Déjà vu all over again: emerging concepts of G protein-coupled receptor (GPCR) function and drug discovery

G protein-coupled cell surface receptors (GPCRs) have served a fundamental role in modern pharmacology, due to their central importance in cell communication, and have been the target for the discovery of a large number of drugs. By one estimate, more than 40% of marketed drugs target GPCRs. Considering that the human genome is believed to express genes for between 800-1,000 different GPCRs and the drugs that are marketed target less than 50 GPCRs, it is safe to speculate that the field of GPCR drug discovery and development is likely to grow in the years ahead.

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GPCRs bind many neurotransmitters and hormones that control the functioning of tissues and organ systems. The binding interaction elicits a cascade of intracellular events in target cells, including changes in levels of second messengers, ionic conductance and several other molecular events that alter cellular activity. Since they couple neurotransmitters and hormones with their target cells, GPCRs are essential for the normal functions of the body and are also important for creating the abnormalities in cellular communication that result in disease. Indeed, GPCRs are involved in a diverse range of normal cellular functions and disease processes. Therapeutics and compounds targeting GPCRs have been developed which treat pain, inflammation, neurobiological disorders and are even used in the food and cosmetics markets to modulate taste and smell.

Drug discovery against GPCRs requires the use of high throughput screening (HTS) assays which primarily utilise cells or fragments of cells. This is due to the extreme difficulty in purifying GPCRs, as they are intrinsically woven into the cell membrane, which is not so much of an issue for other drug targets such as soluble kinase enzymes. Furthermore, there are many GPCRs that have no known ligand termed 'orphans', and it is difficult to screen for compounds which modulate these types of receptor, and often even more difficult to adequately validate the putative GPCR as a druggable target. Cell-based functional assays are a prerequisite for screening these types of orphan receptors. While it is still important to devise new technologies and ways of screening orphans, this class of receptors has proven difficult to develop many therapeutics for due to the reasons mentioned above. A current trend in drug screening of GPCRs is '*déjà vu*' – revisiting older classical drug targets, but now looking for molecules that do not directly bind the natural ligand binding pocket. This brief review will focus on new technologies now allowing development of an entirely new generation of GPCR-based therapeutics such as inverse agonists, allosteric modulators and drugs targeting GPCR heterodimers.

Ligand binding domains

GPCRs have been classified based on the nature of the ligands they bind. Class 1 includes rhodopsin-like receptors for which the β-adrenergic receptor is the prototype. The ligands that activate these receptors are biogenic amines, chemokines, prostanoids and neuropeptides. Class 2 includes secretin-like receptors, and are activated by ligands such as secretin, parathyroid hormone, glucagon, calcitonin gene related peptide, adrenomedullin and calcitonin. Class 3 includes metabotropic-glutamate-receptor-like and calcium sensing receptors.

Much of what is known on the mechanism of binding to GPCRs is based on the seminal studies conducted on the β-adrenergic receptor which binds small molecule ligands in the unique hydrophobic extracellular pocket. Since the endogenous ligands (in the case of the β-adrenergic receptor endogenous ligands epinephrine and norepinephrine) are small they can fit into these binding pockets and are able to interact with sites deep within the core of the protein structure to activate the receptor. GPCRs that recognise larger endogenous ligands, such as peptides, bind their ligands somewhat differently. Peptides interact with recognition sites in the extracellular loops of receptors (relatively few ligands bind to the N-terminus of GPCRs) as well as with residues in the hydrophobic pocket. Ligands that bind to extracellular loops might be expected to induce different conformational changes in the receptor upon binding than small molecules that interact with residues deep within the receptor core.

It has been shown that different ligands targeting the same receptor can produce distinct biological effects, and this has opened up new avenues of research and discovery of novel drugs with desirable therapeutic actions. As described in more detail below, ligands such as partial agonists, inverse agonist and allosteric regulators are becoming important new classes of drugs capable of inducing desired pharmacological effects distinct from classical agonists – and in some cases better. These ligands may induce different conformational effects of GPCRs than classical full agonists and as a consequence produce different pharmacological effects. Technologies that allow us to monitor these diverse activation processes can be used for discovery of these new types of GPCR directed drugs.

GPCRs and G proteins

Intracellular domains of GPCRs contain contact regions for coupling to signal transduction systems. Most prominent among the intracellular effector molecules that these regions interact with are the G proteins which link GPCRs to second messenger systems, such as adenylyl cyclase, phospholipases and ionic conductance channels. G proteins are so named because they interact with GTP and also have inherent GTPase catalytic activity. The G protein super-family consists of heterotrimeric complexes of distinct α, β, and γ subunits. There are numerous Gα, Gβ and Gγ subunits creating a large number of distinct heterotrimeric complexes. These different heteromers have significant specificity both with regards to the GPCRs they interact with and the cellular effector systems they regulate.

The α subunit of G proteins contains the GTPase catalytic activity, as well as many of the receptor contact sites. Agonist binding to the GPCR promotes a conformational change to induce coupling with the G protein. The GPCR/G protein interaction accelerates catalysis of guanosine triphosphate (GTP) to guanosine diphosphate (GDP), providing energy needed for dissociation of the α subunit from the βγ subunits (in general βγ are tightly associated and do not easily dissociate). The free α and βγ subunits then interact with second messengers systems and ionic conductance channels, as well as with other cellular effectors.

Thus, $G\alpha_s$ is known to couple GPCRs to adenylyl cyclase to mediate stimulation of the formation of the second messenger cAMP and subsequent stimulation of a family of cAMP dependent protein kinases. Because nature creates homeostatic systems, there also exists GPCRs which mediate the inhibition of adenylyl cyclase. These receptors, such as the α2–adrenergic, opiate and somatostatin receptors couple to adenylyl cyclase via $G\alpha_i$ which negates the stimulatory actions of $G\alpha_s$.

While $G\alpha_i$ is critical for coupling different families of GPCRs to inhibition of adenylyl cyclase, it is also responsible for modulating other functions of GPCRs. For instance, subtypes of $G\alpha_i$ can link GPCRs to inward rectifying K^+ channels (GIRKs) and stimulation of GPCR/$G\alpha_i$/K^+ channel results in hyperpolarisation of cell membranes. Therefore stimulation of the same receptor/$G\alpha_i$ complex can lead to turning off the cAMP pathway and inhibiting cell firing at the same time.

G proteins also link the receptors to other cellular effector systems. The $G\alpha_o$ subunit has been shown to link GPCRs to Ca^{++} conductance channels to regulate the influx of Ca^{++} to cells. $G\alpha_o$ provides further diversity in function because it can also link GPCRs to phosphoinositol phospholipase Cβ, which catalyses the formation of IP3. This in turn opens IP3 gated calcium channels, causing the release of bound calcium into the cytosol.

The $G\alpha_o$ subunit acts as a critical modulating hub linking GPCRs to regulation of Ca^{++} homeostasis in cells. Importantly, some ligands can stimulate inhibitory GPCR (opiate and somatostatin receptors, for example)/$G\alpha_o$ complexes to inhibit Ca^{++} conductance and influx while at the same time increasing the release of intracellular Ca^{++}. This acts as a subtle regulatory mechanism for cellular Ca^{++} signalling, switching it from one dependent on extracellular Ca^{++} to one dependent only on intracellular Ca^{++}. In addition to $G\alpha_o$, GPCRs also couple to another subfamily of G protein subunits involving $G\alpha_q$. This G alpha subunit, like $G\alpha_o$ is able to link GPCRs to activation of phospholipase C to increase intracellular Ca^{++} release to active protein kinase C. It can also lead to activation of the MAP kinase pathway in cells which is responsible for phosphorylation and activation of transcription factors involved in cell proliferation.

As a result, studies over the past 20 years have revealed that G proteins can link GPCRs to multiple cellular signalling pathways and provide diversity in the functioning of these cell surface receptors. Interestingly, a single GPCR may be capable of coupling to more than one G protein. It has been shown to be the case for some somatostatin receptors as well as for the delta opioid receptors, which are able to associate with several subtypes of $G\alpha_i$, $G\alpha_o$ and $G\alpha_q$. This may explain how agonists at these receptors can inhibit adenylyl cyclase and Ca^{++} conductance while stimulating K^+ conductance in the same cell.

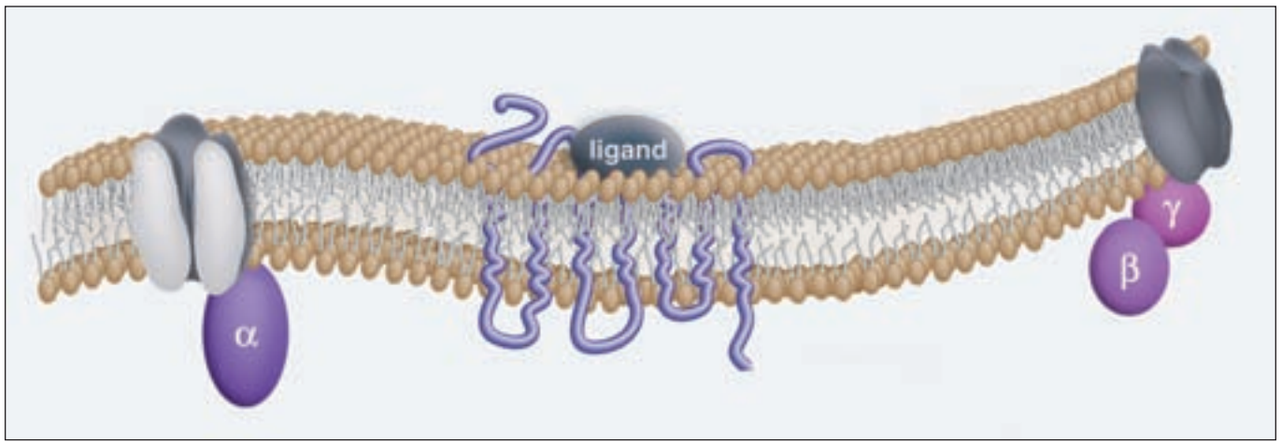
Recent findings indicate that GPCRs can exhibit duality in their coupling to G proteins, and that a given GPCR must be able to generate different intracellular surfaces to attract such functionally and structurally distinct $G\alpha$ subunits. Cell signalling is 'channelled'. The diversity of GPCR/G protein association has interesting implications with regards to drug discovery. If drugs can be identified to change the pattern of GPCR/G protein associations or cause a GPCR to preferentially associate with one G protein and not others, then it would be possible to shift the pharmacological profile and functions of a given receptor. This could be used to remove side-effects associated with activation of a given receptor while maintaining desired therapeutic actions or produce new drug effects via a given GPCR. Since GPCR associations with individual G proteins would require a unique structural basis due to the conformation generated by agonist binding, then developing drugs that reproduce that given conformation could direct GPCRs to coupling to selective G proteins. This could be accomplished either by finding drugs that bind in a unique manner to the ligand binding domain or identifying allosteric regulators.

Allosteric regulators interact with GPCRs as sites that are topographically distinct from the classical ligand binding domains. G proteins act upon allosteric sites to affect GPCR conformation, and it is anticipated that drugs targeting these unique GPCR/G protein contact sites could prevent coupling of the receptor with some G proteins but not others. Such allosteric regulators could be specific for targeting GPCRs and produce selective therapeutic properties. For example, targeting somatostatin type II receptors (SSTR2) in islet beta cells to couple to $G\alpha_q$ and not to $G\alpha_i$ could result in a receptor that stimulates Ca^{++} mobilisation to increase insulin release instead of a receptor that normally inhibits insulin release. Such allosteric regulators of the SSTR2 could be used to treat diabetes.

Allosteric regulators are of interest not only because they are highly selective for a given receptor but can also be used to modulate the maximal effectiveness of full agonists at that receptor. In effect they can tone down the receptor activation. This can be useful in prolonging the activation of the receptor and diminishing desensitisation. There is major interest in the pharmaceutical industry to develop allosteric regulators. Recently Sensipar, an allosteric regulator of the Ca^{++} sensing GPCR, has been approved by the FDA to treat hypoparathyroidism. Furthermore, Caden Biosciences has developed technologies that allow for allosteric regulator drug discovery by targeting drugs at the G protein binding sites of GPCRs. This suggest that allosteric drugs may be a large untapped field of discovery of novel GPCR therapeutics.

GPCR and arrestins

In addition to G proteins, GPCRs also couple with G protein receptor kinases (GRKs) and arrestins (also referred to as β-arrestins) which are involved in desensitisation and termination of receptor activation following prolonged agonist binding. Studies on the β-adrenergic receptor, as well as other GPCRs, have shown that following continued agonist binding to GPCRs, cytosolic GRKs are induced to translocate to GPCRs where they catalyse the phosphorylation of serine or threonine residues on the intracellular loops and C-terminal tail of the receptor. This phosphorylation attracts β-arrestins to the receptors, which compete with G proteins for binding to the cytoplasmic side of the receptor. In effect, β-arrestins uncouple GPCRs from G proteins, terminating signal transduction



Schematic representation of an activated GPCR receptor. Following ligand binding, the G protein subunits α and βγ are stimulating or inhibiting the activity of various effector proteins

via G protein mediated pathways and causing desensitisation of some functional responses of the receptor. Furthermore, β-arrestins serve as adaptor molecules and link GPCRs to clathrin in recycling vesicles to facilitate the internalisation of GPCRs. GPCRs then undergo ubiquitination, which can lead to their targeting to the proteosome and lysosomal compartment. This results in GPCR down-regulation. The translocation of GRKs and β-arrestins to GPCRs is believed to be a universal response to agonist activation and is critical for the inactivation of GPCRs and the termination of neurotransmitter and hormone action.

GRKs and β-arrestins also have also been shown to have *in vivo* physiological roles in mediating the functions of GPCRs, and have been implicated in development of tolerance to and dependence on drugs. GRKs and β-arrestins act as safety mechanisms to prevent the over stimulation of GPCRs. While the translocation and association of β-arrestins to GPCRs is relatively rapid, it is still much slower than typical GPCR activation of G proteins and intracellular signalling pathways. The uncoupling of the receptors from G proteins and GPCR internalisation is sequentially much slower than the processes initiated by acute GPCR stimulation. Thus, the β-arrestins provide a dampening mechanism to preserve GPCR sensitivity to endogenous ligands and to maintain the quick responses evoked by neurotransmitters and hormones.

The importance of GRKs and β-arrestins in mediating GPCR desensitisation suggests that these molecules could be important targets for the development of drugs to prevent tolerance development to established drugs and prolong the therapeutic activity of these agents. Assays are now available to measure translocation of β-arrestin to GPCRs following agonist stimulation. These assays can be employed for drug screening. Thus, if individual β-arrestins interact with unique recognition sites on the intracellular domains of selective populations GPCRs, then it may be possible to identify compounds that selectively block the desensitisation of some receptors, but not others, to prolong the actions of some therapeutically relevant drugs.

β-arrestins, however, do not just simply act to turn off GPCR functions. In fact, more recent evidence suggest that they act as switching mechanisms to convert GPCR function from one dependent on G proteins to one that is not. Thus, β-arrestins can function as adaptor molecules to recruit c-Src to agonist bound GPCRs. c-Src can in turn cause the phosphorylation of critical tyrosine residues on the epidermal growth factor receptor (EGFR). Thus, β-arrestins provide a means of cross-talk between GPCRs and growth factor receptors, and their respective signalling pathways.

Furthermore, β-arrestins can link GPCRs to the MAP kinase pathway. Interestingly, G proteins can also link GPCRs to the MAP kinase system, but via different mechanisms and with different cellular consequences. Thus, G proteins link GPCRs to protein kinase C and A which phosphorylate and activate the multi-component MAP kinase pathway to phosphorylate transcription factors in the nucleus. This effect is rapid, short-lived and involves members of the MAP kinase pathway translocating to the nucleus to phosphorylate transcription factors. In contrast, β-arrestins act as a scaffold to recruit c-Src and other members of the MAP kinase pathway to GPCRs. The β-arrestin associated activation of the MAP kinase pathway is slower in onset and more prolonged, and does not appear to primarily involve phosphorylation of

nuclear transcription factors. This process, rather than inducing rapid changes in gene expression, is focused more on altering cell motility, chemotaxis and to affect apoptoic process in cells.

The duality of GPCR responses via G protein versus β-arrestins mechanisms may provide interesting possibilities for novel drug discovery. As mentioned above, drugs targeting the inhibition of GPCR/β-arrestins associations could be useful in prolonging agonist activation of the GPCR/G protein pathway. Interestingly, inverse agonists at the β-adrenergic receptor, while reducing cAMP basal levels via a G protein mechanism, increased MAP kinase activity via a mechanism independent of G protein and mediated instead by β-arrestins. Thus, ligands acting via the same receptor can produce distinct cellular responses via either G proteins or arrestins. Employing assay systems that can simultaneously measure responses via the G protein pathway (changes in cAMP or IP3 levels) and β-arrestin pathway (translocation of β-arrestins to GPCR) could provide the means to design and develop unique drugs targeting one pathway and not the other.

GPCR oligomerisation

While GPCRs are generally considered as monomeric proteins, there is growing evidence to suggest that GPCRs can exist as oligomers. Importantly, if hetero-oligomers bind ligands differently than homo-oligomers, then the possibility exists to develop drugs that distinguish oligomers which could provide distinct selectivity and therapeutic value not seen in classical drug discovery efforts. In fact, GPCR oligomers may represent a large family of novel targets to discover and develop a new generation of therapeutics.

Oligomerisation has long been recognised as essential for signal transduction via cell surface receptors, in particular for growth factor receptors. In fact, essentially every growth factor receptor consists of either homo- or hetero-oligomers. Oligomerisation usually occurs when extracellular growth factor binds to one subunit, which in turn recruits other subunits. In some cases, the subunit that binds the growth factor does not have inherent kinase activity and the oligomerisation is essential for attracting subunits with kinase activity. In other cases, the growth factor recognition subunit has kinase activity and growth factor binding recruits other subunits that are substrates for the kinase and are essential for intracellular signalling to occur.

Evidence that GPCRs form oligomers first came from biochemical studies in which co-immunoprecipitation of GPCR complexes was detected using antibodies directed against individual receptors in the oligomer. In fact, GPCRs which respond to hormones such as GnRH form disulfide bridges in their N-terminus and exist as dimers. Such biochemical studies have indicated that some GPCRs primarily form homo-oligomers. Substantial evidence exists that homodimers can form constitutively active GPCRs, which are receptors that are active without the presence of ligand. Homodimers can form either by mutations in the GPCR or when receptors are over-expressed. This latter point is important because most of drug screening employing recombinant GPCRs uses cell lines over-expressing the receptors and the expressed receptors are likely to be, in part, constitutively active as well as regulated by agonist. The partial constitutive activity may explain the elevated basal responses such as cAMP or IP3 formation seen in cell lines expressing high levels of recombinant receptor. As a consequence, many of the drugs identified in such screening studies may primarily act on homodimers and may or may not act on monomeric receptors. This can be a problem since it is likely that most GPCRs naturally expressed in the body at relatively low levels are monomeric in function. As a consequence, drugs identified by their interaction with homodimers in drug screening studies may not be effective *in vivo* or produce different effects *in vivo* than *in vitro*.

Because constitutive activity generally raises basal second messenger signalling linked to the receptors, homodimer drug screening may be particularly useful in developing inverse agonists which by definition produce opposite effects compared to classical agonists. There is tremendous interest in the pharmaceutical industry to develop inverse agonists, which have many therapeutic advantages over classical agonists. In addition, practical advantages of inverse agonists are their potential use in treating disorders caused by constitutively active GPCRs.

GPCRs are also able to form hetero-oligomers with other receptors. A number of studies have suggested that heterodimers may have critical functional properties distinct from homodimers, and that hetero-oligomer formation was needed for appropriate sorting of GPCRs. Furthermore, oligomer formation has been suggested to change the G protein coupling and signalling pathways of GPCRs. For instance, the β2/β3 adrenergic receptor heteromer acts via a phospholipase C/Gq pathway to mediate this response, whereas the monomers do not associate with Gq. There is evidence that receptor heteromers may become

dysfunctional in a number of CNS disorders including Schizophrenia, Parkinson's and Huntington's disease, as well as other mental disorders. Thus, drugs that may modulate receptor oligomerisation and either maintain or disrupt such interaction could be therapeutically important in treating some diseases.

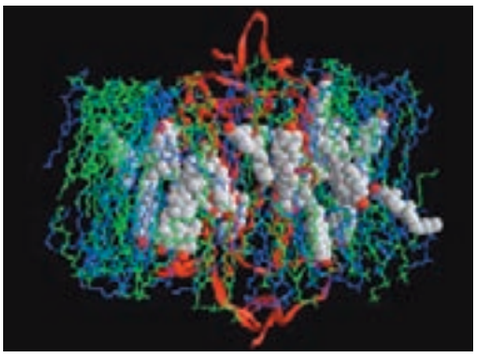
In addition to changing the G protein coupling and signalling of GPCRs and regulation of the receptors, hetero-oligomer formation may also change the ligand binding properties of the receptors. For example, the cannabinoid receptor CB1 and the orexin 1 receptors form hetero-oligomers. It was found that orexin was 100-fold more potent in stimulating the hetero-oligomer than orexin 1 homo-oligomers. This was particularly important because it provides a direct basis for the interaction of the cannabinoid and orexin systems and suggests that CB1 antagonists, which are being proposed as a treatment of obesity may do so by blocking both the actions of endogenous cannabinoids and the orexin system.

These and other findings suggest that the ligand binding pocket of the heteromer may differ from that of the homomer. If this occurs, then it suggests that it may be possible to discover drugs that selectively bind to the heteromer. Developing compounds that selectively bind to heteromers could result in drugs with unique and potentially beneficial therapeutic properties.

Orphan GPCRs as drug discovery targets

Bioinformatic analysis of the human genome has identified a large number of proteins as putative GPCRs based on structural similarities (predicted seven transmembrane structure with consensus sequences such as the DRY amino acid sequence in transmembrane regions) to the 200 or so known GPCRs whose endogenous ligands and functions are known. These putative orphan GPCRs do not have known ligands nor, in most cases, are their functions known. However, in many cases, information on their expression patterns and localisation in the body have provided tantalising evidence of their potential roles in physiology and, as a consequence, much interest has been generated in the pharmaceutical industry for developing drugs against this targets as unique and effective agents to treat disease and other disorders.

Because of this interest for drug discovery, attempts have been made to deorphanise these receptors. While there have been some significant successes in the deorphanisation and identification of novel endogenous ligands for some orphan receptors, many orphan GPCR programmes have failed to yield significant useful therapeutics. The emphasis is transitioning to being able to more carefully characterise the function of a particular novel GPCR and more fully validate its relevance in disease processes prior to initiating full blown drug screening campaigns. However, there is evidence that some recently deorphanised GPCRs, such as orexin receptor, may dimerise or associate with more classical GPCRs. This suggests that there is abundant room for drug discovery against both the orphans themselves and the systems they co-regulate or influence.



G Protein-Coupled Receptors (GPCR) in a membrane environment

GPCR mutations, disease and novel drug discovery

While GPCRs are critical for mediating the normal functions of neurotransmitters and hormones, they also play a role in a number of diseases. Loss of function mutations in GPCRs involved in the control of endocrine systems, such as the receptors for ACTH, FSH, GnRH, GHRH and TRH and TSH, mimic the symptoms found when those hormones are not expressed appropriately. For example, loss of function mutation of the TSH receptor causes congenital hypothyroidism. Furthermore, homozygous loss of function mutations in the type 5 chemokine receptor provides resistance to HIV infection because this receptor is critical for the infectivity of this virus. While explaining how some individuals who have been exposed to the virus do not get the disease, it has also spurred on interest to develop antagonists against this receptor as a potential treatment of HIV infection.

While it may not be easy to develop drugs to compensate for loss of function mutations in GPCRs in which the mutation either prevents agonist binding or activation of the receptor, some studies suggest that drugs may be identified as chemical chaperones able to bind mutant receptors

and relieve defective sorting or processing to treat the disease. In the case of GPCRs, the question arises whether drugs that selectively interact with mutant receptors can be identified to rectify the defect without altering the normal functioning of wild type receptors. Such drugs might be expected to have high selectivity in treating the disease, since they would only bind to a mutant receptor and not the wild type receptor.

In addition to loss of function mutations, gain of function mutations in GPCRs also cause disease. In most cases, the gain of function is related to the conversion of the wild type receptor that is dependent on agonist stimulation to a constitutively active receptor that is not dependent on activating ligand. An example of gain of function disorders is constitutively active rhodopsin, which can cause night blindness. For these diseases, development of inverse agonists could be useful as therapeutics since they would be predicted to selectively block the actions of the constitutively active receptor. These would be especially important if drugs could be developed to target the oligomers and not interact with monomeric receptors so that the drugs would not affect the normal functioning of the GPCRs in the body.

While direct cause and effect links have not been made, it is possible that mutations in GPCRs could be responsible for variations in drug sensitivities among different populations. Such mutations might not only cause variations in effectiveness or potency of GPCR directed drugs, but also drugs that are indirectly active via GPCRs such as neurotransmitter uptake inhibitors. These include ones used to treat depression since changes in sensitivity could determine how the endogenous transmitters activate GPCRs as well as drugs. The influence of GPCR polymorphism on how drugs affect humans could potentially be very important, especially since we primarily view changes in drug sensitivities with regards to variations in metabolism via the P450 system and the P-glycoprotein pathway. Since there is now a much greater effort in pharmacogenetics to link the genetic make-up of an individual to both disease susceptibility and drug responsiveness, then mutation profiles in GPCRs should also be considered in such genetic evaluations, especially since so many of the therapeutics we use today are directed against GPCRs. Clearly a better understanding of the role of GPCR mutations in disease and the consequences of those mutations on the functioning the receptors may be critical in the development of entirely new families of GPCR modulators as therapeutics.

GPCR pharmacology

The fact that nearly 40% of the drugs approved for marketing by the FDA target GPCRs and that there is an ever growing list of GPCR drugs under development suggests that approaches to discover GPCR drugs have been very successful. These methods have advanced and become more sophisticated with the growing need to target more novel receptors and to discover drugs acting in unique ways to affect GPCR signalling. Classical drugs targeting GPCRs generally have fallen into two categories: agonists, which are drugs that mimic the actions of endogenous transmitters and hormones to stimulate GPCRs; and antagonists which have no intrinsic activity of their own but which block activation of the GPCRs by agonists. This somewhat rigid pharmacological terminology has evolved over the years to embrace a wide spectrum of differently acting drugs.

For example, agonists can be distinguished as full agonists, partial agonists and inverse agonists, each with their own advantages and disadvantages as therapeutics. A full agonist is a drug that produces the same maximal effect as the endogenous neurotransmitter or hormone. Partial agonists are drugs which bind to GPCRs in a manner that produces less of an effect than full agonists. Partial agonists can antagonise full agonists. As a consequence, partial agonists exhibit duality in that they bind to GPCRs in a manner similar to both an agonist and an antagonist. Partial agonists are therapeutically important because of their dual nature, and have been used to treat opiate addiction by minimising the effects of the withdrawal reaction.

While full and partial agonists have been know for many years, inverse agonists have only been identified in the last decade. Like partial agonists, inverse agonists are able to block the effects of full agonists at GPCRs. However, the unique property of these ligands is that they induce opposite effects on the same GPCR as full agonists. The inherent activity of an inverse agonist is dependent on the receptor having some level of constitutive basal activity. Inverse agonists may also be useful in pathological conditions where GPCRs undergo constitutive activity *in vivo* either because mutations cause the constitutive activity, or because the receptors become overexpressed.

Summary

GPCRs have been an important target class for drug discovery for many years. The emerging data suggests a high level of complexity in GPCR function, particularly when studied in the cellular environment. Traditionally, most early screening of

GPCRs involved identifying compounds which displaced radioisotopically labelled natural ligands of the receptor using filter binding assays or scintillation proximity techniques. It will be interesting to observe the next generation of GPCR ligands and ultimately therapeutics, as they will be aimed not just at the classical ligand binding interface but also be designed to perturb GPCR function by interaction with other extracellular domains of the receptor. An emerging area which shows considerable promise, both from a biological perspective and clearer intellectual property standpoint, is the use of allosteric modulators. Consequently, numerous new avenues for drug discovery have now opened up in this area, many of which involve the study of GPCR function in the cell. Additionally, there is a drive to develop new technologies which permit interrogation of GPCR function in more biologically relevant contexts, such as primary cell models and in cells expressing the GPCR at endogenous levels. There is also an understanding that different technologies are more suited for screening for inhibitors of particular classes of GPCR. Some Gi coupled receptors continue to be difficult to screen, but with the use of alternate assay markers such as β-arrestin recruitment and ERK activation, they are becoming more therapeutically accessible. This has undoubtedly driven the recent large growth of cell based assays for screening for novel GPCR ligands. DDW

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