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REVIEW

G protein-coupled receptor hetero-dimerization: contribution to pharmacology and function

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The concept that G protein-coupled receptors (GPCRs) can form hetero-dimers or hetero-oligomers continues to gain experimental support. However, with the exception of the $GABA_B$ receptor and the sweet and umami taste receptors few reported examples meet all of the criteria suggested in a recent International Union of Basic and Clinical Pharmacology sponsored review (Pin *et al.*, 2007) that should be required to define distinct and physiologically relevant receptor species. Despite this, there are many examples in which pairs of co-expressed GPCRs reciprocally modulate their function, trafficking and/or ligand pharmacology. Such data are at least consistent with physical interactions between the receptor pairs. In recent times, it has been suggested that specific GPCR hetero-dimer or hetero-oligomer pairs may represent key molecular targets of certain clinically effective, small molecule drugs and there is growing interest in efforts to identify ligands that may modulate hetero-dimer function selectively. The current review summarizes key recent developments in these topics.
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Abbreviations: DOP, δ-opioid peptide; GPCR, G protein-coupled receptor; MOP, μ-opioid peptide; RET, resonance energy transfer

Introduction

G protein-coupled receptors (GPCRs) are the largest family of transmembrane signalling proteins and have been the most tractable target class for the development of therapeutic, small molecule drugs. Until relatively recently GPCRs were considered to exist as monomeric polypeptides. At least in part, this view was based on the monophasic binding characteristics of most antagonist ligands selective for individual GPCRs, while the pharmacology of individual receptors was presumed to provide a detailed and definitive signature of an individual GPCR that would be invariant between tissues. Indeed, prior to the era of cDNA cloning and genome sequencing, subtle variations in ligand structure–activity relationships between tissues, and even between species, was a driver in efforts to subdivide and further characterize GPCRs that responded to the same or overlapping endogenous ligands. In the last decade a series of revolutions in our understanding of protein–protein interactions in cells, and the changes in ligand pharmacology that can be observed with minor sequence variation in individual GPCRs, has altered our view of many of these assumptions. It is now well established that the interaction of a GPCR with intracellular GPCR-interacting proteins can alter the pharmacology, function and/or the trafficking of GPCRs in cells (Milligan and White, 2001; Hall and Lefkowitz, 2002). Furthermore, appreciation that open reading frame polymorphisms of GPCRs can alter pharmacology, function or regulation of a receptor is of crucial importance to the development of concepts of personalized medicine and drug treatment (Tang and Insel, 2005; Insel *et al.*, 2007; Kazius *et al.*, 2008). Equally, appreciation of the variation in pharmacology of ligands at species

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orthologues of GPCRs (Link *et al.*, 1992; Lim *et al.*, 2008) has resulted in consideration of the most appropriate animal models to assess likely efficacy of drugs in man and has ensured that screening and characterization of potential novel therapeutic agents are performed on GPCRs of human origin. Finally, it is becoming increasingly apparent that the basic lexicon of ligand pharmacology: agonist, antagonist and inverse agonist, must be considered to be system- and context-dependent. In a substantial number of cases described in recent years the same ligand can act as an agonist, antagonist or inverse agonist at the same GPCR, dependent upon the assay end point that is measured. This may reflect differential binding modes and the stabilization of distinct conformations of the receptor (Lane *et al.*, 2007; Bhattacharya *et al.*, 2008; Galandrin *et al.*, 2008). Equally the same GPCR may generate distinct signals in different cells. For example, it has recently been shown that the calcium-sensing receptor may cause stimulation or inhibition of secretion of parathyroid hormone-related protein depending on cellular context and coupling to G_s or G_i (Mamillapalli *et al.*, 2008).

As well as each of the issues noted above, the last decade has seen a substantial re-evaluation of the concept that GPCRs exist primarily as monomeric polypeptides, with increasing support for a model in which GPCRs can exist as homo- or hetero-dimers or as homo- or hetero-multimers. The implications of this for ligand pharmacology and its potential for novel drug design will be the focus of this review.

What is the evidence for GPCR homo- and hetero-multimerization?

Homo-dimerization

Protein dimerization is a remarkably common theme in biology (Marianayagam *et al.*, 2004; Mei *et al.*, 2005), and one reason for this is suggested to be the role of protein–protein interfaces in the control of protein folding during synthesis. Furthermore, there may be a propensity for individual GPCR monomers to dimerize or oligomerize in membranes as molecular dynamic simulations of individual molecules of rhodopsin in phospholipid bilayers demonstrate such self-assembly (Periole *et al.*, 2007). Early studies inconsistent with the concept of GPCRs as monomeric species were scattered in the literature and have been reviewed by Salahpour *et al.* (2000). However, in concert with studies that showed that co-expression of two distinct forms of the angiotensin II AT_1 receptor, each unable to bind ligand, resulted in the presence of a ligand binding-competent form of the receptor via protein trans-complementation (Monnot *et al.*, 1996), it was the advent of a series of co-immunoprecipitation studies following the co-expression of pairs of differentially epitope-tagged GPCRs that provided initial biochemical evidence for the presence of multiple copies of each of the β_2-adrenoceptor (Hebert *et al.*, 1996), the dopamine D_2 receptor (Ng *et al.*, 1996) and the δ-opioid peptide (DOP) receptor (Cvejic and Devi, 1997) within a complex. Each of these studies indicated an indeterminate proportion of the GPCR was likely to exist as a dimer and provided impetus for a vast range of related experiments that continue to the present day. Interestingly, each of these early studies also explored aspects of the selectivity, functional consequences, ligand regulation and/or molecular basis of GPCR dimerization and generated data that remain controversial. For example, in the studies of DOP receptor dimerization, Cvejic and Devi (1997) provided evidence to indicate that the addition of certain agonists would inhibit or reverse receptor dimerization and, as a corollary, they concluded that the DOP receptor was likely internalized from the cell surface as a monomer in response to agonist challenge. McVey *et al.* (2001) subsequently used combinations of co-immunoprecipitation studies and two distinct forms of living cell-based resonance energy transfer (RET) techniques to confirm the ability of the DOP receptor to form a homo-multimer that was present at the cell surface. However, efficacious agonists were unable to dissociate the complex. Ng *et al.* (1996) provided evidence that the dopamine D_2 receptor was not only able to homo-dimerize but also to hetero-dimerize with the 5-hydroxytryptamine $5\text{-}HT_{1B}$ receptor, and that certain ligands were selective in binding either dopamine D_2 receptor monomer or dimer. While the prospect of identifying ligands that selectively bind monomers or homo-multimers of the same GPCR remains attractive and would offer great potential for *in situ* identification of monomers and dimers, this has not been verified or extended convincingly. Hebert *et al.* (1996) reported that addition of a synthetic peptide corresponding to transmembrane domain VI of the β_2-adrenoceptor was able to interfere with receptor dimerization and limit agonist activation of adenylyl cyclase, suggesting that the dimer was important for G protein activation and for the function of the receptor. However, recent studies in which monomers of the β_2-adrenoceptor were incorporated into reconstituted high-density lipoprotein phospholipid bilayer particles, together with the stimulatory G protein G_s, demonstrated the capacity of the receptor monomer to produce G protein activation (Whorton *et al.*, 2007). Similar approaches have also indicated the capacity of monomeric rhodopsin to activate transducin (Whorton *et al.*, 2008). Despite this, studies have indicated that the β_2-adrenoceptor (and a number of other receptors) forms dimers/oligomers during protein synthesis and maturation and prior to cell surface delivery (Salahpour *et al.*, 2004) and that the β_2-adrenoceptor is internalized from the cell surface as a homo-dimer in response to binding of a single molecule of agonist to either protomer (Sartania *et al.*, 2007). Such observations question the physiological relevance of the capacity of a purified and reconstituted β_2-adrenoceptor monomer to function in an artificial system (Whorton *et al.*, 2007), despite the elegance of the approach used to demonstrate that a β_2-adrenoceptor monomer is sufficient to cause G protein activation. Furthermore, although the early studies of Hebert *et al.* (1996) indicated a likely central role for transmembrane domain VI as a 'dimer interface' many recent studies, both experimental and theoretical, have implicated transmembrane domains IV and V as key elements in many GPCRs (Lee *et al.*, 2003; Carrillo *et al.*, 2004; Guo *et al.*, 2005; Kim and Jacobson, 2006; Wang *et al.*, 2006; Harikumar *et al.*, 2007; Mancia *et al.*, 2008). However, it should be noted that in various studies, almost every element of one or other GPCR, including the intracellular C-terminal tail and the

extracellular N-terminal region (see Milligan, 2008 for review) and the third intracellular loop (Ciruela *et al.*, 2004) has been suggested to be important for dimerization. There remain skeptics who are unconvinced by the data that support GPCR dimerization/multimerization (Chabre and le Maire, 2005), or who have explored the methods used, particularly those based on RET, and questioned the conclusions reached (James *et al.*, 2006; Meyer *et al.*, 2006). However, a substantial number of reports have attempted to appraise the strengths and weaknesses of the various approaches used (Milligan and Bouvier, 2005; Gandía *et al.*, 2008a), to rebut the criticisms raised (Bouvier *et al.*, 2007; Salahpour and Masri, 2007) or to expand the techniques employed to address this issue (Mesnier and Banères, 2004; Maurel *et al.*, 2008) and support the concept of dimerization. Overall, the vast majority of reports have provided evidence in support of the concept of receptor homo-dimerization (see Milligan, 2004; 2007; 2008; Park *et al.*, 2004; Maggio *et al.*, 2007 for review), while certain studies also indicate the potential for higher-order complexity in GPCR structure (Klco *et al.*, 2003; Carrillo *et al.*, 2004; Park and Wells, 2004; Lopez-Gimenez *et al.*, 2007; Gandía *et al.*, 2008b; Guo *et al.*, 2008) for a number of rhodopsin family receptors although others claim that dimers but not oligomers, of at least the family B secretin receptor (Harikumar *et al.*, 2008) and the family C metabotropic glutamate receptors (Maurel *et al.*, 2008), can be observed.

Hetero-dimerization

If the existence of receptor homo-dimers remains uncertain then clarity over the expression and importance of receptor hetero-dimers is yet more clouded. Although genetic as well as biochemical and pharmacological studies define that the class C $GABA_B$ receptor (Jones *et al.*, 1998; White *et al.*, 1998) and the sweet and umami taste receptors (Zhao *et al.*, 2003) represent constitutively formed hetero-dimers of distinct (but closely related) GPCR polypeptides, the significance of a wide range of other reported hetero-dimers remains unclear. In large part, this reflects that key studies have often been limited to experiments involving the co-transfection of pairs of GPCR cDNAs into heterologous cell lines that are easy to manipulate, or that important observations from native cells and tissues have not always been verified independently. For example, although AbdAlla *et al.* have published a series of interesting papers on angiotensin AT_1 receptor hetero-dimers in pre-eclampsia and in experimental hypertension (AbdAlla *et al.*, 2001; 2005), these studies have not been replicated independently. Equally, fascinating observations of the ability of both angiotensin AT_1 receptor blockers and β-blockers to inhibit downstream signalling via both receptors have been interpreted as reflecting their *in vivo* hetero-dimerization (Barki-Harrington *et al.*, 2003) but, currently, no follow-up of these pharmacologically surprising results has been reported. Many of the early studies on GPCR hetero-dimerization have been reviewed and appraised widely (Milligan, 2006; Szidonya *et al.*, 2008). The International Union of Basic and Clinical Pharmacology has recently suggested guidelines for the range of evidence that might be accumulated prior to claiming evidence of novel GPCR hetero-multimers (Pin *et al.*, 2007). However, in only a limited number of examples has full concordance with these guidelines been achieved (Pin *et al.*, 2007). Although there are many appealing aspects of GPCR hetero-dimers as novel therapeutic targets (Milligan, 2006), until recently the pharmaceutical industry has been unsure of the viability of exploring this avenue (Kent *et al.*, 2007). Not least, this reflects the requirement for more clear-cut validation of the expression of pathophysiologically relevant hetero-dimers, a better understanding of their tissue distribution and the challenges inherent in attempting to screen for and identify small molecule ligands with specificity, or at least significant selectivity, for GPCR hetero-dimers (Milligan, 2006; Eglen *et al.*, 2007; Dalrymple *et al.*, 2008).

Questions relating to the selectivity of GPCR hetero-multimerization, the existence of such complexes in native cells and tissues and their contribution to pathophysiology, the effects of ligands on hetero-dimer behaviour, the molecular basis of dimerization and multimerization and the potential to identify ligands that specifically bind to and regulate GPCR hetero-multimers are currently the most actively researched themes in this area.

Why are receptors dimers?

Facilitation of G protein activation?

It has been suggested, based on the molecular dimensions of the atomic level structures of bovine rhodopsin and of heterotrimeric G proteins, that a GPCR dimer might provide the most appropriate footprint to bind a G protein (Fotiadis *et al.*, 2006). However, despite evidence that the leukotriene B4 BLT_1 receptor–G_i G protein complex is a pentamer consisting of one copy of each of the α, β and γ G protein subunits and two copies of the receptor polypeptide (Banères and Parello, 2003), it is now clear that isolated GPCR monomers can bind and activate G proteins (Whorton *et al.*, 2007; 2008). Indeed, monomers of the neurotensin NTS_1 receptor are reported to activate G protein more effectively than dimers (White *et al.*, 2007). It thus appears unlikely that a requirement for signal generation underlies GPCR dimerization (see Gurevich and Gurevich, 2008 for review).

Control of cell surface delivery?

A number of studies have suggested that GPCR dimerization may be important in cell surface delivery and that dimerization is initiated early in protein synthesis. Taking advantage of clear understanding of the role of the C-terminal endoplasmic reticulum (ER) retention motif of $GABA_{B1}$ in the production and cell surface delivery of the functional $GABA_B$ receptor hetero-dimer (Margeta-Mitrovic *et al.*, 2000), Salahpour *et al.* (2004) replaced the C-terminal tail of the β_2-adrenoceptor with the C-terminal tail of the $GABA_{B1}$ and demonstrated intracellular ER retention of this construct when expressed in human embryonic kidney (HEK)293 cells. When co-expressed with the retained mutant, wild-type β_2-adrenoceptor was also retained intracellularly (Salahpour *et al.*, 2004). Such a 'dominant negative' effect of the chimeric β_2-adrenoceptor–$GABA_{B1}$ construct supports the idea that protein–protein interactions between the engineered ER-retained and wild-type forms of the receptor occurred

during receptor synthesis and maturation. Addition of a distinct, 14 amino acid ER retention motif from the α_{2C}-adrenoceptor to the C-terminal tail of the chemokine CXCR1 receptor resulted in intracellular retention of this chimeric construct in HEK293 cells. This ER-retained construct also limited cell surface transport of co-expressed wild-type forms of both CXCR1 and CXCR2 (Wilson *et al.*, 2005), supporting other evidence of both CXCR1 receptor homo-dimerization and CXCR1–CXCR2 receptor hetero-dimerization (Milligan *et al.*, 2005). The selectivity of such interactions was indicated because the presence of the trapped CXCR1 receptor was without effect on cell surface delivery of a co-expressed α_{1A}-adrenoceptor (Wilson *et al.*, 2005). Equally, by employing a form of the α_{1B}-adrenoceptor containing mutations in both transmembrane domains I and IV that is retained in the ER and fails to mature properly (Canals *et al.*, 2009) and adding distinct bimolecular fluorescence complementation-competent forms of enhanced yellow fluorescent protein to this receptor, 'dimerization' could be shown within the ER (Lopez-Gimenez *et al.*, 2007). The transmembrane domains I and IV mutant of the α_{1B}-adrenoceptor is, however, compromised in dimerization/oligomerization because it displays a reduced effectiveness to generate FRET signals when appropriately tagged forms are co-expressed (Lopez-Gimenez *et al.*, 2007). This mutant was also able to interact with co-expressed wild-type α_{1B}-adrenoceptor and, by acting as a 'dominant negative', retain the wild-type receptor within the ER. As with a number of ER-retained mutant GPCRs, the transmembrane domains I and IV mutant of the α_{1B}-adrenoceptor could be trafficked to the surface of cells by the maintained presence of a 'pharmacological chaperone' (see Conn *et al.*, 2007 for review) in the form of the α_1-adrenoceptor antagonist prazosin (Canals *et al.*, 2009). Cell surface delivery was proceeded by maturation of the N-glycosylation status of the mutant receptor and improved dimerization/oligomerization of the mutant receptor as measured by enhanced FRET signals (Canals *et al.*, 2009). Interestingly, when the transmembrane domains I and IV mutant of the α_{1B}-adrenoceptor was co-expressed with, and caused ER-trapping of, a form of the α_{1B}-adrenoceptor that was mutated to prevent the binding of prazosin but was otherwise wild-type, the maintained presence of prazosin caused cell surface delivery of both the transmembrane domains I and IV mutant and the ligand binding-deficient form of the receptor (Canals *et al.*, 2009). These data are best explained by the generation of dimer/oligomer interface interactions within the ER and the cell surface trafficking of a dimer/oligomer containing both forms of the α_{1B}-adrenoceptor (Figure 1). These studies appear to provide strong support for the concept that GPCRs traffic to the cell surface as dimeric or oligomeric complexes and only after passing ER/Golgi export quality control. Along with real-time FRET-based studies on the location and transport of 5-hydroxytryptamine 5-HT_{2C} receptor dimers/oligomers (Herrick-Davis *et al.*, 2006), such studies help to define ER-to-cell surface trafficking of mammalian class A GPCR homo-dimers or homo-multimers. There have also been a number of studies in which co-expression of pairs of GPCRs promotes surface localization that has been used to support the concept of hetero-dimerization (see Minneman, 2007 for review). In part, such studies have built on the recognition that co-expression of the $GABA_{B2}$ polypeptide was required to interact with the $GABA_{B1}$ polypeptide to allow trafficking of the hetero-dimer complex to the cell surface and functional expression of the $GABA_B$ receptor (Margeta-Mitrovic *et al.*, 2000). This reflects the capacity of the $GABA_{B2}$ subunit to mask an ER retention motif within the C-terminal tail of the $GABA_{B1}$ subunit. In class A receptors, interactions between the α_{1B}-adrenoceptor and the α_{1D}-adrenoceptor promoted cell surface delivery of the α_{1D}-adrenoceptor (Hague *et al.*, 2004b), and a similar effect was produced by co-expression of the α_{1D}-adrenoceptor with the β_2-adrenoceptor (Uberti *et al.*, 2005). Hague *et al.* (2004a) have also demonstrated the capacity of a co-expressed β_2-adrenoceptor to allow cell surface delivery of certain olfactory receptors. Although the physiological significance of this remains to be established, the lack of effectiveness of other adrenoceptors to promote cell surface delivery of the olfactory receptors suggested that it should be straightforward to define the structural determinants of such interactions. Indeed, a follow-up study has suggested a key role for transmembrane domain II of the β_2-adrenoceptor (Bush *et al.*, 2007).

GPCR hetero-dimerization and ligand pharmacology

The ability of a selective ligand at one GPCR to modulate the function of a second, co-expressed GPCR does not inherently imply hetero-dimerization between the two GPCRs. Indeed, such effects often reflect either heterologous sensitization or desensitization or input to common signalling pathways downstream of the receptors in question. For example, Lopez-Gimenez *et al.* (2008) have recently shown that co-activation of a 5-hydroxytryptamine 5-HT_{2A} receptor co-expressed with the μ-opioid peptide (MOP) receptor in HEK293 cells results in the MOP receptor agonist morphine being able to induce each of internalization, desensitization and down-regulation of the MOP receptor. By contrast, morphine was unable to produce any of these effects in the same cells lacking the 5-HT_{2A} receptor, even when 5-hydroxytryptamine was added along with morphine (Lopez-Gimenez *et al.*, 2008). Tagging of the two GPCRs with distinct auto-fluorescent proteins demonstrated that in untreated cells the MOP receptor was present almost exclusively at the cell surface whereas the bulk of the 5-HT_{2A} receptor was present in punctate, intracellular vesicles and could not, therefore, be complexed within a hetero-dimer with the MOP receptor (Lopez-Gimenez *et al.*, 2008). This can be contrasted with functional interactions between the cannabinoid CB_1 receptor and the orexin OX_1 receptor. When expressed alone in HEK293 cells the OX_1 receptor was present at the cell surface, whereas when the cannabinoid CB_1 receptor was expressed alone in such cells it displayed a distribution pattern consistent with rapid, ligand-independent recycling between the cell surface and endosomes (Ellis *et al.*, 2006). Following co-expression, the OX_1 receptor adopted the distribution pattern of the cannabinoid CB_1 receptor and the addition of RET-competent tags to the C-terminal tail of each receptor demonstrated hetero-interactions between the two co-expressed receptors (Ellis *et al.*, 2006). The presence of

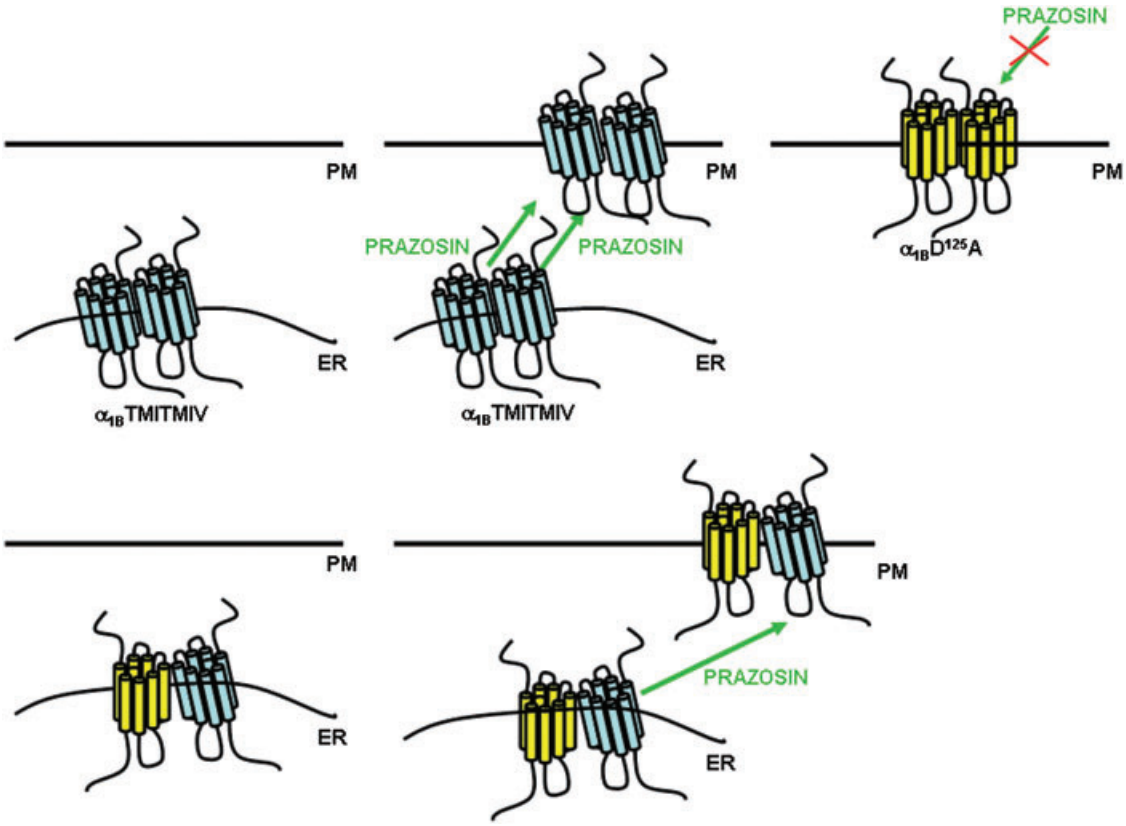

Figure 1 The α_{1B}-adrenoceptor traffics to the cell surface as a dimer/oligomer. A form of the α_{1B}-adrenoceptor containing mutations in transmembrane domains (TM) I and IV (α_{1B}TMITMIV, blue) (Lopez-Gimenez *et al.*, 2007) is retained in the endoplasmic reticulum (ER) when expressed in HEK293 (human embryonic kidney) cells and study employing bimolecular fluorescence complementation indicate that it exists as a dimer/oligomer (Lopez-Gimenez *et al.*, 2007). Sustained treatment of such cells with the α_1-adrenoceptor antagonist prazosin results in maturation of the receptor and its movement to the plasma membrane (PM). A form of the α_{1B}-adrenoceptor that is wild-type except that it contains an $Asp^{125}Ala$ mutation that eliminates its ability to bind prazosin ($\alpha_{1B}D^{125}A$, yellow) is delivered successfully to the PM when expressed. When $\alpha_{1B}D^{125}A$ is co-expressed with α_{1B}TMITMIV, $\alpha_{1B}D^{125}A$ becomes ER-retained because α_{1B}TMITMIV interacts with $\alpha_{1B}D^{125}A$ and functions as a 'dominant negative'. Treatment of these cells with prazosin results in movement of both α_{1B}TMITMIV and $\alpha_{1B}D^{125}A$ to the cell surface. As $\alpha_{1B}D^{125}A$ cannot bind prazosin, these observations indicate that the two forms of the α_{1B}-adrenoceptor move to the PM as a dimer/oligomer (see Canals *et al.*, 2009 for further details).

the cannabinoid CB_1 receptor antagonist/inverse agonist SR-141716A (N-(piperidino-1-yl)-5-(4-chlorophenyl)-1-(2, 4-dichlorophenyl)-4-methyl-pyrazole-3-carboxamide), also known as rimonabant, resulted in redistribution of the CB_1 receptor to the cell surface, whether expressed in isolation or co-expressed with the OX_1 receptor (Ellis *et al.*, 2006). Importantly, in cells co-expressing these two receptors treatment with SR-141716A also caused redistribution of the OX_1 receptor to the cell surface, despite SR-141716A having no significant affinity to bind directly to the OX_1 receptor (Ellis *et al.*, 2006). In an equivalent manner, the selective OX_1 receptor antagonist SB-674042 (1-(5-(2-fluorophenyl)-2-methylthiazol -4-yl)-1-((S)-2-(5-phenyl-[1,3,4]oxadiazol-2-ylmethyl)-pyrrolidin-1-yl)-meth-anone), which displayed no significant affinity to bind directly, caused redistribution of the cannabinoid CB_1 receptor to the surface of cells, but only when the OX_1 receptor was co-expressed (Ellis *et al.*, 2006). The most obvious interpretation of these observations is that when co-expressed, the OX_1 receptor and cannabinoid CB_1 receptor form a stable hetero-dimer complex that is able to bind and be regulated by both cannabinoid CB_1 and OX_1 receptor ligands. A substantial number of studies have shown the ability of selective, usually agonist, ligands to produce co-trafficking of a GPCR that has been co-expressed along with the receptor that binds the selective ligand or to limit internalization of the partner receptor (Jordan *et al.*, 2001; So *et al.*, 2005; Roumy *et al.*, 2007; Ecke *et al.*, 2008; Fiorentini *et al.*, 2008). Although it has been suggested that such ligand-induced 'co-internalization' may reflect other factors (Janoshazi *et al.*, 2007), it is certainly consistent with the concept of 'hetero-dimerization' and internalization of the intact complex from the cell surface.

Selective ligands may also have direct and selective effects on the conformation of the GPCR protomers that comprise the hetero-dimer. In the case of the $GABA_B$ receptor, binding of the neurotransmitter GABA in the N-terminal region of the $GABA_{B1}$ element of the hetero-dimer is transmitted to activation of G protein via the $GABA_{B2}$ protomer. This implies communication between the two elements of the hetero-dimer and a conformational alteration of $GABA_{B2}$ in response to the ligand, although it does not bind directly to this element. Communication between the promoters of a leukotriene B_4 BLT_1 receptor 'homo-dimer', consisting of a wild-type protomer and a modified protomer able to bind the agonist

only weakly, was indicated by alteration in response to an agonist ligand of the fluorescence properties of a 5-hydroxytryptophan introduced into the mutant protomer (Mesnier and Banères, 2004). Equally, based on co-expression of the MOP receptor with a form of the α_{2A}-adrenoceptor able to detect conformational change within this receptor because of the introduction of a pair of FRET-competent reporters, Vilardaga *et al.* (2008) reported that morphine was able to produce a conformational alteration in the α_{2A}-adrenoceptor and that this was associated with inhibition of α_{2A}-adrenoceptor-mediated regulation of extracellular signal-regulated kinase (ERK) mitogen-activated protein (MAP) kinase phosphorylation. Such detailed studies employing biophysical and chemical biology approaches clearly demonstrate that certain GPCR–GPCR interactions can alter receptor structure and, potentially, function. Growing evidence also indicates that GPCR hetero-dimerization can alter receptor pharmacology. If so, it should be possible to detect such interactions in either ligand binding or functional screens (Franco *et al.*, 2008a).

As noted above, co-expression of the cannabinoid CB_1 receptor and the OX_1 receptor results in alterations in the cellular trafficking of both receptors in response to antagonists highly selective for either GPCR (Ellis *et al.*, 2006). The motivation to explore potential hetero-dimerization of these receptors stemmed from earlier studies that showed a marked enhancement of the potency of orexin A to activate the ERK1/2 MAP kinases when the OX_1 receptor was co-expressed with the cannabinoid CB_1 receptor (Hilairet *et al.*, 2003). Furthermore, this effect was blocked by SR-141716. Although different, in that Ellis *et al.* (2006) noted little effect of simply expressing the cannabinoid CB_1 receptor on the potency of orexin A to activate ERK1/2 MAP kinase activity, they also noted that SR-141716 reduced the potency of orexin A in cells co-expressing the two receptors. Given that rimonabant (Acomplia™) is a drug that has been employed clinically to treat obesity, and the cell studies demonstrated its effect to reduce the potency of a signal anticipated to be strongly pro-orexegenic, it is clearly an interesting speculation that rimonabant exerts at least part of its action via a cannabinoid CB_1 receptor–OX_1 receptor hetero-dimer. However, the existence of such a complex *in vivo* remains to be confirmed. It would be of great interest to know the effectiveness of rimonabant in OX_1 receptor knockout mouse models.

As noted earlier, the identification of small molecule ligands specific or highly selective for particular GPCR hetero-dimers would be of great value. As well as being of use as 'proof of concept' agents to promote efforts in screening by the pharmaceutical industry, they would allow the analysis of hetero-dimer expression and function in primary cells, tissues and animal models. Currently, the best-described hetero-dimer-selective ligand is 6′-guanidinonaltrindole (Waldhoer *et al.*, 2005). This simple derivative of a κ-opioid peptide (KOP) receptor ligand is reported to act as a selective agonist in cells co-expressing the KOP and DOP opioid receptors, via the DOP–KOP receptor hetero-dimer. It has also been reported to function as a spinally selective analgesic, despite it being only some sixfold more potent in cells co-expressing the KOP and DOP receptors than in cells expressing only the KOP receptor (Waldhoer *et al.*, 2005). However, as in many initial ligand screening campaigns, the potency data were derived from studies employing the channelling of signals to the elevation of $[Ca^{2+}]$ via use of a promiscuous, chimeric G protein (Milligan and Rees, 1999) rather than via measurement of an end point, such as regulation of ion channel function, which might be directly relevant to opioid function *in vivo*. Despite significant interest in these studies (Park and Palczewski, 2005), independent confirmation of the observations is still awaited. It is difficult to envisage 6′-guanidinonaltrindole spanning the two ligand binding pockets of two GPCR protomers that form a 'contact' DOP–KOP hetero-dimer. However, although they are likely to be less favourable from an energetic standpoint and, therefore, if they exist, may represent only a small proportion of any GPCR dimer (Bakker *et al.*, 2004), 'domain swap' hetero-dimers might be anticipated to generate unique ligand binding pockets that would bind ligands with distinct affinity and produce unique ligand structure–activity relationships. There is, moreover, a substantial literature on alterations in ligand potency with co-expression of pairs of opioid receptors and on ligand pharmacology in native tissues that is not reproduced by the expression of individual opioid receptor subtypes in heterologous cell lines (Levac *et al.*, 2002). Furthermore, the generation of synthetic opioid ligands containing a backbone linker separating two distinct opioid pharmacophores has provided evidence for the organization of co-expressed opioid receptor subtypes as hetero-dimers (Xie *et al.*, 2005).

Another indication of selectivity of ligands for receptor hetero-dimers comes from studies on interactions between co-expressed dopamine D_1 and D_2 receptors. Although the dopamine D_1 receptor is commonly associated with elevation of cAMP levels via activation of G_s and the dopamine D_2 receptor with inhibition of cAMP via activation of G_i-like G proteins, in cells co-expressing these two receptors Rashid *et al.* (2007) demonstrated that either the endogenous agonist dopamine or a combination of selective D_1 and D_2 receptor agonists resulted in elevation of Ca^{2+} via YM254890-sensitive G proteins of the G_q/G_{11} family. Similar results were observed in striatal tissue from wild-type but in neither D_1 nor D_2 knockout mice (Rashid *et al.*, 2007), and SKF83959 (3-methyl-6-chloro-7,8-hydroxy-1-[3-methylphenyl]-2,3,4,5-tetrahydro- 1*H*-3-benzazepine) appeared to function as a hetero-dimer-selective agonist. Interestingly, there are a number of ways in which ligands that show little or no direct affinity for a specific GPCR can regulate the function of that GPCR if it forms a hetero-dimer with a receptor for which the ligand does have affinity. This concept has been discussed in terms of allosteric interactions within hetero-dimers (Milligan and Smith, 2007; Springael *et al.*, 2007). For example, following co-expression of forms of the chemokine CXCR2 receptor and the DOP receptor that allow signal generation only if the two receptor constructs form a hetero-dimer, agonist function at the DOP receptor was enhanced by the presence of the CXCR2 blocker SB-225002 ((N-(2-hydroxy-4-nitrophenyl)-N′-(2-bromophenyl)urea) although this compound has no effect on DOP receptor function in the absence of the CXCR2 receptor (Parenty *et al.*, 2008). Although it was assumed that the CXCR2 blocker functioned as an orthosteric antagonist at the CXCR2 receptor, this is clearly not the only means by which

communication between the protomers of a hetero-dimer can be detected (Milligan and Smith, 2007) and it is, therefore, of considerable interest that certain CXCR1 and CXCR2 blockers appear to bind at an intracellular site on the receptor (Nicholls *et al.*, 2008). It is thus possible that a range of compounds can function as selective allosteric modulators of GPCR hetero-dimers, and molecules of this nature may be uncovered in a relatively straightforward manner if appropriate hetero-dimer screens and homo-dimer counter-screens are established. The concept that knockout animal models may provide an excellent test bed to assess the chemical 'fingerprint' of GPCR hetero-dimers in native tissues has been raised (Franco *et al.*, 2008a,b), and particularly tissue-specific or conditional knockouts may be ideally suited for such studies.

GPCR hetero-dimerization and pathophysiology

A contribution of glutamatergic dysregulation to the aetiology of schizophrenia has long been proposed and has led to approaches other than the standard of antagonism of monoaminergic receptors being championed. In recent times the effectiveness of LY404039 [(-)-(1R,4S,5S,6S)-4-amino-2-sulfonylbicyclo[3.1.0]hexane-4,6-dicarboxylic acid], a selective agonist of $mGlu_{2/3}$ receptors, in the treatment of both positive and negative aspects of schizophrenia (Patil *et al.*, 2007) has garnered considerable attention as a potential novel approach. Hallucinogenic drug models of psychosis have a number of similarities to aspects of schizophrenia, and the contribution of signalling of the 5-hydroxytryptamine $5\text{-}HT_{2A}$ receptor via a pertussis toxin-sensitive, G_i family-initiated pathway is central to the discrimination between hallucinogenic and non-hallucinogenic agonists of this receptor (González-Maeso *et al.*, 2007). Interestingly, González-Maeso *et al.* (2008) have recently shown direct interactions between the $5\text{-}HT_{2A}$ receptor and the $mGlu_2$ but not $mGlu_3$ receptor by combinations of co-immunoprecipitation studies employing human brain tissue and various RET-based studies performed in transfected cell lines. Furthermore, they were able to demonstrate that the affinity of hallucinogenic $5\text{-}HT_{2A}$ receptor agonists to compete with the antagonist [^{3}H]ketanserin for binding to the $5\text{-}HT_{2A}$ receptor in mouse somatosensory cortex was higher in the presence of the $mGlu_{2/3}$ receptor agonist LY379268 [(1R,4R,5S,6R)-4-Amino-2-oxabicyclo [3.1.0]hexane-4,6-dicarboxylic acid], while, by contrast, the affinity of $mGlu_{2/3}$ agonists was reduced in the presence of the hallucinogen 1-(2,5)-dimethoxy-4-indophenyl)-2-aminopropane (DOI). Equally, the potency of DOI to promote binding of [^{35}S]GTPγS to pertussis toxin-sensitive G proteins was greatly enhanced by the presence of the $mGlu_2$ but not $mGlu_3$, while this effect of $mGlu_2$ was not evident when LY379268 was also present. LY379268 also caused a marked reduction of potency of DOI to stimulate binding of [^{35}S]GTPγS to pertussis toxin-sensitive G proteins in membranes prepared from cortical cells maintained in primary culture (González-Maeso *et al.*, 2008). These studies suggest that although $mGlu_2$ agonists appear effective in the treatment of schizophrenia, the effectiveness may stem, at least in part, from regulation of the $5\text{-}HT_{2A}$–$mGlu_2$ hetero-dimer. This is certainly not the only case in which GPCR hetero-dimers have been implicated as therapeutic targets for the treatment of disease. For example, a substantial literature on interactions between dopamine D_2 and adenosine A_{2A} receptors in systems ranging from transfected cells to the brain (Fuxe *et al.*, 2007; Ferré *et al.*, 2008) has highlighted the potential for therapeutic strategies that target this complex. However, despite these interesting observations much more needs to be established in terms of tissue distribution, function and differential pharmacology of distinct sets of GPCR hetero-dimers before they will be considered widely as tractable therapeutic targets.

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Conflict of interest

The author states no conflict of interest.

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