Elucidating GPCR Functional Selectivity and Biased Agonism: Novel Opportunities for Drug Development

DiscoveRx and GDR-3545 European GPCR Technology Symposium

Place & Time: The Hauser Forum, 3 Charles Babbage Road Cambridge, CB3 0GT – Tuesday 12th May 2015 | 09:00 AM – 4:30 PM

Chairman: Ralf Jockers, Research director, Institut Cochin, INSERM U1016
Anthony P. Davenport, Reader in Cardiovascular Pharmacology, University of Cambridge, UK

Agenda

8:30 – 9:00AM	**Registration and Coffee**
9:00 – 9:10AM	**DiscoveRx Welcome and Opening Remarks** **Anthony P. Davenport, Reader in Cardiovascular Pharmacology, University of Cambridge, UK**
9:10 - 9:40 AM	**Jonathan Ellery, Principal Scientist, Takeda Cambridge Ltd.** **The Challenge of Biased Agonism in Drug Discovery** GPCRs are one of the key target classes for which therapeutics have been generated. In certain pathological conditions the activation of a GPCR can have a beneficial effect for patients. The activation of GPCRs, however, leads to multiple signal transduction events and it has been found clinically that in certain cases the activation of particular signalling cascades is associated with clinical benefit whilst the activation of other signalling cascades is associated with undesirable clinical side effects. There is now a growing body of evidence that compounds can be generated which appear to preferentially agonise specific signalling pathways over others. These 'biased' agonists hold the potential to enable therapeutic activation of a GPCR whilst avoiding unwanted side effects. In this talk I will discuss the challenges that are faced in identifying and initially developing such compounds by a Pharmaceutical Company.
9:40 – 10:10 AM	**Patrick Scheerer, Research Group Leader, Charité - University Medicine Berlin** **A common GPCR-binding interface for G protein and arrestin** G-protein-coupled receptors (GPCRs) transmit extracellular signals to activate intracellular heterotrimeric G proteins ($G\alpha\beta\gamma$) and arrestins. For G protein signalling, the $G\alpha$ C-terminus ($G\alpha CT$) binds to a cytoplasmic crevice of the receptor that opens upon activation. Surprisingly, a consensus motif is shared among $G\alpha CT$ from the G_i/G_t family and the 'finger loop' region (ArrFL1–4) of all four arrestins. Our structural and spectroscopic data reflect both the common receptor-binding interface and the divergent biological functions of G proteins and arrestins.

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10:10 – 10:40 AM	**Bernard Mouillac, Research Director, Institut de Génomique Fonctionnelle, Montpellier** **Structural insights into biased agonism of the antidiuretic hormone vasopressin V2 receptor subtype** Recent studies show that some G protein-coupled receptors (GPCRs) signal through both G protein and arrestin pathways in a ligand-dependent manner. Ligands that direct signalling through a specific pathway are known as biased ligands or functionally-selective ligands. The arginine-vasopressin type 2 receptor (V2R), a prototypical peptide-activated GPCR, is an ideal model system to investigate the structural basis of biased signalling. Although the native hormone arginine-vasopressin leads to activation of both the stimulatory Gs protein for adenylyl cyclase and arrestin pathways, synthetic ligands exhibit highly biased signalling through either Gs alone or arrestin alone. We used purified V2R stabilized in neutral amphipols and developed fluorescence-based assays to investigate the structural basis of biased signalling for the V2R. Our studies demonstrate that the G_s-biased agonist stabilize a conformation that is distinct from that stabilized by the arrestin-biased ligand. These results provide unique insights into the structural mechanisms of GPCR activation that may help the design of novel functionally-selective drugs.
10:40 – 11:00 AM	**Coffee**
11:00 – 11:30 AM	**Ralf Jockers, Research Director, Institut Cochin, INSERM U1016** **Functional characterization of biased GPCR variants associated with major diseases – new challenges** Recent large-scale exon sequencing studies revealed the high abundance of rare GPCR mutations in the human population. Many of these rare mutations are suspected to contribute to the risk of common diseases and inter-individual and ethnic differences in drug action. We recently identified 40 rare variants of the melatonin MT2 receptor, a receptor known to regulate circadian rhythms in mammals, that are associated with increased type 2 diabetes (T2D) risk. Given that GPCRs can engage multiple signalling pathways, the objective of this study was to strengthen the functional link between the MT2 receptor and T2D risk by assessing the effects of each mutation on the activation of different pathways and generating a “signaling profile”, thus providing a new insight into possible treatments for T2D harbouring specific MT2 mutations. In conclusion, functional profiling of a large number of GPCR mutants will be necessary to identify those mutants with modified function. Defining the functional defects in carriers of rare GPCR mutations will help to provide refined and personalized therapies to these patients in the future.

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11:30 – 12:00 PM	**David R. Greaves, Professor of Inflammation Biology, University of Oxford** **Chemical Biology Approaches to Identify Novel Agonists Active at the Cannabinoid CB2 Receptor and Study Macrophage Chemotaxis** Activation of the cannabinoid CB2 receptor (CB2) has been reported to have anti-inflammatory effects *in vivo* and induce directed immune cell migration *in vitro*. Initial experiments using the CB2 selective agonist HU-308 were hampered by the extreme hydrophobicity of this tool compound. We therefore used ligand-based virtual screening combined with DiscoveRx cAMP PathHunter kits to identify novel agonists active at the human CB2 receptor. Subsequent medicinal chemistry optimization studies and testing in DiscoveRx GPCR Max and Orphan Max led to the identification of a new class of selective CB2R agonists. Several examples showed high levels of activity (EC_{50}<200 nM) and binding affinity (Ki<200 nM) for CB2, and no detectable activity at CB1. Using a novel real-time chemotaxis assay and a panel of chemically diverse and widely used CB2 agonists, we set out to examine whether CB2 modulates primary murine macrophage chemotaxis. We report that of 12 agonists tested, only JWH133, HU308, L-759,656 and L-759,633 acted as macrophage chemoattractants. Surprisingly, neither pharmacological inhibition nor genetic ablation of CB2 had any effect on CB2 agonist-induced macrophage chemotaxis. As chemotaxis was pertussis toxin sensitive in both WT and CB2R-/- macrophages, we concluded that a non-CB1/CB2, $G_{i}/_{o}$-coupled GPCR must be responsible for CB2 agonist-induced macrophage migration. The obvious candidate receptors GPR18 and GPR55 could not mediate JWH133 or HU308-induced cytoskeletal rearrangement or JWH133-induced β-arrestin recruitment in cells transfected with either receptor, demonstrating that neither are the unidentified GPCR. Taken together our results conclusively demonstrate that CB2 is not a chemoattractant receptor for murine macrophages. Furthermore, we show for the first time that JWH133, HU308, L-759,656 and L-759,633 have off-target effects of functional consequence in primary cells and we believe that our findings have wide ranging implications for the entire cannabinoid field.
12:00 – 12:30PM	**Andrew Green, Senior Drug Discovery Specialist, DiscoveRx Corporation Ltd.** **Compound profiling and screening at the MRGPRX2 Receptor linked to pseudo-allergic drug reactions** Recent data and reports describe a function of the MRGPRX2 receptor. Its expression profile and apparent promiscuous pharmacology suggest a function in mast cells and as a primary effector mechanism in pseudo allergic reactions. New data will be presented comparing DiscoveRx assays for MRGPRX2 receptor signaling and discussion around the importance of revealing any compound liabilities.
12:30 – 2:00PM	**Lunch & Networking**

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2:00 – 2:30PM

Anthony P. Davenport, Reader in Cardiovascular Pharmacology, University of Cambridge

From chemistry to clinic: apelin and biased signalling in the cardiovascular system

Apelin (Pyr1apelin13) binds a single G-protein coupled receptor and has an emerging role in human cardiovascular homeostasis. Apelin causes vasodilation and it is the most potent inotrope discovered to date in isolated human heart. This signalling pathway is down-regulated in cardiovascular disease where short term systemic infusion of apelin to replace missing peptide produces beneficial vasodilation and an increase in cardiac output. These results suggest synthetic agonists would be of therapeutic benefit. A limitation of many agonists acting at GPCRs is that, after signalling via G-proteins to produce a physiological action, the target receptor is internalised via the β-arrestin pathway, limiting the beneficial physiological action.

We hypothesize that a 'biased' agonist preferentially activating the G-protein pathways rather than β-arrestin, will reduce desensitization, produce a more robust response, continued vasodilation and beneficial inotropy.

Using computational chemistry, we have designed and identified a 'biased' apelin agonist, MM07 and shown that it has comparable activity to the native peptide in G-protein mediated pathways *in vitro* but lower β-arrestin and internalization activity. MM07 caused rapid and significant peripheral arterial dilation in the human forearm. The magnitude of the response was significantly greater than the endogenous peptide. Importantly, there was no evidence of desensitization when repeated infusions were given. In the human hand vein, MM07 significantly reversed an established noradrenaline pre-constriction. In anaesthetized rats intravenous boluses of MM07 caused a significant increase in cardiac output, without evidence of positive chronotropy with a magnitude greater than that of the native peptide. Daily intra-peritoneal injects of MM07 prevented the development of pulmonary arterial hypertension in the monocrotaline rat model.

The results suggest MM07 functions as biased agonist *in vitro* and is more effective *in vivo* than apelin in increasing cardiac output and vasodilation. Biased apelin agonists may have a therapeutic advantage in the treatment of conditions such as pulmonary arterial hypertension.

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2:30 – 3:00PM

Franck Vandermoere PhD, CNRS permanent researcher, Institute of Functional Genomics (Montpellier, France)

Phosphoproteomics of 5-HT2A/mGlu2 heteromers: toward new insights into the mechanism of action of hallucinogens and antipsychotics

The serotonin 5-HT2A receptor is the primary target of psychedelic hallucinogens such as LSD, mescaline and psilocybin (agonists), which reproduce some of the core symptoms of schizophrenia and of second-generation antipsychotics such as clozapine, olanzapine and risperidone (antagonists or inverse agonists). Recent findings demonstrate that 5-HT2A receptors form heteromers with metabotropic glutamate mGlu2 receptors, another target of last-generation antipsychotics (agonists or positive allosteric modulators). The association of both receptors has profound consequences on their pharmacology and signal transduction properties as well as on the behavioural effects of drugs that bind to either 5-HT2A receptors or mGlu2 receptors. For instance, 5-HT2A receptor/mGlu2 heteromer formation is essential for the expression of psychotropic-like effects of hallucinogens and imbalanced activity and coupling properties of 5-HT2A and mGlu2 receptors within the heterocomplex might be one of the molecular substrates for a susceptibility to schizophrenia. To get further insight into the mechanism of action of drugs acting at 5-HT2A/mGlu2 heteromers, we explored their impact upon the phosphorylation pattern of each receptor by high-resolution mass spectrometry. We show that hallucinogenic 5-HT2A receptor agonists (LSD, DOI) but not non-hallucinogenic 5-HT2A receptor agonists promote 5-HT2A receptor phosphorylation at Ser280 located in the i3 loop, a region important for receptor desensitization, both in HEK-293 cells and in mice prefrontal cortex. Correspondingly, Ser280 phosphorylation was responsible for the lower capacity of hallucinogens to promote receptor desensitization and internalization, compared with non-hallucinogenic agonists. Conversely, several phosphorylated residues were identified in the C-terminal domain of mGlu2 receptors co-expressed with 5-HT2A receptors in HEK-293 cells. Glutamate treatment increased the phosphorylation state of some of these residues, an effect prevented by the co-application of the synthetic hallucinogen DOI, which alone did not affect mGlu2 phosphorylation. Collectively, these findings reveal novel molecular substrates that might underlie the behavioural effects of drugs acting at each subunit of 5-HT2A/mGlu2 heteromers.

3:00 – 3:30PM

Stéphane A. Laporte, Professor, McGill University

Angiotensin type 1 receptor biased signalling and trafficking

G protein-coupled receptors (GPCRs) can selectively engage specific signalling pathways over others (e.g. biased signalling). Such mode of receptor-directed signalling like in the case of the angiotensin II type 1 receptor (AT1R) can be achieved with specific ligands. Using diverse bioluminescence resonance energy transfer (BRET) sensors we explore the pluridimensionality of signalling and trafficking of AT1R. Our findings underscore differential modes of pathway-specific regulation of AT1R signalling, which include ligand- and receptor dimerization-mediated allosteric control of receptors' responses. The use of new biosensors for vetting receptor endocytosis also unveils the propensities of AT1R ligands to differentially control receptor intracellular trafficking, and allowed us identifying new pharmacological regulators of GPCRs internalization. Our findings identify new mechanisms and means for directing and regulating AT1R signalling, which could help improve cardiovascular drug efficacies. (Supported by the Canadian Institutes of Health Research).

3:30 – 4:00PM

Coffee

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4:00 – 4:30 PM	**Alastair Brown, Head of Pharmacology, Heptares Therapeutics Limited.** **Opportunities and challenges facing biased signalling – a structural and drug discovery perspective** The discovery of biased signalling at G protein-coupled receptors (GPCRs) opens up a wealth of possibilities in the design of new, functionally selective drugs for established targets. However progress in developing effective agents harnessing this pharmacological mechanism has been hampered by the lack of detailed structural information. Emerging structural, biophysical and computational data is beginning to clarify the molecular mechanisms underlying ligand-receptor interactions and conformational changes associated with biased signalling. Heptares' StaR technology allows the isolation of GPCRs in thermally-stabilised and biologically-relevant conformations. Unlike wild-type GPCRs, StaR proteins are compatible with biophysical screening methods and X-ray crystallography, which enables the use of complementary drug discovery techniques such as fragment-based lead generation and structure-based drug design. This discussion will focus on our emerging structural understanding of biased signalling and discuss some of the key aspects of biased signalling that remain to be investigated in order to realise the therapeutic potential of this pharmacological mechanism.
4:30 PM	**Closing Remarks** **Ralf Jockers, Research Director, Institut Cochin, INSERM**

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