Highlights from the Symposium of the Society for Medicines Research, held on March 7, 2002, in Horsham, United Kingdom.

Research Strategies for Orphan G-Protein-Coupled Receptors

by Ian Morris and Robert Williams

Summary

The Society for Medicines Research meeting on orphan G-protein-coupled receptors (GPCRs) was held on March 7, 2002 at the Novartis Research Centre in Horsham, United Kingdom. The dynamic and highly competitive field of GPCR research was the focus of this SMR meeting, featuring speakers from Pharmagene, Manchester University, GlaxoSmithKline, The Royal Danish School of Pharmacy, Pfizer, AstraZeneca, Merck and Synaptic. The meeting attracted a large and enthusiastic audience interested in the research efforts of leading international research teams in the area of GPCR research, whose immediate aim is to evolve the novel molecular targets into lead discovery programs.

In recent years, research activity surrounding the G-protein-coupled receptors (GPCRs) has accelerated greatly. For instance, bibliometric analysis of hits for GPCRs on the database Pub Med shows that more than five times as many publications on GPCRs appeared in 2001 than in 1997. This activity has arisen as a direct result of the phenomenal worldwide effort focused on sequencing of the human genome, a task that has recently been completed. Bioinformatic analysis of genome sequence information has led to the identification of numerous GPCRs, many of which have been termed "orphan receptors," that is, receptors that do not possess a recognized ligand. First estimates suggested that there may be thousands of unpaired GPCRs, although more recently this figure has been revised downward to less than 400. It is perhaps no surprise that the pharmaceutical industry has invested heavily in this area in view of the established success of drugs acting as agonists or antagonists of GPCRs for catecholamines, histamine, 5-HT and so forth, in a wide range of therapeutic areas.

At the Society for Medicines Research (SMR) meeting held on March 7, 2002, at the Novartis Research Centre in Horsham, United Kingdom, Mark Fidock (Pfizer, U.K.) presented an analysis of recent studies identifying ligands for novel GPCRs showing the speed of appearance of studies that have deorphanized receptors (Fig. 1). Thus, the orphan GPCRs may be viewed as a Pandora's box, with the promise of major drugs to be discovered, providing fortune is on the side of the potential adoptive parent. This dynamic and highly competitive field of GPCR research was the focus of the SMR meeting. It attracted a large and enthusiastic audience interested in the research efforts of leading international research teams in this area whose immediate aim is to evolve these novel molecular targets into lead discovery programs.

The scene was set with an overview by Dr. Bob Coleman (Pharmagene, U.K.) who has spent more than 35 years "drug hunting" in the GPCR area. Many of those years were spent at Glaxo, a company with a rich history in the successful identification of GPCR-modulating drugs, which have become the most fruitful class of molecular targets for the pharmaceutical industry. In 1968, Allen & Hanbury launched **AH-3365** (**salbutamol**, ***Ventolin***®) as a bronchodilator for the control of asthma. This drug, a β_2-GPC–adrenoceptor agonist, trans-

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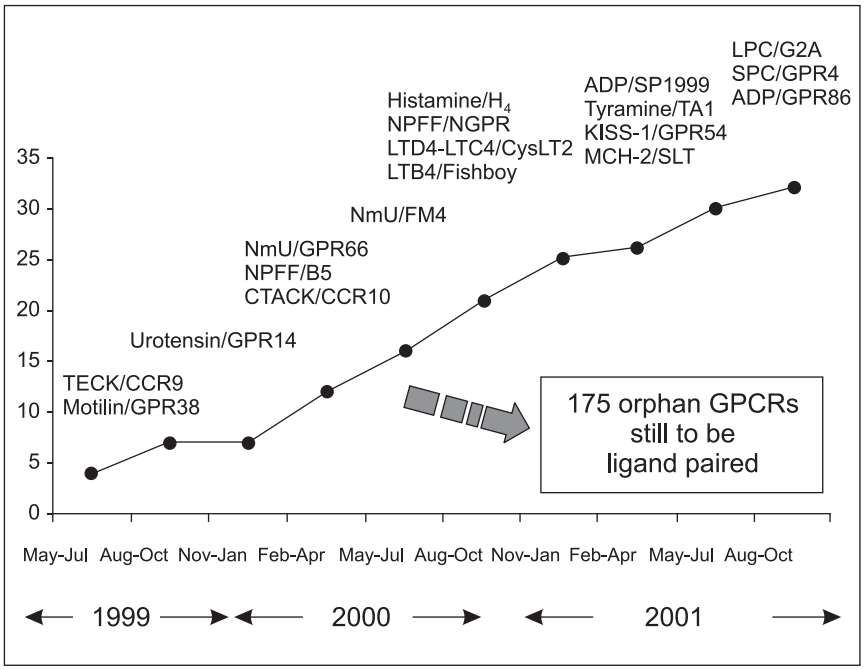

Fig. 1. The trend and importance of orphan G-protein-coupled receptors (GPCRs): ligand pairing. The number of ligands reported to have been paired to an orphan receptor has been plotted against the date of the report. With permission from Dr. M. Fidock.

formed the company from an organization known for manufacturing pastilles to a drug discovery company. Salbutamol was the first of Glaxo Group Research's raft of successful drugs modulating GPCRs, which include **ranitidine** (***Zantac***), **labetalol** (***Trandate***), **sumatriptan** (***Imigran***), **ondansetron** (***Zofran***®) and **salmeterol** (***Serevent***®). There were, however, frustrations among the success stories, with efforts to develop drugs acting at prostanoid receptors proving unsuccessful. Even so, in 2000, 26 of the world's 100 top-selling drugs targeted GPCRs, amassing annual sales in excess of $20 billion. Dr. Coleman finished his overview by stressing the importance of examining human rather than animal tissue distribution of novel GPCRs when looking for clues regarding potential areas of future therapeutic utility. Using a bank of over 70 tissue-specific mRNAs and quantitative RT-PCR technology, Pharmagene has mapped relative receptor concentrations of many GPCRs. The robustness of this technique was demonstrated with reference to prostanoid EP_3 and dopamine-D1 receptor mRNA distribution, which correlated well with established pharmacology.

Merck Research Laboratories' GPCR research strategy of searching genomic sequences, cloning novel open reading frames and expressing the mRNA in systems suited to the application of aequorin and aurora β-lactamase screening technologies was described by Dr. Jim Liu. This approach led to the identification of neuromedin U as a ligand for the novel GPCRs FM3 and FM4. The neuropeptide NPVF was identified as a novel ligand for the receptors GPCRB5 and HG31 (also known as FF1/FF2). Once the ligands had been identified, they were used in tissue distribution studies to reveal discrete patterns of receptor expression in brain and spinal cord. NPVF, when administered intrathecally, was found to potentiate opiate-induced analgesia via activation of FF2 receptors. In contrast, administration of NPVF into the cerebral ventricles antagonized opiate-induced analgesia via an FF1-mediated mechanism. These receptors are currently a focus for efforts aimed at deriving novel, pain-modulating drugs. However, many neuropeptides play a role in multiple physiological pathways, and the discovery that neuromedin U modulates food intake has provided the opportunity to pursue another goal to develop novel antiobesity drugs.

Bioinformatics has provided the foundation for many of the GPCR research programs, although many scientists often do not appreciate the limitations of this approach. Prof. Terri Attwood (Manchester University, U.K.) set the scene for her presentation with the introductory slide "Which (witch) craft is best," supported by pictures of bubbling cauldrons. The presentation drew to the audience's attention the complexities of the *in silico* world of bioinformatics and highlighted the need for caution when interpreting the output of database interrogation. The challenge of family analysis is to distinguish between highly divergent families with a single function or super families with many diverse functions. Using the rhodopsin, opiate and muscarinic GPCRs as examples, Prof. Attwood showed how a number of databases (e.g., BLOCKS, PRINTS, PROSITE, PROFILE, PFAM and eMOTIF) could be accessed for "motif-based" searching, based upon the sequences of the transmembrane and loop domains. A particular problem commented on was the generation of false-negative data when using pattern matching based on single motifs, which can be over 20%. The PRINTS program, developed at Manchester University, uses multiple motif diagnostic "fingerprinting" that characterizes protein function. The overall message was clear: There are a lot of databases, and searching strategies need to be carefully formulated in the attempt to annotate sequences. Using a basic BLAST and PROSITE approach is likely to derive a significant amount of misleading information because of false negatives, false positives, annotation errors and so forth. The user must also be cautious when using so-called "expert systems," such as Genquiz, Magpie and Pedant, which may rely heavily on BLAST and PROSITE. An integrated approach to finding a consensus is likely to be the most successful method, although it may often be more demanding in its execution. The

reality check was that three-dimensional structures of proteins are still difficult to predict from DNA sequence information, and confirmation of biological activity will still require the witchcraft of *in vivo* and *in vitro* biological experimentation.

Dr. Alan Wise (GlaxoSmithKline, U.K.) addressed the use of functional genomics to identify novel ligand-orphan receptor pairings to family A nonsensory GPCRs. The multidisciplinary approach for effective target discovery was clear in this and other presentations from the impressive array of collaborators in bioinformatics and gene, cell and tissue biology, as well as technologists well versed in high-throughput screening. The genealogy of this family shows interesting group homologies such as those shared by the leukotrienes and the PAF-like ligands, or the adenosine and biogenic amine ligands, and represent a wide variety of functions from olfaction to contraction of smooth muscle. Many of these receptors are not present in flies or worms, which may be indicative of a role in the mammalian central nervous or immune systems. After *in silico* identification and selection of putative therapeutic targets, GlaxoSmithKline's typical orphan strategy focuses on receptor expression in mammalian (CHO, HEK293), yeast or Xenopus cells and screening against a panel of putative ligands. Functional readouts include yeast cell proliferation, calcium mobilization and aequorin and pigment dispersion in melanophores. An advantage of using yeast as an experimental system is that experiments can be performed against a "GPCR null" background. Yeast experiments identified GPR41 and GPR43 as receptors for short chain fatty acids. GPR41 is strongly expressed in adipose tissue and inhibits lipolysis, but the role of GPR43 is more difficult to define, as the tissue expression profile is more variable.

Another interesting line of research was the identification of the GPCR Axor35, which was phylogenetically classified as an aminergic receptor closely related to the muscarinic GPCR and subsequently found to be a novel histamine receptor. When expressed in HEK293 cells, the receptor was liganded by agonists (**imetit**) and antagonists (**thioperamide**) previously thought to be H_3-selective. However expression profiling helped to identify Axor35 as a novel histamine receptor (H_4) that was originally described pharmacologically in human eosinophils in 1994. Dr. Wise concluded his presentation by illustrating the rapid advances in the field using the OGR1 GPCR and orthologues, which are activated by lipids such as sphingosylphosphorylcholine. Predicted future work would reveal many interesting opportunities in this very competitive field.

In a complimentary presentation, Prof. Hans Bräuner-Osborne (The Royal Danish School of Pharmacy, Denmark) focused on the family C GPCRs that are characterized by a large extracellular ligand-binding N-terminal domain. This family includes receptors for the neurotransmitters GABA and glutamine as well as for calcium. Novel receptors were sought using the sequence of GluR2 as a BLAST query that generated around 80 hits. Receptors identified using this strategy include GPRC5B, GPRC5C, GPRC5D and RAIG1, which were subsequently cloned for further investigation. The role in cancer has been speculated on the basis of reports that a chromosomal locus containing RAIG1 and GPRC5D is deleted in a number of tumors and also that transcription of these receptors can be induced by retinoic acid, a treatment used for certain leukemias. However, the identification of the endogenous ligands was complicated because of the wide tissue expression, the small extracellular domain and the low sequence identity to the liganded family C GPCRs. Even so, using chimeric Ca and orphan GPCRs, it was possible to delineate some of the intracellular signal transduction pathways of the receptors. Screening of more than 1,000 putative ligands using a FLIPR (fluorescent imaging plate reader)-based approach has, however, failed to identify an activating ligand for these receptors. The deorphanization of this group of GPCRs is likely to be challenging.

Pfizer's global research into orphan GPCRs has been established in seven centers located throughout the world, reflecting the intense competition in this area. Dr. Mark Fidock (Pfizer, U.K.) described an orphan GPCR strategy not dissimilar to earlier speakers in the meeting, that is, expression of receptors in a variety of host systems followed by screening of putative activating ligands. "Reverse pharmacology" (i.e., identification of the receptor target before the generation of an agonist or antagonist) is the modern paradigm for drug discovery, being especially amenable to high-throughput screening. The development of a robust screening platform was essential to efficiency of the program and aimed to ensure that activation of all receptors resulted in the same measurable end point. To achieve this, chimeric G proteins have been engineered to ensure signal transduction passes through the Pi pathway. The Aurora technology was a useful integrated method resulting in the activation of transcription of the β-lactamase reporter gene that lies at the watershed of several transduction cascades. Dr. Fidock indicated that the strategic rationale for this was that the approach capitalized on efficiencies of scale, use of high-throughput technologies and synergies in medicinal chemistry. Even so, the task and competition is so great that partnerships are essential for the rapid development of any drug discovery program, and Pfizer's strategy has included associations with Celera, Incyte, Gennaisance, Deltagen, Inpharmatica, Lifespan, Genelogic and Affymetrix.

The GPCR database built up by Lifespan is especially well developed and can provide data mining and curative services as well as tissue-specific and disease-associated information. The tie between bioinformatics and immunohistochemical localization proved to be especially useful.

Deltagen's high-throughput knockout mice technology can quickly generate analyses of the phenotypic functions of a particular gene. Dr. Fidock presented data to show how the use of knockout animals in *in vivo* screening for antipsychotic drugs can greatly facilitate the discovery process. Dr. Fidock rounded off his presentation by demonstrating the utility of the gene to drug target strategy using the GPCR orphans for histamine-H_4 and NmU2R as potential drug targets in eosinophil function and appetite control, respectively.

GPCRs in respiratory inflammatory disease were established as a target with the advent of **salbutamol**. However, Dr. Graeme Wilkinson (AstraZeneca, U.K.) pointed out that the β adrenoceptor is not the only opportunity for intervention in respiratory inflammatory disease. Any disease process is the sum of pathological changes taking place in numerous cells involving, in this case, mast cells, eosinophils, dendritic cells and T cells. An understanding of the functions of these cells in asthma underpins the focus of the AstraZeneca program. Cytokines play a key role in allergic diseases and GPCR-mediated control of cytokine production in T-helper cell subsets (Th0, Th1 and Th2 cell clones) is a key focus. A novel type-B GPCR was found to be differentially expressed in T-helper cells. *In silico* data mining identified 16 GPCRs of interest, some of which were highly expressed in eosinophils and other inflammatory cells. Using this information, the group has discovered a novel neuropeptide-like ligand. Further evaluation of peptides, however, often requires the generation of antibodies as biological tools, which can delay research programs. Dr. Wilkinson outlined the use of phage display, which offered a faster, more controlled method for the generation of monoclonal reagents. With the use of phage generated antibodies, an unspecified orphan GPCR was localized to the cell membrane when expressed in HEK 293 cells. The orphan GPCR was also coupled to intracellular calcium in the FLIPR assay and could be activated by agonists identified in screening programs.

The final speaker of the meeting, Dr. Tom Blackburn, crossed the Atlantic from Synaptic Pharmaceuticals, New Jersey, United States, to give an evocative talk called "Trace amine receptors—A new GPCR family from a neoclassic pharmacological era." Although the trace amines have a defined neurotransmitter role in invertebrates, the presence of β-phenylethylamine (β-PEA), tryptamine, tyramine and octopamine in the mammalian nervous system was, until recently, something of a mystery. Synaptic Pharmaceuticals identified 15 GPCRs for the trace amines and so established a new family and new functions for the trace amines in their own right. Dr. Blackburn sought to convince the audience of the biological importance of trace amines by suggesting that, among the pharmacologically active ingredients of chocolate, the trace amines β-PEA, tyramine and tryptamine may account for the popularity of this confection. However, his description of the association of headache with tryptamine intake was perhaps more convincing, as this amine is present in high concentration in Chianti red wine!

A human and rodent receptor for these biogenic amines, TA1, when expressed in Cos-7 cells binds tyramine with high affinity and specificity. However, there are some interesting species differences. For instance, the K_i (nM) for the human compared with the rat TA1 receptor is 8 cf. 57 for β-PEA, 57 cf. 176 for amphetamine and 1084 cf. 7 for tryptamine. Perhaps disappointingly, an antagonist at the rat receptor was weakly active when tested against the human receptor. Expression of the mRNA is also species specific, so while rat, mouse and human stomach express TA1 mRNA, TA1 expression in the amygdala is only observed in mouse. Interestingly the genes for the GPCRs TA2–5 are located within a susceptibility locus for schizophrenia on chromosome 6. β-PEA, a TA receptor agonist, increases monoamine oxidase release and has been found to be low in attention deficiency disorder and depression and high in paranoid schizophrenia. β-PEA has been proposed to be an endogenous amphetamine linked to the antidepressive effects of exercise. This and other data suggests that the TA family have potential roles in food intake, depression, anxiety, gastrointestinal function, electrolyte balance and hypertension. Concluding, Dr. Blackburn eluded to the fact that TA1 knockout mice are phenotypically normal, but display an "interesting pharmacology" on administration of trace amines—a story for another day!

The meeting was closed by the Society for Medicines Research chairman, Dr. Malcolm Duckworth, who expressed thanks to Novartis for their generosity in hosting the symposium. The speakers had impressively shown that orphan GPCRs are an important and exciting target for drug discovery. However, Dr. Duckworth spoke for many in the audience in expressing the view that progress in pairing new ligands to the orphan GPCRs and discovery of synthetic agonists and antagonists seemed to be disappointingly slow. Perhaps the groundwork has been laid, and fervent in-house activities are currently shielded from public view. The next time that novel, genomically derived GPCRs are visited as an SMR conference topic, the lineup of speakers may consist of a band of medicinal chemists reviewing the success of their lead identification programs.

Dr. Ian Morris, Manchester University, and Dr. Robert Williams, Prolifix Ltd., are members of The SMR Committee, which organizes conferences on behalf of the Society for Medicines Research four times a year. These one-day conferences are of a multidisciplinary nature, therapeutically focused and normally staged in or around London. Details about forthcoming meetings can be obtained from: SMR Secretariat, Triangle House, Broomhill Road, London, SW18 4HX, U.K. Tel: +44 (0)20 8875-2431; Fax: +44 (0)20 8875-2424; http://www.socmr.org; e-mail: secretariat@socmr.org.