Institution: University of Nottingham

Unit of Assessment: 5 - School of Life Sciences

Title of case study:

Discovery of GPCR 'biased signalling' as a novel pharmacological concept, enabling development of pathway-selective therapeutic drugs.

1. Summary of the impact

Members of the Pharmacology Research Group identified hitherto unknown properties of G protein Coupled Receptors (GPCRs): that ligands can signal differentially through both G-protein-coupled and β-arrestin pathways. This led to the concept of GPCR 'biased signalling' and development of fluorescent reporters to quantify β-arrestin signalling. These discoveries have been adopted widely by the pharmaceutical industry, attracting R&D investment and collaborative research funding, to drive discovery of new drugs operating through 'biased signalling'. The commercial opportunity has also been exploited by screening reagent providers and contract screening organisations. These discoveries will ultimately produce better drugs to treat GPCR-based diseases to improve human health.

2. Underpinning research

At the time of the original underpinning research, the basic concept in receptor pharmacology was that agonists at a particular G protein-coupled-receptor (GPCR) had a particular potency and efficacy for stimulating a response that was always via a specific G protein-mediated signalling pathway. The adenosine A_1-receptor is a GPCR that normally couples to Gi-proteins to mediate inhibition of intracellular cyclic AMP formation. However, work from the laboratory of Professor Steve Hill in the Pharmacology group, funded by the Wellcome Trust[7,8] provided early evidence that specific adenosine A_1-agonists could direct signalling to other G-protein (e.g. Gs, Gi) signalling pathways[1,2]. However, the concept of signalling pathway-dependent responses, or 'biased signalling' was taken to a new level by research on the β2-adrenoceptor performed by Professor Jillian Baker, also in the Pharmacology group, during her Wellcome Trust-funded[9] work (2000-2003) undertaken under the supervision of Professor Hill. This work showed that the prototypical β-blocker, propranolol, acted as an inverse agonist (antagonist) at the β2-adrenoceptor on Gs-mediated increases in cyclic AMP accumulation but it acts as an agonist of G-protein-independent β-arrestin signalling at the same receptor, signalling via MAP kinase to alter gene expression. The key discovery of the dual agonist / inverse agonist action of propranolol at β2-adrenoceptors was first published as a poster abstract in 2002 (Baker, Hall and Hill (2002) *Br. J. Pharmacol.* **136:** 5P) and then as a full paper in 2003[3]. The observation of 'dual efficacy' agonists acting via different, G-protein dependent and β-arrestin signalling pathways was independently confirmed in concurrent research that was reported in 2003 by the research groups of M Bouvier for β-adrenoceptors and RJ Lefkowitz for angiotensin II receptors, thus jointly establishing for the first time the principle of 'biased signalling' or 'biased agonism' in GPCR pharmacology. The group in Nottingham has since contributed to the development of new assay formats to investigate the coupling of GPCRs to β-arrestin pathways[4,5].

Prof Baker's expertise in β-adrenoceptor pharmacology, further funded by a Wellcome Trust Clinician Scientist Fellowship[10], was also applied to the elucidation of a high resolution β-adrenoceptor crystal structure[6] – a significant milestone for structure-based GPCR drug design. This subsequently resulted in Wellcome Trust funding[11] of £2.88 million (2008-2012) to Prof Baker and Prof Hill, with Prof Peter Fischer and Prof Barrie Kellam (School of Pharmacy) to develop new β1-selective β-blockers for treatment of cardiac disease with concurrent respiratory disease.

3. References to the research

Key Publications (UoN authors in bold, key author(s) underlined)

1. **Cordeaux Y**, **Briddon SJ**, **Megson AE**, **McDonnell J**, **Dickenson JM**, **Hill SJ** (2000). Influence of receptor number on functional responses elicited by agonists acting at the human

adenosine A(1) receptor: Evidence for signalling pathway-dependent changes in agonist potency and relative intrinsic activity. Mol. Pharmacol. 58, 1075-1084. DOI: 10.1124/mol.58.5.1075

2. **Cordeaux Y**, Ijzerman AP, **Hill SJ** (2004). Coupling of the Human A1 Adenosine Receptor to Different Heterotrimeric G-Proteins; Evidence for Agonist-Specific G-protein Activation. Br. J. Pharmacol 143: 705-714. DOI: 10.1038/sj.bjp.0705925
3. **Baker JG**, **Hall IP**, **Hill SJ**. (2003) Agonist and inverse agonist actions of "β-blockers" at the human β2-adrenoceptor provide evidence for agonist-directed signalling. Mol. Pharmacol 64: 1357-1369. DOI: 10.1124/mol.64.6.1357
4. **Carter AA**, **Hill SJ** (2005) Characterization of isoprenaline- and salmeterol-stimulated interactions between beta2-adrenoceptors and beta-arrestin 2 using beta-galactosidase complementation in C2C12 cells. J Pharmacol Exp Ther 315: 839-48. DOI: 10.1124/jpet.105.088914.
5. **Kilpatrick LE**, **Briddon SJ**, **Hill SJ**, **Holliday ND** (2010). Quantitative analysis of neuropeptide Y receptor association with β-arrestin2 measured by bimolecular fluorescence complementation. Br J Pharmacol 160: 892-906. DOI: 10.1111/j.1476-5381.2010.00676.x.
6. Warne T, Serrano-Vega MJ, **Baker JG**, Moukhametzianov R, Edwards PC, Henderson R, Leslie AG, Tate CG, Schertler GF. (2008) Structure of a β1-adrenergic G-protein coupled receptor. Nature 454: 486-491. DOI: 10.1038/nature07101

Key Research Grants Awarded

7. **Hill SJ**: Molecular mechanisms underlying the coupling of adenosine A1-receptors to phospholipase C. Wellcome Trust (1996-2002); **£672,863**
8. **Hill SJ**, **Kellam B**: The pharmacological characteristics of the human A1-adenosine receptor at the single molecular level. Wellcome Trust (2002-2005); **£226,310**
9. **Baker JG:** Wellcome Trust Clinical Training Fellowship (2000 – 2003), supervised by **Prof Ian Hall** and **Prof SJ Hill**
10. **Baker JG:** Molecular mechanisms underlying the agonist and antagonist effects of β-adrenoceptor ligands at the human β1- and β2-adrenoceptors. Wellcome Trust Clinician Scientist Fellowship (2004-2010); **£601,728**
11. **Baker JG**, **Hill SJ**, **Fischer PM**, **Kellam B**: Development of highly-selective β1-adrenoceptor antagonists for therapeutic application in patients with concomitant cardiovascular and respiratory disorders. Wellcome Trust Seeding Drug Discovery Initiative (2008-2012); **£2,880,146**

4. Details of the impact

Impact 1: Worldwide Pharmaceutical Industry

As a consequence of the underpinning research by Professors Baker and Hill, and others, in 2003 that identified "dual efficacy" ligands at different GPCRs, the novel pharmacological concept – that drugs acting at the same GPCR could *selectively* influence different downstream signalling pathways – became accepted worldwide by the academic and pharmaceutical communities. This led to the major pharmaceutical companies screening compounds in parallel for G-protein mediated and G-protein independent signalling[A], in particular via β-arrestin proteins, in order to identify pathway-selective GPCR drug candidates. It also led to the establishment of speciality 'biased ligand' drug discovery companies, e.g. Trevena Inc (www.trevenainc.com) founded in 2008, seeking biased ligands to avoid signalling pathways that lead to detrimental side effects[B]. Indeed, the first such 'pathway-biased' ligands have reached clinical trials (SM DeWire & JD Violin (2011) *Circ Res* 109, 205-16).

Impact 2: Commercial Development

The demand for GPCR assays that can be used to assess compound activity at different G-protein- and β-arrestin-dependent signalling pathways has generated a new market for companies to develop cell lines, reagents and assay systems to facilitate this new mode of screening for drugs

and compounds that selectively signal through the β-arrestin pathway. Assay platforms have been developed by DiscoveRx (β-arrestin PathHunter™)[D], Molecular Devices (TransFluor™)[E] and Life Technologies (Tango™)[F]. In 2005, Prof Hill's group contributed to characterisation of the GPCR-β-arrestin assay of Applied Biosystems that was based on galactosidase complementation. This was later developed into the GPCR-β-arrestin-PathHunter screening platform[5] by DiscoveRx using a different-sized galactosidase fragment. DiscoveRx launched the PathHunter β-arrestin assay technology and a range of β-arrestin-coupled GPCR cell lines, mid-2006. Their β-arrestin-coupled GPCR cell line catalogue was expanded over the following 2-3 years, and the screening technology was subsequently partnered in 2009 as a 3-way alliance[C] with MRC Technology and GSK to identify natural substances as ligands for orphan GPCRs. In addition, contract research organisations such as BioFocus[F] began to provide β-arrestin screening services using DiscoveRx assays (introduced from 2009).

The G-protein-independent GPCR functional assay screening technology market (specifically including β-arrestin assays) had already reached 11.5% of all GPCR functional screening assays in 2008 and continued to increase over subsequent years, with DiscoveRx already taking 26% of the market in 2008 (HTS-Tec, 2008)[G]. In 2010, the cell-based screening consumables market (of which β-arrestin reagents are a part) was estimated to be $405million, with a compound annual growth rate of 6% (HTS-Tec, 2009)[G]. DiscoveRx had a market share of ~ 3.2% (~$13million) of this worldwide cell-based screening market in 2008 – of which sales of GPCR β-arrestin screening reagents are a significant proportion (HTS-Tec, 2009)[G]. DiscoveRx (UK) reported £4.2 million European sales revenue in 2011, an increase of nearly 40% over the previous year[C]. This indicates the impact of G-protein independent GPCR signalling on the drug discovery and compound screening market in the period from the original discovery of biased signalling in 2003, through the launch of the first β-arrestin screening reagents in 2006, to widespread adoption of the technology by the pharma industry from 2007-2008 onwards.

Impact 3: Healthcare Benefits

Drugs that operate specifically through biased signalling pathways have been avidly sought by the pharma industry (e.g. Flordellis CS (2012): *Curr Pharm Des.* **18**:145-60). As examples, Trevena[B] have: 1) an angiotensin II receptor β-arrestin-biased ligand in Phase II clinical development for heart failure (http://www.clinicaltrials.gov; study identifiers: NCT01187836, NCT01444872) a condition affecting an estimated 23 million people worldwide and for which the current drug market was estimated at $11.2 billion in 2010 and predicted to grow to $18.6 billion by 2016)[H]; and 2) a G-protein-dependent biased ligand for the mu-opioid receptor in Phase I for acute post-operative pain (http://www.clinicaltrials.gov, study identifier: NCT01514578), addressing an opioid analgesic market predicted to reach $17 billion by 2015[I]. The company also has a number of other projects in preclinical development and lead optimisation. Thus, arguably, patients in the Phase II trial are already beginning to benefit from the first of these biased signalling drugs. With the usual timeframe for drug discovery to new drug regulatory approval being 10 – 15 years, the majority of new biased ligand drugs are likely to have an impact on human health within the next 5 - 10 years.

Impact Beneficiaries

The principal beneficiaries of the impact of this research have been the global Pharma and Biotech drug discovery industry (e.g. GSK, Novartis, AZ, Heptares), reagent providers to that industry (e.g. DiscoveRx, Molecular Devices, Perkin-Elmer, Life Technologies) and contract research organisations providing compound screening services (e.g. BioFocus). For the latter two categories, the direct benefits have been increases in revenue from the enhanced range of products and/or services they have provided as a consequence. For the pharmaceutical and biotech industry the benefits have been an improved likelihood of successful drug development.

5. Sources to corroborate the impact

A. Corroborative statement from Novartis Horsham Research Centre, Novartis (UK) about the importance of biased signalling to Novartis held on file and available on request
B. Corroboration of Trevena Inc foundation and goal to develop GPCR biased ligands: http://www.trevenainc.com/about.php; and drug development pipeline: http://www.trevenainc.com/pipeline.php

C. http://www.discoverx.com/technologies-platforms/enzyme-fragment-complementation-technology/pathhunter-efc-cell-based-assay-platform/protein-protein-interactions/gpcrs-b-arrestin
For the DiscoveRx initiative with MRCT and GSK: http://www.mrctechnology.org/discoverx-gsk-mrct-collaborate-to-de-orphanise-gpcrs/
For news item on DiscoveRx company turnover: http://www.birminghampost.co.uk/news/local-news/science-park-based-discoverx-reports-surge-3920972; pdfs of press releases held on file and available on request
D. http://www.moleculardevices.com/Products/Assay-Kits/GPCRs/Transfluor.html
E. http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Drug-Discovery/Target-and-Lead-Identification-and-Validation/g-protein_coupled_html/GPCR-Cell-Based-Assays/Tango.html
F. Joint BioFocus-DiscoveRx press announcement: http://www.newswiretoday.com/news/58829/;
G. HTS-Tec reports: GPCR Screening & Profiling Trends (2008); Cellular Assay Reagents Trends (2009), held on file and available on request
H. Corroboration of Congestive Heart Failure patient and drug market estimates:
http://www.marketresearch.com/Kalorama-Information-v767/Congestive-Heart-Failure-Worldwide-Drug-729795/
http://www.bccresearch.com/report/congestive-heart-failure-drugs-treatment-phm102a.html
I. Corroboration of Post-operative Analgesia drug market estimates:
http://www.researchandmarkets.com/reports/1417852/the_global_pain_therapeutics_market_4th_edition