawa K & Uezono Y 2008 μ-Opioid receptor forms a functional heterodimer with cannabinoid CB1 receptor: electrophysiological and FRET assay analysis. *Journal of Pharmacological Sciences* **108** 308–319.

Houston DB & Howlett AC 1998 Differential receptor-G-protein coupling evoked by dissimilar cannabinoid receptor agonists. *Cellular Signalling* **10** 667–674.

Howlett AC 1985 Cannabinoid inhibition of adenylate cyclase. Biochemistry of the response in neuroblastoma cell membranes. *Molecular Pharmacology* **27** 429–436.

Howlett AC 2005 Cannabinoid receptor signalling. *Handbook of Experimental Pharmacology* **168** 53–79.

Howlett AC & Fleming RM 1984 Cannabinoid inhibition of adenylate cyclase. Pharmacology of the response in neuroblastoma cell membranes. *Molecular Pharmacology* **26** 532–538.

Howlett AC, Qualy JM & Khachatrian LL 1986 Involvement of G$_i$ in the inhibition of adenylate cyclase by cannabimimetic drugs. *Molecular Pharmacology* **29** 307–313.

Jin W, Brown S, Roche JP, Hsieh C, Celver JP, Kovoor A, Chavkin C & Mackie K 1999 Distinct domains of the CB1 cannabinoid receptor mediate desensitization and internalization. *Journal of Neuroscience* **19** 3773–3780.

Johns DG, Behm DJ, Walker DJ, Ao Z, Shapland EM, Daniels DA, Riddick M, Dowell S, Staton PC, Green P *et al.* 2007 The novel endocannabinoid receptor GPR55 is activated by atypical cannabinoids but does not mediate their vasodilator effects. *British Journal of Pharmacology* **152** 825–831.

Katona I, Sperlagh B, Sik A, Kafalvi A, Vizi ES, Mackie K & Freund TF 1999 Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *Journal of Neuroscience* **19** 4544–4558.

Kearn CS, Blake-Palmer K, Daniel E, Mackie K & Glass M 2005 Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors enhances heterodimer formation: a mechanism for receptor cross-talk? *Molecular Pharmacology* **67** 1697–1704.

Kenakin T 2007 Functional selectivity through protean and biased agonism: who steers the ship? *Molecular Pharmacology* **72** 1393–1401.

Korzh A, Keren O, Gafni M, Bar-Josef H & Sarne Y 2008 Modulation of extracellular signal-regulated kinase (ERK) by opioid and cannabinoid receptors that are expressed in the same cell. *Brain Research* **1189** 23–32.

Kouznetsova M, Kelley B, Shen M & Thayer SA 2002 Desensitization of cannabinoid-mediated presynaptic inhibition of neurotransmission between rat hippocampal neurons in culture. *Molecular Pharmacology* **61** 477–485.

Kunos G, Osei-Hyiaman D, Batkai S, Sharkey KA & Makriyannis A 2008 Should peripheral CB(1) cannabinoid receptors be selectively targeted for therapeutic gain? *Trends in Pharmacological Sciences* **30** 1–7.

Lauckner JE, Hille B & Mackie K 2005 The cannabinoid agonist WIN55,212-2 increases intracellular calcium via CB1 receptor coupling to G$_{q/11}$ G proteins. *PNAS* **102** 19144–19149.

Lauckner JE, Jensen JB, Chen HY, Lu HC, Hille B & Mackie K 2008 GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. *PNAS* **105** 2699–2704.

Limbird LE & Lefkowitz RJ 1976 Negative cooperativity among beta-adrenergic receptors in frog erythrocyte membranes. *Journal of Biological Chemistry* **251** 5007–5014.

Liu J, Gao B, Mirshahi F, Sanyal AJ, Khanolkar AD, Makriyannis A & Kunos G 2000 Functional CB1 cannabinoid receptors in human vascular endothelial cells. *Biochemical Journal* **346** 835–840.

Mackie K & Hille B 1992 Cannabinoids inhibit N-type calcium channels in neuroblastoma–glioma cells. *PNAS* **89** 3825–3829.

Mackie K, Devane WA & Hille B 1993 Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells. *Molecular Pharmacology* **44** 498–503.

Mackie K, Lai Y, Westenbroek R & Mitchell R 1995 Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *Journal of Neuroscience* **15** 6552–6561.

MacLennan SJ, Reynen PH, Kwan J & Bonhaus DW 1998 Evidence for inverse agonism of SR141716A at human recombinant cannabinoid CB1 and CB2 receptors. *British Journal of Pharmacology* **124** 619–622.

Marcellino D, Carriba P, Filip M, Borgkvist A, Frankowska M, Bellido I, Tanganelli S, Muller CE, Fisone G, Lluis C *et al.* 2008 Antagonistic cannabinoid CB1/dopamine D2 receptor interactions in striatal CB1/D2 heteromers. A combined neurochemical and behavioral analysis. *Neuropharmacology* **54** 815–823.

Martin B, Stevenson LA, Pertwee RG, Breivogel CS, Williams W, Mahadevan A & Razdan RK 2002 Agonists and silent antagonists in a series of cannabinoid sulfonamides. Symposium on the Cannabinoids. Burlington, Vermont, International Cannabinoid Research Society, 2.

Martin BR, Razdan RK & Pertwee RG 2005 Sulfonamide cannabinoid agonists and antagonists. United States Patent 7279500.

Marullo S & Bouvier M 2007 Resonance energy transfer approaches in molecular pharmacology and beyond. *Trends in Pharmacological Sciences* **28** 362–365.

Matsuda LA, Lolait SJ, Brownstein MJ, Young AC & Bonner TI 1990 Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346** 561–564.

McAllister SD, Griffin G, Satin LS & Abood ME 1999 Cannabinoid receptors can activate and inhibit G protein-coupled inwardly rectifying potassium channels in a *Xenopus* oocyte expression system. *Journal of Pharmacology and Experimental Therapeutics* **291** 618–626.

McPartland JM, Glass M & Pertwee RG 2007 Meta-analysis of cannabinoid ligand binding affinity and receptor distribution: interspecies differences. *British Journal of Pharmacology* **152** 583–593.

Milligan G & Smith NJ 2007 Allosteric modulation of heterodimeric G-protein-coupled receptors. *Trends in Pharmacological Sciences* **28** 615–620.

Milligan G, Canals M, Pediani JD, Ellis J & Lopez-Gimenez JF 2006 The role of GPCR dimerisation/oligomerisation in receptor signalling. *Ernst Schering Foundation Symposium Proceedings* **2** 145–161.

Molina-Holgado F, Pinteaux E, Heenan L, Moore JD, Rothwell NJ & Gibson RM 2005 Neuroprotective effects of the synthetic cannabinoid HU-210 in primary cortical neurons are mediated by phosphatidylinositol 3-kinase/AKT signalling. *Molecular and Cellular Neurosciences* **28** 189–194.

Mukhopadhyay S & Howlett AC 2005 Chemically distinct ligands promote differential CB1 cannabinoid receptor–G$_i$ protein interactions. *Molecular Pharmacology* **67** 2016–2024.

Mukhopadhyay S, McIntosh HH, Houston DB & Howlett AC 2000 The CB(1) cannabinoid receptor juxtamembrane C-terminal peptide confers activation to specific G proteins in brain. *Molecular Pharmacology* **57** 162–170.

Mukhopadhyay S, Shim JY, Assi AA, Norford D & Howlett AC 2002 CB(1) cannabinoid receptor-G protein association: a possible mechanism for differential signalling. *Chemistry and Physics of Lipids* **121** 91–109.

Munro S, Thomas KL & Abu-Shaar M 1993 Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365** 61–65.

Neu A, Foldy C & Soltesz I 2007 Postsynaptic origin of CB1-dependent tonic inhibition of GABA release at CCK-positive basket cell to pyramidal cell synapses in the CA1 region of the rat hippocampus. *Journal of Physiology* **578** 233–247.

Nie J & Lewis DL 2001 Structural domains of the CB1 cannabinoid receptor that contribute to constitutive activity and G-protein sequestration. *Journal of Neuroscience* **21** 8758–8764.

Oka S, Nakajima K, Yamashita A, Kishimoto S & Sugiura T 2007 Identification of GPR55 as a lysophosphatidylinositol receptor. *Biochemical and Biophysical Research Communications* **362** 928–934.

Pacher P, Batkai S & Kunos G 2006 The endocannabinoid system as an emerging target of pharmacotherapy. *Pharmacological Reviews* **58** 389–462.

Pan X, Ikeda SR & Lewis DL 1996 Rat brain cannabinoid receptor modulates N-type Ca^{2+} channels in a neuronal expression system. *Molecular Pharmacology* **49** 707–714.

Paradisi A, Pasquariello N, Barcaroli D & Maccarrone M 2008 Anandamide regulates keratinocyte differentiation by inducing DNA methylation in a CB1 receptor-dependent manner. *Journal of Biological Chemistry* **283** 6005–6012.

Pertwee RG 2005 Inverse agonism and neutral antagonism at cannabinoid CBI receptors. *Life Sciences* **76** 1307–1324.

De Petrocellis L, Marini P, Matias I, Moriello AS, Starowicz K, Cristino L, Nigam S & Di Marzo M 2007 Mechanisms for the coupling of cannabinoid receptors to intracellular calcium mobilization in rat insulinoma beta-cells. *Experimental Cell Research* **313** 2993–3004.

Prather PL, Martin NA, Breivogel CS & Childers SR 2000 Activation of cannabinoid receptors in rat brain by WIN 55212-2 produces coupling to multiple G protein alpha-subunits with different potencies. *Molecular Pharmacology* **57** 1000–1010.

Rajesh M, Mukhopadhyay P, Hasko G & Pacher P 2008 Cannabinoid CB1 receptor inhibition decreases vascular smooth muscle migration and proliferation. *Biochemical and Biophysical Research Communications* **377** 1248–1252.

Rhee MH, Bayewitch M, Avidor-Reiss T, Levy R & Vogel Z 1998 Cannabinoid receptor activation differentially regulates the various adenylyl cyclase isozymes. *Journal of Neurochemistry* **71** 1525–1534.

Rinaldi-Carmona M, Le Duigou A, Oustric D, Barth F, Bouaboula M, Carayon P, Casellas P & Le Fur G 1998 Modulation of CB1 cannabinoid receptor functions after a long-term exposure to agonist or inverse agonist in the Chinese hamster ovary cell expression system. *Journal of Pharmacology and Experimental Therapeutics* **287** 1038–1047.

Rios C, Gomes I & Devi LA 2006 μ opioid and CB1 cannabinoid receptor interactions: reciprocal inhibition of receptor signalling and neuritogenesis. *British Journal of Pharmacology* **148** 387–395.

Ross RA 2009 The enigmatic pharmacology of GPR55. *Trends in Pharmacological Sciences* **30** 156–163.

Rubovitch V, Gafni M & Sarne Y 2002 The cannabinoid agonist DALN positively modulates L-type voltage-dependent calcium-channels in N18TG2 neuroblastoma cells. *Brain Research. Molecular Brain Research* **101** 93–102.

Rueda D, Galve-Roperh I, Haro A & Guzman M 2000 The CB(1) cannabinoid receptor is coupled to the activation of c-Jun N-terminal kinase. *Molecular Pharmacology* **58** 814–820.

Ruiu S, Pinna GA, Marchese G, Mussinu JM, Saba P, Tambaro S, Casti P, Vargiu R & Pani L 2003 Synthesis and characterization of NESS 0327: a novel putative antagonist of the CB1 cannabinoid receptor. *Journal of Pharmacology and Experimental Therapeutics* **306** 363–370.

Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J, Elebring T, Nilsson K, Drmota T & Greasley PJ 2007 The orphan receptor GPR55 is a novel cannabinoid receptor. *British Journal of Pharmacology* **152** 1092–1101.

Sarnataro D, Pisanti S, Santoro A, Gazzerro P, Malfitano AM, Laezza C & Bifulco M 2006 The cannabinoid CB1 receptor antagonist rimonabant (SR141716) inhibits human breast cancer cell proliferation through a lipid raft-mediated mechanism. *Molecular Pharmacology* **70** 1298–1306.

Savinainen JR, Saario SM, Niemi R, Jarvinen T & Laitinen JT 2003 An optimized approach to study endocannabinoid signalling: evidence against constitutive activity of rat brain adenosine A1 and cannabinoid CB1 receptors. *British Journal of Pharmacology* **140** 1451–1459.

Seifert R & Wenzel-Seifert K 2002 Constitutive activity of G-protein-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn-Schmiedeberg's Archives of Pharmacology* **366** 381–416.

Sim LJ, Hampson RE, Deadwyler SA & Childers SR 1996 Effects of chronic treatment with delta9-tetrahydrocannabinol on cannabinoid-stimulated [^{35}S]GTPgammaS autoradiography in rat brain. *Journal of Neuroscience* **16** 8057–8066.

Steinberg BA & Cannon CP 2007 Cannabinoid-1 receptor blockade in cardiometabolic risk reduction: safety, tolerability, and therapeutic potential. *American Journal of Cardiology* **100** 27P–32P.

Straiker A, Stella N, Piomelli D, Mackie K, Karten HJ & Maguire G 1999 Cannabinoid CB1 receptors and ligands in vertebrate retina: localization and function of an endogenous signalling system. *PNAS* **96** 14565–14570.

Sugiura T, Kodaka T, Kondo S, Tonegawa T, Nakane S, Kishimoto S, Yamashita A & Waku K 1996 2-Arachidonoylglycerol, a putative

endogenous cannabinoid receptor ligand, induces rapid, transient elevation of intracellular free Ca^{2+} in neuroblastoma×glioma hybrid NG108-15 cells. *Biochemical and Biophysical Research Communications* **229** 58–64.

Sugiura T, Kodaka T, Kondo S, Nakane S, Kondo H, Waku K, Ishima Y, Watanabe K & Yamamoto I 1997 Is the cannabinoid CB1 receptor a 2-arachidonoylglycerol receptor? Structural requirements for triggering a Ca^{2+} transient in NG108-15 cells *Journal of Biochemistry* **122** 890–895.

Sugiura T, Kishimoto S, Oka S & Gokoh M 2006 Biochemistry, pharmacology and physiology of 2-arachidonoylglycerol, an endogenous cannabinoid receptor ligand. *Progress in Lipid Research* **45** 405–446.

Szidonya L, Cserzo M & Hunyady L 2008 Dimerization and oligomerization of G-protein-coupled receptors: debated structures with established and emerging functions. *Journal of Endocrinology* **196** 435–453.

Turu G, Simon A, Gyombolai P, Szidonya L, Bagdy G, Lenkei Z & Hunyady L 2007 The role of diacylglycerol lipase in constitutive and angiotensin AT1 receptor-stimulated cannabinoid CB1 receptor activity. *Journal of Biological Chemistry* **282** 7753–7757.

Turu G, Varnai P, Gyombolai P, Szidonya L, Offertaler L, Bagdy G, Kunos G & Hunyady L 2009 Paracrine transactivation of the CB1 cannabinoid receptor by AT1 angiotensin and other $G_{q/11}$ protein-coupled receptors. *Journal of Biological Chemistry* **284** 16914–16921.

Vasquez C & Lewis DL 1999 The CB1 cannabinoid receptor can sequester G-proteins, making them unavailable to couple to other receptors. *Journal of Neuroscience* **19** 9271–9280.

Wager-Miller J, Westenbroek R & Mackie K 2002 Dimerization of G protein-coupled receptors: CB1 cannabinoid receptors as an example. *Chemistry and Physics of Lipids* **121** 83–89.

Waldeck-Weiermair M, Zoratti C, Osibow K, Balenga N, Goessnitzer E, Waldhoer M, Malli R & Graier WF 2008 Integrin clustering enables anandamide-induced Ca^{2+} signalling in endothelial cells via GPR55 by protection against CB1-receptor-triggered repression. *Journal of Cell Science* **121** 1704–1717.

Waldhoer M, Fong J, Jones RM, Lunzer MM, Sharma SK, Kostenis E, Portoghese PS & Whistler JL 2005 A heterodimer-selective agonist shows *in vivo* relevance of G protein-coupled receptor dimers. *PNAS* **102** 9050–9055.

Wilson RI & Nicoll RA 2001 Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* **410** 588–592.

Yin H, Chu A, Li W, Wang B, Shelton F, Otero F, Nguyen DG, Caldwell JS & Chen YA 2009 Lipid G protein-coupled receptor ligand identification using β-arrestin PathHunter assay. *Journal of Biological Chemistry* **284** 12328–12338.

Zhu PJ & Lovinger DM 2005 Retrograde endocannabinoid signalling in a postsynaptic neuron/synaptic bouton preparation from basolateral amygdala. *Journal of Neuroscience* **25** 6199–6207.