BJP British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01731.x
www.brjpharmacol.org

COMMENTARY

So what do we call GPR18 now?

SPH Alexander

School of Biomedical Sciences, University of Nottingham Medical School, Nottingham, UK

Correspondence
SPH Alexander, School of Biomedical Sciences, University of Nottingham Medical School, Nottingham NG7 2UH, UK.
E-mail: steve.alexander@nottingham.ac.uk

Keywords
cannabinoid receptor; GPR18; anandamide; *N*-arachidonoylglycine; tetrahydrocannabinol; AM251

Received
6 October 2011
Accepted
7 October 2011

The further characterization of the orphan GPCR GPR18 conducted by McHugh *et al.* in this issue of the *British Journal of Pharmacology* has generated a pharmacological profile that raises some interesting questions about the nomenclature of this receptor and may also prompt some questions about the pharmacological definition of the classical cannabinoid receptors, CB_1 and CB_2.

LINKED ARTICLES

This article is a commentary on McHugh *et al.*, pp. 2414–2424 of this issue and is part of a themed section on Cannabinoids in Biology and Medicine. To view McHugh *et al.* visit http://dx.doi.org/10.1111/j.1476-5381.2011.01497.x. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2012.165.issue-8. To view Part I of Cannabinoids in Biology and Medicine visit http://dx.doi.org/10.1111/bph.2011.163.issue-7

Abbreviations

2-AG, 2-arachidonoylglycerol; AEA, anandamide; CBD, cannabidiol; NAGly, *N*-arachidonoylglycerol; THC, Δ^9-tetrahydrocannabinol

In this issue of the *British Journal of Pharmacology*, the second associated with a celebration of the 80th birthday of Raphael Mechoulam, Doug McHugh, Heather Bradshaw and colleagues describe their investigations into the orphan GPCR, GPR18 (McHugh *et al.*, 2012). They observed that *N*-arachidonoylglycine (NAGly, an endogenous fatty acid: amino acid conjugate; see Figure 1A), Δ^9-tetrahydrocannabinol (THC, the major psychoactive component of the cannabis plant; see Figure 1A) and anandamide (AEA, an endogenous cannabinoid agonist; see Figure 1A) function as 'full' agonists at GPR18 expressed heterologously in HEK293 cells, as measured by phosphorylation of ERK1/2, with that rank order of potency. This leads me to pose the question: what do we call GPR18 now?

The short answer to that question, of course, is that we wait for a decision from the appropriate Nomenclature Committee of IUPHAR – the Union of Basic and Clinical Pharmacology (http://www.iuphar.org/nciuphar.html). So what options are open to them? Guidance from NC-IUPHAR suggests naming the receptor after the endogenous ligand or group of ligands. Of the ligands examined for activity at recombinant GPR18, NAGly was the most potent in the current study (McHugh *et al.*, 2012). Is GPR18, therefore, a NAGly (or potentially a $NAGly_1$ or NAG_1) receptor?

NAGly as an endogenous entity

GPR18 was initially cloned and deorphanized from a human T-cell line in a search for novel chemokine-like receptors (Kohno *et al.*, 2006). Using the recombinant receptor, NAGly was observed to be an agonist, with 10 μM of this lipoamino acid evoking a rapid and transient elevation of intracellular calcium ions. Additionally, NAGly evoked a concentration-dependent, pertussis toxin-sensitive inhibition of cAMP formation, with a calculated IC_{50} value of 20 nM (Kohno *et al.*, 2006), similar to its potency in the McHugh *et al.* study. NAGly has been reported to be ineffective as an agonist at either CB_1 or CB_2 cannabinoid receptors (Sheskin *et al.*, 1997; Huang *et al.*, 2001), despite a marked structural similarity to the endogenous cannabinoid anandamide (Figure 1A). Indeed, it has been suggested that NAGly biosynthesis might involve anandamide as a precursor, with oxidation at the terminal alcohol by cytochrome *c* (McCue *et al.*, 2008) or alcohol dehydrogenase (Bradshaw *et al.*, 2009). NAGly inhibits fatty acid amide hydrolase activity (Ghafouri *et al.*, 2004) but not monoacylglycerol activity, suggesting selective impairment of amide endocannabinoid, but not glyceride endocannabinoid, hydrolysis (Ghafouri *et al.*, 2004). It has also been suggested that NAGly is formed through the action of fatty acid amide hydrolase in 'reverse mode' (Bradshaw

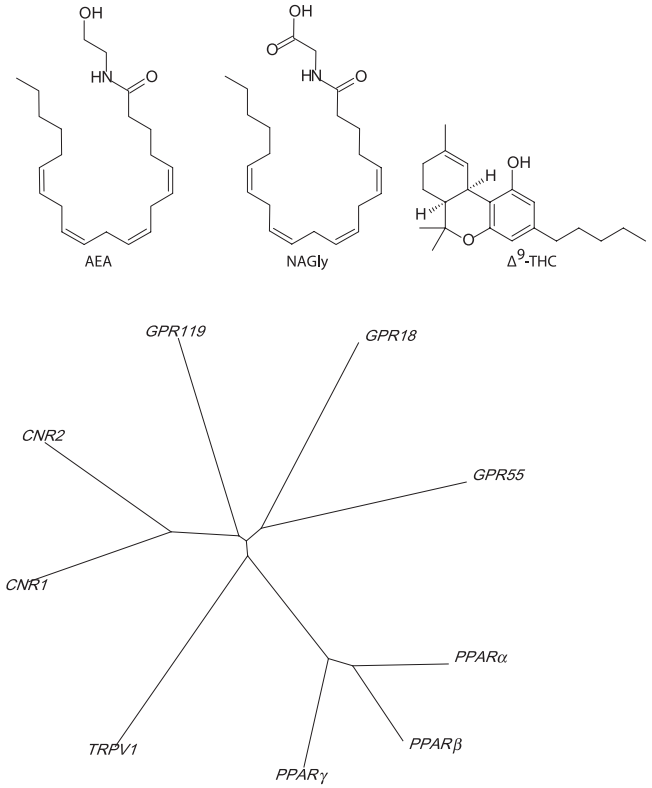

Figure 1

An evaluation of GPR18 pharmacology (A) and structure (B). The structures shown in (A) are of AEA, an endogenous cannabinoid; NAGly, the putative endogenous agonist for GPR18 receptors; and Δ^9-THC, the archetypal natural cannabinoid from the *Cannabis* plant. An unrooted dendrogram in (B) illustrates sequence similarities for putative cannabinoid and cannabinoid-like receptors in man (conducted using ClustalX2).

et al., 2009). NAGly is also a substrate for COX-2, producing prostaglandin conjugates of glycine (Prusakiewicz *et al.*, 2002), although the relevance of this potential biotransformation is unclear.

Levels of NAGly in *ex vivo* tissues were initially observed to be highest in spinal cord and small intestine, with intermediate levels in brain and testes, and low levels in blood, spleen and heart (Huang *et al.*, 2001). Further studies suggested that brain levels of NAGly were similar to those of AEA but appreciably lower than 2-arachidonoylglycerol a further endogenous cannabinoid agonist (Bradshaw *et al.*, 2006). As yet, there are no reports demonstrating whether NAGly levels may be altered with pharmacological or pathological interventions.

Tissue distribution of GPR18

The original cloning of GPR18 suggested high levels of expression in man in testes and spleen (Gantz *et al.*, 1997). An effect on immune function was suggested, with detectable levels of expression in the thymus, peripheral white blood cells and small intestine. Many other tissues appear to be devoid of GPR18 expression, including the brain, heart, lung, liver, kidney, pancreas, colon, skeletal muscle, ovary, placenta, prostate, adrenal medulla and cortex. In cultured cells, GPR18 expression has been identified in human lymphoid cells (Kohno *et al.*, 2006), BV2 murine microglial cells (McHugh *et al.*, 2010), metastatic melanoma (Qin *et al.*, 2011) and now HEC-1B human endometrial cells (McHugh *et al.*, 2012).

There appears, therefore, to be a slight disconnection between tissues expressing significant amounts of NAGly and the expression of the putative NAGly receptor, GPR18. Further work on the distribution of GPR18 and NAGly needs to be done to confirm co-localization (or not). Of course, it is quite conceivable that NAGly performs other functions apart from acting as a GPR18 agonist. NAGly was initially suggested to act as an agonist at GPR92, at the time another orphan receptor (Oh *et al.*, 2008); however, a later study suggested that NAGly is a poor ligand at this receptor, while lysophosphatidic acid is a much more potent agonist (Williams *et al.*, 2009). GPR92 has been re-named as the LPA_5 receptor. Further suggested actions of NAGly include an inhibitory action at GlyT2/*SLC6A5* glycine transporters (Wiles *et al.*, 2006) and voltage-gated calcium channels (Barbara *et al.*, 2009). So this may explain the discrepancy in distribution between agonist and receptor.

Is GPR18 a novel cannabinoid receptor?

McHugh *et al.* report that two cannabinoid agonists, AEA and THC, are full agonists at GPR18, while a further *Cannabis*-derived compound cannabidiol (CBD) is a low efficacy agonist (McHugh *et al.*, 2012). Intriguingly, AEA is a partial agonist at CB_1 cannabinoid receptors, as is THC (Pertwee *et al.*, 2010). At CB_2 cannabinoid receptors, both THC and AEA also have very low efficacy (Pertwee *et al.*, 2010). CBD is a poor ligand at both CB_1 and CB_2 cannabinoid receptors (Pertwee *et al.*, 2010). These data could be taken as evidence to suggest that GPR18 should be considered a third cannabinoid receptor, a suggestion considered premature only a year ago (Pertwee *et al.*, 2010). Comparing primary sequences of putative cannabinoid receptors with CB_1 and CB_2 cannabinoid receptors suggests very little overlap in structure between the archetypal cannabinoid receptors and GPR18 (or indeed with GPR55, GPR119, TRPV1, PPARα, PPARβ and PPARγ, see Figure 1B).

As yet, there is a single report investigating the possible action of 2-arachidonoylglycerol at GPR18, where it was reported to be ineffective (Yin *et al.*, 2009). In counterpoint, though, it should be noted that this report suggested that NAGly was also ineffective as a GPR18 agonist. What would make the suggestion that GPR18 is a further cannabinoid receptor more compelling would be a response evoked by a cannabinoid *in vivo* that could be shown to be mediated by GPR18, and not CB_1 or CB_2 cannabinoid receptors. Although a transgenic mouse model has yet to be described in which the *gpr18* gene is disrupted, a double knockout of CB_1 and CB_2 receptors has been described, which might well be an attrac-

tive topic for future research involving GPR18. Another fruitful area of research would be to investigate whether changes in GPR18 expression or coupling result when the classical cannabinoid receptors are disrupted.

Furthermore, the antagonist action of CBD at GPR18 described by McHugh *et al*. (2012) may well cause a re-examination, if not re-evaluation, of the literature in which combinations of AEA and CBD or THC and CBD have been investigated. Additionally, McHugh *et al*. showed that the widely used CB_1-selective antagonist AM251 was a very weak partial agonist at GPR18, with a calculated potency of approximately 100 μM. However, AM251 was able to evoke a concentration-dependent inhibition of THC-evoked responses at GPR18 with a potency in the mid-nanomolar range. Since THC and AM251 are often used in combination to suggest a role for CB_1 cannabinoid receptors *in vivo*, this interpretation may need to be more cautious in the future.

To return to my initial question about how we refer to GPR18, for the moment, the most pragmatic solution would be to retain the GPR18 nomenclature but to allow the cannabinoid community to foster this orphan, at least until further research allows a more definitive decision to be made.

References

Barbara G, Alloui A, Nargeot J, Lory P, Eschalier A, Bourinet E *et al*. (2009). T-type calcium channel inhibition underlies the analgesic effects of the endogenous lipoamino acids. J Neurosci 29: 13106–13114.

Bradshaw HB, Rimmerman N, Krey JF, Walker JM (2006). Sex and hormonal cycle differences in rat brain levels of pain-related cannabimimetic lipid mediators. Am J Physiol Regul Integr Comp Physiol 291: R349–R358.

Bradshaw HB, Rimmerman N, Hu SS, Benton VM, Stuart JM, Masuda K *et al*. (2009). The endocannabinoid anandamide is a precursor for the signaling lipid N-arachidonyl glycine through two distinct pathways. BMC Biochem 10: 14.

Gantz I, Muraoka A, Yang YK, Samuelson LC, Zimmerman EM, Cook H *et al*. (1997). Cloning and chromosomal localization of a gene (GPR18) encoding a novel seven transmembrane receptor highly expressed in spleen and testis. Genomics 42: 462–466.

Ghafouri N, Tiger G, Razdan RK, Mahadevan A, Pertwee RG, Martin BR *et al*. (2004). Inhibition of monoacylglycerol lipase and fatty acid amide hydrolase by analogues of 2-arachidonoylglycerol. Br J Pharmacol 143: 774–784.

Huang SM, Bisogno T, Petros TJ, Chang SY, Zavitsanos PA, Zipkin RE *et al*. (2001). Identification of a new class of molecules, the arachidonyl amino acids, and characterization of one member that inhibits pain. J Biol Chem 276: 42639–42644.

Kohno M, Hasegawa H, Inoue A, Muraoka M, Miyazaki T, Oka K *et al*. (2006). Identification of N-arachidonylglycine as the endogenous ligand for orphan G-protein-coupled receptor GPR18. Biochem Biophys Res Commun 347: 827–832.

McCue JM, Driscoll WJ, Mueller GP (2008). Cytochrome c catalyzes the in vitro synthesis of arachidonoyl glycine. Biochem Biophys Res Commun 365: 322–327.

McHugh D, Hu SSJ, Rimmerman N, Juknat A, Vogel Z, Walker JM *et al*. (2010). N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. BMC Neurosci 11: 44.

McHugh D, Page J, Dunn E, Bradshaw HB (2012). Δ^9-THC and N-arachidonyl glycine are full agonists at GPR18 and cause migration in the human endometrial cell line, HEC-1B. Br J Pharmacol 165: 2414–2424.

Oh DY, Yoon JM, Moon MJ, Hwang JI, Choe H, Lee JY *et al*. (2008). Identification of farnesyl pyrophosphate and N-arachidonylglycine as endogenous ligands for GPR92. J Biol Chem 283: 21054–21064.

Pertwee RG, Howlett AC, Abood ME, Alexander SPH, Di Marzo V, Elphick MR *et al*. (2010). International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. Pharmacol Rev 62: 588–631.

Prusakiewicz JJ, Kingsley PJ, Kozak KR, Marnett LJ (2002). Selective oxygenation of N-arachidonylglycine by cyclooxygenase-2. Biochem Biophys Res Commun 296: 612–617.

Qin Y, Verdegaal EM, Siderius M, Bebelman JP, Smit MJ, Leurs R *et al*. (2011). Quantitative expression profiling of G-protein-coupled receptors (GPCRs) in metastatic melanoma: the constitutively active orphan GPCR GPR18 as novel drug target. Pigment Cell Melanoma Res 24: 207–218.

Sheskin T, Hanus L, Slager J, Vogel Z, Mechoulam R (1997). Structural requirements for binding of anandamide-type compounds to the brain cannabinoid receptor. J Med Chem 40: 659–667.

Wiles AL, Pearlman RJ, Rosvall M, Aubrey KR, Vandenberg RJ (2006). N-Arachidonyl-glycine inhibits the glycine transporter, GLYT2a. J Neurochem 99: 781–786.

Williams JR, Khandoga AL, Goyal P, Fells JI, Perygin DH, Siess W *et al*. (2009). Unique ligand selectivity of the GPR92/LPA5 lysophosphatidate receptor indicates role in human platelet activation. J Biol Chem 284: 17304–17319.

Yin H, Chu A, Li W, Wang B, Shelton F, Otero F *et al*. (2009). Lipid G-protein-coupled receptor ligand identification using β-arrestin pathhunter assay. J Biol Chem 284: 12328–12338.