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REVIEW

Cannabinoid CB_1 receptor-interacting proteins: novel targets for central nervous system drug discovery?

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The main pharmacological effects of marijuana, as well as synthetic and endogenous cannabinoids, are mediated through G-protein-coupled receptors (GPCRs), including CB_1 and CB_2 receptors. The CB_1 receptor is the major cannabinoid receptor in the central nervous system and has gained increasing interest as a target for drug discovery for treatment of nausea, cachexia, obesity, pain, spasticity, neurodegenerative diseases and mood and substance abuse disorders. Evidence has accumulated to suggest that CB_1 receptors, like other GPCRs, interact with and are regulated by several other proteins beyond the established role of heterotrimeric G-proteins. These proteins, which include the GPCR kinases, β-arrestins, GPCR-associated sorting proteins, factor associated with neutral sphingomyelinase, other GPCRs (heterodimerization) and the novel cannabinoid receptor-interacting proteins: $CRIP_{1a/b}$, are thought to play important roles in the regulation of intracellular trafficking, desensitization, down-regulation, signal transduction and constitutive activity of CB_1 receptors. This review examines CB_1 receptor-interacting proteins, including heterotrimeric G-proteins, but with particular emphasis on non-G-protein entities, that might comprise the CB_1 receptosomal complex. The evidence for direct interaction with CB_1 receptors and potential functional roles of these interacting proteins is discussed, as are future directions and challenges in this field with an emphasis on the possibility of eventually targeting these proteins for drug discovery.
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Abbreviations: Δ^9-THC, Δ^9-tetrahydrocannabinol; 2-AG, 2-arachidonoylglycerol; AC, adenylyl cyclase; BRET, bioluminescence resonance energy transfer; CNS, central nervous system; CRIP, cannabinoid receptor-interacting protein; DA, dopamine; eCB, endogenous cannabinoid; FAN, factor associated with neutral sphingomyelinase; FRET, fluorescence resonance energy transfer; GASP, GPCR-associated sorting protein; GIRK, G-protein-coupled inwardly rectifying potassium channel; GPCR, G-protein-coupled receptor; GRK, G-protein-coupled receptor kinase; GTPγS, guanosine-5′-*O*-[γ-^{35}S]-triphosphate; JNK, c-Jun N-terminal kinase; LAMP, lysosome-associated membrane protein; MAPK, mitogen-activated protein kinase; PDZ, PSD-95/Disc-large-protein/ZO-1; PLA2, phospholipase A2; PLC, phospholipase C; PTX, *Pertussis* toxin; TNF, tumour necrosis factor

Cannabis, or marijuana, has been used for centuries, but its major psychoactive constituent, Δ^9-tetrahydrocannabinol (Δ^9-THC), was not identified until the 1960s (Gaoni and Mechoulam, 1964). The biological actions of Δ^9-THC and synthetic cannabinoids are mediated primarily by CB_1 and CB_2 receptors[1], which are heptahelical G-protein-coupled receptors (GPCRs) that activate G-proteins mainly of the *Pertussis* toxin (PTX)-sensitive $G_{i/o}$ family (Howlett *et al.*, 2002). CB_1 receptors are highly expressed in the central nervous system (CNS), with low to moderate expression in the periphery (Howlett *et al.*, 2002). CB_2 receptor expression is high in the immune system, with much lower and more restricted distribution in the CNS (Howlett *et al.*, 2002; Van Sickle *et al.*, 2005).

Although there is indirect evidence to suggest additional cannabinoid receptors exist, none beyond $CB_{1/2}$ have been

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[1]Molecular target nomenclature conforms to specifications presented in the Guide to Receptors and Channels (Alexander *et al.* 2008).
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definitively identified and cloned (Mackie and Stella, 2006). The best characterized is GPR55 (Sawzdargo *et al*., 1999). Activation of GPR55 by methanandamide, anandamide or THC increases calcium in dorsal root ganglion neurons, whereas other cannabinoid agonists had no effect (Lauckner *et al*., 2008). A broader profile of cannabinoids, including THC, anandamide, 2-arachidonoylglycerol (2-AG), noladin ether, virhodamine, CP55,940 and HU210-stimulated G-protein activity in GPR55-expressing human embryonic kidney (HEK-293) cells (Ryberg *et al*., 2007). However, the physiological function of GPR55 and its role in the endocannabinoid system has not been clearly defined.

Within the CNS, CB_1 receptors are densely distributed in the basal ganglia, hippocampus, cerebral cortex and cerebellum, with low to moderate expression in the diencephalon, brainstem and spinal cord (Herkenham *et al*., 1991; Glass *et al*., 1997). The acute *in vivo* effects of cannabinoids in humans and laboratory animals reflect the functions of these CNS regions and include short-term memory impairment, mood alteration, hypomotility, reward, catalepsy, decreased motor coordination, hypothermia and anti-nociception (Dewey, 1986; Hollister, 1986). Studies in CB_1 receptor null mice have confirmed that the CNS effects of Δ^9-THC are mediated by CB_1 receptors (Ledent *et al*., 1999; Zimmer *et al*., 1999).

Endogenous cannabinoids (eCBs) that activate cannabinoid receptors have been discovered. The major known eCBs are eicosanoids, including arachindonyl ethanolamide (anandamide) and 2-AG (Ahn *et al*., 2008). 2-AG is synthesized in a calcium-dependent manner in post-synaptic neurons and participates in several forms of synaptic plasticity (Kano *et al*., 2009). A number of other eCB ligands have been identified, including 2-arachidonyl-glyceryl ether (noladin), *O*-arachidonoyl-ethanolamine (virhodamine) and *N*-arachidonoyl-dopamine (NADA), but less is known regarding their function (Piomelli, 2003).

Although the biological effects of CB_1 receptors are mediated largely through activation of heterotrimeric G-proteins, in recent years it has become clear that GPCRs can interact with a number of additional signalling, scaffolding and regulatory proteins (Bockaert *et al*., 2004; Ritter and Hall, 2009). Some of these proteins interact with many GPCR types, including β-arrestins and the Ca^{2+}-binding protein, calmodulin. Others appear to be selective for particular groups of receptors, such as the A-kinase anchoring proteins and spinophilin, which interact with certain monoamine receptors. Finally, some GPCR-interacting proteins are selective for particular receptor subtypes, such as the Homer proteins that regulate certain isoforms of metabotropic glutamate receptors. Most GPCR-interacting proteins modulate intracellular signalling, trafficking or ligand selectivity of GPCRs, and many serve as adaptor or scaffolding proteins that link GPCRs to other signalling or regulatory proteins. The concept that GPCRs exist in functional complexes of macromolecules that contact each other directly or indirectly lead led to the terms 'receptosomes' or 'signalosomes', which describe microdomains containing receptors and their interacting proteins. The present review will focus on proteins that interact with CB_1 receptors and discuss the possibility that these proteins offer potential targets for future drug discovery. To place these findings in perspective, the first three sections of this review will briefly discuss CNS drug discovery in the cannabinoid system, canonical G-protein-mediated signalling by CB_1 receptors and intracellular trafficking of CB_1 receptors and their adaptation to prolonged ligand occupancy. The following sections will discuss CB_1 receptor-interacting proteins and evidence for their roles in CB_1 receptor signalling and regulation, and future directions and challenges in this field.

CB_1 receptors, eCBs and CNS drug discovery

Given the widespread CNS distribution of CB_1 receptors and the variety of *in vivo* effects produced by cannabinoids, it is not surprising that numerous potential therapeutic effects of marijuana have been reported both anecdotally and in laboratory studies. In fact, several states in the USA have decriminalized marijuana for medicinal purposes with a physician's permission. Drug formulations that contain Δ^9-THC either with or without cannabidiol (e.g. Sativex or Marinol, respectively), or synthetic cannabinoids (e.g. Nabilone) are approved in some countries. Uses of these drugs include treatment of nausea, vomiting, cachexia, spasticity and neuropathic pain (Pertwee, 2009). Other proposed therapeutic effects of cannabinoids include analgesia, anti-tumour effects, mood elevation, relief of insomnia and treatment of neurodegenerative disorders (Pertwee, 2009). However, clinical use of cannabinoids has been limited by psychoactive side effects, including abuse liability, and the development of tolerance with repeated administration. There is also interest in the potential therapeutic benefits of increasing eCB levels, for example by inhibiting eCB degradative enzymes (Cravatt and Lichtman, 2003). This approach might provide therapeutic benefit with reduced side effects. More recently, new approaches have focused on modulation of CB_1 receptor activity by allosteric modulators, which act at receptor sites outside of the orthosteric ligand-binding domain (Pertwee, 2005).

CB_1 antagonists provide an alternate strategy for modulating CB_1 receptors by inhibiting activity of this system. Rimonabant (SR141716A) was the first selective CB_1 receptor antagonist developed (Rinaldi-Carmona *et al*., 1994). The mechanism of action for rimonabant could be antagonism of eCB activity *in vivo*, or inverse agonism that inhibits constitutive activity of the CB_1 receptor (Bouaboula *et al*., 1997; Landsman *et al*., 1997). Rimonabant reduces food intake and produces weight loss in animals, and clinical trials showed its effectiveness in treating obesity and dyslipidemia (Di Marzo, 2008). However, clinical data revealed serious side effects, notably psychiatric disturbances, limiting the therapeutic usefulness of rimonabant and similar compounds (Janero and Makriyannis, 2009).

CB_1 receptors and eCBs also mediate the rewarding properties of other drugs, in part by modulating dopamine (DA) release in the mesocorticolimbic system, which is activated by most addictive drugs (Lupica *et al*., 2004; Maldonado *et al*., 2006). The role of the cannabinoid system in the motivational effects of drugs including morphine, nicotine, alcohol and cocaine has been demonstrated in studies that showed reduced drug self-administration/preference in CB_1 receptor

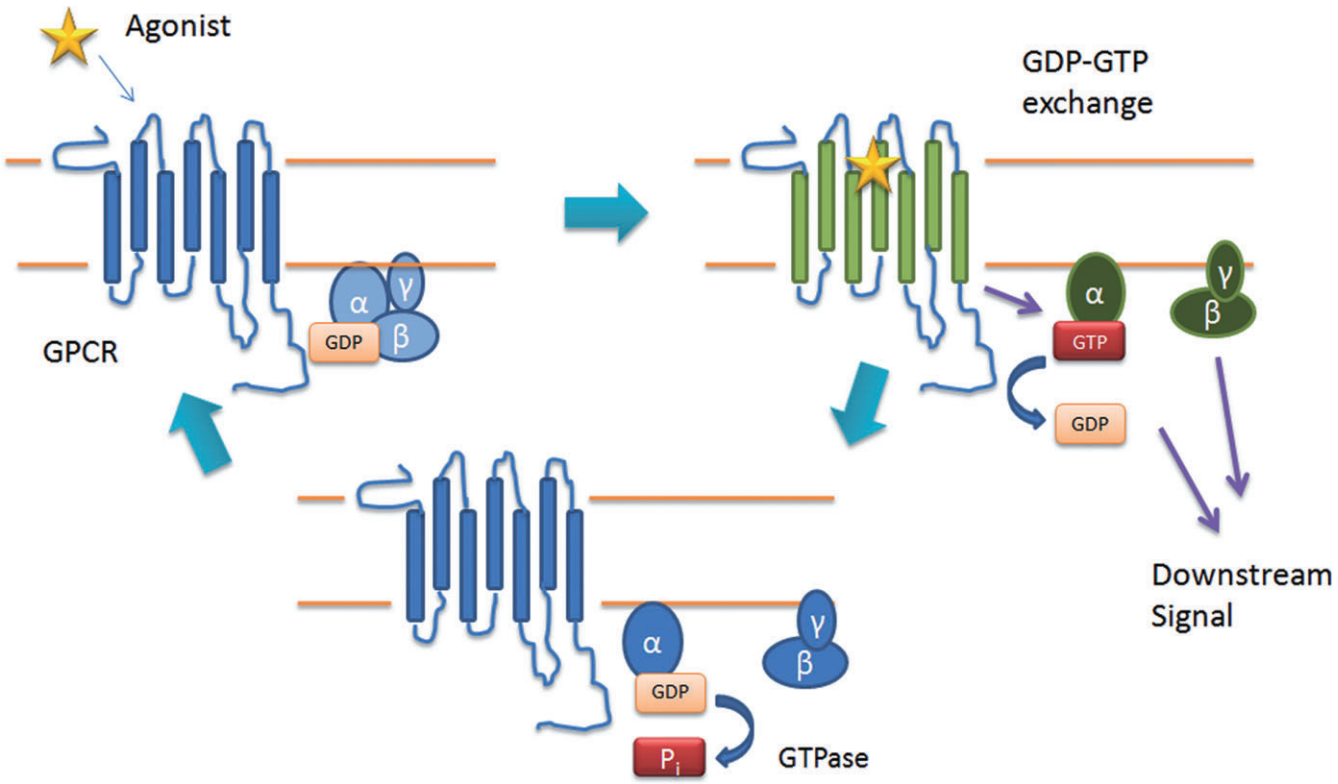

Figure 1 G-protein-coupled receptor (GPCR)-mediated G-protein activation. In the inactive state, G-proteins exist in the form of an αβγ heterotrimer, with the Gα subunit bound to GDP. Upon receptor activation, either by the binding of agonist or constitutively, the receptor changes to an active conformation (green), thereby activating G-proteins by promoting the exchange of GDP for GTP. The Gα-GTP and Gβγ dimer functionally dissociate from one another and the receptor and are free to modulate downstream effectors. The cycle concludes when the GTPase activity of the Gα subunit hydrolyses GTP to GDP, allowing the Gα subunit to return to its resting confirmation and reassociate with Gβγ.

null mice (Maldonado *et al.*, 2006). Consistent with these findings, rimonabant decreases opioid self-administration (Navarro *et al.*, 2001) and conditioned place preference in rodents (De Vries *et al.*, 2003) and is a potential treatment for drug addiction (Beardsley *et al.*, 2009). Rimonabant is also effective in smoking cessation (Fernandez and Allison, 2004), possibly by decreasing reinforcement, as shown in nicotine self-administration studies (Le Foll *et al.*, 2008). Rimonabant also reduces conditioned reinstatement of ethanol-seeking behaviour in rats (Cippitelli *et al.*, 2005) and decreases cocaine relapse after cocaine re-exposure (De Vries and Schoffelmeer, 2005). Thus, attenuating CB_1 receptor function may be a pharmacotherapeutic strategy for the treatment of multiple substance abuse disorders.

CB_1 receptor signalling through canonical G-protein-mediated pathways

CB_1 receptors, like other GPCRs, have seven transmembrane α-helical domains, an extracellular N-terminus and an intracellular C-terminus (Matsuda *et al.*, 1990). CB_1 receptors activate the PTX-sensitive G_i/G_o subfamily of proteins (Howlett *et al.*, 1986), of which there are four types: $G\alpha_i1$, $G\alpha_i2$, $G\alpha_i3$ and two splice variant subtypes of $G\alpha_o$ (Hildebrandt, 1997). CB_1 receptor-mediated downstream signals not blocked by PTX involve alternative CB_1-mediated pathways, such as the signal switching to $G\alpha_s$ seen when CB_1 receptors are stimulated concurrently with DA D_2 receptors (Glass and Felder, 1997; Kearn *et al.*, 2005), or non-G-protein-mediated signalling possibly through β-arrestin or other GPCR-interacting proteins (Pierce *et al.*, 2001; Ritter and Hall, 2009).

The basic mechanism of GPCR-mediated G-protein activation has previously been reviewed (Gilman, 1987; Hildebrandt, 1997) and is shown in Figure 1. GPCRs, including CB_1 receptors, act catalytically such that each receptor can activate multiple G-proteins over time, and the resulting accumulation of activated G-proteins provides signal amplification (Gierschik *et al.*, 1989; Sim *et al.*, 1996b; Breivogel *et al.*, 1997). Even in the absence of agonist, GPCRs exhibit some degree of spontaneous activity that is referred to as constitutive activity (Seifert and Wenzel-Seifert, 2002). Constitutively active GPCRs can increase basal G-protein activity and subsequent modulation of downstream effectors, and this activity is reversible by inverse agonists. However, when analysing a GPCR for constitutive activity, determination of endogenous ligands within the study system is important to rule out their contribution to apparent basal activity (Morisset *et al.*, 2000).

G-protein activation couples CB_1 receptors to the modulation of multiple downstream signalling pathways, including inhibition of adenylyl cyclase (AC) (Howlett *et al.*, 1986), phosphorylation of p42/p44 mitogen-activated protein

kinases (MAPK) (Bouaboula *et al*., 1995; Derkinderen *et al*., 2001; Galve-Roperh *et al*., 2002), inhibition of N-type and P/Q type voltage-dependent Ca^{2+} channels (Pan *et al*., 1996; Twitchell *et al*., 1997), stimulation of inward rectifying K^+ (GIRK) channels (Mackie *et al*., 1995; Vasquez *et al*., 2003), inhibition of Na^+ channels (Nicholson *et al*., 2003), stimulation of phospholipases C and A2 (PLC, PLA2) (Hunter *et al*., 1986) and activation of c-Jun N-terminal kinase (JNK) and p38 kinase (Rueda *et al*., 2002). CB_1 receptors can modulate AC activity via either $G\alpha_i$ or $G\beta\gamma$, including inhibition of AC types 3, 5, 6 and 8 by $G\alpha_i$ and of type 1 by both $G\alpha_i$ and $G\beta\gamma$ (Rhee *et al*., 2000; Howlett *et al*., 2002; Offermanns, 2003). CB_1 receptors can also stimulate certain AC types (2, 4 and 7) via $G\beta\gamma$ (Rhee *et al*., 1998).

The ability of cannabinoid ligands to act as a full, partial or inverse agonists has been determined in G-protein activation assays measuring receptor-mediated binding of the hydrolysis-resistant GTP analogue guanosine-5′-*O*-[γ-^{35}S]-triphosphate ([^{35}S]GTPγS) in the presence of excess GDP, in which the maximal stimulation of [^{35}S]GTPγS binding indicates ligand efficacy (Sim *et al*., 1996a; Burkey *et al*., 1997; Breivogel *et al*., 1998). Examples of high-efficacy CB_1 agonists include the aminoalkylindole WIN55,212-2, the bicyclic CP55,940, the dimethylheptyl side-chain THC analogue, HU210 and the eCB 2-AG. CB_1 partial agonists include the phytocannabinoid Δ^9-THC and the eCB anandamide and its stable analogue methanandamide. CB_1 receptor inverse agonists include rimonabant and its analogues (Bouaboula *et al*., 1997; Landsman *et al*., 1997).

G-proteins interact with the C-terminus (Nie and Lewis, 2001a) and the third intracellular loop (Mukhopadhyay *et al*., 2000) of the CB_1 receptor. Distinct G-protein types appear to interact specifically with certain regions of the CB_1 receptor. For example, $G\alpha_i1$ and $G\alpha_i2$ interact with third cytosolic loop of the CB_1 receptor (Mukhopadhyay *et al*., 2000; Mukhopadhyay and Howlett, 2001) whereas $G\alpha_i3$ and $G\alpha_o$ interact with the C-terminus (Mukhopadhyay *et al*., 2000). Furthermore, specific agonists can differentially activate specific $G\alpha_{i/o}$ proteins, such that full agonists maximally activate a greater number of $G\alpha_{i/o}$ subtypes than partial agonists (Glass and Northup, 1999; Mukhopadhyay and Howlett, 2005). These studies suggest that there are multiple active conformations of the CB_1 receptor that can be differentially stabilized by distinct ligands, as recently indicated by plasmon waveguide resonance spectroscopy (Georgieva *et al*., 2008). Overall, this evidence implies that selective pharmacological targeting of CB_1 receptors could be used to promote therapeutic pharmacological effects while potentially minimizing side effects. Moreover, if CB_1 receptor-G-protein coupling specificity is modulated by endogenous proteins, then these proteins can also be pharmacologically targeted for the same purpose.

The use of inverse agonists has allowed determination of structural elements in CB_1 receptors that play a role in constitutive activity. A highly conserved aspartate residue in the second transmembrane domain, denoted II:14D [transmembrane domain II, amino acid position 14, Asp (D)] (Baldwin *et al*., 1997) or D164 (Asp at CB_1 amino acid position 164), is critical to CB_1 receptor constitutive activity. Mutation of this residue abolished constitutive activity without disrupting agonist-mediated inhibition of Ca^{2+} channels (Nie and Lewis, 2001b). However, mutation of II:14D disrupted agonist-stimulated activation of GIRK channels or inhibition of cAMP formation and prevented agonist-induced internalization (Tao and Abood, 1998; Roche *et al*., 1999). A role for this residue in CB_1 receptor activation is not surprising, because II:14D is responsible for allosteric regulation of GPCRs by sodium (Horstman *et al*., 1990; Ceresa and Limbird, 1994), which diminishes constitutive GPCR activity and affects the relative efficacy of ligands (Koski *et al*., 1982; Seifert and Wenzel-Seifert, 2002). Thus, like sodium, proteins that allosterically modulate the basal activation state of CB_1 receptors would be expected to modulate the relative efficacies of cannabinoid ligands.

Intracellular regulatory proteins can interact with the C-terminus of some GPCRs to regulate constitutive activity (Bockaert *et al*., 2004; Ritter and Hall, 2009). Interestingly, Nie and Lewis (2001b) found that truncation of the distal C-terminus of the CB_1 receptor at amino acid 417 enhanced its constitutive activity. This finding raises the possibility that a protein binds to the distal C-terminal tail that attenuates the constitutive activity of the CB_1 receptor.

CB_1 receptor adaptation and trafficking

Persistent agonist occupancy induces CB_1 receptor desensitization (attenuated receptor-mediated G-protein and effector activity), which reduces sensitivity to subsequent activation by agonists. For example, recording of CB_1-mediated GIRK activation in CB_1-expressing *Xenopus oocytes* showed that these receptors desensitize acutely (<10 min) to application of agonist (Jin *et al*., 1999). Agonist-induced desensitization of CB_1-mediated inhibition of synaptic transmission in cultured hippocampal neurons required longer (>2 h) agonist treatment (Kouznetsova *et al*., 2002).

Desensitization of CB_1 receptor-mediated G-protein activation has also been reported in the brain after chronic, but not acute, administration of Δ^9-THC, WIN55,212-2 or CP55,940 (Sim-Selley, 2003; Martin *et al*., 2004). CB_1 receptor desensitization appears as a decrease in maximal agonist-induced stimulation of [^{35}S]GTPγS binding in brain membrane homogenates or brain sections (autoradiography). Cannabinoid-stimulated [^{35}S]GTPγS autoradiography in brains from rodents treated with Δ^9-THC or synthetic cannabinoids has shown decreased agonist-stimulated binding in almost all brain regions (Sim *et al*., 1996a; Sim-Selley, 2003). Interestingly, the magnitude and time course of desensitization are region-dependent, perhaps reflecting regional differences in the co-localization of CB_1 receptors with various regulatory proteins (Sim-Selley, 2003). CB_1 receptors in the hippocampus generally exhibit the greatest/fastest desensitization, whereas nuclei in the basal ganglia show less/slower desensitization.

Prolonged agonist treatment can also reduce CB_1 receptor levels (down-regulation). CB_1 receptor down-regulation, measured as decreased radioligand binding in autoradiography or reduced B_{max} values in brain membrane homogenates, has been demonstrated in rodent brain after prolonged treatment with Δ^9-THC or synthetic cannabinoid agonists (Sim-Selley, 2003). [^{3}H]SR141716A binding is also decreased in the hippocampus, striatum/basal ganglia and mesencephalon of

brains from regular marijuana users compared with non-users (Villares, 2007). The magnitude of CB_1 receptor down-regulation varies among brain regions in rodent in a similar anatomical distribution as desensitization; down-regulation is greatest/fastest in hippocampus, cortex, cerebellum followed by caudate-putamen, with the least/slowest down-regulation seen in substantia nigra and globus pallidus (Sim-Selley, 2003).

Transcriptional down-regulation could contribute to region-specific differences because decreased CB_1 receptor mRNA has been measured in striatum but not hippocampus or cerebellum (Sim-Selley, 2003). However, immunoblot analysis indicated that CB_1 receptor down-regulation in brain is primarily due to a loss in receptor because the time course of recovery from CB_1 receptor down-regulation after cessation of chronic Δ^9-THC treatment was more closely associated with levels of CB_1 receptor protein and [^{3}H]SR141716A-binding sites than CB_1 receptor mRNA levels (Sim-Selley *et al*., 2006). Interestingly, CB_1 receptors were not down-regulated in cultured hippocampal neurons (Coutts *et al*., 2001) or N18TG2 neuroblastoma cells (McIntosh *et al*., 1998) in response to agonist, while down-regulation of heterologously expressed CB_1 receptors was reported in some cell lines (Shapira *et al*., 2003) but not others (Rinaldi-Carmona *et al*., 1998). Differences in rates and magnitudes of agonist-induced CB_1 receptor desensitization and down-regulation among CNS regions and cell types suggest differential expression profiles of regulatory proteins interacting with CB_1 receptors.

Agonist exposure can also induce CB_1 receptor trafficking among subcellular compartments. For example, CB_1 receptors heterologously expressed in cell lines undergo endocytosis (internalization) in response to agonists (Hsieh *et al*., 1999; Wu *et al*., 2008). CB_1 receptors can undergo endocytosis mediated by either clathrin-coated pits or caveolae (Keren and Sarne, 2003; Bari *et al*., 2008; Wu *et al*., 2008). Agonist dose, exposure time and post-endocytic sorting are important factors in determining the fate of internalized CB_1 receptors. Once internalized, GPCRs are either dephosphorylated and recycled back to the cell surface (resensitization) or targeted to lysosomes for degradation (down-regulation) (Reiter and Lefkowitz, 2006). Rapid recycling of CB_1 receptors heterologously expressed in cell lines occurs after short agonist exposures (minutes) and requires dephosphorylation and endosomal acidification (Hsieh *et al*., 1999). CB_1 receptor endocytosis is important in resensitization, as indicated by the finding that blockade of endocytic recycling enhances WIN55,212-2-induced desensitization of CB_1 receptor-mediated cAMP inhibition (Wu *et al*., 2008). Longer agonist exposure (1.5 h) promotes down-regulation of CB_1 receptors (Hsieh *et al*., 1999), as can briefer exposure to very high concentrations of agonists (Keren and Sarne, 2003; Martini *et al*., 2007). CB_1 receptor down-regulation is associated with co-localization of the receptor with lysosomal markers, lysosome-associated membrane protein (LAMP)1 and 2 (Martini *et al*., 2007). These findings suggest that proteins that regulate the rate and magnitude of CB_1 receptor endocytosis, or post-endocytic sorting of CB_1 receptors, can modulate desensitization and down-regulation of these receptors.

Constitutive activity of CB_1 receptors can also modulate their subcellular localization. CB_1 receptors were spontaneously internalized and recycled back to the cell surface, a process blocked by inverse agonists (Leterrier *et al*., 2004). Although evidence that constitutive activity may not be necessary for constitutive CB_1 internalization has also been reported (McDonald *et al*., 2007), constitutive internalization of CB_1 receptors can play a role in axonal targeting in neurons (Leterrier *et al*., 2006; McDonald *et al*., 2007). Therefore, regulatory proteins that modulate constitutive internalization of CB_1 receptors, whether by modulating constitutive activity or through alternative mechanisms, could regulate axonal targeting of these receptors in the CNS.

Localization of CB_1 receptors within microdomains of the plasma membrane could also influence CB_1 receptor function. Plasma membranes contain discrete regions that are rich in cholesterol and sphingolipids, termed lipid rafts (Barnett-Norris *et al*., 2005). Many GPCRs localize to lipid rafts, and agonists can promote GPCR entry into, or exit from, lipid raft microenvironments (Patel *et al*., 2008). There is evidence that lipid rafts can limit signal transduction by CB_1 receptors. For example, treatment of C6 glioma cells with a lipid raft disruptor increased G-protein activation and downstream signalling by anandamide (Bari *et al*., 2005). Moreover, we have reported that sphingosine, a major component of lipid rafts, can act as a CB_1 receptor antagonist with modest affinity (Paugh *et al*., 2006). Finally, lipid rafts have been associated with trafficking and metabolism of eCBs (Barnett-Norris *et al*., 2005; Dainese *et al*., 2007). Thus, regulatory proteins that modulate the trafficking of CB_1 receptors into and out of lipid rafts could be important modifiers of CB_1 receptor activity.

GPCR-interacting proteins that mediate CB_1 receptor desensitization and down-regulation

Several GPCR-interacting proteins regulate signalling, trafficking and degradation of GPCRs, including the G-protein-coupled receptor kinase (GRK)/arrestin pathway. The mechanism of GRK/arrestin-mediated regulation of GPCRs has been reviewed (Pitcher *et al*., 1998). Briefly, activated GPCRs are phosphorylated on Ser/Thr residues, generally in the C-terminal tail or third intracellular loop, by one of several GRKs. The phosphoylated receptor recruits the cytoplasmic proteins arrestin2/3 (β-arrestin1/2).

Several of these proteins contribute to CB_1 receptor regulation. Acute CB_1 receptor desensitization of GIRK channel activation was enhanced by co-expression of GRK3 and β-arrestin-2 (Jin *et al*., 1999). Phosphorylation at residues 426 and 430 in the CB_1 receptor C-terminus was required for this effect. Likewise, desensitization of CB_1-mediated inhibition of glutamatergic neurotransmission in hippocampal neurons was blocked by expression of dominant negative mutants of GRK2 or β-arrestin2 (Kouznetsova *et al*., 2002). Further support for a role of GRK/β-arrestin in the regulation of CB_1 receptors is the finding that chronic THC treatment enhanced the expression of GRK2 and 4 and β-arrestin-1 and 2 in some mouse brain regions (Rubino *et al*., 2006). Moreover, *in vivo* evidence for a role of β-arrestin-2 in regulating acute signalling by CB_1 receptors was obtained in studies of β-arrestin-2 null mice, in which sensitivity to THC was greater in tests of

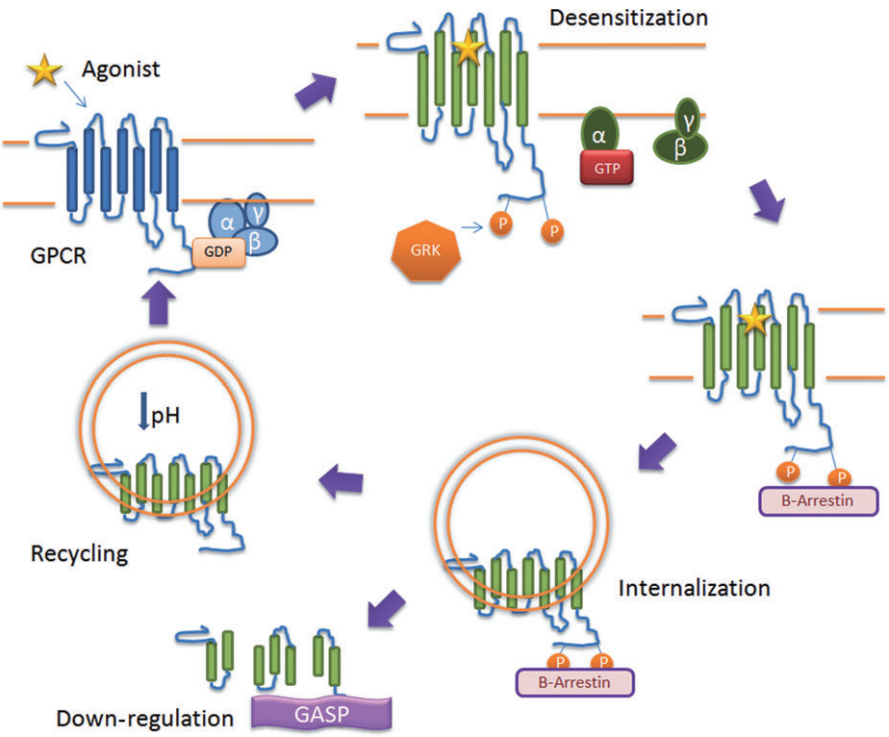

Figure 2 G-protein-coupled receptor (GPCR) desensitization, internalization and down-regulation. Upon activation of the GPCR, GPCR kinase (GRK) phosphorylates the receptor, generally on C-terminal Ser/Thr residues. Once phosphorylated, β-arrestin can bind to the GPCR, desensitizing the receptor and causing the receptor to internalize via clathrin-coated pits. Once internalized, GPCRs may be recycled back to the cell surface following dephosphorylation in acidified endosomal compartments. Alternatively, GPCRs can be trafficked to lysosomes and degraded (down-regulation), a process that is facilitated by GPCR-associated sorting protein (GASP)1.

anti-nociception and hypothermia compared with wild-type mice (Breivogel *et al.*, 2008). However, sensitivity to other cannabinoid agonists was unaffected in the mutant mice, suggesting that β-arrestin-2 effects on acute CB_1 receptor function are ligand-selective.

Although mutation of putative phosphorylation sites in the distal C-terminus of CB_1 blocked agonist-induced internalization (Hsieh *et al.*, 1999), a definitive role for the GRK/β-arrestin regulatory pathway has not been established for internalization. In fact, despite the evidence for a role of GRK/β-arrestin in negatively regulating CB_1 receptor signalling, little direct evidence for CB_1 receptor interaction with GRK or β-arrestin has been reported. One study showed that a synthetic peptide corresponding to residues 419–439 of the CB_1 receptor interacted with β-arrestin-2 in solution using NMR approaches (Bakshi *et al.*, 2007). However, a recent study using bioluminescence resonance energy transfer (BRET), which provides direct evidence of close proximity, found only weak interaction between CB_1 and β-arrestin-2 (Vrecl *et al.*, 2009). Furthermore, GRK-mediated phosphorylation of CB_1 receptors has not been examined, and co-immunoprecipitation of CB_1 receptors with GRK or β-arrestin isoforms has not been demonstrated. Thus, although existing evidence suggests that GRK and β-arrestins play a role in CB_1 regulation, evidence of direct interaction between these proteins and CB_1 receptors is minimal.

The GPCR-associated sorting protein (GASP1) is a large (~170 kD) protein that participates in post-endocytic sorting of certain GPCRs, including δ-opioid and DA D_2 receptors, and targets them for lysosomal degradation (Whistler *et al.*, 2002; Bartlett *et al.*, 2005). Like many other GPCR-interacting proteins, GASP1 binds to the C-terminus and a likely binding domain has been identified in the proximal C-terminus, homologous to rhodopsin helix-8, in several GPCRs (Simonin *et al.*, 2004). GASP1 interacts with the CB_1 receptor C-terminus and targets CB_1 receptors to LAMP1/2-positive lysosomes (Martini *et al.*, 2007; Tappe-Theodor *et al.*, 2007). Moreover, CB_1 receptors co-localize with GASP1 in rat striatal, hippocampal and spinal cord neurons and co-immunoprecipitated with GASP1 from rat brain extracts. Furthermore, expression of a dominant negative construct, cGASP1, inhibited agonist-induced CB_1 receptor targeting to lysosomes and its degradation. Importantly, virally mediated expression of cGASP1 in rat spinal cord dorsal horn reduced CB_1 receptor down-regulation induced by repeated WIN55,212-2 treatment, and this effect was associated with reduced anti-nociceptive tolerance (Tappe-Theodor *et al.*, 2007). Thus, there is relatively strong evidence for direct interaction of CB_1 receptors with GASP1, which appears to play a significant role in CB_1 receptor down-regulation. Figure 2 shows a summary of putative CB_1 receptor regulation by GRKs, β-arrestin and GASP1.

CB_1 receptor-interacting proteins that mediate G-protein-independent signalling

It has become evident that several GPCR-interacting proteins can mediate signal transduction independently of G-proteins.

For example, β-arrestins serve as scaffolds for assembly of signalling complexes, in addition to mediating desensitization and receptor trafficking (Pierce *et al*., 2001). For example, internalized GPCRs that cannot couple to G-proteins activate MAPK in a β-arrestin-dependent manner in some cell types. However, no direct evidence links CB_1 receptor-mediated activation of MAPK to β-arrestin, rather most evidence suggests a role for G-protein-mediated activation of phosphoinositide-3-kinase or inhibition of AC in the MAPK response (Derkinderen *et al*., 2001; Galve-Roperh *et al*., 2002).

Sphingomyelin hydrolysis, which generates ceramide, can be activated by cannabinoids in a G-protein-independent manner in astrocytes but not neurons (Velasco *et al*., 2005). This response was mediated by the factor associated with neutral sphingomyelinase (FAN), a protein that was previously shown to couple tumour necrosis factor (TNF) receptors to sphingomyelin hydrolysis (Adam-Klages *et al*., 1996). FAN is a WD repeat-containing protein, similar to G-protein β-subunits, which suggests its participation in numerous protein–protein interactions. CB_1 receptors in astrocytes co-immunoprecipitated with FAN in the presence of Δ^9-THC (Sanchez *et al*., 2001). Moreover, expression of a dominant negative mutant of FAN blocked Δ^9-THC-induced sphingomyelin hydrolysis, but pretreatment with PTX did not. Although the region of the CB_1 receptor that interacts with FAN has not been conclusively demonstrated, amino acid residues 431–435 in the CB_1 receptor C-terminus contains a homologous motif (DCLHK) to that associated with FAN activation in the TNF receptor (DSAHK). Interestingly, this sequence is conserved in CB_1 receptors among mammals, but is not found in CB_2 receptors. These findings indicate that, at least in astrocytes, CB_1 receptors can activate the ceramide signalling pathway via direct interaction of the receptor with FAN.

Novel CB_1 receptor-interacting proteins, $CRIP_{1a}$ and $CRIP_{1b}$

The finding that truncation of the distal C-terminal tail of the CB_1 receptor enhanced constitutive receptor activity (Nie and Lewis, 2001b) lead to a search for a protein that might bind to the CB_1 receptor C-terminus and inhibit this constitutive activity. Two novel proteins, termed cannabinoid receptor-interacting proteins 1a and b ($CRIP_{1a}$ and $CRIP_{1b}$) were recently discovered by Lewis *et al*. (Niehaus *et al*., 2007) via yeast two-hybrid screening of a human brain cDNA library, using the last 55 amino acids (418–472) of the CB_1 receptor C-terminal tail as bait. These novel proteins are encoded by the *Cnrip* gene, which is found on human chromosome 22. Alternative splicing produces $CRIP_{1a}$ (exons 1, 2 and 3a) and $CRIP_{1b}$ (exons 1, 2 and 3b), which are 164 and 128 amino acids respectively. The role of $CRIP_{1b}$ is unknown; it is found only in primates and its effects on CB_1 receptor function are unclear. However, $CRIP_{1a}$ appears to decrease the constitutive activity of the CB_1 receptor, as discussed below.

The region of the CB_1 receptor required for CRIP interaction was determined using yeast two-hybrid screening of CB_1 receptor C-terminal tail mutants as bait and $CRIP_{1b}$ as prey (Niehaus *et al*., 2007). The last nine amino acids of the CB_1 receptor were required for $CRIP_{1b}$ interaction. $CRIP_{1b}$ did not interact with amino acid sequences containing either the putative phosphorylation sites required for desensitization (419–438) or internalization (460–463) of the CB_1 receptor. Furthermore, bacterially expressed $CRIP_{1a}$ bound specifically to immobilized GST-tagged CB_1 C-terminal tail. *In vivo* interaction of $CRIP_{1a}$ and CB_1 receptors was inferred from co-immunoprecipitation of $CRIP_{1a}$ with CB_1 receptors from rat brain homogenates. Interestingly, $CRIP_{1a}$ did not interact with CB_2 receptors, as the distal C-terminus of this receptor exhibits low homology to CB_1 receptors. Likewise, homology searching found low homology between this motif in CB_1 versus other GPCRs, suggesting that $CRIP_{1a}$ is CB_1-selective, although such selectivity has not been definitively demonstrated.

Tissue profiling showed that $CRIP_{1a}$ was highly expressed in mouse brain and was also detected in heart, lung and intestine. Confocal microscopy of cDNA-microinjected rat superior cervical ganglion neurons found that $CRIP_{1a}$ was co-localized with CB_1 receptors near the plasma membrane. Co-expression of $CRIP_{1a}$ in HEK or CHO cells stably expressing CB_1 receptors showed that $CRIP_{1a}$ did not affect total CB_1 receptor expression, and that $CRIP_{1a}$ immunoreactivity was present in the membrane fraction.

Comparative genomic analysis indicated that $CRIP_{1a}$ is conserved throughout the vertebrates (Niehaus *et al*., 2007). $CRIP_{1a}$ contains no transmembrane domains, as determined by hydropathy analysis, but does contain a predicted palmyitoylation site, which may aid its association with the plasma membrane. The C-terminal tail of $CRIP_{1a}$ contains a predicted PSD-95/Disc-large-protein/ZO-1 (PDZ) class I ligand, which could allow it to interact with PDZ domain-containing proteins. This finding suggests that $CRIP_{1a}$, like many other proteins that interact with PDZ modules, may be important for regulating CB_1 receptor signalling, scaffolding or trafficking. Interestingly, many GPCR-interacting proteins contain PDZ domains and several GPCRs contain PDZ ligand sequences, suggesting that $CRIP_{1a}$ could indirectly link CB_1 receptors to other GPCRs.

Electrophysiological recordings of calcium currents in rat superior cervical ganglion neurons microinjected with CB_1 cDNA, with and without co-microinjection of $CRIP_{1a}$ cDNA, showed that $CRIP_{1a}$ attenuated constitutive CB_1-mediated inhibition of calcium channels that was blocked by the inverse agonist, rimonabant (Niehaus *et al*., 2007). However, WIN55,212-2-induced inhibition of calcium currents was unaffected by $CRIP_{1a}$ expression. These results pose the intriguing possibility that $CRIP_{1a}$ modulates constitutive CB_1 receptor activity in the CNS, which might affect receptor subcellular localization (Leterrier *et al*., 2006) or basal levels of neurotransmitter release (Kano *et al*., 2009).

One group has examined the potential role of $CRIP_{1a}$ in the brain to date. Ludanyi *et al*. (2008) postulated that expression of proteins in the endocannabinoid system might be altered in pathologic neuronal excitability because of the putative protective role of eCBs. To address this hypothesis, they utilized quantitative PCR to evaluate mRNA levels of CB_1 receptor and $CRIP_{1a}$ in epileptic versus healthy post-mortem human hippocampal tissue. Human sclerotic hippocampi exhibited a reduction in $CRIP_{1a}$ gene expression in tandem

with reduced CB_1 receptor expression, although only CB_1 receptor mRNA was decreased in non-sclerotic tissue (Ludanyi *et al*., 2008). The implications of this study are unclear, but might suggest a role for modulation of CB_1 receptor function by $CRIP_{1a}$ in the pathogenesis of or in response to epilepsy. However, the co-localization of $CRIP_{1a}$ and CB_1 receptors in the CNS still remains to be demonstrated, complicating the interpretation of these results.

CB_1 receptor heterodimerization

Evidence has accumulated that GPCRs can exist as dimeric or multimeric complexes with themselves (homodimers/oligomers) or other GPCRs (heterodimers/oligomers) (Gomes *et al*., 2001; Milligan, 2010), as demonstrated using co-immunoprecipitation, BRET or fluorescence resonance energy transfer (FRET) imaging. For example, CB_1 receptors can exist as homodimers (Wager-Miller *et al*., 2002; Mackie, 2005), but their functional relevance has not been defined.

Heterodimerization of CB_1 receptors with DA D_2 receptors has been best characterized (Mackie, 2005). Glass and Felder (1997) demonstrated in cultured striatal neurons that simultaneous activation of CB_1 and D_2 receptors switched their signalling from inhibition to stimulation of AC activity. Subsequent studies in cells heterologously co-transfected with CB_1 and D_2 receptors also showed CB_1 'signal switching' even in the absence of D_2 agonist (Jarrahian *et al*., 2004). In both studies, PTX treatment enhanced the effect, suggesting involvement of non-$G_{i/o}$ proteins, presumably G_s. Further studies demonstrated that CB_1 and D_2 receptors co-expressed in cells could be co-immunoprecipitated as heterodimers, and simultaneous activation of both receptors increased heterodimer formation (Kearn *et al*., 2005). Moreover, heterodimerization was associated with PTX-resistant stimulation of cAMP formation and MAPK phosphorylation, suggesting that earlier observations of D_2-mediated CB_1 receptor signal switching were due to heterodimerization. In agreement, CB_1–D_2 heterodimerization has recently been shown using FRET/BRET approaches in co-transfected cells (Marcellino *et al*., 2008; Navarro *et al*., 2008). Although *in vivo* implications of these interactions are unclear, evidence indicates co-localization (Pickel *et al*., 2006) and reciprocal modulation of ligand binding and signalling by CB_1 and D_2 receptors in striatum (Meschler and Howlett, 2001; Marcellino *et al*., 2008). Moreover, CB_1 and D_2 agonists appear to have antagonistic or synergistic effects on locomotor activity in a species-specific manner (Meschler *et al*., 2000a,b; Marcellino *et al*., 2008), although the role of heterodimerization is unknown.

CB_1 receptor heterodimerization has also been reported with μ-, κ- and δ-opioid (Rios *et al*., 2006; Hojo *et al*., 2008), adenosine A_{2A} (Carriba *et al*., 2007; Navarro *et al*., 2008) and orexin-1 (Ellis *et al*., 2006) receptors. Simultaneous activation of CB_1 and μ-opioid receptors attenuated activation of G-proteins or MAPK compared with activation of either receptor alone. A subsequent study demonstrated intracellular Ca^{2+} elevation by μ-CB_1 heterodimers in an oocyte model (Hojo *et al*., 2008). The *in vivo* significance of CB_1 opioid receptor heterodimerization is unknown, but there is extensive literature on cannabinoid–opioid interactions that could result in part from heterodimerization (Vigano *et al*., 2005; Robledo *et al*., 2008; Welch, 2009).

CB_1 receptor heterodimerization with A_{2A} receptors has been reported (Carriba *et al*., 2007; Navarro *et al*., 2008). A_{2A} antagonists decreased CB_1 receptor-mediated inhibition of AC in cells endogenously co-expressing these receptors (Carriba *et al*., 2007). CB_1 and A_{2A} receptors were also co-localized in rat striatum, and co-administration of an A_{2A} antagonist decreased cannabinoid-induced locomotor depression. Furthermore, heterotrimeric association between CB_1, D_2 and A_{2A} receptors has been reported (Navarro *et al*., 2008), and administration of an A_{2A} antagonist decreased the ability of a cannabinoid agonist to attenuate D_2 agonist-induced hyperlocomotion in rats (Marcellino *et al*., 2008).

Heterodimerization of CB_1 with orexin-1 receptors was demonstrated in heterologously co-transfected cells (Ellis *et al*., 2006). Co-expression with CB_1 receptors spontaneously internalized orexin-1 receptors, which could be reversed by antagonists of either receptor. Likewise, antagonism of either receptor decreased the potency of agonists of the other receptor to activate MAPK. Thus, interactions between these two receptors affected both intracellular trafficking and signalling. Heterodimerization of these receptors might regulate appetite (Viveros *et al*., 2008), but their interactions in the brain have not been examined.

Future directions and challenges

A number of proteins interact with the CB_1 receptor, as summarized in Figure 3. GRKs, β-arrestins and GASP1 are likely to play distinct roles in desensitization, intracellular trafficking and down-regulation of CB_1 receptors; however, direct interaction with CB_1 receptors has only been demonstrated for GASP1. FAN can mediate CB_1 receptor coupling to sphingomyelin hyrolysis in glia, but the factors that regulate CB_1 receptor-mediated activation of FAN are unclear. The significance of CB_1 association with FAN in glia but not neurons may be related to cell proliferation, which is limited in adult CNS neurons. Whether β-arrestin also plays a direct role in intracellular signalling by CB_1 receptors remains to be determined. CB_1 receptor heterodimerization with other GPCRs occurs in cell models, with distinct functional consequences, but it is uncertain whether heterodimerization occurs in the brain or contributes to *in vivo* drug interactions by agonists that activate these receptors.

The novel protein $CRIP_{1a}$ appears to inhibit constitutive activity of the CB_1 receptor in coupling to Ca^{2+} channels, but many questions remain about its role. These include whether $CRIP_{1a}$ co-localizes with CB_1 receptors *in vivo*, and whether $CRIP_{1a}$ is highly selective for CB_1 or has other roles in receptor signalling, as suggested by its PDZ ligand. Although $CRIP_{1a}$ had no effect on CB_1 agonist-mediated inhibition of Ca^{2+} channels, it is unknown whether it modulates other effectors or G-protein activation directly. Moreover, whether $CRIP_{1a}$ modulates CB_1 receptor trafficking and adaptation is also unknown. Finally, the function of $CRIP_{1b}$, which occurs only in primates, is also unknown. Because $CRIP_{1b}$ has an alternate C-terminus that lacks a PDZ ligand, it is tempting to speculate that this

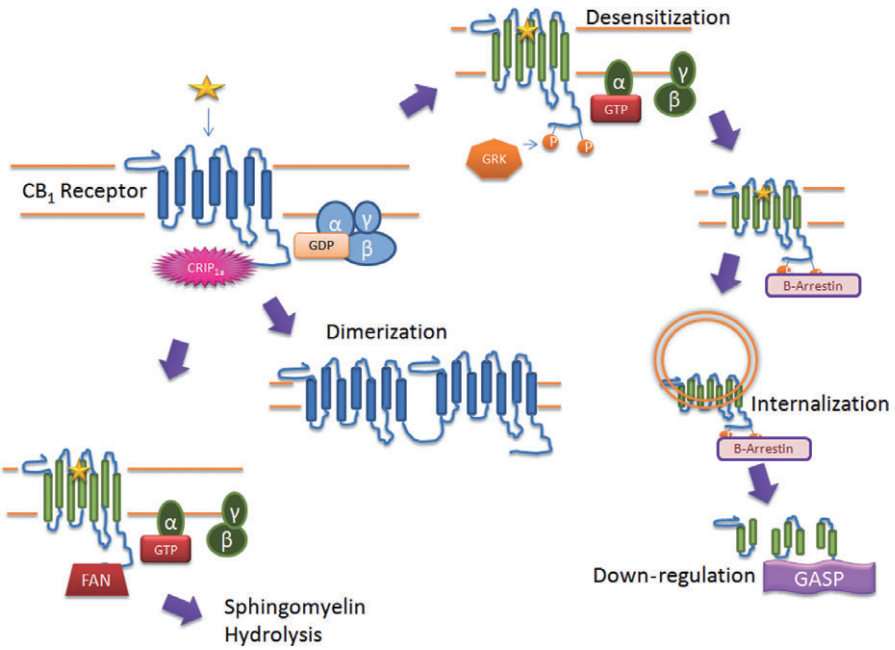

Figure 3 Schematic summary of CB_1 receptor-interacting proteins. CB_1 receptors can be bound by cannabinoid receptor-interacting protein $(CRIP)_{1a}$ (or $CRIP_{1b}$ in primates) on their distal C-terminus, which might stabilize the receptors in an inactive state. Once activated, such as by the binding of an agonist, CB_1 receptors can activate $G_{i/o}$-proteins in many cells types and could also activate FAN (factor associated with neutral sphingomyelinase) in a G-protein-independent manner in astrocytes. Activated CB_1 receptors also might become a substrate for G-protein-coupled receptor kinase (GRK)-mediated phosphorylation, presumably in the C-terminus. GRK-phosphorylated CB_1 receptors could recruit β-arrestin, thereby undergoing desensitization and clathrin-dependent internalization, followed in some cell types by G-protein-coupled receptor-associated sorting protein (GASP)1-mediated lysosmal degradation. CB_1 receptors might in some cells types form heterodimers with other GPCRs, such as dopamine D_2, adenosine A_{2A}, μ-, δ- or κ-opioid, or orexin-1, which could have numerous effects on their signalling and intracellular trafficking.

isoform could act as a dominant negative modulator of $CRIP_{1a}$ function. A similar scenario has been demonstrated for different Homer isoforms in modulating metabotropic glutamate receptor function (Bockaert *et al*., 2004).

Demonstration of direct interaction between CB_1 receptors and associated interacting proteins is technically challenging. High-affinity protein–protein interactions can be demonstrated by co-immunoprecipitation or pull-down approaches, but specificity must be confirmed. Moreover, co-immunoprecipitation does not verify direct interaction between proteins. Proteomic approaches (mass spectrometry, two-dimensional gel electrophoresis) are useful to identify multiple proteins in a precipitated complex, as are controls such as PTX pretreatment to rule out indirect association through $G_{i/o}$-proteins (Law *et al*., 2005). Imaging approaches that determine close proximity can suggest direct interaction, although there are cavaets to these approaches (Mackie, 2005). The quantification of low-affinity protein–protein interactions are especially challenging, particularly for membrane-bound proteins that require detergent for co-precipitation, which can disrupt protein–protein interactions. Low-affinity interactions can be assessed using chemical cross-linking, but additional supportive evidence is required due to the likelihood of detecting indirect interactions. Plasmon waveguide resonance spectroscopy of purified proteins is also useful for low-affinity interactions (Hruby *et al*., 2010).

Most functional characterization of CB_1-interacting proteins has been obtained from systems with heterologous or overexpression of one or both proteins. However, loss-of-function approaches in physiologically relevant systems will be important to determine the role of these protein–protein interactions. Conditional genetic knockout is the best established for approach for addressing *in vivo* function of a protein. RNA targeting with small interfering or antisense RNA to reduce protein expression, and transgenic or virally mediated expression of dominant negative constructs has also proven useful.

Understanding the physical and functional relationships between CB_1 receptors and interacting proteins could provide novel targets for drug discovery. However, identifying small molecules with 'drug-like' physiochemical properties to specifically disrupt protein–protein interactions is challenging. Nonetheless, these challenges are surmountable with modern drug discovery approaches. For example, molecular modelling of protein–protein interacting domains, combined with site-directed mutagensis, allows design of peptidomimetics to target these domains. Alternatively, high-throughput functional screening of large diverse chemical libraries can provide hit compounds to be optimized by traditional medicinal chemistry approaches. Perhaps the greatest challenge is identifying the relevant target proteins for specific purposes. Many GPCR-interacting proteins are multi-functional and interact with multiple GPCRs. For example, targeting GRKs, β-arrestins or GASP1 might inhibit tolerance to cannabinoids, but these proteins interact with multiple receptors and can mediate certain *in vivo* effects of additional GPCRs (Schmid and Bohn, 2009). Moreover, rapid development of tolerance to side effects can be desirable. The potential CB_1 selectivity of

some GPCR-interacting proteins, such as $CRIP_{1a/b}$, provides an opportunity for specific targeting of this system, but much remains to be learned about the function and selectivity of these novel proteins. Ultimately, the systems biological challenges in this field are likely to be the rate-limiting factor in drug discovery.

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Conflict of interest

None.

References

Adam-Klages S, Adam D, Wiegmann K, Struve S, Kolanus W, Schneider-Mergener J *et al*. (1996). FAN, a novel WD-repeat protein, couples the p55 TNF-receptor to neutral sphingomyelinase. *Cell* **86**: 937–947.

Ahn K, McKinney MK, Cravatt BF (2008). Enzymatic pathways that regulate endocannabinoid signaling in the nervous system. *Chem Rev* **108**: 1687–1707.

Alexander SP, Mathie A, Peters JA (2008). Guide to Receptors and Channels (GRAC), 3rd edition. *Br J Pharmacol* **153** (Suppl. 2): S1–S209.

Bakshi K, Mercier RW, Pavlopoulos S (2007). Interaction of a fragment of the cannabinoid CB1 receptor C-terminus with arrestin-2. *FEBS Lett* **581**: 5009–5016.

Baldwin JM, Schertler GF, Unger VM (1997). An alpha-carbon template for the transmembrane helices in the rhodopsin family of G-protein-coupled receptors. *J Mol Biol* **272**: 144–164.

Bari M, Battista N, Fezza F, Finazzi-Agro A, Maccarrone M (2005). Lipid rafts control signaling of type-1 cannabinoid receptors in neuronal cells. Implications for anandamide-induced apoptosis. *J Biol Chem* **280**: 12212–12220.

Bari M, Oddi S, De Simone C, Spagnolo P, Gasperi V, Battista N *et al*. (2008). Type-1 cannabinoid receptors colocalize with caveolin-1 in neuronal cells. *Neuropharmacology* **54**: 45–50.

Barnett-Norris J, Lynch D, Reggio PH (2005). Lipids, lipid rafts and caveolae: their importance for GPCR signaling and their centrality to the endocannabinoid system. *Life Sci* **77**: 1625–1639.

Bartlett SE, Enquist J, Hopf FW, Lee JH, Gladher F, Kharazia V *et al*. (2005). Dopamine responsiveness is regulated by targeted sorting of D2 receptors. *Proc Natl Acad Sci USA* **102**: 11521–11526.

Beardsley PM, Thomas BF, McMahon LR (2009). Cannabinoid CB1 receptor antagonists as potential pharmacotherapies for drug abuse disorders. *Int Rev Psychiatry* **21**: 134–142.

Bockaert J, Roussignol G, Becamel C, Gavarini S, Joubert L, Dumuis A *et al*. (2004). GPCR-interacting proteins (GIPs): nature and functions. *Biochem Soc Trans* **32**: 851–855.

Bouaboula M, Poinot-Chazel C, Bourrie B, Canat X, Calandra B, Rinaldi-Carmona M *et al*. (1995). Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem J* **312** (Pt 2): 637–641.

Bouaboula M, Perrachon S, Milligan L, Canat X, Rinaldi-Carmona M, Portier M *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. *J Biol Chem* **272**: 22330–22339.

Breivogel CS, Sim LJ, Childers SR (1997). Regional differences in cannabinoid receptor/G-protein coupling in rat brain. *J Pharmacol Exp Ther* **282**: 1632–1642.

Breivogel CS, Selley DE, Childers SR (1998). Cannabinoid receptor agonist efficacy for stimulating [35S]GTPgammaS binding to rat cerebellar membranes correlates with agonist-induced decreases in GDP affinity. *J Biol Chem* **273**: 16865–16873.

Breivogel CS, Lambert JM, Gerfin S, Huffman JW, Razdan RK (2008). Sensitivity to delta9-tetrahydrocannabinol is selectively enhanced in beta-arrestin2 -/- mice. *Behav Pharmacol* **19**: 298–307.

Burkey TH, Quock RM, Consroe P, Ehlert FJ, Hosohata Y, Roeske WR *et al*. (1997). Relative efficacies of cannabinoid CB1 receptor agonists in the mouse brain. *Eur J Pharmacol* **336**: 295–298.

Carriba P, Ortiz O, Patkar K, Justinova Z, Stroik J, Themann A *et al*. (2007). Striatal adenosine A2A and cannabinoid CB1 receptors form functional heteromeric complexes that mediate the motor effects of cannabinoids. *Neuropsychopharmacology* **32**: 2249–2259.

Ceresa BP, Limbird LE (1994). Mutation of an aspartate residue highly conserved among G-protein-coupled receptors results in nonreciprocal disruption of alpha 2-adrenergic receptor-G-protein interactions. A negative charge at amino acid residue 79 forecasts alpha 2A-adrenergic receptor sensitivity to allosteric modulation by monovalent cations and fully effective receptor/G-protein coupling. *J Biol Chem* **269**: 29557–29564.

Cippitelli A, Bilbao A, Hansson AC, del Arco I, Sommer W, Heilig M *et al*. (2005). Cannabinoid CB1 receptor antagonism reduces conditioned reinstatement of ethanol-seeking behavior in rats. *Eur J Neurosci* **21**: 2243–2251.

Coutts AA, Anavi-Goffer S, Ross RA, MacEwan DJ, Mackie K, Pertwee RG *et al*. (2001). Agonist-induced internalization and trafficking of cannabinoid CB1 receptors in hippocampal neurons. *J Neurosci* **21**: 2425–2433.

Cravatt BF, Lichtman AH (2003). Fatty acid amide hydrolase: an emerging therapeutic target in the endocannabinoid system. *Curr Opin Chem Biol* **7**: 469–475.

Dainese E, Oddi S, Bari M, Maccarrone M (2007). Modulation of the endocannabinoid system by lipid rafts. *Curr Med Chem* **14**: 2702–2715.

De Vries TJ, Schoffelmeer AN (2005). Cannabinoid CB1 receptors control conditioned drug seeking. *Trends Pharmacol Sci* **26**: 420–426.

De Vries TJ, Homberg JR, Binnekade R, Raaso H, Schoffelmeer AN (2003). Cannabinoid modulation of the reinforcing and motivational properties of heroin and heroin-associated cues in rats. *Psychopharmacology (Berl)* **168**: 164–169.

Derkinderen P, Ledent C, Parmentier M, Girault JA (2001). Cannabinoids activate p38 mitogen-activated protein kinases through CB1 receptors in hippocampus. *J Neurochem* **77**: 957–960.

Dewey WL (1986). Cannabinoid pharmacology. *Pharmacol Rev* **38**: 151–178.

Di Marzo V (2008). CB(1) receptor antagonism: biological basis for metabolic effects. *Drug Discov Today* **13**: 1026–1041.

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. *J Biol Chem* **281**: 38812–38824.

Fernandez JR, Allison DB (2004). Rimonabant Sanofi-Synthelabo. *Curr Opin Investig Drugs* **5**: 430–435.

Galve-Roperh I, Rueda D, Gomez del Pulgar T, Velasco G, Guzman M (2002). Mechanism of extracellular signal-regulated kinase activation by the CB(1) cannabinoid receptor. *Mol Pharmacol* **62**: 1385–1392.

Gaoni Y, Mechoulam R (1964). Isolation, structure, and partial synthesis of an active constituent of hashish. *J Am Chem Soc* **86**: 1646–1647.

Georgieva T, Devanathan S, Stropova D, Park CK, Salamon Z, Tollin G *et al*. (2008). Unique agonist-bound cannabinoid CB1 receptor conformations indicate agonist specificity in signaling. *Eur J Pharmacol* **581**: 19–29.

Gierschik P, Sidiropoulos D, Jakobs KH (1989). Two distinct Gi-proteins mediate formyl peptide receptor signal transduction in human leukemia (HL-60) cells. *J Biol Chem* **264**: 21470–21473.

Gilman AG (1987). G proteins: transducers of receptor-generated signals. *Annu Rev Biochem* **56**: 615–649.

Glass M, Felder CC (1997). Concurrent stimulation of cannabinoid CB1 and dopamine D2 receptors augments cAMP accumulation in striatal neurons: evidence for a Gs linkage to the CB1 receptor. *J Neurosci* **17**: 5327–5333.

Glass M, Northup JK (1999). Agonist selective regulation of G-proteins by cannabinoid CB1 and CB2 receptors. *Mol Pharmacol* **56**: 1362–1369.

Glass M, Dragunow M, Faull RLM (1997). Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* **77**: 299–318.

Gomes I, Jordan BA, Gupta A, Rios C, Trapaidze N, Devi LA (2001). G protein coupled receptor dimerization: implications in modulating receptor function. *J Mol Med* **79**: 226–242.

Herkenham M, Lynn AB, Johnson MR, Melvin LS, de Costa BR, Rice KC (1991). Characterization and localization of cannabinoid receptors in rat brain: a quantitative *in vitro* autoradiographic study. *J Neurosci* **11**: 563–583.

Hildebrandt JD (1997). Role of subunit diversity in signaling by heterotrimeric G proteins. *Biochem Pharmacol* **54**: 325–339.

Hojo M, Sudo Y, Ando Y, Minami K, Takada M, Matsubara T *et al*. (2008). mu-Opioid receptor forms a functional heterodimer with cannabinoid CB1 receptor: electrophysiological and FRET assay analysis. *J Pharmacol Sci* **108**: 308–319.

Hollister LE (1986). Health aspects of cannabis. *Pharmacol Rev* **38**: 1–20.

Horstman DA, Brandon S, Wilson AL, Guyer CA, Cragoe EJ, Jr, Limbird LE (1990). An aspartate conserved among G-protein receptors confers allosteric regulation of alpha 2-adrenergic receptors by sodium. *J Biol Chem* **265**: 21590–21595.

Howlett AC, Qualy JM, Khachatrian LL (1986). Involvement of Gi in the inhibition of adenylate cyclase by cannabimimetic drugs. *Mol Pharmacol* **29**: 307–313.

Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA *et al*. (2002). International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* **54**: 161–202.

Hruby VJ, Alves I, Cowell S, Salamon Z, Tollin G (2010). Use of plasmon waveguide resonance (PWR) spectroscopy for examining binding, signaling and lipid domain partitioning of membrane proteins. *Life Sci* **86**: 569–574.

Hsieh C, Brown S, Derleth C, Mackie K (1999). Internalization and recycling of the CB1 cannabinoid receptor. *J Neurochem* **73**: 493–501.

Hunter SA, Burstein S, Renzulli L (1986). Effects of cannabinoids on the activities of mouse brain lipases. *Neurochem Res* **11**: 1273–1288.

Janero DR, Makriyannis A (2009). Cannabinoid receptor antagonists: pharmacological opportunities, clinical experience, and translational prognosis. *Expert Opin Emerg Drugs* **14**: 43–65.

Jarrahian A, Watts VJ, Barker EL (2004). D2 dopamine receptors modulate Galpha-subunit coupling of the CB1 cannabinoid receptor. *J Pharmacol Exp Ther* **308**: 880–886.

Jin W, Brown S, Roche JP, Hsieh C, Celver JP, Kovoor A *et al*. (1999). Distinct domains of the CB1 cannabinoid receptor mediate desensitization and internalization. *J Neurosci* **19**: 3773–3780.

Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M (2009). Endocannabinoid-mediated control of synaptic transmission. *Physiol Rev* **89**: 309–380.

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? *Mol Pharmacol* **67**: 1697–1704.

Keren O, Sarne Y (2003). Multiple mechanisms of CB1 cannabinoid receptors regulation. *Brain Res* **980**: 197–205.

Koski G, Streaty RA, Klee WA (1982). Modulation of sodium-sensitive GTPase by partial opiate agonists. An explanation for the dual requirement for Na+ and GTP in inhibitory regulation of adenylate cyclase. *J Biol Chem* **257**: 14035–14040.

Kouznetsova M, Kelley B, Shen M, Thayer SA (2002). Desensitization of cannabinoid-mediated presynaptic inhibition of neurotransmission between rat hippocampal neurons in culture. *Mol Pharmacol* **61**: 477–485.

Landsman RS, Burkey TH, Consroe P, Roeske WR, Yamamura HI (1997). SR141716A is an inverse agonist at the human cannabinoid CB_1 receptor. *Eur J Pharmacol* **334**: R1–R2.

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. *Proc Natl Acad Sci USA* **105**: 2699–2704.

Law PY, Erickson-Herbrandson LJ, Zha QQ, Solberg J, Chu J, Sarre A *et al*. (2005). Heterodimerization of mu- and delta-opioid receptors occurs at the cell surface only and requires receptor-G protein interactions. *J Biol Chem* **280**: 11152–11164.

Le Foll B, Forget B, Aubin HJ, Goldberg SR (2008). Blocking cannabinoid CB1 receptors for the treatment of nicotine dependence: insights from pre-clinical and clinical studies. *Addict Biol* **13**: 239–252.

Ledent C, Valverde O, Cossu G, Petitet F, Aubert JF, Beslot F *et al*. (1999). Unresponsiveness to cannabinoids and reduced addictive effects of opiates in CB1 receptor knockout mice. *Science* **283**: 401–404.

Leterrier C, Bonnard D, Carrel D, Rossier J, Lenkei Z (2004). Constitutive endocytic cycle of the CB1 cannabinoid receptor. *J Biol Chem* **279**: 36013–36021.

Leterrier C, Laine J, Darmon M, Boudin H, Rossier J, Lenkei Z (2006). Constitutive activation drives compartment-selective endocytosis and axonal targeting of type 1 cannabinoid receptors. *J Neurosci* **26**: 3141–3153.

Ludanyi A, Eross L, Czirjak S, Vajda J, Halasz P, Watanabe M *et al*. (2008). Downregulation of the CB1 cannabinoid receptor and related molecular elements of the endocannabinoid system in epileptic human hippocampus. *J Neurosci* **28**: 2976–2990.

Lupica CR, Riegel AC, Hoffman AF (2004). Marijuana and cannabinoid regulation of brain reward circuits. *Br J Pharmacol* **143**: 227–234.

McDonald NA, Henstridge CM, Connolly CN, Irving AJ (2007). An essential role for constitutive endocytosis, but not activity, in the axonal targeting of the CB1 cannabinoid receptor. *Mol Pharmacol* **71**: 976–984.

McIntosh HH, Song C, Howlett AC (1998). CB1 canabinoid receptor: cellular regulation and distribution in N18TG2 neuroblastoma cells. *Brain Res Mol Brain Res* **53**: 163–173.

Mackie K (2005). Cannabinoid receptor homo- and heterodimerization. *Life Sciences* **77**: 1667–1673.

Mackie K, Stella N (2006). Cannabinoid receptors and endocannabinoids: evidence for new players. *AAPS J* **8**: E298–E306.

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. *J Neurosci* **15**: 6552–6561.

Maldonado R, Valverde O, Berrendero F (2006). Involvement of the endocannabinoid system in drug addiction. *Trends Neurosci* **29**: 225–232.

Marcellino D, Carriba P, Filip M, Borgkvist A, Frankowska M, Bellido I *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 BR, Sim-Selley LJ, Selley DE (2004). Signaling pathways involved in the development of cannabinoid tolerance. *J Pharmacol Sci* **25**: 325–330.

Martini L, Waldhoer M, Pusch M, Kharazia V, Fong J, Lee JH *et al*. (2007). Ligand-induced down-regulation of the cannabinoid 1 receptor is mediated by the G-protein-coupled receptor-associated sorting protein GASP1. *FASEB J* **21**: 802–811.

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.

Meschler JP, Howlett AC (2001). Signal transduction interactions between CB1 cannabinoid and dopamine receptors in the rat and monkey striatum. *Neuropharmacology* **40**: 918–926.

Meschler JP, Conley TJ, Howlett AC (2000a). Cannabinoid and dopamine interaction in rodent brain: effects on locomotor activity. *Pharmacol Biochem Behav Behavior* **67**: 567–573.

Meschler JP, Kraichely DM, Wilken GH, Howlett AC (2000b). Inverse agonist properties of N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2, 4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide HCl (SR141716A) and 1-(2-chlorophenyl)-4-cyano-5-(4-methoxyphenyl)-1H-pyrazole-3-carboxyl ic acid phenylamide (CP-272871) for the CB(1) cannabinoid receptor [In Process Citation]. *Biochem Pharmacol* **60**: 1315–1323.

Milligan G (2010). The role of dimerisation in the cellular trafficking of G-protein-coupled receptors. *Curr Opin Pharmacol* **10**: 23–29.

Morisset S, Rouleau A, Ligneau X, Gbahou F, Tardivel-Lacombe J, Stark H *et al*. (2000). High constitutive activity of native H3 receptors regulates histamine neurons in brain. *Nature* **408**: 860–864.

Mukhopadhyay S, Howlett AC (2001). CB1 receptor-G protein association. Subtype selectivity is determined by distinct intracellular domains. *Eur J Biochem* **268**: 499–505.

Mukhopadhyay S, Howlett AC (2005). Chemically distinct ligands promote differential CB1 cannabinoid receptor-Gi protein interactions. *Mol Pharmacol* **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. *Mol Pharmacol* **57**: 162–170.

Navarro G, Carriba P, Gandia J, Ciruela F, Casado V, Cortes A *et al*. (2008). Detection of heteromers formed by cannabinoid CB1, dopamine D2, and adenosine A2A G-protein-coupled receptors by combining bimolecular fluorescence complementation and bioluminescence energy transfer. *ScientificWorldJournal* **8**: 1088–1097.

Navarro M, Carrera MR, Fratta W, Valverde O, Cossu G, Fattore L *et al*. (2001). Functional interaction between opioid and cannabinoid receptors in drug self-administration. *J Neurosci* **21**: 5344–5350.

Nicholson RA, Liao C, Zheng J, David LS, Coyne L, Errington AC *et al*. (2003). Sodium channel inhibition by anandamide and synthetic cannabimimetics in brain. *Brain Res* **978**: 194–204.

Nie J, Lewis DL (2001a). The proximal and distal C-terminal tail domains of the CB1 cannabinoid receptor mediate G protein coupling. *Neuroscience* **107**: 161–167.

Nie J, Lewis DL (2001b). Structural domains of the CB1 cannabinoid receptor that contribute to constitutive activity and G-protein sequestration. *J Neurosci* **21**: 8758–8764.

Niehaus JL, Liu Y, Wallis KT, Egertova M, Bhartur SG, Mukhopadhyay S *et al*. (2007). CB1 cannabinoid receptor activity is modulated by the cannabinoid receptor interacting protein CRIP 1a. *Mol Pharmacol* **72**: 1557–1566.

Offermanns S (2003). G-proteins as transducers in transmembrane signalling. *Prog Biophys Mol Biol* **83**: 101–130.

Pan X, Ikeda SR, Lewis DL (1996). Rat brain cannabinoid receptor modulates N-type Ca2+ channels in a neuronal expression system. *Mol Pharmacol* **49**: 707–714.

Patel HH, Murray F, Insel PA (2008). G-protein-coupled receptor-signaling components in membrane raft and caveolae microdomains. *Handb Exp Pharmacol* 167–184.

Paugh SW, Cassidy MP, He H, Milstien S, Sim-Selley LJ, Spiegel S *et al*. (2006). Sphingosine and its analog, the immunosuppressant 2-amino-2-(2-[4-octylphenyl]ethyl)-1,3-propanediol, interact with the CB1 cannabinoid receptor. *Mol Pharmacol* **70**: 41–50.

Pertwee RG (2005). The therapeutic potential of drugs that target cannabinoid receptors or modulate the tissue levels or actions of endocannabinoids. *AAPS J* **7**: E625–E654.

Pertwee RG (2009). Emerging strategies for exploiting cannabinoid receptor agonists as medicines. *Br J Pharmacol* **156**: 397–411.

Pickel VM, Chan J, Kearn CS, Mackie K (2006). Targeting dopamine D2 and cannabinoid-1 (CB1) receptors in rat nucleus accumbens. *J Comp Neurol* **495**: 299–313.

Pierce KL, Luttrell LM, Lefkowitz RJ (2001). New mechanisms in heptahelical receptor signaling to mitogen activated protein kinase cascades. *Oncogene* **20**: 1532–1539.

Piomelli D (2003). The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci* **4**: 873–884.

Pitcher JA, Freedman NJ, Lefkowitz RJ (1998). G protein-coupled receptor kinases. *Annu Rev Biochem* **67**: 653–692.

Reiter E, Lefkowitz RJ (2006). GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling. *Trends Endocrinol Metab* **17**: 159–165.

Rhee MH, Bayewitch M, Avidor-Reiss T, Levy R, Vogel Z (1998). Cannabinoid receptor activation differentially regulates the various adenylyl cyclase isozymes. *J Neurochem* **71**: 1525–1534.

Rhee MH, Nevo I, Avidor-Reiss T, Levy R, Vogel Z (2000). Differential superactivation of adenylyl cyclase isozymes after chronic activation of the CB(1) cannabinoid receptor. *Mol Pharmacol* **57**: 746–752.

Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C *et al*. (1994). SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Letters* **350**: 240–244.

Rinaldi-Carmona M, Le Duigou A, Oustric D, Barth F, Bouaboula M, Carayon P *et al*. (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. *J Pharmacol Exp Ther* **287**: 1038–1047.

Rios C, Gomes I, Devi LA (2006). Mu opioid and cannabinoid receptor interactions: reciprocal inhibition of receptor signaling and neuritogenesis. *Br J Pharmacol* **148**: 387–395.

Ritter SL, Hall RA (2009). Fine-tuning of GPCR activity by receptor-interacting proteins. *Nat Rev Mol Cell Biol* **10**: 819–830.

Robledo P, Berrendero F, Ozaita A, Maldonado R (2008). Advances in the field of cannabinoid – opioid cross-talk. *Addict Biol* **13**: 213–224.

Roche JP, Bounds S, Brown S, Mackie K (1999). A mutation in the second transmembrane region of the CB1 receptor selectively disrupts G protein signaling and prevents receptor internalization. *Mol Pharmacol* **56**: 611–618.

Rubino T, Vigano D, Premoli F, Castiglioni C, Bianchessi S, Zippel R *et al*. (2006). Changes in the expression of G protein-coupled receptor kinases and beta-arrestins in mouse brain during cannabinoid tolerance: a role for RAS-ERK cascade. *Mol Neurobiol* **33**: 199–213.

Rueda D, Navarro B, Martinez-Serrano A, Guzman M, Galve-Roperh I (2002). The endocannabinoid anandamide inhibits neuronal progenitor cell differentiation through attenuation of the Rap1/B-Raf/ERK pathway. *J Biol Chem* **277**: 46645–46650.

Ryberg E, Larsson N, Sjogren S, Hjorth S, Hermansson NO, Leonova J *et al*. (2007). The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* **152**: 1092–1101.

Sanchez C, Rueda D, Segui B, Galve-Roperh I, Levade T, Guzman M (2001). The CB(1) cannabinoid receptor of astrocytes is coupled to sphingomyelin hydrolysis through the adaptor protein fan. *Mol Pharmacol* **59**: 955–959.

Sawzdargo M, Nguyen T, Lee DK, Lynch KR, Cheng R, Heng HHQ *et al*. (1999). Identification and cloning of three novel human G

protein-coupled receptor genes GPR52, GPR53 and GPR55: GPR55 is extensively expressed in human brain. *Mol Brain Res* **64**: 193–198.

Schmid CL, Bohn LM (2009). Physiological and pharmacological implications of beta-arrestin regulation. *Pharmacol Ther* **121**: 285–293.

Seifert R, Wenzel-Seifert K (2002). Constitutive activity of G-protein-coupled receptors: cause of disease and common property of wild-type receptors. *Naunyn Schmiedebergs Arch Pharmacol* **366**: 381–416.

Shapira M, Gafni M, Sarne Y (2003). Long-term interactions between opioid and cannabinoid agonists at the cellular level: cross desensitization and downregulation. *Brain Res* **960**: 190–200.

Sim LJ, Hampson RE, Deadwyler SA, Childers SR (1996a). Effects of chronic treatment with delta9-tetrahydrocannabinol on cannabinoid-stimulated [35S]GTPgammaS autoradiography in rat brain. *J Neurosci* **16**: 8057–8066.

Sim LJ, Selley DE, Xiao R, Childers SR (1996b). Differences in G-protein activation by mu- and delta-opioid, and cannabinoid, receptors in rat striatum. *Eur J Pharmacol* **307**: 97–105.

Sim-Selley LJ (2003). Regulation of cannabinoid CB1 receptors in the central nervous system by chronic cannabinoids. *Crit Rev Neurobiol* **15**: 91–119.

Sim-Selley LJ, Schechter NS, Rorrer WK, Dalton GD, Hernandez J, Martin BR *et al*. (2006). Prolonged recovery rate of CB1 receptor adaptation after cessation of long-term cannabinoid administration. *Mol Pharmacol* **70**: 986–996.

Simonin F, Karcher P, Boeuf JJ, Matifas A, Kieffer BL (2004). Identification of a novel family of G protein-coupled receptor associated sorting proteins. *J Neurochem* **89**: 766–775.

Tao Q, Abood ME (1998). Mutation of a highly conserved aspartate residue in the second transmembrane domain of the cannabinoid receptors, CB1 and CB2, disrupts G-protein coupling. *J Pharmacol Exp Ther* **285**: 651–658.

Tappe-Theodor A, Agarwal N, Katona I, Rubino T, Martini L, Swiercz J *et al*. (2007). A molecular basis of analgesic tolerance to cannabinoids. *J Neurosci* **27**: 4165–4177.

Twitchell W, Brown S, Mackie K (1997). Cannabinoids inhibit N- and P/Q-type calcium channels in cultured rat hippocampal neurons. *J Neurophysiol* **78**: 43–50.

Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K *et al*. (2005). Identification and functional characterization of brainstem cannabinoid CB2 receptors. *Science* **310**: 329–332.

Vasquez C, Navarro-Polanco RA, Huerta M, Trujillo X, Andrade F, Trujillo-Hernandez B *et al*. (2003). Effects of cannabinoids on endogenous K+ and Ca2+ currents in HEK293 cells. *Can J Physiol Pharmacol* **81**: 436–442.

Velasco G, Galve-Roperh I, Sanchez C, Blazquez C, Haro A, Guzman M (2005). Cannabinoids and ceramide: two lipids acting hand-by-hand. *Life Sci* **77**: 1723–1731.

Vigano D, Rubino T, Parolaro D (2005). Molecular and cellular basis of cannabinoid and opioid interactions. *Pharmacol Biochem Behav* **81**: 360–368.

Villares J (2007). Chronic use of marijuana decreases cannabinoid receptor binding and mRNA expression in the human brain. *Neuroscience* **145**: 323–334.

Viveros MP, de Fonseca FR, Bermudez-Silva FJ, McPartland JM (2008). Critical role of the endocannabinoid system in the regulation of food intake and energy metabolism, with phylogenetic, developmental, and pathophysiological implications. *Endocr Metab Immune Disord Drug Targets* **8**: 220–230.

Vrecl M, Norregaard PK, Almholt DL, Elster L, Pogacnik A, Heding A (2009). Beta-arrestin-based Bret2 screening assay for the 'non'-beta-arrestin binding CB1 receptor. *J Biomol Screen* **14**: 371–380.

Wager-Miller J, Westenbrook R, Mackie K (2002). Dimerization of G protein-coupled receptors: CB1 cannabinoid receptors as an example. *Chem Phys Lipids* **121**: 83–89.

Welch SP (2009). Interaction of the cannabinoid and opioid systems in the modulation of nociception. *Int Rev Psychiatry* **21**: 143–151.

Whistler JL, Enquist J, Marley A, Fong J, Gladher F, Tsuruda P *et al*. (2002). Modulation of postendocytic sorting of G protein-coupled receptors. *Science* **297**: 615–620.

Wu DF, Yang LQ, Goschke A, Stumm R, Brandenburg LO, Liang YJ *et al*. (2008). Role of receptor internalization in the agonist-induced desensitization of cannabinoid type 1 receptors. *J Neurochem* **104**: 1132–1143.

Zimmer A, Zimmer AM, Hohmann AG, Herkenham M, Bonner TI (1999). Increased mortality, hypoactivity, and hypoalgesia in cannabinoid CB1 receptor knockout mice. *Proc Natl Acad Sci USA* **96**: 5780–5785.