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Themed Section: Molecular Pharmacology of GPCRs

EDITORIAL

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This themed issue of the British Journal of Pharmacology stems from the 7th in the series of meetings on the Molecular Pharmacology of G Protein-Coupled Receptors (MPGPCR) held at the Monash Institute of Pharmaceutical Sciences in Melbourne Australia from the 6th–8th December 2012.

LINKED ARTICLES

This article is part of a themed section on Molecular Pharmacology of GPCRs. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2014.171.issue-5

The most recent MPGPCR meeting was wide ranging and included sessions on GPCR drug discovery, a technical workshop, signaling, chemical biology, integrated physiology and *in vivo* pharmacology as well as early career and student oral prize presentations. In spite of some last minute changes to the programme resulting from two of our keynote speakers attending an alternative occasion in Stockholm the meeting was a great success with lively and informative talks.

GPCRs continue to be studied enthusiastically by many groups driven by their fascinating biology, their potential as therapeutic targets and most recently by the rapidly expanding information on GPCR structural biology. The paper by *Barrie Kellam and colleagues* (Vernall *et al.*, 2014) details the development of fluorescent molecules for the study of GPCRs, outlining the properties that are required for selectivity and potency of the conjugate compounds. They provide a critical assessment of the fluorescent ligands that have been synthesised to label GPCRs and suggest in a number of cases how they may be improved. The section on fluorescent probes for the adenosine receptor highlights how informed development can result in tools that not only can replace radioligands in receptor binding studies but are suitable for high resolution imaging. Here the fluorescent ligands often have distinct advantages including emission of a signal only when binding to receptors in membranes being quenched in solution making measurements at equilibrium feasible and making the development of high affinity ligands less critical. When the structures of fluorescent ligands have been optimised correctly they have clear potential for the study of receptors by flow cytometry, confocal fluorescence microscopy and FRET. *Larry Miller and colleagues* (Dong *et al.*, 2014) bring together a large amount of material informing structure-function relationships for the relatively small group of class B GPCRs that nevertheless have important therapeutic potential. Although superficially similar to class A GPCRs, the class B GPCRs have a number of important structural differences including the helical bundle and N terminal domain. This review concentrates on information obtained on the N terminal domain and extracellular loop regions of class B GPCRs that include 4 subgroups – secretin, CGRP, corticoliberin and PTH receptors. They have analysed structure-function relationships for studies involving site directed mutagenesis, ligand modification, photoaffinity labelling and cysteine trapping. While there seems to be no consistent theme that can be applied across class B GPCRs for binding of ligands, it is expected that there will be a common molecular mechanism involved in the activation of signalling cascades. At the time of writing the review there were high-resolution NMR studies of regions of class B GPCRs but subsequently there have been structures published for the

glucagon receptor (GCGR) (Siu *et al.*, 2013) and the corticotropin-releasing factor 1 receptor (Hollenstein *et al.*, 2013) that includes detailed mutagenesis studies and promises to inform structural modelling of interactions between class B GPCRs and their peptide ligands. The next review by *Sebastian Furness and colleagues* (Pabreja *et al.*, 2014) provides an excellent example of how one of the class B GPCRs is being developed as a therapeutic target and the difficulties associated with this exercise. The GLP-1 receptor is a secretin group class B GPCR that responds to a variety of peptides including GLP-1, GLP-2, oxyntomodulin and glicentin secreted from intestinal L cells following food ingestion. The receptor is regarded as an important therapeutic target since activation of GLP-1 receptors in pancreatic β-cells increases glucose-dependent insulin secretion, increases their mass and improves function. Successful treatment of type II diabetes has utilised GLP-1 receptor agonists or dipeptidyl peptide IV (DPPIV) inhibitors that slow the breakdown of naturally produced GLP-1. The molecular mechanisms underlying GLP-1 receptor activation are discussed in depth and these are particularly interesting given the number of endogenous ligands that activate the receptor and the evidence that they display biased signalling profiles. Currently all of the GLP-1 receptor agonists that are used therapeutically are peptides limiting them to parenteral use. They mainly differ in potency and duration of action but do display other differences that may point to signalling bias. The DPPIV inhibitors although more convenient, are less effective and may be associated with a wider spectrum of side effects. The review emphasises the importance of a better understanding of the pleiotropic nature of GLP-1 receptor signalling and how this can be harnessed by exploiting biased signalling profiles to produce the therapeutic effect without undesirable side effects. *Stephen Hill and his colleagues* (Hill *et al.*, 2014) detail recent advances in the understanding of adenosine receptors brought about by the use of fluorescent ligands that allow studies of the effects of allosteric modulation of receptors at the single cell level. The review highlights the therapeutic potential of adenosine receptors and illustrates how a better understanding of the biology is bringing this closer to fruition. Some of the earliest examples of allosteric modulators were described for this system but it is now being recognised that not only do allosteric modulators have different effects on affinity and efficacy of an orthosteric agonist at the A_3 adenosine receptor but the modulation of efficacy is functionally biased. Similar effects have been reported for the A_1 adenosine receptor. The authors go on to describe some of their own work using well characterised fluorescent probes to examine allosteric effects on dissociation kinetics at the single cell level. A clear advantage of this approach is the ability to examine dissociation kinetics by infinite dilution thus avoiding possible interference from addition of an excess of competing ligand. They provide some excellent examples of how dissociation kinetics can be influenced by ligand addition that can also influence cooperativity across the homodimeric interface. Clearly an intricate knowledge of allosteric modulators, biased signalling and oligomer formation enhances the potential for exploitation of adenosine receptors therapeutically. *Gunnar Schulte and co-workers* (Dijksterhuis *et al.*, 2014) summarise current thinking in the challenging field of WNT/Frizzled signalling. Classified as GPCRs on the basis of seven transmembrane spanning regions and evidence for coupling to G proteins but grouped separately from class A, B and C GPCRs by IUPHAR-NC based on substantial structural differences, FZDs continue to be poorly understood from a mechanistic standpoint. While FZD signalling clearly plays an important role in cell proliferation, differentiation, fate and migration and is critical for development, only recently has information started to emerge on signalling mechanisms activated by WNTs. Currently there are 10 mammalian FZDs and 19 WNTs but systematic studies of specificity and bias in what is clearly a pleiotropic signalling system is lacking. It is also clear that there is crosstalk between FZD signalling pathways and formation of signalling complexes. Signalling pathways have been grouped into β-catenin-dependent and β-catenin-independent pathways and opinion is somewhat divided on whether β-catenin-dependent signalling involves G proteins. However, evidence is emerging that implicates G proteins in a wider spectrum of FZD signalling responses than was originally supposed. In addition, in common with many other GPCRs, FZDs interact with scaffold proteins that have important roles in signalling. Structurally FZDs resemble other GPCRs in having conserved cysteine residues in extracellular loops 1 and 2 but lack the DRY motif in TM3 thought to be important for G protein coupling. The recent crystal structure of the related smoothened (SMO) confirms that features common to class A GPCRs are not present in FZD receptors. Although crystal structures of regions of FZDs have been solved and provide useful information a complete FZD structure has yet to be solved. The authors then provide a detailed analysis of the information available to date on all of the FZDs and their responses to WNTs followed by discussion of their physiological relevance. It is clear that this important group of GPCRs is now beginning to yield new information that will inform the development of new tools and potentially therapeutic intervention. *Hans Bräuner-Osborne and his group* (Clemmensen *et al.*, 2014) write about another 'difficult' GPCR, the class C GPRC6A that has potential for development as a target for drugs treating metabolic disease. This is a timely review and while there is general agreement that GPRC6A is activated by basic and aliphatic L amino-acids and modulated by divalent cations there is less general agreement regarding activation by osteocalcin and testosterone. The receptor appears to be involved in inflammation, metabolism and endocrine functions and the GPRC6A knockout mouse has an interesting phenotype suggesting that the receptor may be a therapeutic target. The evidence that GPRC6A likely forms dimers in the same way as most other class C receptors is presented in detail. The difficulties arise in the study of the signalling pathways activated by GPRC6A possibly due to poor cell surface expression. While good intracellular expression is observed very little of the protein seems to be trafficked to the cell membrane. This problem has to some extent been overcome by expression of a chimeric human/goldfish GPRC6A that does traffic to the cell surface an enables signalling studies to be performed. There has been relatively little success in developing selective agonist and antagonist tools based on the orthosteric binding site and the actions of osteocalcin and testosterone at the receptor are controversial. There has been some success however in developing allosteric modulators although this would be assisted by the develop-

ment of a bioassay utilising the human GPRC6A. The review then goes on to present the evidence that GPRC6A is widely expressed and as judged by the phenotype of knockout mice has a number of important functions. However the unlocking of its full potential will depend on the development of specific tools to help fully understand its physiological roles. *Arthur Conigrave and collaborators* (Leach *et al.*, 2014) evaluate the calcium-sensing receptor (CaSR) another class C GPCR but one that has been more amenable to study than GPRC6A. Like other class C GPCRs, the CaSR has a large extracellular N terminal domain that includes a Venus fly trap (VFT) domain linked to the transmembrane spanning regions by a cysteine rich domain. The VFT domain contains binding sites for divalent cations although these ligands are also able to activate the receptor via the transmembrane spanning regions. The binding sites have been identified and there are allosteric modulators, both calcimimetics and calcilytics, some of which have therapeutic applications. The CaSR exhibits pleiotropic signalling with coupling demonstrated to $G_{q/11}$, $G_{i/o}$ and $G_{12/13}$ and even in some contexts Gs. This together with the existence of multiple ligands for the receptor make it an ideal candidate for biased signalling that it indeed displays. The authors then describe the role of the CaSR in Ca^{2+} homeostasis, bone and cartilage turnover, vascular function, metabolism and in the gut epithelium. There are many mutations and polymorphisms of the CaSR many of which are linked to human disease. Interestingly some of these disorders can be corrected by biased agonists acting at the CaSR and there is great potential for further development of this approach. The authors also outline the profiles that are likely required for future drugs acting allosterically or as biased agonists to treat particular conditions. It will be very interesting to see how this plays out in the development of new therapeutic options. *Nigel Bunnett and co-workers* (Lieu *et al.*, 2014) introduce us to the recently identified GPBA (TGR5) receptor for bile acids that is involved in obesity and pruritis but has physiological roles in thermogenesis, inflammation, digestive functions and sensory transduction making it a potential therapeutic target for metabolic, inflammatory, digestive and sensory disorders. The authors provide a concise description of the mechanisms that underpin the wide variation in bile acid concentrations with feeding, fasting, diseases and therapy together with an outline of the structure, expression pattern and relative potency of ligands for the GPBA. Signalling pathways have yet to be fully explored but evidence exists for coupling to $G\alpha_s$ and $G\alpha_q$ whereas studies that examine coupling to β-arrestins are equivocal. GPBA also has roles in energy and glucose metabolism with some interesting links to changes in metabolism produced by bariatric surgery that invite further study. There is also evidence supporting roles for GPBA in liver, biliary and intestinal function as well as sensory transduction and as targets for neurosteroids in the brain. Clearly this is a promising area for future research. *Justin Ludeman and Martin Stone* (Ludeman and Stone, 2014) examine the post-translational modification of chemokine receptors by tyrosine sulfation and the effects on function. Although quite a number of GPCRs undergo tyrosine sulfation this is a relatively underappreciated area of GPCR research. Chemokine receptors are primarily expressed in leukocytes and activation is associated with adhesion and migration. In addition they may be involved in the metastasis of tumours and in invasion of cell by pathogens. These properties make chemokine receptors important targets for cancer and diseases such as HIV and malaria. The review describes studies in which sulfopeptides based on the N-terminus of chemokine receptors have been used to investigate chemokine recognition by sulfotyrosine residues. The studies show that sulfation of a receptor peptide can not only change the affinity of chemokine binding but also the relative affinity of a variety of ligands. In addition binding to receptor sulfopeptides can alter the oligomerisation state of the chemokines further influencing interaction with the receptor. Biophysical and structural studies using sulfopeptides and eventually intact receptors promise to reveal insights into the complex interactions between chemokines and their targets. *Morley Hollenberg and his colleagues* (Hollenberg *et al.*, 2014) explore the recent recognition that in addition to the endogenous tethered ligands for proteinase-activated receptors (PARs) and synthetic receptor-activating peptides there are peptide agonists and antagonists that display biased signalling. The review summarises the implications of these findings for the roles of PARs in inflammation. The discovery of PARs is concisely discussed emphasising the layers of selectivity associated with these receptors at the level of proteases, ligands and biased signalling. Early studies showed that mutations in the tethered ligand sequence altered Ca^{2+} and MAP kinase signalling in a biased manner leading to the identification of other biased ligands. Extension of these studies revealed that interaction with β-arrestin and internalisation equated to Ca^{2+} plus MAP kinase signalling whereas non-internalisation was associated with selective activation of MAP kinase signalling. Focussing on PAR2, the authors describe their recent work that shows that GB88, developed as a PAR2 antagonist, is a biased ligand that blocks Ca^{2+} signalling while simultaneously activating MAP kinases. Given that GB88 also has anti-inflammatory properties this creates a degree of optimism that biased signalling at PAR2 receptors may be converted to useful therapeutic ends.

Recent years have seen remarkable progress in GPCR research. There have been major strides in our understanding of agonist and antagonist bound, and most recently allosteric modulator and β-arrestin-bound, GPCR structures. One of the features reflected in these brief reviews is the growing interest in allosteric and biased ligand interactions with GPCRs. The stage is set for translation of these findings into novel therapeutics that impact some of the major disease conditions affecting society.

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