British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01679.x
www.brjpharmacol.org

Themed Section: Molecular Pharmacology of GPCRs

EDITORIAL

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Keywords
molecular pharmacology < A; general fields; GPCRs; other neuropeptides; gastrointestinal pharmacology; endocrine pharmacology; anti-inflammatory drugs < B; mediators; receptors; transporters; channels & ligands; drug receptor mechanisms; anticancer drugs; neuropharmacology < C; drug classes; apoptosis < D; functional systems GPCRs

Received
24 August 2011
Revised
31 August 2011
Accepted
2 September 2011

As our knowledge and understanding of the way in which GPCRs operate continues to grow rapidly, many new opportunities are emerging to develop novel therapeutic agents. This themed issue of the *British Journal of Pharmacology* contains a series of papers that cover recent developments and identify approaches that may help determine future directions. Many of these papers contain material that was presented at the 6th International Molecular Pharmacology of G Protein-Coupled Receptors meeting held at the Monash Institute of Pharmaceutical Sciences in Melbourne Australia in late 2010.

LINKED ARTICLES

This article is part of a themed section on the Molecular Pharmacology of G Protein-Coupled Receptors (GPCRs). To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2012.165.issue-6. To view the 2010 themed section on the same topic visit http://onlinelibrary.wiley.com/doi/10.1111/bph.2010.159.issue-5/issuetoc

This themed issue on GPCRs follows on from a previous issue (*Br J Pharmacol* (2010), 159: 983–1186) that stemmed from one of a series of meetings on the Molecular Pharmacology of G Protein-Coupled Receptors (MPGPCR). The latest meeting emphasized that new developments in the field of GPCRs have continued apace, and invitations to produce up to the minute reviews for the *British Journal of Pharmacology* on key areas of interest were received enthusiastically. The focus of the meetings is on recent discoveries and advances, and there are now more than 2 days of key presentations from leading researchers from around the world describing novel concepts in GPCR pharmacology and drug discovery. The major themes covered at the meeting included new technologies for the study of GPCRs, recent advances in the knowledge of agonist and antagonist-bound GPCR structures, the importance of membrane microdomains and protein complexes in GPCR signalling, ligand-directed signalling bias, allosteric modulation of receptors and GPCR signalling, regulation and structure/function relationships. The 7th MPGPCR meeting will be held on December 2012 in Melbourne.

While GPCRs are the most studied group of cell surface receptors and one of the most exploited in terms of successful therapeutic applications, recent advances in key facets of GPCR biology have great potential for translation into novel therapeutic agents. The paper by Paul Insel and colleagues (Insel *et al.*, 2012) takes a broad view and reminds us that many individual cell types may express >100 different GPCRs, many of which are not targeted as potential therapeutic targets. Cognate ligands for as many as 25% of known human GPCRs have yet to be identified and even when they are identified turn out to be rather unexpected molecules. In academic studies, microarrays are increasingly being used to

identify GPCR receptor expression patterns. Although many of the GPCRs identified are olfactory receptors that have often been assumed to be of little interest for drug development, this is probably not the case as recent evidence strongly suggests that many of these receptors can influence functional responses in a much more conventional manner. The authors go on to suggest ways in which the data obtained from GPCR expression studies can be approached in order to provide information that will be valuable for future drug development. It is clear that for many of the potential targets. there is a paucity of tools available to study them and effort should be put into development of validated antibodies, radioligands, agonists and antagonists, histological methods to determine localized sites of expression as well as 'biologicals' including peptides, proteins and nucleic acid derivatives.

Rob Leurs and colleagues (Scholten *et al*., 2012) show how a number of current concepts in GPCR research including computer-assisted modelling, allosteric interactions, functional selectivity (ligand-directed signalling bias) and receptor oligomerization have been brought together to study the modulation of chemokine receptors that are important targets for the treatment of a number of immune-related diseases. They begin with a concise overview of chemokines and their receptors with examples of their importance in inflammatory responses associated with many diseases. They go on to suggest that apparent redundancy in the chemokine receptor system where a single chemokine receptor will bind several ligands, and individual ligands may bind to several receptors may in fact reflect functional selectivity. Evidence is now accumulating that particular chemokines may display their own pattern of activation of signalling pathways, suggesting that different chemokines may play different roles. The complex mode of interaction of chemokines with their receptors is explained using the two-step model in which the core region of the chemokine binds to a site formed by the N-terminus and ECLs of the receptor followed by the interaction of the chemokine N-terminus with a second site formed by parts of the ECLs and TM domains, which leads to receptor activation. Interestingly, the binding or function of relatively large chemokines can often be disrupted by small MWt ligands that are increasingly being recognised as interacting with the receptor in an allosteric manner. The effects of these ligands are saturable and probe-dependent, and their potential as therapeutic agents is now being realized, and allosteric chemokine receptor antagonists acting at CCR5 and CXCR4 have promise for the treatment of HIV/AIDS. In addition, the authors describe the extensive screening programs for chemokine receptor antagonists that also serendipitously led to the discovery of small molecule agonists, some of which displayed biased signalling characteristics and may have therapeutic potential. They also include the rapidly expanding use of biologicals (monoclonal antibodies) as high affinity and potency ligands for chemokine receptors that can either directly block receptors or trigger indirect biological activity. Antibody fragments or nanobodies have now been developed that are highly potent antagonists of chemokine receptors. The authors go on to describe the impact of the recently solved CXCR4 receptor structure on structure-based drug design and its limitations. The review closes with an examination of the evidence for chemokine receptor oligomer formation and concludes that there is good evidence to suggest that oligomers are formed intracellularly to facilitate folding and transport to the cell surface but then may fall apart and re-form as homomers or as heteromers with other chemokine receptors or with other receptors such as opiate receptors. This may have implications for drug screening and development as the ligand recognition and regulatory properties may vary in heteromers. It is clear that chemokine receptors are GPCRs with considerable therapeutic potential that is beginning to be realised.

Michelle Halls (Halls, 2012) presents evidence that certain GPCRs form signalling complexes in the cell membrane termed signalosomes that display extraordinary receptor sensitivity. The formation of signalosomes allows compartmentalisation of signalling with second messengers acting in a specific and orchestrated manner. The concept is illustrated using the RXFP1 receptor, the cognate receptor for the hormone relaxin, that displays a complex signalling profile and is involved in a variety of physiological responses. Previous studies have shown that RXFP1 couples to at least three G-proteins and when activated causes increases in intracellular levels of cAMP. More recently, Halls and her coworkers have shown that RXFP1 expression induces a constitutively active and tightly regulated signalosome that consists of RXFP1 scaffolded to AC2 by AKAP79. The cAMP produced by the signalling scaffold is in turn regulated by PDE4D3 scaffolded to the receptor C-terminus by β-arrestin-2. The signalosome is quite distinct from the conventional signalling pathways and is sensitive to attomolar concentrations of relaxin. Interestingly, the application of higher concentrations of relaxin causes dissociation of the signalosome complex and cAMP generation via the conventional pathways. While the full physiological significance of this elegant signalling paradigm has yet to be demonstrated, it may provide the key to understanding how low circulating concentrations of relaxin are able to exert profound physiological effects.

Characteristically, Terry Kenakin (Kenakin, 2012) has produced a thought-provoking article on the inherently allosteric nature of GPCRs. He reminds us that GPCRs evolved to be highly flexible proteins where the binding of molecules at one site affects the binding of other molecules in other parts of the receptor, and that this paradigm can explain many recently described behaviours such as allosteric ligands (the nomenclature for allosteric and orthosteric sites may be a misnomer), receptor oligomerisation and signalling bias. A model of functional allostery can be used to provide parameters that can describe the activity of any ligand acting at a GPCR. These types of quantitative measurements are fundamental in the characterization of the properties of ligands and essential for the development of pharmacological profiles that underpin successful drug development.

Gerda Breitwieser and colleagues (Cavanaugh *et al*., 2012) examine the important and unusual calcium-sensing receptors (CaSR), GPCRs that respond to a wide variety of ligands and can be regarded as metabolic sensors. CaSR have several endogenous allosteric modulators and were the first GPCR for which a clinically useful allosteric modulator (cinacalcet) was developed. Cinacalcet is a potent calcimimetic that reduces PTH secretion and is likely the precursor of many drugs with potential to modulate CaSR function. Although not so advanced, research into calcilytics that cause the parathyroid gland to sense an apparent fall in plasma Ca^{2+} has also pro-

vided some promising leads. It is hoped that calcilytics will prove useful for the treatment of osteoporosis and in patients with gain of function CaSR mutations or cancers characterized by increased expression of CaSR. One interesting property of allosteric modulators of CaSR is their capacity to act as pharmacological chaperones. The calcimimetic NPS R-568 stabilizes CaSR and causes increases in net and plasma membrane levels of the receptor. In contrast, the calcilytic NPS 2143 has the opposite effect. This property has potential for the treatment of diseases associated with mutations of the CaSR of which there are many. Loss of function mutants can be rescued by treatment with calcimimetics, whereas gain of function mutants can be 'normalized' by treatment with calcilytics.

Marc Laburthe and Thierry Voisin (Laburthe and Voisin, 2012) outline the potential of the orexin OX_1R as a target for the treatment of colon cancer. While OX_1R are not expressed in normal colonic epithelial cells, they appear in primary colorectal tumours and in metastases. Human colon cancer cells in culture respond to orexins with apoptosis as do xenografts in nude mice. Even cells that are resistant to 5-FU respond to orexins, suggesting that OX_1R agonists may be useful for the treatment of colon cancer. The apoptotic mechanism involves coupling of OX_1R to Gq but not the activation of PLC but rather the released βγ subunits that activate Src-like tyrosine kinases. These in turn phosphorylate tyrosines located in the immunoreceptor tyrosine-based inhibitory and switch motifs of the OX_1R that recruit and activate SHP-2, a trigger for apoptosis. Mutation of either of these tyrosines in OX_1R abolishes apoptosis mediated by activation of this receptor. The authors suggest that this mechanism represents a new paradigm of GPCR signalling.

Mark Wheatley and colleagues (Wheatley *et al.*, 2012) focus on the role of the extracellular loops of GPCRs in signalling. While GPCRs display a common architecture comprising seven transmembrane (TM) spanning helices linked by alternating extracellular loops (ECLs) and intracellular loops, they do display remarkable diversity in ligand binding and function. For family A and B GPCRs, the binding site for biogenic amines usually lies within the TM helices and for peptides in the N-terminal region, whereas many signalling proteins bind to receptor domains associated with the intracellular loops and C-terminus. Given this scenario, a role for ECLs might seem somewhat unlikely, but there are now many examples that demonstrate their importance in various aspects of GPCR function. The authors outline experimental approaches that are available for the study of ECLs, highlighting their inherent flexibility and the difficulty of using modelling approaches even when knowledge of the structure is available. However, a variety of indirect methods have yielded useful data on key residues in the ECLs. Comparison of ECL2 from a number of GPCRs reveals a variety of different functions from a 'lid' structure in rhodopsin to a highly structured α-helix in β-adrenoceptors and adenosine A_{2A} receptors that in the latter case contribute to the ligand binding pocket. Another common feature of many GPCRs is the disulphide bond between Cys residues in ECL2 and the top of TM3, which is necessary for preservation of structural integrity. Removal often results in a marked reduction in ligand affinity. There is no single function that is associated with ECL2 in the majority of GPCRs and mutating residues in this region can result in a variety of effects including alteration of subtype selectivity of ligands, conversion of an antagonist to an agonist or modulation of agonist-induced receptor internalization. ECL2 has also been identified as a common site of interaction with allosteric modulators. Fewer studies have been carried out with ECL1 and ECL3, but there is also evidence that they can influence function. The authors go on to describe the contributions to our understanding made by the crystal structures of agonist and antagonist-bound GPCRs but add a note of caution reminding us that these structures contain modifications to impair the flexibility of the GPCR to improve thermostability and facilitate crystallization. The modifications may therefore limit or prevent conformational changes in ECL. The article concludes by describing the early progress with describing the role of ECLs in the function of family B GPCRs, which is clearly at a much earlier stage of understanding. However, given their ability to activate multiple signalling cascades and to exhibit ligand-biased signalling, it is likely that ECLs will also play an important role in the signalling of family B GPCRs.

Mac Christie and Vu Dang (Dang and Christie, 2012) explore the mechanisms of analgesia and tolerance to opioids. They describe the differential signalling efficacies of agonists acting at μ-opioid receptors for G-protein coupling, desensitization and endocytosis and the involvement of these processes in the development of opioid tolerance with a view to developing opioids that are analgesic but do not display tolerance. They provide evidence that greater opioid tolerance develops to agonists with low (morphine and related alkaloids) versus high (enkephalin-related peptides, sufentanyl, etorphine, etc.) differential efficacy for endocytosis. The effects on morphine tolerance of genetically ablating trafficking proteins (β-arrestin-2 k.o.) or constructing μ-opioid receptor mutants that recycle efficiently with morphine both strongly suggest that while μ-opioid receptor desensitization, endocytosis and recycling are important for the development of tolerance, some of the assumptions underpinning explanations of how this works are incomplete or incorrect. For instance, β-arrestin-2 binding and endocytosis are not necessary to produce desensitization of μ-opioid receptors and other mechanisms can very efficiently desensitize the receptor. In addition, μ-opioid receptors dephosphorylate and resensitize as or more efficiently when endocytosis is blocked, regardless of the agonist used. There is now evidence that suggests that opioids may be developed that stabilize μ-opioid receptors in conformations that signal to G-proteins but cannot desensitize, and that this will limit tolerance.

Steve Ferguson and colleagues (Magalhaes *et al.*, 2012) examine the interactions of GPCRs with a variety of other proteins that regulate their processing in the endoplasmic reticulum, trafficking to the cell surface, localization to membrane microdomains, endocytosis and coupling to G-protein-independent signalling pathways. Many GPCRs not only couple to multiple G-proteins to activate a variety of signalling pathways but also act as scaffolds for other proteins that can either modulate this signalling or trigger their own signalling cascades. One of the better characterized GPCR interacting proteins are the β-arrestins that were identified for their role in receptor desensitization. G-protein receptor kinase phosphorylated receptors recruit β-arrestin, which facilitates uncoupling from G-proteins and promotes receptor endocytosis. More recently, however, it has been recognized

that β-arrestins also scaffold a wide variety of kinases, small GTPases, guanine nucleotide exchange factors, phosphodiesterases and transcription factors. The review goes on to describe concisely the interactions of many interacting proteins such as receptor activity-modifying proteins; regulators of G-protein signalling; GPCR -associated sorting proteins; homer proteins; small G-proteins; and PDZ proteins on the regulation and signalling profile of GPCRs. A better understanding of these complex interactions has the potential to lead to development of novel drugs that channel GPCR activity along particular pathways to achieve clinically useful outcomes without undesirable side effects.

Recent years have seen remarkable progress in GPCR research. There have been major strides in our understanding of both agonist and antagonist-bound GPCR structure, the pleiotropic nature of signalling and how this is influenced by ligands acting at orthosteric and allosteric binding sites. The importance of scaffolding proteins in the regulation and signalling properties of receptors has also been clearly established. 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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