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MINIREVIEW

G Protein-Coupled Receptor Dimerization: Function and Ligand Pharmacology

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ABSTRACT

It is now generally accepted that G protein-coupled receptors (GPCRs) can exist as dimers or as part of larger oligomeric complexes. Increasing evidence suggests that a dimer is the minimal functional structure, but considerable variation exists between reports of the effects of agonist ligands on quaternary structure. Many studies have intimated the existence of heterodimeric GPCR pairings. Key questions that remain to be addressed effectively include the prevalence and relevance of these in native tissues and the implications of heterodimerization for pharmacology and, potentially, for drug design.

Although the generation of functional complexes via dimerization and oligomerization is a common theme for polypeptides including membrane proteins (Woolf and Linderman, 2003), for many years G protein-coupled receptors (GPCRs) were assumed to exist and function as monomeric species. In many regards, this was understandable. Both the expression of single DNA species encoding GPCRs and functional reconstitution studies employing artificial lipid vesicles and purified proteins indicated that the product of a single gene and single polypeptides chains were able to generate the anticipated pharmacology and function. Of course, such studies did not address, or indeed seek to address, whether the GPCR functioned as a monomer or function required quaternary structure. In the recent past, the concept that GPCRs can exist as dimers or higher-order oligomers has advanced rapidly (for reviews, see Angers et al., 2002; Lee et al., 2003; Milligan et al., 2003a). There is also growing appreciation that GPCR dimerization may be a requisite for function. A wide range of approaches has contributed to the view that individual GPCRs can exist as homodimers. These include the analysis of ligand binding studies (Armstrong and Strange, 2001), the tendency of GPCRs to migrate, even in SDS-polyacrylamide gels, as complexes with the molecular mass anticipated for dimers and/or higher-order structures (Nimchinsky et al., 1997), the ability to coimmunoprecipitate coexpressed but differentially epitope-tagged forms of a GPCR (Hebert et al., 1996; Cjevic and Devi, 1997), and the capacity to reconstitute function after the coexpression of distinct pairs of mutants that are both individually nonfunctional (Lee et al., 2002; Carrillo et al., 2003). As well as reductionist techniques, a range of studies have employed methods such as fluorescence resonance energy transfer (FRET) (Overton and Blumer 2000; Rocheville et al., 2000a, McVey et al., 2001; Dinger et al., 2003; Stanasila et al., 2003), bioluminescence resonance energy transfer (BRET) (Angers et al., 2000; Cheng and Miller, 2001; Kroeger et al., 2001; McVey et al., 2001; Ayoub et al., 2002; Issafras et al., 2002; Babcock et al., 2003) and imaging of functional reconstitution (Carrillo et al., 2003) to explore such interactions in living cells, either at the population level or in single cells. Essentially in parallel with these efforts, studies using similar techniques have noted the capacity of many pairs of GPCRs to form heterodimers or hetero-oligomers (Marshall, 2001; George et al., 2002). This is clearly not a random process (Milligan et al., 2004); in certain cases [e.g., the GABAb receptor (Marshall et al., 1999; Mohler and Fritschy, 1999) and taste receptors (Zhao et al., 2003; Matsunami and Amrein, 2004)], selective dimerization defines ligand pharmacol-

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ABBREVIATIONS: GPCR, G protein-coupled receptor; FRET, fluorescence resonance energy transfer; BRET, bioluminescence resonance energy transfer.

ogy and response. However, although both direct experiments (Ramsay et al., 2002) and computational strategies (Filizola et al., 2002; Soyer et al., 2003) have been employed to analyze such interactions, understanding of the molecular basis of heterodimerization and of the relative propensity of GPCRs to heterodimerize is currently in its infancy.

One key question that remains contentious is how, or indeed whether, receptor ligands alter the dimerization or organizational structure of GPCRs. The growing literature on GPCR heterodimerization has also resulted in attempts to address whether ligand pharmacology or function at heterodimer pairs is distinct from that at the corresponding homodimers. This might provide molecular explanations for tissue pharmacology that is not easily explained by heterologous expression of individual GPCRs. Neither of these questions can be addressed fully at this stage. However, in this commentary, I review data pertinent to these points and try to encapsulate key issues that require further study. As many of the approaches used cannot effectively discriminate between dimers and higher-order oligomers, I have used the term 'dimer' except in situations in which higher-order organization seems likely.

Are All GPCRs Dimers?

The most convincing demonstration that members of the rhodopsin-related, family A GPCRs exist as dimers in situ, and may indeed be further arranged into oligomeric arrays, derives from the application of atomic force microscopy to the organization of rhodopsin in rod outer segment membranes (Liang et al., 2003). Although some reservations have been expressed (Chabre et al., 2003) about the preparation procedures employed and the implications for molecular organization of biophysical experiments conducted a number of years ago, these studies demonstrated rhodopsin to be arranged in *para*-crystalline arrays. Models based on these pictures were inferred to indicate that intradimer links might be provided by contacts between transmembrane helices IV and V, with further organizational structure derived from interactions involving transmembrane helices I and II. Only occasional rhodopsin monomers were observed. It could be argued that the high density of rhodopsin in the rod outer segment might impose regularity and organization beyond that required for GPCRs expressed at much lower density. However, virtually all of the basic functions and mechanisms of rhodopsin have subsequently been found to have parallels in other family A GPCRs and as such, it has provided an excellent template to explore and understand other members of this family.

It is difficult to ascertain the fraction of any family A GPCR that exists as dimers. Although GPCR aggregates with molecular mass corresponding to dimers often survive resolution by SDS-polyacrylamide gel electrophoresis, the relative fraction of apparent monomers to dimers observed in such studies may indicate more about the relative interaction affinities of GPCR pairs than the fraction present as dimers in intact cells. Attempts at quantitation derived from FRET-based studies on neuropeptide Y receptor subtypes suggested that some 26% of neuropeptide Y Y2 receptor and 44% of neuropeptide Y Y1 receptor constructs were able to generate effective FRET signals. These numbers are likely to represent minimum estimates of dimerization in that they were calculated relative to an neuropeptide Y Y2 receptor construct with a FRET positive control attached to its sequence (Dinger et al., 2003). Studies employing saturation BRET, in which various ratios of *Renilla reniformis* luciferase and green fluorescent protein-tagged forms of the β_2-adrenoceptor were coexpressed (Mercier et al., 2002), estimated that more than 80% of this GPCR was present in dimers. Because GPCR dimerization is difficult to analyze in native cells and tissues, it has also been suggested that some examples of GPCR dimerization may, at least in part, be artifacts derived from the high levels of expression that can often be achieved in heterologous cell systems. Although Mass-Action implies this issue must be taken seriously, Mercier et al. (2002) estimated that the extent of dimerization of the β_2-adrenoceptor was unchanged over a 20-fold range of GPCR expression levels. Likewise, although it is often convenient to monitor signals in resonance energy transfer experiments with higher levels of expression, constitutive dimerization of the CCR5 chemokine/HIV coentry receptor was observed at levels comparable with those expressed endogenously (Issafras et al., 2002).

Although the preceding discussion has concentrated on the family A GPCRs because they are by far the largest grouping, key information on constitutive dimerization and the inherent requirement of this for membrane delivery has been gleaned from study of the metabotropic glutamate-like family C GPCRs that all possess long N-terminal sequences. Although these share no homology with the family A GPCRs, the recognition that the GABAbR1 GPCR is not trafficked effectively to the cell surface in the absence of expression of the GABAbR2 (Marshall et al., 1999) and that the extracellular domain of the metabotropic glutamate receptor 1a forms a constitutive dimer (Kunishima et al., 2000) was integral to more widespread appreciation of the general nature of GPCR dimerization. The intrinsic requirement of dimerization of the family C GPCRs for function is so firmly established that this is no longer a point of contention.

Why Should GPCRs Be Dimers?

There is growing evidence that GPCR-GPCR interactions occur initially during biosynthesis. Maturing forms of most family A GPCRs become terminally *N*-glycosylated and this is probably a key 'quality control' step before export from the Golgi apparatus. At least for the family C GABAb receptor (White et al., 1998), this seems to require protein-protein interactions provided by dimerization. In studies of oxytocin and vasopressin receptors, Terrillon et al., (2003) demonstrated immature forms of these GPCRs were present as dimers while still present in the endoplasmic reticulum. This again suggests that dimerization may be an integral aspect of GPCR maturation and that dimerization is an early commitment step in the production of functional GPCRs. It is also noteworthy that both artificially produced and naturally occurring GPCR truncation and splice variants can act to limit cell surface delivery of GPCRs (Coge et al., 1999; Karpa et al., 2000; Lee et al., 2000). This is likely to reflect interactions that do not result in final maturation of the dimer.

Models generated from the structural organization of rhodopsin and the actual molecular size of both this GPCR and G proteins indicate that a dimer would be required to provide an appropriate 'footprint' for the binding of a single heterotrimeric G protein consisting of transducin α and the $\beta_1 + \gamma_1$

complex (Liang et al., 2003). It is clear that GPCRs are able to bind G protein α and β/γ subunits independently and that β/γ interactions contribute substantially to the ability of agonists to activate G protein α subunits (Liu et al., 2002). Biophysical studies after expression in *Escherichia coli*, denaturation and refolding of the leukotriene B4 BLT1 receptor followed by reconstitution of the GPCR with a heterotrimeric G protein consisting of $G\alpha_{i2}$, β_1, and γ_2 also provided compelling evidence for the overall assembly of a pentameric $(BLT1)_2$ $G\alpha_{i2}$, β_1, and γ_2 complex (Baneres and Parello, 2003). It is interesting that the structure of arrestins (Han et al., 2001) is also consistent with the binding of a single arrestin across the cytoplasmic face of a GPCR dimer. Thus, binding of a single arrestin might be expected to desensitize a GPCR dimer.

One early attempt to disrupt GPCR dimerization involved the use of a peptide corresponding to transmembrane helix VI of the β_2-adrenoceptor. As monitored by the relative proportions of immunodetected GPCR migrating through SDS-PAGE in positions consistent with monomer and dimer, the proportion of dimer was decreased by this peptide in a concentration-dependent manner (Hebert et al., 1996). This correlated with a reduction in adenylyl cyclase activity mediated by agonists but not forskolin or NaF (Hebert et al., 1996). Such observations would certainly be consistent with the idea that high-affinity binding of G protein, and therefore effective G protein-mediated signal transduction, would require the GPCR dimer. An essential dimer interface in the *Saccharomyces cerevisiae* α-factor GPCR involves the glycophorin A-like GXXXG dimerization sequence that is present in transmembrane helix I (Overton et al., 2003). Mutation of this sequence inhibited protein-protein interactions as monitored in FRET studies and also resulted in a marked reduction in ligand-mediated signal generation. This was argued not simply to reflect poor membrane delivery of the mutated receptor and shown not to reflect poor agonist affinity. Early studies on dimerization of the dopamine D2 receptor indicated that in addition to a peptide corresponding to transmembrane region VI, an equivalent peptide corresponding to transmembrane region VII was also able to reduce the proportion of dimer (Ng et al., 1996). Few subsequent studies or models of GPCR dimers have suggested a likely role for transmembrane region VII as a dimer interface. However, a recent study directly introduced peptides corresponding to this region from a variety of GPCRs into rats and recorded a remarkable range of loss of physiological functions that would be anticipated to reflect activation of the corresponding receptors (George et al., 2003). These authors (Lee et al., 2003b) and others (Guo et al., 2003) have indicated a key role for transmembrane domain IV in dimerization of the dopamine D2 receptor. Thus, although it is difficult to rationalize the molecular basis of these results, they would be consistent with reduction or loss of GPCR function resulting from disruption of specific GPCR homodimers. Such studies would certainly benefit from independent replication.

Are GPCRs Permanent Dimers?

As discussed above, emerging evidence indicates that dimerization occurs early in the biogenesis of at least certain GPCRs. This supports a model in which GPCRs are delivered to the plasma membrane as dimers. Reports indicating that ligands can regulate GPCR dimerization do not seem consistent with this scenario and will be discussed later. The genetic tractability of *S. cerevisiae* resulted in early evidence that the α-factor GPCR is internalized as a dimeric complex (Yesilaltay and Jenness, 2000). The intracellular complement of GPCRs, such as the α_{1a}-adrenoceptor, that constitutively internalize at a rapid rate in the absence of ligands also seems to be composed of dimers (D. Ramsay, I. C. Carr, J. Pediani, J. F. Lopez-Gimenez, R. Thurlow, M. Fidock, and G. Milligan, submitted). There is therefore growing evidence that the entire life cycle of some GPCRs takes place within a dimeric complex.

The concept that GPCRs internalize as dimers has been used to attempt to explore the selectivity of GPCR heterodimerization. For example, after coexpression of the α_{1a}- and α_{1b}-adrenoceptors, the α_{1a}-selective agonist oxymetazoline was able to induce cointernalization of the two GPCRs. However, this agonist did not produce cointernalization of either the NK1 tachykinin receptor or the CCR5 chemokine receptor when they were coexpressed with the α_{1a}-adrenoceptor (Stanasila et al., 2003). This suggests that the α_{1a}- and α_{1b}-adrenoceptors form a high-affinity heterodimer but that the other pairs do not. In a similar vein, coexpression of the NK1 receptor with the MOP opioid receptor resulted in cointernalization of these two GPCRs in response to addition of selective ligands for either receptor (Pfeiffer et al., 2003). It has also been reported that when the β_2-adrenoceptor is coexpressed with the DOP opioid receptor, internalization of the opioid receptor is seen in response to isoproterenol (Jordan et al., 2001). However, when the β_2-adrenoceptor was coexpressed with the KOP opioid receptor, isoproterenol treatment failed to induce β_2-adrenoceptor internalization (Jordan et al., 2001). These observations are of considerable interest but also pose problems of interpretation. The β_2-adrenoceptor-DOP opioid receptor data indicate a capacity of these two GPCRs to interact and that agonist-occupation of one element of the heterodimer is sufficient to induce internalization. The KOP opioid receptor is much more resistant to homologous ligand-induced internalization than the DOP opioid receptor. A reasonable interpretation of these results is that the production of a KOP opioid receptor-β_2-adrenoceptor heterodimer restrains or blocks ligand-induced internalization. It is interesting, however, that direct measurements indicate the propensity of the β_2-adrenoceptor and KOP opioid receptors to form a heterodimer is much less than for the KOP opioid receptor to form a homodimer or a heterodimer with the DOP opioid receptor (Ramsay et al., 2002). KOP opioid receptor-β_2-adrenoceptor interactions can be measured, but only at high expression levels (Ramsay et al., 2002). This raises questions about the physiological relevance of such a dimer. If two co-expressed GPCRs, A and B, have equivalent propensity to form homo and heterodimers, then 50% of the population should be represented by A-A and B-B. Therefore, if the KOP opioid receptor and the β_2-adrenoceptor are coexpressed but have only relatively low mutual affinity, then it must be anticipated that the majority of dimers formed would be β_2-adrenoceptor-β_2-adrenoceptor and KOP opioid receptor-KOP opioid receptor homodimers. There is no reason to suppose that a β_2-adrenoceptor-β_2-adrenoceptor homodimer would not internalize in response to isoproterenol. Although this is pure speculation now, the data of Jordan et al. (2001) may indicate the production of a

larger oligomeric complex containing copies of both GPCRs. When the β_2-adrenoceptor and the DOP opioid receptor were stably coexpressed at relatively modest levels in human embryonic kidney 293 cells, agonists at both GPCRs were required to produce internalization of the two polypeptides (Tsao and von Zastrow, 2000). It is notable that the trafficking patterns of the two GPCRs could be resolved (Tsao and von Zastrow, 2000). These observations suggest that if the GPCRs are permanent dimers, then the homodimers predominate or if there is a significant heterodimer population, these are separated into monomers during intracellular trafficking. Other data, discussed below, would allow the second hypothesis. Interactions with β-arrestins to allow clathrin-mediated internalization are consistent with a trimeric $(GPCR)_2$-β-arrestin complex (and therefore the GPCR dimer) persisting at least until removal of β-arrestin within acidified endosomal vesicles. This concept also raises intriguing questions about the fate of GPCR heterodimers that incorporate so-called type A and B GPCRs. These are separated according to their affinity of interactions with β-arrestins. The type A GPCRs have relatively poor β-arrestin affinity and are rapidly separated from it after internalization. The type B GPCRs have substantially higher affinity for, and generally cointernalize with, β-arrestins into endocytic vesicles (Shenoy and Lefkowitz, 2003). Coexpression of pairs of related GPCRs, such the thyrotropin releasing hormone receptors 1 and 2, resulted in altered patterns of β-arrestin subtype trafficking than seen for either GPCR alone (Hanyaloglu et al., 2002). Terrillon et al., (2004) have recently reported similar data after coexpression of the type B vasopressin V2 receptor and the type A vasopressin V1a receptor. This clearly is an area requiring further detailed analysis. However, the capacity of β-arrestin-2 to act as a scaffolding protein for some pathways of GPCR-mediated ERK MAP kinase activation (Shenoy and Lefkowitz, 2003) may underlie observations of altered regulation of this pathway after coexpression of pairs of GPCRs. This has been a popular endpoint measure used to support potential distinct functions of GPCR heterodimers.

What Is the Propensity of GPCRs to Form Heterodimers?

Before we can attempt to rationalize potential variations in ligand function and pharmacology with GPCR heterodimerization, we must understand the expression pattern of the GPCR family. The best estimates of the number of functional GPCR genes in humans predict 339 encoding olfactory receptors (Malnic et al., 2004) and approximately 400 others (Fredriksson et al., 2003; Vassilatis et al., 2003). Assuming that olfactory GPCRs do indeed form dimers (and difficulties in expressing these in heterologous systems have restricted analysis of this issue) then at least in the olfactory system, they are restricted to homodimers because each olfactory neuron expresses only one member of the family. Although not yet fully confirmed, microarray and related studies have indicated a surprisingly widespread distribution of a number of olfactory GPCRs (Hakak et al., 2003). Microarray-based information has confirmed general suspicions that individual organs and small regions of tissue may express up to 100 distinct GPCRs (Hakak et al., 2003). However, at least in the public domain, such information is lacking for individual cells. Despite this, it is clear from the sensitivity of widely used cell culture lines to chemicals used in high-throughput screens that a substantial number of GPCRs may be coexpressed in detectable amounts. Although transport of mRNAs to different cellular locations before translation in terminally differentiated and polarized cells may prevent cotemporal and spatial synthesis of some potential GPCR dimer pairings, the above information does suggest the possibility of a potentially bewildering set of combinations.

One enduring and frustrating aspect of studies of GPCR dimerization selectivity is the difficulty in producing clear and unambiguous negative controls. For example, in coimmunoprecipitation studies, Salim et al. (2002) reported the capacity of all GPCRs tested to be coimmunoprecipitated with the serotonin 5-HT_{1A} receptor. In studies of a number of family A GPCRs, negative controls have used family C GPCRs, such as the GABAbR1, that have no inherent homology. However, even this may not be absolute. Interactions have been reported between the family A adenosine A2A receptor and the family C metabotropic Glu5 receptor (Ferre et al., 2002), although the molecular basis for this interaction remains unknown. The chemokine CCR5 receptor has also been a popular negative control for nonchemokine GPCRs. Relatively few studies have tried to assess relative interaction affinities directly, but saturation BRET techniques (Mercier et al., 2002) offer one approach. The most obvious hypothesis is that more homologous GPCRs would show higher interaction affinities. This is clearly too simplistic. Saturation BRET studies indicate the β_1-adrenoceptor and β_2-adrenoceptor can form heterodimers with similar affinity as the corresponding homodimers (Mercier et al., 2002). Likewise, KOP opioid receptor-DOP opioid heterodimers form at least as effectively as KOP opioid receptor homodimers (Ramsay et al., 2002). However, the inability of the α_{1a}- and α_{1d}-adrenoceptors to form heterodimers (Uberti et al., 2003), the reported lack of interactions between KOP and MOP opioid receptors (Jordan and Devi, 1999), and the ability of only some somatostatin receptor subtypes to generate heterodimers (Rocheville et al., 2000b) clearly points to greater complexity. Informational studies have suggested a basis for the opioid receptor results (Filizola et al., 2002), but this remains to be validated and expanded to a general case model. In the medium term, it is likely that relative GPCR interaction affinities will have be measured directly on a case by case basis.

Do Ligands Alter GPCR Dimerization?

Although this might seem a relatively easy question to answer, the range of observations on this topic is wide and confusing. Early studies on the β_2-adrenoceptor indicated a substantial level of constitutive dimerization and small but significant increases in this with addition of isoproterenol (Hebert et al., 1996; Angers et al., 2000). In contrast, initial studies on the DOP opioid receptor suggested that ligands capable of inducing receptor internalization also caused monomerization of the receptor (Cvejic and Devi, 1997). This conclusion was in marked contrast to the case of the *S. cerevisiae* α-factor receptor, which showed a lack of effect of ligand (Overton and Blumer, 2000) and, as noted above, where the dimer is internalized. A substantial number of

resonance energy transfer-based experiments have shown small effects of agonist ligands above substantial signals corresponding to constitutive dimers/oligomers (for review, see Milligan, 2004). Despite this, an emerging consensus favors family A GPCRs with small endogenous ligands that bind within the seven transmembrane domains predominantly as constitutive dimers. Observed effects of ligands are then most probably caused by conformational alterations within the complex, resulting in small changes in the orientation or distance between the resonance energy transfer reporters (Ayoub et al., 2002).

This generalization cannot currently be extended to family A GPCR groupings in which larger ligands bind, at least partially, to elements of the N-terminal domain and the extracellular loops. One of the most interesting groupings is the chemokine receptors. Early studies indicated that the chemokine SDF-1α produces dimerization of the CXCR4 receptor that was almost undetectable in the absence of the ligand (Vila-Coro et al., 1999). In complete contrast, combinations of BRET and sedimentation studies indicated the CXCR4 receptor to be a constitutive dimer that was completely unaffected by the presence of SDF-1α (Babcock et al., 2003). Ligand-independent dimerization of both the CXCR2 receptor (Trettel et al., 2003) and the CCR5 receptor (Issafras et al., 2002) has also been reported. Ligand-induced dimerization, however, was also recorded for the CCR2 receptor on addition of monocyte chemoattractant protein-1 (Rodriguez-Frade et al., 1999). As well as the effect of agonist, function was induced by addition of a bivalent anti-chemokine receptor monoclonal antibody but not by Fab fragments derived from the antibody. These authors drew direct parallels between the ability of chemokine receptor agonists to induce GPCR dimerization and the effects of cytokines on their non-GPCR receptor targets. This group has also reported that the capacity of HIV-1 to use the CCR5 receptor as a 'coreceptor' for cell entry was blocked by dimerization of the receptor by an antibody or by chemokine agonists at the receptor (Vila-Coro et al., 2000). Although not directly related, in the same studies that reported the CXCR4 receptor as a constitutive dimer, the CCR5 receptor was indicated to be a monomer (Babcock et al., 2003). Furthermore, in the studies that showed ligand-independent CXCR2 dimerization, the closely related CXCR1 receptor, which also uses interleukin 8 as a ligand, was reported not to dimerize (Trettel et al., 2003). Heterodimerization has also been reported between some (Mellado et al., 2001; Rodriguez-Frade et al., 2004) but not other (Babcock et al., 2003; Trettel et al., 2003) chemokine receptor pairs; a clear pattern therefore remains difficult to discern. One issue may be the comparison of the effects of chemokine ligands and monoclonal antibodies. It is possible that the reported effects of antibodies relate to clustering and capping of receptors rather than alterations in dimerization per se (Issafras et al., 2002).

It is not only the chemokine receptors at which substantial and divergent effects of ligands have been reported. Neuropeptide Y Y4 receptor (Berglund et al., 2003), thyroid-stimulating hormone receptor (Latif et al., 2001), and type A cholecystokinin receptor (Cheng and Miller, 2001) dimers have been reported to be constitutively formed but dissociated by agonists. In light of the discussion of the capacity of arrestins to bridge GPCRs dimers, it might be expected that ligand-induced dissociation of a GPCR dimer would limit or prevent interactions with arrestins and, therefore, both desensitization and arrestin-dependent internalization. Direct analysis of this issue would be of interest. By contrast, gonadotropin-releasing hormone (Cornea et al., 2001), lutropin (Tao et al., 2004), and a number of other receptors have been reported to increase in aggregation state in response to agonists, whereas the C5a complement receptor is a constitutive dimer that is unaffected by agonist (Floyd et al., 2003).

Does GPCR Dimerization Alter Ligand Pharmacology or Function?

With the caveats noted above that suggest some reported GPCR heterodimer pairs may have limited physiological significance, perhaps the most important questions in terms of relevance of GPCR heterodimers relate to whether they generate novel receptors in terms of either ligand pharmacology or function. This is a highly complex area, but for certain heterodimer pairs, at least, this does seem likely. This issue has been addressed most directly for opioid receptor pairings. This reflects a combination of the large number of subtype-selective ligands available and the long appreciated view that opioid tissue pharmacology cannot be explained only by the molecularly defined and characterized receptor subtypes. Many of the key experiments have been reviewed in detail (Jordan et al., 2000; Devi, 2001), and more recent studies have expanded this (Pan et al., 2002). Likewise, the idea of opioid receptor interactions has influenced thinking on the molecular basis of opioid tolerance (He et al., 2002). Despite clear variations in opioid pharmacology after coexpression of opioid receptor pairs, it has been difficult to fully replicate opioid tissue pharmacology using this approach. It remains unclear whether this is true simply because receptor expression ratios need to be more fully titrated and controlled because, as discussed earlier, coexpression of the MOP and DOP opioid receptors, for example, will not result in production of a pure population of MOP-DOP heterodimers. Ligands able to selectively target heterodimer pairs need to be identified and their function analyzed in physiological settings. The greatest progress in identifying such ligands undoubtedly stems from work on GPCRs that have historically attracted great efforts in medicinal chemistry. For example, a number of antiparkinsonian agents may have significantly higher affinity at dopamine D3/D2 heterodimers than at the corresponding homodimers (Maggio et al., 2003). A substantial number of other studies have reported unique pharmacology associated with the coexpression of pairs of GPCRs (e.g., Yoshioka et al., 2001). Likewise, the ability of β-blockers to interfere with angiotensin AT1-mediated signaling and of the AT1 receptor blocker valsartan to reduce catecholamine-induced elevation in heart rate (Barki-Harrington et al., 2003) may indicate functional angiotensin AT1-β-adrenoceptor interactions in vivo. Such studies, although fascinating, are indirect and may reflect cross-talk between the signaling pathways at a level downstream of receptor activation. Such interactions between G_s and G_q-mediated signals are well established (Cordeaux and Hill, 2002). Strategies that allow the detection of ligand binding or function only by a heterodimer pair in the presence of the corresponding homodimers are required to allow rapid and effective screening and detection of ligands with these characteristics. Only with such ligands at

hand will it be possible to tease out the physiological relevance of GPCR heterodimerization.

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