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REVIEW

GPR18 in microglia: implications for the CNS and endocannabinoid system signalling

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A review of what is presently known about the G protein coupled receptor GPR18 in terms of its expression and distribution, pharmacology and potential implications for central nervous system and endocannabinoid system signalling.

LINKED ARTICLES

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Abbreviations

2-AG, 2-arachidonoyl glycerol; 7TM, seven transmembrane; Abn-CBD, abnormal cannabidiol; ACPA, arachidonoyl cyclopropylamide; AM251, 1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-N-(1-piperidyl)pyrazole-3-carboxamide; AM630, [6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl](4-methoxyphenyl)-methanone; AEA, *N*-arachidonoyl ethanolamine; BV-2, mouse microglial cell line BV-2; CBD, cannabidiol; CB₁, cannabinoid receptor 1; CB₂, cannabinoid receptor 2; CP55940, 2-[(1R,2R,5R)-5-hydroxy-2-(3-hydroxypropyl) cyclohexyl]-5-(2-methyloctan-2-yl)phenol; CysL1, cysteinyl receptor 1; CysL2, cysteinyl receptor 2; Δ⁹-THC, Δ⁹-tetrahydrocannabinol; EB12, Epstein-Barr virus-induced receptor 2; eCBS, endogenous cannabinoid system; FAAH, fatty acid amide hydrolase; FACS, fluorescence activated cell sorting; FBS, fetal bovine serum; fMLP, *N*-formyl-methionine-leucine-phenylalanine; FPR, formyl peptide receptor 1; FPRL-1, formyl peptide receptor-like 1; GFP, green fluorescence protein; HEC-1B, human endometrial cell line 1-B; JWH-015, (2-methyl-1-propyl-1H-indol-3-yl)-1-naphthalenylmethanone; JWH-133, (6aR,10aR)-3-(1,1-dimethylbutyl)-6a,7,10,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran; LPA, lysophosphatidic acid; NAGly, *N*-arachidonoyl glycine; O-1602, *trans*-4-[3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-5-methyl-1,3-benzenediol; O-1918, 1,3-dimethoxy-5-methyl-2-[(1R,6R)-3-methyl-6-(1-methylethenyl)-2-cyclohexen-1-yl]-benzene; PTX, pertussis toxin; qPCR, quantitative PCR; RAW264.7, mouse macrophage-derived cell line RAW264.7; R1-methAERA, *N*-(2-hydroxy-1R-methylethyl)-5Z,8Z,11Z,14Z-eicosatetraenamide; RT-PCR, reverse transcriptase PCR; simRNA, short interfering messenger RNA; siRNA, short interfering RNA; SR141 (SR141716A; rimonabant), *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboximide hydrochloride; SR144 (SR144528), 5-(4-chloro-3-methylphenyl)-1-[(4-methylphenyl)methyl]-*N*-[(1S,4R,6S)-1,5,5-trimethyl-6bicyclo[2.2.1]heptanyl]pyrazole-3-carboxamide; WIN55212-2, (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone; URB597, (3'-(aminocarbonyl)[1,1'-biphenyl]-3-yl)-cyclohexylcarbamate

Introduction

In normal brain, microglia possess a characteristic ramified morphology that facilitates continuous CNS surveillance. Following CNS injury or inflammation, receptor-initiated signalling cascades recruit microglia to sites of damage, where the

local microenvironment governs the bespoke morphological and phenotypic characteristics they manifest (Wake *et al.*, 2009). The molecular mechanisms that recruit and instruct microglia to adapt their behaviour in this fashion are poorly understood. Neurons are known to use the endogenous cannabinoid system (eCBs) to communicate with each other

(Schlicker and Kathmann, 2001; Pertwee and Ross, 2002), and Walter *et al.* (2003) reported the involvement of the eCB signalling in recruiting microglia towards dying neurons *in vitro*. The majority of cannabinoid effects on microglia have been conducted using the immortalized primary microglial cell line (BV-2). While this cell line is not always fully representative of *in situ* microglia, it has been shown to retain most of the morphological, phenotypical and functional properties described for freshly isolated active microglial cells (Blasi *et al.*, 1990). Indeed, Walter and colleagues demonstrated that pathological stimulation of both neurons and microglia dramatically and selectively increased the production of 2-arachidonoyl glycerol (2-AG), which, together with other structurally related lipids, triggered microglial cell migration by engaging CB₂ and abnormal cannabidiol (Abn-CBD) receptors (Walter *et al.*, 2003). N-arachidonoyl glycine (NAGly)-GPR18 signalling has been recently introduced as an important 'new player' in microglial-neuronal communication, providing a novel mechanism (both receptor and ligand) for directed migration and phenotypic switches in microglia (McHugh *et al.*, 2010). The published data strongly support a significant role for NAGly and GPR18 in regulating microglia in the CNS and, together with subsequent work, have wider implications for our understanding of the eCB system. This review will outline what is presently known about GPR18, its pharmacology and the potential impact of NAGly-GPR18 signalling on the field.

GPR18 expression and distribution

Mammalian GPCRs constitute a superfamily of diverse proteins with hundreds of members, each having seven transmembrane domains (Bockaert and Pin, 1999). On the basis of shared sequence motifs, they are grouped into four classes: A, B, C and F/S (Horn *et al.*, 1998). The established cannabinoid receptors, CB₁ and CB₂, belong to class A (i.e. rhodopsin-like) and share considerable structural and phylogenetic homology (see Pertwee *et al.*, 2010 for review). Another GPCR that is activated by the phytocannabinoid, Δ^9 -tetrahydrocannabinol (Δ^9 -THC), and by an oxygenated metabolite of the endogenous cannabinoid anandamide (NAGly) has been recently identified (Bradshaw *et al.*, 2009; McHugh *et al.*, 2010; 2012). This GPCR, GPR18, likewise belongs to class A; however, its structural and phylogenetic background are dissimilar to CB₁ and CB₂ (see Pertwee *et al.*, 2010 for review).

The first reports to describe GPR18 arose unanticipated from various broad expression studies of GPCRs. In 1997, while exploiting relaxed stringency PCR to identify a receptor for gastrin-releasing hormone, Gantz *et al.* inadvertently isolated fragments of a novel 7TM GPCR, 331 amino acids long, from canine gastric mucosa and the human colonic cancer Colo 320DM cell line. Subsequent cloning and genomic library screening identified this gene as *GPR18* (designated according to human gene mapping workshop nomenclature) and indicated that the human and canine clones were highly conserved. Fluorescence *in situ* hybridization was used to localize GPR18 to human chromosome 13q32, where it clusters with Epstein-Barr virus-induced

receptor 2 (EBI2) and the lipid receptors, cysteinyl leukotriene receptor 1 and 2 (CysLT₁ and CysLT₂) (Gantz *et al.*, 1997; Rosenkilde *et al.*, 2006). Northern blot analysis conducted by Gantz *et al.* in multiple human tissues reported GPR18 transcripts in spleen, thymus, peripheral blood leucocytes, small intestine, appendix and lymph node – suggesting a possible role for GPR18 in immune system regulation. However, the most abundant expression observed was in the testis, where transcripts were found in several cell types. GPR18 mRNA was detected in gametes of all levels of differentiation, with the highest in the most terminally differentiated cells. The tissues Gantz *et al.* reported to lack any apparent GPR18 mRNA included brain, heart, lung, liver, kidney, pancreas, colon, skeletal muscle, ovary, placenta, prostate, adrenal medulla and adrenal cortex.

Five years later, Vassilatis *et al.* (2003) published a study that had examined the GPCR repertoires of humans and mice via real-time (RT)-PCR tissue profiling. They reported the following four different expression levels for GPR18: *no expression* – amygdala, frontal cortex, hippocampus, liver and muscle; *low expression* – cortex, thalamus, adrenal tissue, colon, intestine, kidney, prostate, skin, spleen, stomach and uterus; *moderate expression* – lung, ovary, testis, thymus and striatum; *strong expression* – hypothalamus, thyroid, peripheral blood leucocytes, cerebellum and brain stem (Vassilatis *et al.*, 2003).

In 2006, while searching for chemokine receptors and GPCRs expressed in adult T-cell leukaemia (ATL) cells, Kohno *et al.* found *GPR18* amongst the genes of relevance. After cloning and further analysis, the study reported that GPR18 was more highly expressed in lymphocytes (CD4⁺, CD4⁺CD45RA⁺, CD4⁺CD45RO⁺, CD8⁺ and CD19⁺) in comparison with monocytes. Stably transfected GPR18-expressing cell lines were created and used in conjunction with the Bioactive Lipid Library, Ca²⁺ mobilization and cAMP assays to subsequently identify NAGly as an endogenous ligand for GPR18. Kohno *et al.* observed concentration-dependent inhibition of forskolin-stimulated cAMP production in GPR18-transfected CHO cells from 1 nM–10 μ M NAGly, with an IC₅₀ value of 20 \pm 8 nM. The NAGly-mediated inhibition was completely abolished by pertussis toxin (PTX), indicating GPR18 coupling to G α_i (Kohno *et al.*, 2006). Studies by McHugh *et al.* have shown a similar potency and PTX sensitivity (McHugh *et al.*, 2010; 2012).

Yin *et al.* (2009) screened a large number of newly 'deorphaned' receptors against a collection of ~400 receptors using the β -arrestin PathHunter™ assay system (DiscoverX, Fremont, CA, USA) and HEK293 cells stably expressing β -arrestin2- β -gal-EA fusion protein. NAGly was not part of their original lipid collection but was later tested on GPR18. The authors reported that they found NAGly to be inactive at GPR18 but did not present their data or details regarding the concentration(s) of NAGly employed in their GPR18 experiment. A number of possible explanations may account for this negative result: firstly, GPCRs are promiscuous. Their coupling partners vary a lot and include both β -arrestin-dependent and β -arrestin-independent pathways. GPR18 mediated effects of NAGly occurring, although a β -arrestin-independent pathway would be missed by the screening system used in the Yin *et al.* study. Secondly, it is the experience of multiple investigators that GPR18 is difficult to suc-

cessfully transfect (unpublished observations); low or no expression of GPR18 would also result in a lack of response to NAGly. Thirdly, the concentration of NAGly may have been inappropriately high or low. Without further information to provide a context, a final interpretation of this piece of data is problematic.

The most current of the PCR-based GPCR screening investigations to involve GPR18 was published in 2010 by Qin *et al.*, who performed a comprehensive array-based, quantitative PCR analysis of the expression profile of 130 genes in three typical sites of melanoma metastases. A comparison between metastases and benign nevi revealed 16 genes that were significantly differentially expressed. Of these, GPR18 and the chemokine ligand CCL4 had the greatest changes in expression levels, which were 24.1- and 27.4-fold higher, respectively. Subsequently, functional experiments in yeast and melanoma were designed to test the hypothesis that GPR18 was able to mediate proliferative or anti-apoptotic signalling. They found that the GPR18 sequence deviated from other GPCRs at position 3.35, where an alanine is present in place of a normally highly conserved asparagine. Asparagine to alanine mutations at 3.35 have been previously shown to result in constitutive activity in CXCR3 and CXCR4 chemokine receptors, precluding the requirement of an agonist ligand to activate them (Ballesteros and Weistein, 1995). Qin *et al.* reported that mutating the alanine back to asparagine at 3.35 resulted in the loss of constitutive activity of GPR18. This is of interest given that malignant cells are dependent on the constitutive or overexpression of driver genes for maintenance of cell survival or inhibition of apoptosis. Qin *et al.* (2010) found that *in vitro* siRNA-mediated knockdown of GPR18 in human melanoma cells enhanced death via apoptosis in further support of this hypothesis.

Using comparative gene microarray analysis, a recent study by Juknat *et al.* (2012) characterized differential transcriptional profiles in BV-2 microglia when exposed to 10 μ M concentrations of CBD and Δ^9 -THC for 6 h. Relative levels of CB₁, CB₂, GPR18, TRPV2 and fatty acid amide hydrolase (FAAH) were not significantly altered; however, Juknat and colleagues did find 1298 transcripts that were differentially regulated by the treatments (Juknat *et al.*, 2012). CBD affected many more genes than Δ^9 -THC, emphasizing that Δ^9 -THC and CBD can signal through different pathways in BV-2 microglia. This study lays the groundwork for potential insights regarding the signalling mechanisms of GPR18 in BV-2 microglia by identifying transcriptional targets of CBD and Δ^9 -THC in BV-2.

In summary, on the basis of mRNA transcripts, there is evidence of GPR18 expression in gastrointestinal, immune and testicular tissues, as well as various brain structures and metastatic melanoma. Using GPR18-targeting antibodies, kindly donated by Dr Ken Mackie (Indiana University), McHugh *et al.* provided evidence of GPR18 receptors in BV-2 microglia. Further immunohisto- and immunocytochemistry in the field will help solidify the pattern of GPR18 expression in humans and the commonly used animal models. This important next step is currently awaiting the availability of GPR18^{-/-} animals in order to definitively verify selectivity of custom or commercially available GPR18 antibodies.

Pharmacology of GPR18

Two recently published papers have described the pharmacology of NAGly, various cannabinoids and other signalling ligands at GPR18. The first investigated the relationship between GPR18 and Abn-CBD receptor in BV-2 microglia (McHugh *et al.*, 2010). The Abn-CBD receptor is a prominent non-CB₁/non-CB₂ cannabinoid receptor, discriminated by means of various pharmacological and genetic tools and implicated in the modulation of microglial, endothelial and glioma cell migration, and various cardiovascular responses (Járai *et al.*, 1999; Franklin and Stella, 2003; Offertáler *et al.*, 2003; Walter *et al.*, 2003; Mo *et al.*, 2004; Begg *et al.*, 2005; Vaccani *et al.*, 2005; Mackie and Stella, 2006). Using Boyden chamber migration experiments, yellow tetrazolium (MTT) conversion, in-cell Western, qPCR and immunocytochemistry, it was reported that NAGly at sub-nanomolar, and Abn-CBD and O-1602 at low nanomolar, concentrations potently induced directed cell migration in both BV-2 microglia and HEK293-GPR18-transfected cells, but not in non-transfected HEK293 wild-type cells; the migration effects were blocked or attenuated in both systems by the Abn-CBD receptor antagonist O-1918, and its low efficacy agonist, cannabidiol; NAGly promoted proliferation and activation of MAP kinases in BV-2 microglia and HEK293-GPR18 cells at low nanomolar concentrations – cellular responses correlated with microglial migration; and BV-2 microglia displayed GPR18 immunocytochemical staining and abundant GPR18 mRNA, while qPCR demonstrated that primary microglia, likewise, express abundant amounts of GPR18 mRNA (McHugh *et al.*, 2010). Further work to fully characterize GPR18 expression and function in primary microglia and validate the data arising from BV-2 microglia will be a welcome next step for the field. At the 2011 symposium of the International Cannabinoid Research Society (ICRS), data were presented from siRNA GPR18 knockdown studies showing that migration induced by NAGly, O-1602 and Abn-CBD was significantly attenuated in GPR18 knockdown BV-2 cells compared with the control, whereas migration to vehicle and fMLP (a formylated tripeptide chemoattractant ligand known to stimulate migration through its own distinct GPCRs) remained unchanged (McHugh *et al.*, 2011). Collectively, these data provide definitive evidence that these compounds, characteristic of Abn-CBD receptor pharmacology, are acting via GPR18 in BV-2 microglia.

The second recent publication presented evidence that the endocannabinoid system plays a regulatory role in human endometrial HEC-1B cell migration. Endogenous and phytocannabinoid effects implied a signalling mechanism mediated through CB₂ receptors and, to a greater extent, GPR18, which echoes the CB₂/Abn-CBD dual expression first described in microglia by Walter *et al.* (2003). The most effective activator of endometrial cell migration was NAGly, and RT-qPCR revealed that HEC-1B endometrial cells express GPR18 mRNA (McHugh *et al.*, 2012). In addition, the specificity of cannabinoid ligands including traditional CB₁ and CB₂ receptor agonists and antagonists at GPR18 was screened via p44/42 MAPK activation in stably transfected HEK293-GPR18 cells. In order of potency, NAGly, O-1602, Abn-CBD, Δ^9 -THC, N-arachidonoyl ethanolamide (AEA) and arachidonylcyclopropylamide (ACPA) are full agonists at GPR18; CBD and

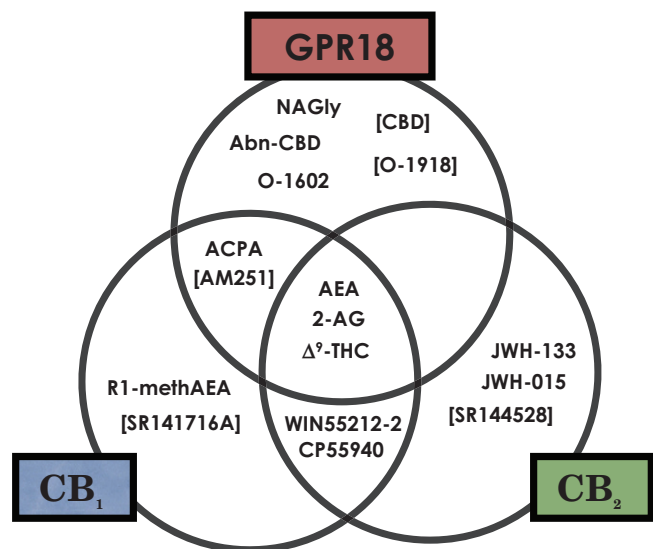


Figure 1

Venn diagram indicating the agonist and antagonist activity that is selective for or shared between CB₁, CB₂ and GPR18 receptors with regard to various cannabinoid or related compounds. Compounds in square parentheses act as antagonists. CB₁, CB₂ and GPR18 receptors were stably transfected in HEK293 cells (see McHugh *et al.*, 2010 and McHugh *et al.*, 2012 for further details).

AM251 weak GPR18 partial agonists/antagonists; and WIN55212-2, CP55940, R1-methAEA, JWH-133 and JWH-015 had no effect (for EC₅₀ values, refer to McHugh *et al.*, 2012). Figure 1 shows a Venn diagram demonstrating the overlap of AEA, 2-AG and Δ⁹-THC for all three receptors as well as the specificity of agonists and antagonists at each receptor. Table 1 summarizes the pharmacology of cannabinoids and related compounds at GPR18 known at present.

The background activity of GPR18 receptors in metastatic melanoma reported by Qin *et al.* (2010), together with the pharmacological profile of CBD and AM251, raises the possibility that low efficacy partial agonist/antagonist or inverse agonist compounds may reduce tonic signalling even in the absence of an agonist. This would be in keeping with the majority of CB₁ and CB₂ antagonists, including SR141, AM251, SR144 and AM630, which have been reported to behave as inverse agonists. Whether or not this would be expected for GPR18 in a given cell or tissue type depends on the extent of GPR18 receptor expression, the degree to which a subpopulation of GPR18 receptors is constitutively active (i.e. coupled spontaneously to their signalling pathways) and the binding affinity of the ligand in question for the 'active' or 'inactive' GPR18 receptor state. No binding affinity or quantitative expression data currently exist for GPR18 in microglia.

Takenouchi *et al.* (2012) have recently examined the effects of NAGly on the mouse macrophage-derived cell line, RAW264.7, and primary mouse peritoneal macrophages. They reported that 30 μM NAGly caused an ~70% significant reduction in the cell viability of RAW264.7 cells via apoptosis after 24 h. Caspase-3 was activated after the addition of

30 μM NAGly for 12 h, while the Bax/Bcl-2 ratio (an indicator of apoptosis) was increased. Apoptosis was suppressed by pretreatment with PTX and transfection with GPR18-specific siRNA. With regard to the primary mouse cells, 3 μM NAGly significantly reduced the cell viability of M1 and M2 peritoneal macrophages by ~40 and ~10%, respectively. Notably, the M1 macrophages exhibited a higher expression of GPR18 mRNA than the M2 cells. The study also demonstrated activation of p44/42 (ERK1/2), p38, and to a lesser extent, JNK MAPKs by NAGly, and that treatment with LPS and interferon-γ augmented GPR18 mRNA levels when compared with treatment with IL-4 or no stimulation (Takenouchi *et al.*, 2012). Collectively, these data confirm the previous findings of Kohno *et al.* (2006) and McHugh *et al.* (2012), and suggest that NAGly might function as an anti-inflammatory factor to reduce pro-inflammatory macrophages.

The Takenouchi *et al.* (2012) study confirms a report by Burstein *et al.* (2011) regarding the resolution of inflammation by NAGly in a mouse peritonitis model. Here, 0.3 and 1.2 mg·kg⁻¹ oral doses of NAGly significantly reduced the migration of inflammatory leucocytes following injection of the pro-inflammatory agent, thioglycollate, by greater than 50% (Burstein *et al.*, 2011). The authors also report that treatment of GPR18-transfected HEK293 cells with 5 μM NAGly caused a 40-fold increase in the concentration of the prostaglandin, PGJ₂, in culture media. Addition of polyclonal anti-GPR18 antibody reduced this response to fourfold, suggesting that GPR18 mediates this action. The 3 μM NAGly treatment of HEK293-GPR18 cells caused a 1.78-fold increase in trypan blue staining (an indicator of reduced cell viability), which was blocked by pretreatment with polyclonal anti-GPR18 antibody. FACS analysis following annexin staining of NAGly-treated HEK293-GPR18 supported the occurrence of programmed cell death (i.e. apoptosis) (Burstein *et al.*, 2011).

GPR18 in microglia: implications for neuron–glia interactions

Directed migration, selective phagocytosis and free radical production are critical functions of microglia that have a significant impact on overall CNS stability, both from an acute and a long-term perspective. In both developmental and post-developmental contexts, microglia discriminately engulf and eliminate dead or dying neurons (Ferrer *et al.*, 1990; Wake *et al.*, 2009). These processes must be tightly controlled in order to sustain the least possible collateral damage to adjacent neurons. Indeed, microglial activity is not only orchestrated to exacting standards, but the coupling between the death of neurons and their degradation by microglia is both striking and swift (Peri and Nusslein-Volhard, 2008). This remarkable relationship suggests a fast-acting communication between neurons and microglia, such that the microglia are forewarned of the specific task (i.e. apoptosis, infection or damage). Yet, microglia are also implicated in virtually all CNS neuropathological processes, where they become highly reactive to dying neurons and provoke sustained secondary neurotoxicity (Graeber *et al.*, 1988a,b; Konno *et al.*, 1989; Rao and Lund, 1989; Flaris and Hickey, 1992; Morioka *et al.*, 1992; Berman *et al.*, 1999; Rezaie and

Table 1

Activation of GPR18, CB₁ or CB₂ receptors stably transfected into HEK293 cells induced p44/42 MAP kinase phosphorylation by cannabinoids and related lipids

Compound	Activity at GPR18	EC ₅₀ values (95% confidence limits)	E _{max} values (95% confidence limits)
NAGly	Full agonist	44.5 nM (32.4–61.0)	101.6% (98.4–104.9)
O-1602	Full agonist	65.3 nM (49.3–86.5)	102.1% (98.9–105.3)
Abn-CBD	Full agonist	835.7 nM (579.6–1205)	98.6% (92.6–104.6)
Δ ⁹ -THC	Full agonist	0.96 μM (0.43–2.12)	104.9% (88.7–121.2)
AEA	Full agonist	3.83 μM (2.11–6.90)	109.0% (97.3–120.8)
ACPA	Full agonist	13.5 μM (9.03–20.4)	105.5% (95.8–115.3)
CBD	Partial agonist/antagonist	51.1 μM (35.8–73.2)	61.9% (46.8–77.0)
AM251	Partial agonist/antagonist	96.4 μM (n/a)	50.3% (n/a)
WIN55212-2	None	–	–
CP55,940	None	–	–
R1-methAEA	None	–	–
JWH-133	None	–	–
JWH-015	None	–	–
SR141716A	None	–	–
SR144528	None	–	–

Compound	Activity at CB ₁	EC ₅₀ values (95% confidence limits)	E _{max} values (95% confidence limits)
CP55,940	Full agonist	4.56 nM (3.60–5.79)	97.3% (94.8–98.5)
ACPA	Full agonist	29.5 nM (25.1–34.8)	98.2% (94.9–100.3)
WIN55212-2	Full agonist	35.9 nM (30.7–42.0)	97.1% (95.0–99.2)
R1-methAEA	Full agonist	108.1 nM (97.6–120.0)	96.7% (94.8–98.9)

Compound	Activity at CB ₂	EC ₅₀ values (95% confidence limits)	E _{max} values (95% confidence limits)
JWH-133	Full agonist	95.7 nM (68.0–115.9)	98.1% (95.7–100.4)
JWH-015	Full agonist	207.5 nM (158.7–245.6)	98.9% (97.3–101.6)

EC₅₀ and E_{max} values (percentage of p44/42 MAP kinase activation induced by 10 μM ionomycin) were calculated from sigmoidal concentration–response curves constructed in GraphPad Prism 4 (GraphPad Software, San Diego, CA, USA). The data represent the mean with 95% confidence limits, *n* = 3 (McHugh *et al.*, 2012).

Lantos, 2001; Segal, 2003; Games *et al.*, 2006; Gowing *et al.*, 2006; Czigler *et al.*, 2007; Denes *et al.*, 2007; Yasuda *et al.*, 2007). This extreme dichotomy of behaviour underscores the importance of understanding the specific signalling systems that both recruit and instruct microglia to adapt their phenotype selectively in response to damaged/dying/apoptotic neurons, and which are foundational to our ability to monitor and manage dys-regulated microglial activity.

Microglial migration and phenotypic modification are induced by various ligands, including bacterial peptides (e.g. formyl-methionyl-leucyl-phenylalanine, fMLP), lysophospholipids (e.g. lysophosphatidic acid, LPA) and endocannabinoids (Lorton *et al.*, 2000; Schilling *et al.*, 2004). The potential importance of NAGly-GPR18 signalling as a ‘new player’ in

microglial–neuronal communication can be appreciated when compared with other typical signalling molecules. Picomolar concentrations of NAGly elicit a response twice that produced by 1 μM fMLP or 1 μM LPA. Additionally, NAGly is 30- and 700-fold more potent than the endocannabinoids, 2-AG and AEA, respectively, and more efficacious than either (McHugh *et al.*, 2010). To date, there are no Food and Drug Administration-approved pharmacological therapies that target microglial–neuronal receptor–ligand interactions. Some promising results have been described regarding the neuroprotective effects of minocycline, a broad spectrum tetracycline antibiotic, against a host of neurodegenerative diseases. Minocycline disrupts T cell–microglia signalling, impairing T-cell-induced TNF-α production by microglia and

also blocks activation of microglia via inhibition of NF- κ B nuclear translocation (Baptiste *et al.*, 2005; Giuliani *et al.*, 2005; Maier *et al.*, 2007). In line with this, elucidating the NAGly-GPR18 neuronal–microglial communication system has the potential to lead to novel pharmacotherapies centred on enhancing (optimized GPR18 ligands) or suppressing (optimized GPR18 antagonists) microglial activation in the CNS.

GPR18 and N-arachidonoyl glycine: potential impact on the eCB system

A quarter of a century ago, Dr Raphael Mechoulam and Dr Yehiel Gaoni's key breakthrough in isolating Δ^9 -THC eventually led to the molecular cloning of CB₁ and CB₂ in the early 1990s. The identification of these two distinct GPCRs, in turn, triggered a search for an endogenous cannabinoid, which culminated in the identification of the lipid molecule N-arachidonoyl ethanolamide or anandamide (AEA; Devane, 1992). Brain-derived NAGly is synthesized primarily from AEA via a fatty acid amide hydrolase (FAAH)-dependent pathway and can be prevented by URBS97, an irreversible inhibitor of FAAH (Bradshaw *et al.*, 2009). NAGly has been reported to be ineffective as an agonist at either CB₁ or CB₂ receptors (Sheskin *et al.*, 1997; Huang *et al.*, 2001), despite the obvious structural overlap with AEA.

As described earlier, AEA and Δ^9 -THC are full agonists (although neither are especially potent) at GPR18 receptors, while CBD behaves as a low efficacy partial agonist/antagonist. This pharmacology of established cannabinoids at GPR18 receptors, together with the precursor relationship between AEA and NAGly, has raised the prospect that NAGly and GPR18 are either additional pieces of the endocannabinoid system puzzle or components of a distinct signalling mechanism that readily interacts with the eCB system. Further work is necessary before we can discern which hypothesis is correct.

In the meantime, GPR18 signalling has important and immediate implications with regard to how to interpret past literature and current studies (see Alexander, 2012 for further commentary). The potential role of GPR18 must be brought to the table and considered when interpreting the effects of AEA or Δ^9 -THC as being due to either or both CB₁/CB₂; making use of CBD in combination with AEA or Δ^9 -THC; or employing the CB₁-versus-CB₂ selective agonist ACPA (also a GPR18 agonist) or CB₁-versus-CB₂ selective antagonist AM251 (also GPR18 antagonist) to suggest a role for CB₁.

Conclusions

We are now in the midst of major advances in biochemistry/physiology associated with therapeutic actions of the endocannabinoids, including fertility, neurodegeneration and neuroprotection, learning and memory, anxiety, pain relief, treatment of cancer, anti-nausea, appetite and obesity, and drug abuse. The discovery of a new endogenous ligand (NAGly) and cannabinoid receptor (GPR18) is a valuable contribution to the field's understanding of the molecular

mechanisms responsible for the effects of both endogenous and phytocannabinoids. Indeed, further investigation of NAGly-GPR18 signalling in microglia will greatly enhance our understanding of the extent to which endocannabinoids and related endogenous lipids initiate and maintain neuroimmunological events via microglia.

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Conflicts of interest

The author has no conflicts of interest.

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