

REVIEW

Novel cannabinoid receptors

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Cannabinoids have numerous physiological effects. In the years since the molecular identification of the G protein-coupled receptors CB₁ and CB₂, the ion channel TRPV₁, and their corresponding endogenous ligand systems, many cannabinoid-evoked actions have been shown conclusively to be mediated by one of these specific receptor targets. However, there remain several examples where these classical cannabinoid receptors do not explain observed pharmacology. Studies using mice genetically deleted for the known receptors have confirmed the existence of additional targets, which have come to be known collectively as non-CB₁/CB₂ receptors. Despite intense research efforts, the molecular identity of these non-CB₁/CB₂ receptors remains for the most part unclear. Two orphan G protein-coupled receptors have recently been implicated as novel cannabinoid receptors; these are GPR119, which has been proposed as a receptor for oleoylethanolamide, and GPR55 which has been proposed as a receptor activated by multiple different cannabinoid ligands. In this review I will present an introduction to non-CB₁/CB₂ pharmacology, summarize information on GPR55 and GPR119 currently available, and consider their phylogenetic origin and what aspects of non-CB₁/CB₂ pharmacology, if any, they help explain.

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Abbreviations: abn-cbd, abnormal cannabidiol; AEA, arachidonoyl ethanolamide; 2-AG, 2-arachidonoylglycerol; CBD, cannabidiol; CBN, cannabinol; EPSC, excitatory postsynaptic current; GPCR, G protein-coupled receptor; LPA, lysophosphatidic acid; LPC, lysophosphatidylcholine; NAAA, N-acyl ethanolamine-hydrolysing acid amidase; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; PPAR, peroxisome proliferator-activated receptor; PTX, pertussis toxin; S1P, sphingosine-1-phosphate; THC, Δ^9 -tetrahydrocannabinol; TRPV₁, transient receptor potential vanilloid type 1 receptor

Introduction

Cannabinoids, which include the bioactive constituents of the marijuana plant *Cannabis sativa*, as well as endogenous lipids (endocannabinoids) and synthetic compounds with cannabinoid-like activity, interact with specific receptors to cause their effects on target tissues (for review see Pacher *et al.*, 2006). To date, three receptors have been identified by molecular cloning; these are the transient receptor potential vanilloid type 1 receptor (TRPV₁) ion channel, and the G protein-coupled receptors (GPCRs) cannabinoid type 1 receptor (CB₁) and cannabinoid type 2 receptor (CB₂). At the phylogenetic level, CB₁ and CB₂ are most related to the family of lipid receptors, formerly EDG receptors, which are activated by the sphingolipids sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA; a phylogenetic tree of human family A GPCRs is shown in Figure 1). CB₁ and CB₂ are also lipid receptors, and recognize acylethanolamide

analogues, typified by anandamide (arachidonoyl ethanolamide, AEA), and 2-arachidonoylglycerol (2-AG). TRPV₁ is activated by various lipids including acylethanolamides such as AEA (Starowicz *et al.*, 2007).

Cannabinoid type 1 receptor is highly expressed in brain and mediates many of the neurobehavioural and psychotropic effects of Δ^9 -tetrahydrocannabinol (THC), the primary bioactive cannabinoid of *C. sativa*. CB₁ is also present at lower levels in testis, heart, vascular tissue, and in immune cells. The fundamental importance of the CB₁-endocannabinoid axis is reflected in the ongoing development of high-affinity CB₁ antagonists and inverse agonists as therapeutic drugs for diabetes, metabolic syndrome and drug dependence. The first of these, SR141716A (rimonabant, Accomplia), is now marketed for treatment of obesity. CB₂ plays a role in inflammatory reactions and the immune response and is expressed predominantly by immune and haematopoietic cells and is also present in the CNS during neuroinflammatory states (Elmes *et al.*, 2004; Klein, 2005). CB₂^{-/-} mice lack the helper T-cell-activating response to THC observed in wild type and are deficient in particular subsets of B and T cells (Buckley *et al.*, 2000; Ziring *et al.*, 2006). Activation of both CB₁ and CB₂ contribute to the

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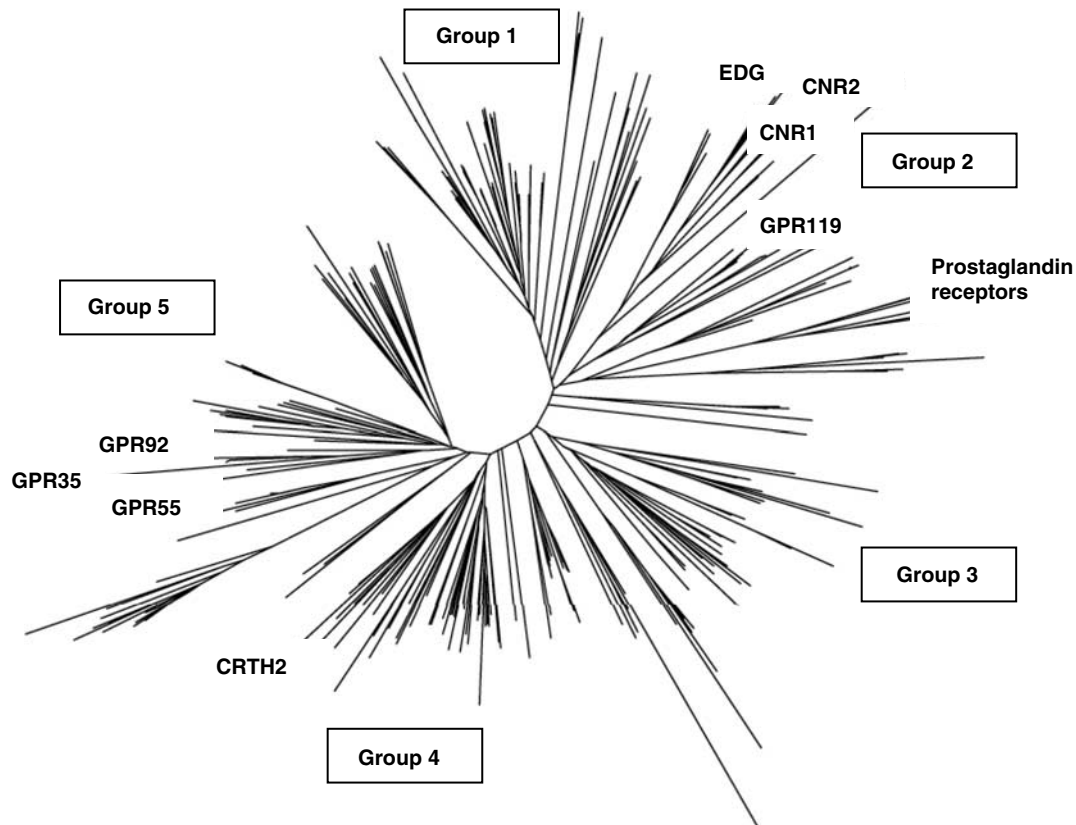


Figure 1 Phylogenetic analysis of all non-sensory human family A G protein-coupled receptors (GPCRs; adapted from Foord, 2007). The neighbour-joining method was used, after forced alignment according to common amino-acid motifs. Each line represents a GPCR; known and putative cannabinoid receptors and other receptors described in this review are identified. Clusters have been assigned to groups (Foord, 2007): group 1, the monoamine-like receptors; group 2, a diverse group containing opsins and glycoprotein/leucine-rich repeat (LRG) type as well as cannabinoid, prostaglandin and lipid receptors; group 3, brain/gut peptide receptors; group 4, chemokine receptors; and group 5, metabolic receptors including purinergic, thrombin and free-fatty acid receptors. Cross-species comparisons show that groups 4 and 5 are not found in nematodes and insects. Abbreviations: CNR1 and CNR2 are the genes encoding cannabinoid type 1 receptor (CB₁) and cannabinoid type 2 receptor (CB₂), respectively. EDG indicates the sphingolipid receptor cluster, which comprises LPA₁/EDG2, LPA₂/EDG4 and LPA₃/EDG7 and the sphingosine-1-phosphate (S1P) receptors (S1P₁ to S1P₅). LPA, lysophosphatidic acid.

antinociceptive and immunomodulatory effects of cannabinoid ligands, and both CB₂-selective and non-selective agonists have entered clinical development for pain. However, studies performed with CB₁^{-/-} and CB₂^{-/-} mice have indicated that certain effects of cannabinoids on tissues are mediated by neither CB₁ nor CB₂ (for review see Begg *et al.*, 2005; Mackie and Stella, 2007). Some effects appeared still to involve GPCRs rather than other potential targets such as ion channels or intracellular lipid receptors such as the PPAR (peroxisome proliferator-activated receptor) family, as deduced from the stimulation of GTP binding or sensitivity to pertussis toxin (PTX). Indeed, only a subset of cannabinoids has affinity for CB₁ or CB₂. Two naturally occurring acylethanolamides: oleoylethanolamide (OEA) and palmitoylethanolamide (PEA), and two phytocannabinoids: cannabiol (CBN) and cannabidiol (CBD), all lack affinity at CB₁/CB₂ but evoke pharmacological effects. Recently, two orphan GPCRs have emerged as candidate non-CB₁/CB₂ receptors. These are GPR119, which is reportedly a receptor for OEA (Overton *et al.*, 2006), and GPR55, which is reportedly activated by various cannabinoids. Initially, little information on GPR55 was available in the public domain, and its identification as a cannabinoid receptor came solely

from patent and meeting abstracts (that is, non-peer-reviewed sources; Baker *et al.*, 2006). In this issue, Johns *et al.* (2007) have described cardiovascular phenotyping of GPR55^{-/-} mice as well as preliminary studies of the pharmacology of recombinantly expressed GPR55. Also, Staton *et al.* (2006) have explored the phenotype of the same GPR55^{-/-} line in models of inflammatory and neuropathic pain. This review will evaluate whether, based on current data, GPR119 and GPR55 can be considered as novel cannabinoid receptors and, if so, whether they might explain any observed non-CB₁/CB₂ effects.

Non-CB₁/CB₂ sites in the vasculature

The most extensively studied non-CB₁/CB₂ site occurs in resistance arteries of the mesenteric vasculature, where AEA and analogues, but not synthetic cannabinoid agonists, cause vasodilatation (Begg *et al.*, 2005). This effect is abolished by endothelial denudation and is sensitive to blockade by SR141716A, but not by the chemically similar CB₁ antagonist AM251, and only at concentrations higher than required to inhibit CB₁ (Begg *et al.*, 2005). Abnormal

cannabidiol (abn-cbd), which is a synthetic structural analogue of cannabidiol, is a selective agonist at this endothelial site, inducing vasodilatation, but having negligible activity at CB₁ or CB₂ (Jarai *et al.*, 1999). Cannabidiol itself and the analogue O-1918 are selective antagonists, blocking vasodilatation induced by AEA or abn-cbd but having negligible affinity or activity at CB₁ or CB₂ (Offertaler *et al.*, 2003). Several but not all studies report the mesenteric vasodilator effect of abn-cbd to be PTX-sensitive (Begg *et al.*, 2005; Hiley and Kaup, 2007). A similar ligand profile is detected in human umbilical vein endothelial cells, where abn-cbd activates p42/44 MAP kinase and potentiates voltage-gated K⁺ currents via BK_{Ca} channels, and O-1918 or PTX block these effects (Begg *et al.*, 2003). The endothelial abn-cbd-sensitive site may be related to another non-CB₁/CB₂ site in heart, which mediates the hypotensive response to systemic bacterial endotoxin that is thought to result from release of AEA by activated macrophages. Endotoxin-evoked hypotension is sensitive to blockade by SR141716A but not AM251, and is comparable in wild-type, CB₁^{-/-} or CB₁^{-/-}/CB₂^{-/-} mice (Begg *et al.*, 2005). There may also be a non-CB₁/CB₂ site on smooth muscle cells, as THC causes PTX-sensitive vasodilatation that is endothelium-independent (O'Sullivan *et al.*, 2005).

Non-CB₁/CB₂ sites in the CNS

Breivogel *et al.* (2001) showed that AEA and the aminoalkyl-indole WIN55212-2, but not HU210 or THC, can stimulate [³⁵S]-GTPγS binding in brain slices and in membrane preparations from CB₁^{-/-} mice. In separate studies, WIN55212-2 and also CP55940 inhibited glutaminergic excitatory postsynaptic currents (EPSCs) on hippocampal CA1 pyramidal cells from CB₁^{-/-} mice, allowing electrophysiological characterization of this site (Hajos *et al.*, 2001). These two putative non-CB₁/CB₂ sites in the CNS share with the vascular endothelial site sensitivity to blockade by PTX, or by high concentrations of SR141716A and not by AM251. However, they appear pharmacologically distinct from the endothelial site since the synthetic cannabinoids WIN55212-2 and/or CP55940 were efficacious at the CNS site(s) whereas neither stimulated the endothelial site. Conversely abn-cbd had no effect on glutaminergic EPSCs but activated the endothelial site. Hints about the role of hippocampal non-CB₁/CB₂ receptors have come from the demonstration that SR141716A but not AM251 has anxiolytic effects in CB₁^{-/-} mice, hence the non-CB₁/CB₂ site might mediate anxiogenesis, opposing the anxiolytic effects of CB₁ (Haller *et al.*, 2004).

Non-CB₁/CB₂ sites on immune cells

As in the CNS and cardiovascular systems, many of the effects of cannabinoids on the immune system are induced by binding to CB₁ and/or CB₂ receptors, particularly CB₂ which is abundantly expressed by macrophages, dendritic cells, and B cells (Massi *et al.*, 2006). However studies using CB₁^{-/-} and CB₂^{-/-} mice again support the existence of additional receptor targets. Resting T-cells exhibit robust

elevations of intracellular calcium ([Ca²⁺]_i) in response to THC, CBN and HU-210 but not CP55940, an effect which is similar in splenocytes isolated from either wild-type or CB₁^{-/-}/CB₂^{-/-} mice (Rao and Kaminski, 2006). Both SR141716A and SR144528 blocked these effects, though only at concentrations (1–5 μM) higher than their reported affinities for CB₁ and CB₂. This observation serves to highlight the challenges inherent in using these agents, which are well established to be selective (for CB₁ over CB₂, or vice versa), but whose specificity over other, novel cannabinoid receptors, is less well defined. Non-CB₁/CB₂ effects have also been characterized on activated T cells, where 2-AG suppresses production of interleukin-2, though this seems to occur via intracellular PPARγ (Rockwell *et al.*, 2006). On neutrophils, high-micromolar concentrations of CP55940 but not AEA nor the non-hydrolysable analogue methanandamide reduced superoxide production from chemoattractant-stimulated cells (Kraft *et al.*, 2004).

Orphan endocannabinoids: PEA and OEA

Palmitoylethanolamide reduces the pain behaviour resulting from immune challenge, an effect blocked by SR144528 (Calignano *et al.*, 1998; Jaggar *et al.*, 1998). Since PEA lacks affinity for CB₂, its effects have been postulated to involve indirect activation of CB₂, either through inhibition of AEA hydrolysis, which is catalysed by fatty-acid amide hydrolase (FAAH), or by coupling of a putative PEA receptor to PLC and diacylglycerol lipase, resulting in increased 2-AG production. However, PEA has anti-inflammatory effects not blocked by SR144528 (Lo Verme *et al.*, 2005b). In tissues, production of PEA can occur independently of production of AEA and 2-AG, and a PEA-specific hydrolytic enzyme has been isolated, named *N*-acylethanolamine-hydrolysing acid amidase (NAAA), distinct from FAAH. NAAA is ~30-fold selective for PEA over AEA, differs in its optimal pH for hydrolytic activity, and has unique tissue distribution and subcellular location. Hence, PEA and its receptor target have the characteristics of a parallel endocannabinoid-signalling pathway, distinct from CB₁, CB₂, AEA and 2-AG (Mackie and Stella, 2007). OEA also has characteristics of a paracrine- or endocrine-signalling mediator. OEA has been linked to satiety and mechanisms controlling food intake. In the mucosal layer of the small intestine, levels of OEA as well as OEA-synthesizing and OEA-degrading activities change in proportion to food intake (Nielsen *et al.*, 2004). OEA is detected in other tissues but at levels unaffected by feeding (Fu *et al.*, 2007). Dosing OEA to rodents prolongs the time between feeding activity and decreases overall food intake (Rodriguez de Fonseca *et al.*, 2001). Mechanistically, the effects of both OEA and PEA are believed to involve PPARα. OEA binds with high affinity to purified ligand-binding domain of PPARα, and both ligands activate PPARα in cell-based assays with EC₅₀ values 120 nM and 3.1 μM for OEA and PEA, respectively (Fu *et al.*, 2003; Lo Verme *et al.*, 2005a, b). PPARα is a familiar regulator of energy balance and lipid metabolism and is expressed in the small intestine (Bocher *et al.*, 2002). Synthetic PPARα agonists have similar behavioural effects on feeding as OEA, and dosing either

synthetic PPAR α agonists or OEA to PPAR α ^{-/-} mice fails to induce hypophagic effects (Fu *et al.*, 2003). Likewise, activation of PPAR α reduces inflammation (Chinetti *et al.*, 2000), and PEA lacks its anti-inflammatory activity in PPAR α ^{-/-} mice (Lo Verme *et al.*, 2005a). Hence, the anti-nociceptive effects of PEA may be due to synergistic activity on both cannabinoid and PPAR α systems (Russo *et al.*, 2007).

GPR55

GPR55 was identified as an orphan GPCR in the purinergic subfamily, most closely related to two other orphans, GPR35 and GPR23, and the purinoceptor P2Y₅ (Figure 1; Sawzdargo *et al.*, 1997). The first association between GPR55 and cannabinoids appeared in a patent from GlaxoSmithKline describing expression of human GPR55 in yeast host strains that coexpressed yeast/human chimeric G proteins (Brown and Wise, 2001). These cells are engineered to grow only under conditions of receptor activation, allowing sensitive and specific detection of either receptor constitutive activity, or receptor activation by agonist ligands (Brown *et al.*, 2000). Owing to the lack of endogenous GPCRs in the yeast cells, this approach is a useful method to identify endogenous or other ligands for orphan GPCRs (Dowell and Brown, 2002). GPR55-expressing yeast were activated by AM251 and SR141716A (see Table 1; Brown and Wise, 2001). A subsequent patent from AstraZeneca (London, UK) corroborated the link between GPR55 and cannabinoids, showing that membranes from HEK293 cells transiently transfected with GPR55 bound [³H]CP55940 and [³H]SR141716A, but not [³H]WIN55212-2 (Drmotá *et al.*, 2004). [³⁵S]GTP γ S binding in response to a panel of chemically diverse cannabinoids was also determined in these membranes. Several cannabinoids including AEA, PEA, 2-AG, THC, virodhamine and CP55940 behaved as potent agonists. SR141716A, AM251, SR144528 and OEA also activated GPR55 (see Table 1). Virodhamine, an endogenously occurring isomer of anandamide in which arachidonic acid is linked to ethanolamide via an ester moiety, appeared to have the greatest intrinsic activity (Drmotá *et al.*, 2004). Johns *et al.* (2007) describe similar findings for several of these ligands. [³⁵S]GTP γ S binding was insensitive to PTX or cholera toxins, implicating a G protein distinct from G_i or G_s (Drmotá *et al.*, 2004). Consistent with this, GPR55-constitutive activity in yeast was only detected in the presence of a chimeric G protein α -subunit incorporating the C terminus of G α_{13} (Brown and Wise, 2001). Further abstracts presented by the AstraZeneca group using G protein-specific peptides or antibodies to inhibit [³⁵S]GTP γ S binding also implicate G₁₃ as the coupling partner of GPR55 (Baker *et al.*, 2006). The association with G₁₃ tends to indicate that GPR55 is not responsible for any of the known PTX-sensitive non-CB₁/CB₂ effects. However, all other GPCRs known to activate G₁₃ also activate other G proteins (Riobo and Manning, 2005), so further G protein-signalling pathways (potentially, PTX-sensitive) for GPR55 may remain to be discovered.

The function of GPR55 remains an open question. Similarities between GPR55 and the endothelial vasodilator site (AEA, abn-cbd and O-1602 as agonists; cannabidiol as

antagonist; Drmotá *et al.*, 2004) led Johns *et al.* (2007) to evaluate the cardiovascular phenotype of GPR55^{-/-} mice. Blood pressure and heart rate were not significantly different between GPR55^{-/-} and wild-type littermates, and both genotypes showed similar decreases in blood pressure following systemic dosing with O-1602. Isolated mesenteric resistance arteries from GPR55^{-/-} mice had normal contractile responses to carbachol, and pre-contracted arteries from GPR55^{-/-} and wild-type mice were relaxed by O-1602 with identical potencies. This did not disprove the hypothesis that GPR55 may be present and functional in the vasculature, but did indicate that GPR55 was unlikely to be the vasodilator site-of-action of abn-cbd (Johns *et al.*, 2007). This conclusion was consistent with the efficacy of CP55940 at recombinant GPR55 but not at the endothelial site (Begg *et al.*, 2005). Also, the endothelial site appears to signal through G_i whereas G₁₃-linked receptors are more commonly associated with vasoconstrictor effects (Lee *et al.*, 2004; Begg *et al.*, 2005). Currently, there are no published data measuring GPR55 expression in vascular tissue, though abstracts have suggested the presence of GPR55 in vascular smooth muscle (Baker *et al.*, 2006).

Human GPR55 mRNA is expressed in brain, most abundantly in the caudate nucleus and putamen, with lesser levels in the hippocampus, thalamus, pons, cerebellum and frontal cortex (Sawzdargo *et al.* (1997) and data not shown). In rat brain, GPR55 mRNA was detected by *in situ* hybridization in hippocampus, thalamic nuclei and regions of the mid-brain. GPR55 mRNA is also present in spleen in both human and rodent (Sawzdargo *et al.*, 1997). Robust data on the location of GPR55 protein await the generation of antibodies reactive with mouse GPR55 and their validation on tissues from GPR55^{-/-} and wild-type littermates. However, the overall pattern of expression in CNS and immune cells leads to the proposition of GPR55 as a putative non-CB₁/CB₂ site in these tissues. WIN55212-2 activates the non-CB₁/CB₂ site in CNS (Breivogel *et al.*, 2001), whereas WIN55212-2 reportedly lacks activity at GPR55 (Drmotá *et al.*, 2004), suggesting GPR55 does not mediate the non-CB₁/CB₂ effects of this ligand. In an abstract, Staton *et al.* (2006) described a study of GPR55^{-/-} mice in models of inflammatory and neuropathic pain. The hypersensitivity to mechanical nociception observed in wild-type mice following either injection of Freund's complete adjuvant (FCA) into the hind paw, or surgery to partially constrict the sciatic nerve, was completely absent in GPR55^{-/-} animals at all time points observed (1–28 days). Increased plasma cytokine levels resulting from the FCA challenge were not significantly different between GPR55^{-/-} and wild-type mice on day 1, though at day 14, a subset of cytokines including interferon- γ remained significantly elevated in GPR55^{-/-} relative to wild-type mice. These results suggest a role for GPR55 in pain signalling, though whether the site-of-action is neuronal, immune cell, or other, remains to be determined.

GPR55: outstanding questions

A focus of current research is to understand which tissues express functional GPR55 protein. This arises from the

Table 1 Activity of cannabinoid ligands at GPR55

Ligand	GPR55 EC ₅₀ (nM) GTPγS binding	GPR55 EC ₅₀ (nM) yeast reporter	GPR119 fold induction of yeast reporter
<i>Endocannabinoids</i>			
AEA	18 ^a	Inactive ^b	2 ^c
Noladin ether	11 ^a		
PEA	3 ^a		20 ^c
Virodhamine	10 ^a	Inactive ^b	
2-AG	3 ^a	Inactive ^b	Inactive ^c
OEA	420 ^a	Inactive ^b	60 ^c
<i>Phytocannabinoids</i>			
THC	8 ^a		
CBD	350^a (IC ₅₀)		
CBN	> 30 000 ^a		
<i>Synthetic cannabinoids</i>			
Methanandamide		Inactive ^b	Inactive ^c
CP55940	7 ^a	20^b (pA ₂)	Inactive ^c
WIN55212-2	> 30 000, ^a > 1000 ^d	Inactive ^b	Inactive ^c
HU210	33 ^a	Inactive ^b	
JWH015	4 ^a		
JWH133	> 30 000 ^a		Inactive ^c
abn-cbd	2780, ^a 2.5 ^d		
SR144528	≈ 1000 ^a		
SR141716A	≈ 600 ^a	3000 ^b	
AM251	39 ^e	3000 ^b	
AM630	≈ 1500 ^a	Inactive ^b	
O-1602	13, ^e 1.4 ^d		

Abbreviations: abn-cbd = abnormal cannabidiol; AEA = arachidonylethanolamide; 2-AG = 2-arachidonoylglycerol; CBD = cannabidiol; CBN = cannabinol; OEA = oleoylethanolamide; PEA = palmitoylethanolamide; THC = Δ⁹-tetrahydrocannabinol.

The table shows reported ligand activities at the putative cannabinoid receptors, GPR55 and GPR119. For GPR55, data show agonist EC₅₀ (nM) or IC₅₀/pA₂ (in bold), where the compound acts as an antagonist. For SR141716A, SR144528 and AM251, EC₅₀ values in GTPγS binding have been estimated from graphical data (Drmota *et al.*, 2004). For GPR119, data show estimated fold induction of a yeast reporter gene in the presence of 30-μM compound.

^aDrmota *et al.* (2004).

^bGlaxoSmithKline abstract (see Baker *et al.*, 2006).

^cOverton *et al.* (2006).

^dJohns *et al.* (2007).

^eAstraZeneca abstract (see Baker *et al.*, 2006).

apparent paradox between the strong binding of [³H]CP55940 to GPR55 *in vitro* (Drmota *et al.*, 2004), and autoradiographic studies that show a lack of binding of this ligand to mouse brain after genetic deletion of CB₁ (Zimmer *et al.*, 1999). This might be explained by a lower affinity of GPR55 for CP55940, compared with CB₁. Repetition of these studies at higher concentrations of radioligand, and comparison of CB₁^{-/-} with CB₁^{-/-}/GPR55^{-/-} mice, may in future reveal a GPR55-mediated component. Also, Buckley *et al.* (2000) were unable to detect specific binding of [³H]CP55940 to spleen membranes derived from CB₂^{-/-} mice even at high radioligand concentrations (30 nM). Further investigation is also required to understand whether G₁₃ is important for GPR55 signalling *in vivo*, or whether other G protein-mediated or G protein-independent mechanisms are involved, and to explain the discrepancies in pharmacology between GPR55 expressed recombinantly in yeast or mammalian hosts (see Table 1).

The endogenous ligand of GPR55 is unclear. The association of both GPR55 and PEA with inflammatory pain signalling reinforces speculation that PEA, in addition to its action at PPARα described above, might also activate GPR55 *in vivo* (Mackie and Stella, 2007). The activity of CBD at GPR55 is also noteworthy, since CBD is reported to have anticonvulsive, anxiolytic, antipsychotic, antiemetic and

antiarthritic properties. CBD does not bind to CB₁ or CB₂, and its effects have not been conclusively attributed to any receptor or other target, though its ability to inhibit AEA transport and degradation or its antioxidant capacity have been suggested to mediate *in vivo* activity (Mechoulam *et al.*, 2002).

For any orphan receptor, it is usual to consider whether homologues offer any clues to pharmacology. The closest relative of GPR55 in phylogenetic clustering is GPR35 (Figure 1), which shares low (30%) amino-acid identity. In human and rodent, GPR35 mRNA is detected in immune tissues and isolated immune cells, gastrointestinal tract, and in the case of rat, in neural tissue from the dorsal root ganglia (Wang *et al.*, 2006; Taniguchi *et al.*, 2006). This profile is somewhat similar to that of GPR55, except that little GPR35 was detected in brain. However, ligand specificity of GPR35 appears divergent from GPR55. GPR35 is activated by kynurenic acid, a metabolite of tryptophan (Wang *et al.*, 2006), and by zaprinast, a synthetic PDE inhibitor (Taniguchi *et al.*, 2006). Chemically, zaprinast is a xanthine analogue of cGMP, and was a lead molecule in the development of sildenafil (Viagra). Both of these ligand pairings have been corroborated by expression in yeast, where human GPR35 was activated by kynurenic acid and zaprinast with potencies comparable to literature reports (SJ Dowell, personal communication).

Neither structure bears obvious chemical similarity to proposed ligands of GPR55 nor are cannabinoids reported to activate GPR35, suggesting little similarity between GPR55 and GPR35 at the level of pharmacophore.

If GPR55 is a receptor for AEA, its phylogenetic divergence from CB₁ and CB₂ suggests that it may have arisen by convergent evolution towards the pre-existing signalling molecule. Is there precedence for such convergent evolution in the GPCR superfamily? The answer is affirmative—two examples are worthy of note. First, GPR23 and GPR92 were originally orphans but recently have been shown to respond to LPA and designated LPA₄ and LPA₅, respectively (Noguchi *et al.*, 2003; Lee *et al.*, 2006). Phylogenetically, they are only distally related to the other known LPA receptors (LPA₁/EDG2, LPA₂/EDG4 and LPA₃/EDG7; Figure 1). GPR23/LPA₄ and GPR92/LPA₅ cluster proximal to GPR55 in the purinergic subfamily of evolutionarily recent origin, whereas LPA_{1–3} cluster proximal to S1P receptors (S1P_{1–5}) and CB₁ and CB₂ in a much more ancient branch of family A GPCRs. Hence, LPA₄ and LPA₅ appear to have diverged not from a pre-existing LPA receptor but from an unrelated receptor. McPartland *et al.* (2006) noted these relationships, and using data from point-mutation studies on LPA receptors (Fujiwara *et al.*, 2005), extrapolated by alignment of GPR55, GPR23, CB₁ and LPA₁ sequences to propose four candidate residues or motifs potentially involved in the GPR55 ligand-binding site. These will form a starting point for point-mutational analysis of GPR55, once robust mammalian cell-based assays for this target are available. The second example of putative convergent evolution is among prostaglandin receptors. The eight originally identified prostaglandin receptors (EP_{1–4}, TP, FP, DP₁ and IP; Figure 1) are evolutionarily ancient and have related primary sequences. The ninth and most recently described receptor, originally named CRTH2 (for chemoattractant receptor on T_H2 cells) but now renamed DP₂, is activated by prostaglandin D₂ (Hirai *et al.*, 2001) but bears minimal sequence similarity to DP₁ beyond motifs common to all family A GPCRs. Residues involved in ligand-binding in DP₁ are not conserved in DP₂ (Hata *et al.*, 2005), and DP₂ clusters with chemoattractant receptors (FPR, FPRL-1) in the purinergic subfamily.

GPR119

GPR119 is an orphan receptor originally identified in genome-sequencing efforts and expressed predominantly in the pancreas and gastrointestinal tract (Fredriksson *et al.*, 2003). Immunohistochemical staining colocalized GPR119 to cells expressing pancreatic polypeptide (PPY) within mouse pancreatic islets; no immunoreactivity was detected in α - or β -cells (Sakamoto *et al.*, 2006). This striking expression pattern prompted the initiation of drug screening, which yielded selective synthetic small-molecule agonists of GPR119, including AR-231453 (EC₅₀ = 25 nM; Arena Pharmaceuticals patent WO 2004065380), and PSN632408 (EC₅₀ = 5 μ M; Overton *et al.*, 2006). GPR119 behaves as a G_s-coupled receptor: transfection of GPR119 into mammalian cells resulted in elevation of intracellular cAMP levels and sensitization to forskolin (Sakamoto *et al.*, 2006), and

small-molecule agonists evoke concentration-dependent increases in cAMP in GPR119-transfected cells (Overton *et al.*, 2006). When dosed systemically into rodent, both small-molecule agonists have effects on metabolic status. In acute studies, PSN632408 reduced cumulative food intake in free-feeding rats for 24-h post-dosing. Longer term oral dosing of PSN632408 to fat-fed male rats reduced both cumulative food intake and body weight gain (Overton *et al.*, 2006). AR-231453 elevated plasma insulin in a glucose-dependent fashion in normal mice and improved glucose tolerance in both normal and diabetic mouse models, but not in mice genetically deleted for GPR119 (Jones, 2006). These studies strongly suggest a role for GPR119 in the pancreas, regulating energy balance. It is still unclear whether this regulation is secondary to effects on PPY, a peptide known to inhibit pancreatic exocrine secretion and attenuate the elevation of post-prandial glucose, since Jones (2006) described increased cAMP levels in isolated pancreatic β -cells treated directly with AR-231453.

The identification of GPR119 as a putative cannabinoid receptor comes from reports of activation of GPR119 by OEA. Overton *et al.* (2006) described activation of yeast expressing either human or mouse GPR119 by OEA. Significant induction of the yeast reporter gene was achieved only at >10 μ M OEA; PEA was more weakly active, and other cannabinoids tested including AEA were inactive (see Table 1). Host cells lacking GPR119 failed to respond to OEA, though the control, of OEA tested on yeast expressing unrelated receptors, was not described. HEK293 cells stably expressing tetracycline-inducible human GPR119 (Overton *et al.*, 2006), or HEK293 or COS-7 cells transiently transfected with mouse GPR119 (Sakamoto *et al.*, 2006) were also reported to respond to >1 μ M OEA with increases in intracellular cAMP. The hypothesis that OEA is the endogenous ligand of GPR119 is initially compelling, given the hyperphagic effects of OEA (Rodriguez de Fonseca *et al.*, 2001). However, it is unclear whether the high concentrations of OEA required to activate recombinant GPR119 occur (patho)physiologically, or whether another as-yet unidentified ligand with greater potency might be the endogenous ligand. Not all groups have observed specific agonism of GPR119 by OEA, using mammalian cell-based assays in which small-molecule agonists exhibit high potencies (H Sauls, personal communication). Given the evidence that OEA acts via PPAR α , it will be crucial to test whether the characteristic effects of OEA on feeding remain after genetic ablation of GPR119. Soga *et al.* (2005) also report activation of GPR119 by oleoyl- and palmitoyl-lysophosphatidylcholine (18:1-LPC and 16:0-LPC) in the micromolar range (EC₅₀ = 1.5 and 1.6 μ M, respectively), whereas Overton *et al.* (2006) detected activation by 18:1-LPC only at higher concentrations (EC₅₀ > 30 μ M). LPC has a history of misassignment as a ligand of orphan GPCRs possibly due to pleiotropic effects on host cells; LPC was described as an agonist at the orphan G2A but these reports were later retracted (Kabarowski *et al.*, 2001; Witte *et al.*, 2005), and it now appears that direct activators of G2A are oxidized free-fatty acids including 9-hydroxyoctadecadienoic acid (9-HODE; Obinata *et al.* (2005) and SJ Dowell and AJ Brown, unpublished data).

Conclusion

It is now established that phytocannabinoids, endocannabinoids, and synthetic cannabinoids have multiple *in vivo* sites-of-action additional to CB₁, CB₂ and TRPV₁. Some of the observed non-CB₁/CB₂ effects may be explained by cross-activity at PPARs or other known receptors, but novel targets, particularly GPCRs, likely also play a part. On the basis of the variety of observed pharmacological profiles, these have been estimated to number three or more (Mackie and Stella, 2007). Two orphan GPCRs, GPR55 and GPR119, have so far been reported as novel cannabinoid receptors. GPR55 has been demonstrated to interact with chemically unrelated cannabinoid ligands, in both mammalian and non-mammalian recombinant expression systems, and by independent research groups. Clearly, there is some relationship between the ligand-binding sites of GPR55 and CB₁/CB₂; however, the endogenous agonist and physiological relevance of GPR55 are not yet clear. On the basis of the reasonable assumption that GPR55 might mediate the unexplained vasodilatory effects of AEA and abn-cbd, Johns *et al.* (2007) evaluated cardiovascular phenotypes of GPR55^{-/-} mice, but found no differences to wild-type mice. A more speculative pain study showed a clear phenotype in GPR55^{-/-} animals, of reduced mechanical nociception following inflammatory or neuropathic challenge (Staton *et al.*, 2006). By analogy, CB₂ was readily linked to inflammatory pain signalling in whole-animal studies using selective ligands (Clayton *et al.*, 2002; Ibrahim *et al.*, 2003; Giblin *et al.*, 2007). However, defining the effects of CB₂ activation at the cellular level and how they relate to mechanisms of inflammation has been a far greater challenge. Different hypotheses hold that the antinociceptive site-of-action of CB₂ may be directly on immune cells to reduce local release of inflammatory mediators, or neuronal, or on other cells such as keratinocytes, acting through release of β -endorphins (Cheng and Hitchcock, 2007). For GPR55, the generation of selective pharmacological tools suitable for *in vivo* studies will confirm whether this GPCR is associated with pain signalling in wild-type animals. If this holds to be the case, detailed studies will be required to define mechanisms involved, with the initial focus being on immune regulation. The second reported novel cannabinoid receptor, GPR119, has been the focus of intensive screening efforts in several pharmaceutical laboratories, and is strongly implicated in the regulation of energy balance and body weight. However, further corroborating data of the activity of acylethanolamides at GPR119 will be required before it can be regarded unequivocally as a cannabinoid receptor. Overall, it appears none of the known non-CB₁/CB₂ effects is associated with these putative novel cannabinoid receptors. Ironically, even though the original precept was that ligand fishing was expected to identify novel cannabinoid GPCRs, such as that mediating abn-cbd-evoked vasodilatation, this approach has instead yielded novel cannabinoid receptors involved in diverse other aspects of physiology. The implication is that further novel cannabinoid receptors mediating non-CB₁/CB₂ effects remain to be identified.

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Conflict of interest

The author is an employee of GlaxoSmithKline.

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