GPCRs come of age in San Diego

Roger Crossley, BioFocus, Sittingbourne Research Centre, Sittingbourne, Kent, UK ME9 8AZ; tel: +44 1795 412 300, fax: +44 1795 471 123, e-mail: rcrossley@biofocus.com

IBC Life Sciences' 7th annual *G-Protein Coupled Receptors* meeting (14–16 October 2002; San Diego, CA, USA) showed just how much the area has developed in recent years. The early conferences in the series concentrated on the expansion of the area as a result of new technology and the effect of genomics, whereas this meeting was notable for a move towards applications in drug discovery, as well as the revolutionary changes in our appreciation of the structure and function of GPCRs.

Two's company

An exemplary description of the use of Bioluminescence Resonance Energy Transfer (BRET) and BRET^2 from Michel Bouvier of the University of Montreal (http://www.umontreal.ca/) to elucidate the functional composition of GPCRs has shown the importance of receptor dimerization. Some receptors, those in Family C such as gamma-aminobutyric acid (GABA) and glutamate, are covalently associated with one half apparently sensing interactions with ligands and the other half effecting the signalling to the G-protein. In other cases, dimerization is non-covalent but can still be important. For example, in some receptors, dimerization is required for the normal trafficking of newly synthesized receptors to the plasma membrane from the endoplasmic reticulum. For others, heterodimerization is important in cross-talk regulation and enables greater pharmacological diversity. The question then arises as to whether it is necessary to consider dimerization as a prelude to drug screening and, if so, how? The current state of knowledge for many receptors does not enable such considerations to have an impact, but the rules and relevance will in time become more evident. Also intriguing is the possibility that cells can govern the generation of homo- or heterodimers in two ways: one by a physical separation of the newly synthesized receptors and the other by the temporal separation of their synthesis.

Other friends

Superimposed on the potential to dimerize is the potential for different signalling pathways to be used by different heterodimers. Again, it is not yet evident how to use this phenomenon in the design of assays or drugs, nor indeed its pharmacological importance. Thue Schwartz of the University of Copenhagen (http://www.ku.dk/) and 7TM Pharma (http://www.7tm.com/) extended the consideration of dimerization to consider other proteins with which the GPCRs might interact. There are several of these, some of which are functional, such as receptor kinases and arrestins, but there are also a large number of cytosolic adaptor and scaffolding proteins, all of which affect the GPCR responses by helping to recruit different signal transduction complexes (signal transductosomes). These, in theory, provide different molecular pharmacological phenotypes that could be considered as drug targets; it is likely that differential pharmacology could be explained in terms of such complexes. Nevertheless, the crucial role of arrestins in the internalization of GPCRs, and hence their inactivation, is likely to be important as well. Structural studies of arrestins by May Han of Millennium Pharmaceuticals (http://www.millennium.com) were amplified by subsequent discussions on dimeric GPCRs. Arrestins are essentially dimers with a binding site equivalent to one GPCR. The intriguing possibility, therefore, is that GPCRs owe their trafficking to the surface to their ability to form dimers and rely on dimerization to be readsorbed following their interaction with arrestins.

Cell-based assays

The more recent thoughts on the functioning of GPCRs open up new possibilities for drug interaction and this was illustrated in the *Cell-Based Assays* session, which was run as an annex on the third day, in a presentation by Thomas H. Large of Lilly (http://www.lilly.com/). Family C GPCRs are a group of receptors that act as dimers, linked by a Cys-Cys bond, with one unit of the dimer recognizing the ligand and the other doing the signalling through the G-protein. Recognition of the natural ligand, for example GABA or glutamate, is effected by a large N-terminal extracellular binding domain and competitive antagonists act at this site. The group at Lilly has produced some allosteric modulators (LY508869 and LY566332) that act as mGluR2 potentiators by interacting within the transmembrane helices. Mapping the site indicates the importance of transmembrane (TM) helices 4 and 5 in interaction with these molecules with Asp735 in TM5 being required for full potentiation. Although the two molecules are close analogues, they occupy overlapping but distinct binding sites. Competitive (orthosteric) modulators at the metabotropic

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glutamate receptor have so far failed to live up to their initial promise as therapeutic agents and it will be interesting to see if allosteric modulators fare better.

Whatever the outcome for the metabotropic glutamate receptor, allosteric modulation, either positive or negative, does open up new possibilities for therapeutic intervention at GPCRs. Potentators might be more physiological than direct-acting agonists in that they should have little effect in the absence of neurotransmitter; rather they enhance its effect and thus maintain temporal and spatial coding of synaptic activity. Allosteric compounds are thought to stabilize the receptor in various states and there is the possibility that multiple allosteric domains could exist on each receptor. The difficulty then lies in developing approaches to discover these domains – functional assays have a crucial part in discovering allosteric compounds but, depending on the readout, managing the medicinal chemistry might not be so straightforward.

Targets and leads

Previous meetings in the series have concentrated more on the biological aspects of GPCRs, and in 2001, Graham Milligan of The University of Glasgow (http://www.gla.ac.uk/) remarked on the need to extend the conference to include a more medicinal chemical input. Although still biased to the advances in biology, some of the parallel advances in medicinal chemistry were also tackled this time. These mainly concerned the design of compounds, either in the case of Rodney D. Bush of Procter and Gamble (http://www.pg.com/), who described how the use of medicinal chemistry, together with modelling, had led to the design of nanomolar ligands for melanin concentrating hormone (MCH) and melanocortin 4 (MC4) receptors, or in the design of libraries of compounds directed at GPCRs.

Three significantly different approaches were described in this regard. A technology workshop from Stanley A. Lang of Chemical Diversity Labs (http://www.chemdiv.com/) outlined their approach, which is based on a scoring scheme for the classification of molecules into GPCR-ligand-like and non-GPCR-ligand-like. The method is based on the use of statistical treatments using neural networks to classify compounds from 450 combinatorial libraries using 50 descriptors, and has produced a collection of 35,000 compounds predicted to be active at GPCRs. A second approach, described by Wim Meutermans of Alchemia (http://www.alchemia.com.au/), used sugar chemistry to produce five libraries that were designed to target selected GPCRs using biased pharmacophoric groups, scaffolds and substituent patterns, and which showed hit rates ranging up to 99% against selected receptors. A third approach to library design, Thematic Analysis™, in use at BioFocus (http://www.biofocus.co.uk) was described by Roger Crossley.

In terms of new and interesting targets, David E. Cummings of the University of Washington (http://www.washington.edu/) made a convincing argument for the importance of ghrelin, a recently discovered orexigenic enteric hormone. As well as being implicated in growth processes, Cummings maintains that ghrelin's more important role is in governing feeding. The rise in ghrelin levels before, and the fall after, every meal is seen as a key factor in the promotion of the eating response. Crucially, the rise in ghrelin levels seen following weight loss caused in several ways implicates it as the method by which the body strives to regain the weight lost during periods of dieting. Two methods of effecting weight loss, which do not affect ghrelin levels, are low-fat diets and the rather extreme gastric bypass surgery. Neither of these elicits compensatory rise in ghrelin levels and the significant weight-reducing efficacy of these methods is thought to be related to their lack of effect on ghrelin.

Concluding remarks

Drugs that target GPCRs represent over 45% of all currently marketed drugs, and a third of all research done by pharmaceutical companies is directed at producing more. GPCRs are likely to remain supreme for years to come. This meeting showed that the opportunities for GPCRs are increasing as a result of new discoveries and that the tools to produce the next generation of GPCR drugs are becoming more sophisticated and effective.