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# Mini-review

**G protein-coupled receptor dimerization: function and ligand pharmacology**

Graeme Milligan

Molecular Pharmacology Group

Division of Biochemistry and Molecular Biology

Institute of Biomedical and Life Sciences

University of Glasgow

Glasgow G12 8QQ

Scotland, U.K.

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Address all correspondence to:

Graeme Milligan, Molecular Pharmacology Group, Davidson Building, University of

Glasgow, Glasgow G12 8QQ Scotland, U.K.

Tel: 44 (0) 141 330 5557

FAX: 44 (0) 141 330 4620

e-mail: g.milligan@bio.gla.ac.uk

[1] Abbreviations: FRET; fluorescence resonance energy transfer, BRET;

bioluminescence resonance energy transfer, GPCR; G protein–coupled receptor.

MOLPHARM 2004 0497

## 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 hetero-dimeric GPCR pairings. Key questions that remain to be addressed effectively include the prevalence and relevance of these in native tissues and the implications of hetero-dimerization for pharmacology and, potentially, for drug design.

## Introduction

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 [1]) 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, nor indeed seek to address, whether the GPCR functioned as a monomer or if function required quaternary structure. In the recent past the concept that GPCRs can exist as dimers or higher-order oligomers has advanced rapidly (see Angers et al., 2002, Lee et al., 2003, Milligan et al., 2003, for reviews). There is also growing appreciation that GPCR dimerization may be a requisite for function. A

3

wide range of approaches has contributed to the view that individual GPCRs can exist

as homo-dimers. 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 co-immunoprecipitate co-expressed

but differentially epitope-tagged forms of a GPCR (Hebert et al., 1996, Cjevic and

Devi, 1997) and the capacity to reconstitute function following the co-expression of

distinct pairs of mutants that are both individually non-functional (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, McVey et al., 2001, Cheng and Miller, 2001, Kroeger 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 hetero-dimers or -oligomers (Marshall, 2001, George et al., 2002). This is

clearly not a random process (Milligan et al., 2004) and in certain cases, for example

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 pharmacology 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 analyse such interactions, understanding of

MOLPHARM 2004 0497

the molecular basis of hetero-dimerization and of the relative propensity of GPCRs to

hetero-dimerize is currently in its infancy.

One key question that remains contentious is how, or indeed if, receptor ligands alter

the dimerization or organizational structure of GPCRs. The growing literature on

GPCR hetero-dimerization has also resulted in attempts to address if ligand

pharmacology or function at hetero-dimer pairs is distinct from that at the

corresponding homo-dimers. It is possible 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 appears

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 about the preparation procedures

employed and the implications for molecular organization of biophysical experiments

conducted a number of years ago (Chabre et al., 2003) these studies demonstrated

rhodopsin to be arranged in para-crystalline arrays. Models based on these pictures were inferred to indicate that intra- dimer 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 above and 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-PAGE, 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 NPY Y2 receptor and 44% of NPY Y1 receptor constructs were able to generate effective FRET signals. These numbers are likely to represent minimum estimates of dimerization as they were calculated relative to an NPY 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* luciferase and GFP-tagged forms of the $\beta_2$-adrenoceptor were co-expressed

(Mercier et al., 2002), estimated that more than 80% of this GPCR was present in dimers. Because GPCR dimerization is difficult to analyse in native cells and tissues it has also been suggested that some examples of GPCR dimerization may, at least in part, be artefacts 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 $\beta_2$-adrenoceptor was unchanged over a 20 fold range of GPCR expression levels. Equally, 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 co-entry receptor was observed at levels comparable to 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, both 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) were 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.

MOLPHARM 2004 0497

**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 likely a key 'quality control' step prior to 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 whilst 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, Lee et al., 2000, Karpa 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 $\alpha$ and the $\beta_1 + \gamma_1$ complex (Liang et al., 2003).  It is

clear that GPCRs are able to bind G protein $\alpha$ and $\beta/\gamma$ subunits independently and that

$\beta/\gamma$ interactions contribute substantially to the ability of agonists to activate G protein

$\alpha$ subunits (Liu et al., 2002).  Biophysical studies following expression in *E. Coli*,

denaturation and refolding of the leukotriene B4 BLT1 receptor followed by

reconstitution of the GPCR with a heterotrimeric G protein consisting of $G\alpha_{i2}$, $\beta_1$ and

MOLPHARM 2004 0497

$\gamma_2$ also provided compelling evidence for the overall assembly of a pentameric

$(BLT1)_2$ $G\alpha_{i2}$, $\beta_1$ and $\gamma_2$ complex (Baneres and Parello, 2003). Interestingly, 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 $\beta_2$-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 agonist (but not forskolin or NaF)–mediated adenylyl

cyclase activity (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. A key dimer interface

in the *Saccharomyces cerevisiae* $\alpha$-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 as well as 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). Both these authors (Lee et al., 2003) and others (Guo et al., 2003) have

also 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 homo-dimers. Such studies would

certainly benefit from independent replication.


**Are GPCRs permanent dimers?**

As discussed above, emerging evidence indicates dimerization to occur 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 *Saccharomyces 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 $\alpha_{1a}$-

adrenoceptor, that constitutively internalize at a rapid rate in the absence of ligands,

also appears to be composed of dimers (Ramsay et al., 2004). There is therefore

growing evidence that the entire life-cycle of some GPCRs takes place within a

dimeric complex.

MOLPHARM 2004 0497

The concept that GPCRs internalize as dimers has been utilized to attempt to explore

the selectivity of GPCR hetero-dimerization. For example, following co-expression

of the $\alpha_{1a}$- and $\alpha_{1b}$-adrenoceptors, the $\alpha_{1a}$-selective agonist oxymetazoline was able to

induce co-internalization of the two GPCRs. However, this agonist did not produce

co-internalization of either the NK1 tachykinin receptor or the CCR5 chemokine

receptor when they were co-expressed with the $\alpha_{1a}$-adrenoceptor (Stanasila et al.,

2003). This suggests that the $\alpha_{1a}$- and $\alpha_{1b}$-adrenoceptors form a high affinity hetero-

dimer but that the other pairs do not. In a similar vein, co-expression of the NK1

receptor with the MOP opioid receptor resulted in co-internalization 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 $\beta_2$-adrenoceptor is co-expressed with

the DOP opioid receptor, internalization of the opioid receptor is seen in response to

isoproterenol (Jordan et al., 2001). However, when the $\beta_2$-adrenoceptor was co-

expressed with the KOP opioid receptor, isoproterenol treatment failed to induce $\beta_2$-

adrenoceptor internalization (Jordan et al., 2001). These observations are of

considerable interest but also pose problems of interpretation. The $\beta_2$-adrenoceptor-

DOP opioid receptor data indicate a capacity of these two GPCRs to interact and that

agonist-occupation of one element of the hetero-dimer 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-$\beta_2$-

adrenoceptor hetero-dimer restrains or blocks ligand-induced internalization.

Interestingly, however, direct measurements indicate the propensity of the $\beta_2$-

adrenoceptor and KOP opioid receptors to form a hetero-dimer is much less than for

the KOP opioid receptor to form a homo-dimer or a hetero-dimer with the DOP

opioid receptor (Ramsay et al., 2002). KOP opioid receptor-$\beta_2$-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 hetero-

dimers, then 50% of the population should be represented by A-A and B-B.

Therefore, if the KOP opioid receptor and the $\beta_2$-adrenoceptor are co-expressed but

have only relatively low mutual affinity then it must be anticipated that the majority

of dimers formed would be $\beta_2$-adrenoceptor-$\beta_2$-adrenoceptor and KOP opioid

receptor-KOP opioid receptor homo-dimers. There is no reason to suppose that a $\beta_2$-

adrenoceptor-$\beta_2$-adrenoceptor homo-dimer would not internalize in response to

isoproterenol. Although at the current time this is pure speculation, the data of Jordan

et al. (2001) may therefore indicate the production of a larger oligomeric complex

containing copies of both GPCRs. When the $\beta_2$-adrenoceptor and the DOP opioid

receptor were stably co-expressed at relatively modest levels in HEK293 cells,

agonists at both GPCRs were required to produce internalization of the two

polypeptides (Tsao and von Zastrow, 2000). Interestingly, 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 homo-dimers predominate

or if there is a significant hetero-dimer population these are separated into monomers

during intracellular trafficking. Other data, discussed later, would allow the second

hypothesis. Interactions with $\beta$-arrestins to allow clathrin-mediated internalization are

consistent with a trimeric $(GPCR)_2$-$\beta$-arrestin complex (and therefore the GPCR

dimer) persisting at least until removal of $\beta$-arrestin within acidified endosomal

vesicles. This concept also raises intriguing questions about the fate of GPCR hetero-dimers that incorporate so called type A and type 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 following internalization. The type B GPCRs have substantially higher affinity for, and generally co-internalise with, β-arrestins into endocytic vesicles (Shenoy and Lefkowitz, 2003). Co-expression 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 following co-expression 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 following co-expression of pairs of GPCRs. This has been a popular endpoint measure used to support potential distinct functions of GPCR hetero-dimers.

**What is the propensity of GPCRs to form hetero-dimers?**

Before we can attempt to rationalize potential variations in ligand function and pharmacology with GPCR hetero-dimerization we must understand the expression pattern of the GPCR family. The best estimates of the number of functional GPCR genes in man are for some 339 encoding olfactory receptors (Malnic et al., 2004) and

in the region of 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 homo-dimers because each olfactory neurone

expresses only one member of the family. Although not yet fully confirmed,

microarray and related studies have, however, 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 co-expressed

in detectable amounts. Although transport of mRNAs to different cellular locations

prior to translation in terminally differentiated and polarized cells may prevent co-

temporal 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 co-

immunoprecipitation studies Salim et al., (2002) reported the capacity of all GPCRs

tested to be co-immunoprecipitated with the serotonin 5-HT$_{1A}$ receptor. In studies of

a number of family A GPCRs negative controls have utilised family C GPCRs, such

as the GABAbR1, that have no inherent homology. However, even this may not be

14

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 non-chemokine 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 $\beta_1$-adrenoceptor and $\beta_2$-adrenoceptor can form hetero-dimers with similar affinity as the corresponding homo-dimers (Mercier et al., 2002). Equally, KOP opioid receptor-DOP opioid hetero-dimers form at least as effectively as KOP opioid receptor homo-dimers (Ramsay et al., 2002). However, the inability of the $\alpha_{1a}$-adrenoceptor and $\alpha_{1d}$-adrenoceptors to form hetero-dimers (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 hetero-dimers (Rocheville et al., 2000b) clearly points to greater complexity. Informatic 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.

MOLPHARM 2004 0497

**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 $\beta_2$-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 *Saccharomyces cerevisiae* $\alpha$-factor receptor that showed a lack of effect of

ligand (Overton and Blumer, 2000) and, where as noted above, the dimer is

internalized. A substantial number of RET-based experiments have shown small

effects of agonist ligands above substantial signals corresponding to constitutive

dimers/oligomers (see Milligan, 2004 for review). Despite this, an emerging

consensus favours 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 likely due to conformational alterations within the

complex resulting in small changes in the orientation or distance between the RET

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 the chemokine SDF-1$\alpha$ to produce 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-1$\alpha$ (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 recently. Ligand-induced dimerization

was, however, 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. Fascinatingly, this group have also reported that the capacity of HIV-1 to

utilise the CCR5 receptor as a 'co-receptor' 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). Hetero-dimerization 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 and at this time a clear pattern is

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. Each of neuropeptide Y Y4 receptor (Berglund et al., 2003), TSH 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, the GnRH (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 hetero-dimer pairs may have limited physiological significance, perhaps the most important questions in terms of relevance of GPCR hetero-dimers relate to whether they generate novel receptors in terms of either ligand pharmacology or function. This is a highly complex area, but at least for certain hetero-dimer pairs, this does appear 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). Equally, 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 following

co-expression of opioid receptor pairs, it has been difficult to fully replicate opioid

tissue pharmacology using this approach. It remains unclear if this is simply because

receptor expression ratios need to be more fully titrated and controlled because, as

discussed earlier co-expression of, for example, the MOP and DOP opioid receptors

will not result in production of a pure population of MOP-DOP hetero-dimers.

Ligands able to selectively target hetero-dimer pairs need to be identified and then

their function analysed 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 anti-parkinsonian

agents may have significantly higher affinity at dopamine D3/D2 hetero-dimers than

at the corresponding homo-dimers (Maggio et al., 2003). A substantial number of

other studies have reported unique pharmacology associated with the co-expression of

pairs of GPCRs (e.g. Yoshioka et al., 2001). Equally, the ability of β-blockers to

interfere with angiotensin AT1-mediated signalling 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 signalling pathways at a level downstream of receptor activation. Such

interactions between $G_s$ and $G_q$-mediated signals are well established to occur

(Cordeaux and Hill, 2002). Strategies that allow the detection of ligand binding or

function only by a hetero-dimer pair in the presence of the corresponding homo-

dimers are required to allow rapid and effective screening and detection of ligands

with these characteristics. Only with such ligands to hand will it be possible to tease

out the physiological relevance of GPCR hetero-dimerization.

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MOLPHARM 2004 0497

**Footnotes**

Address all correspondence to:

Graeme Milligan

Molecular Pharmacology Group

Davidson Building

University of Glasgow

Glasgow G12 8QQ

Scotland, U.K.


Tel: 44 (0) 141 330 5557

FAX: 44 (0) 141 330 4620

e-mail: g.milligan@bio.gla.ac.uk