Oligomerisation of G-protein-coupled receptors

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Summary

A range of approaches have recently provided evidence that G-protein-coupled receptors can exist as oligomeric complexes. Both homo-oligomers, comprising multiple copies of the same gene product, and hetero-oligomers containing more than one receptor have been detected. In several, but not all, examples, the extent of oligomerisation is regulated by the presence of agonist ligands, and emerging evidence indicates that receptor hetero-oligomers can display distinct pharmacological characteristics. A chaperonin-like role for receptor oligomerisation in effective delivery of newly synthesised receptors to the cell surface is a developing concept, and recent studies have employed a series of energy-transfer techniques to explore the presence and regulation of receptor oligomerisation in living cells. However, the majority of studies have relied largely on co-immunoprecipitation techniques, and there is still little direct information on the fraction of receptors existing as oligomers in intact cells.

Key words: Receptor, G protein, Signal transduction

Introduction

The family of G-protein-coupled receptors (GPCRs) is one of the largest in the human genome. They act as the recognition elements for a vast range of hormones, transmitters and modulators (Bockaert and Pin, 1999). They have therefore been widely studied and are considered the most tractable class of proteins for drug design by the pharmaceutical industry (Wilson et al., 1998). However, in most cells and tissues individual GPCRs are expressed at low levels. In concert with widely appreciated difficulties in purification and crystallisation of membrane proteins, atomic-level structural information on these proteins is currently available only for the photon receptor, rhodopsin (Palczewski et al., 2000). GPCRs share a common topology: they have seven transmembrane-spanning helices linked by three short extracellular loops and three intracellular loops, which, particularly for the third intracellular loop, can vary markedly in size. The C-terminal tail of GPCRs is intracellular, whereas the N-terminal region is extracellular, is frequently N-glycosylated and, at least for the class III GPCRs, can be a major structural feature in its own right (Bockaert and Pin, 1999). Kunishima et al. have recently crystallised the ligand-binding region of this section of the metabotropic glutamate receptor 1 as a dimeric complex both with and without glutamate bound (Kunishima et al., 2000): the dimeric complex can bind two molecules of glutamate, one to each element of the dimer.

GPCRs interact directly with heterotrimeric G proteins to allow transduction of information to classes of ion channels and enzymes that alter the rate of synthesis or degradation of intracellular second messengers. Furthermore, a rapidly emerging literature has begun to identify a range of other proteins that can interact with GPCRs (Hall et al., 1999; Heuss and Gerber, 2000). Among a range of functions, such interactions are likely to determine the cellular compartmentalisation of certain GPCRs, to ensure proximity to effector systems and to regulate the trafficking of GPCRs within the cell. However, the recognition that GPCRs can exist as dimers or higher oligomers has recently initiated some of the most fevered debate in the area.

GPCR oligomerisation: an old story?

Until last year virtually every cartoon of GPCR structure and function had depicted the protein as a monomer. However, a small but persistent literature had provided evidence that they might be present as oligomeric arrays. Many of the key early studies have recently been reviewed (Salahpour et al., 2000) and include data from ligand-binding studies, target-size irradiation and antibody-induced receptor activation. Particularly compelling were studies in which co-expression of two distinct, non-functional mutants of the angiotensin II AT_1 receptor resulted in the appearance of a functional receptor (Monnot et al., 1996). However, the recent acceleration in interest derived from two main efforts: (1) co-immunoprecipitation studies; and (2) efforts to understand the lack of appropriate cellular targeting and function of a GPCR gene product that seemed to encode the γ-aminobutyric $acid_B$ $(GABA)_B$ receptor. Whether such studies are indicative of the formation and presence of only dimers or also higher oligomers is unclear, and the apparent interchangeability of these terms in the literature reflects this uncertainty.

Co-immunoprecipitation studies

When forms of the human β_2-adrenoceptor were differentially modified at the N-terminus to incorporate either HA (haemaglutinin) or Myc epitope tags and then co-expressed in insect Sf9 cells, addition of either anti-HA or anti-Myc antibodies co-precipitated both forms of the GPCR. These receptor complexes were not disrupted by sodium dodecyl sulphate (SDS; Hebert et al., 1996). Equivalent studies using histamine H2 (Fukushima et al., 1997), δ opioid (Cvejic and

Devi, 1997), m3 muscarinic acetylcholine (Zeng and Wess, 1999), chemokine CCR2 (Rodriguez-Frade et al., 1999) and other receptors expressed in either insect or mammalian cell systems were also able to identify dimeric species, although the stability of these to reducing SDS-PAGE varied between individual GPCRs; this indicated potential differences in the role of disulphide bonds in the dimerisation process or their stability. Equivalent studies in both yeast (Yesilaltay and Jenness, 2000) and brain membranes (Nimchinsky et al., 1997; Zeng and Wess, 1999) demonstrated that GPCR complexes can be immunoprecipitated from native cells.

Such co-immunoprecipitation studies and other co-isolation approaches have allowed detection of a range of hetero-oligomeric interactions. These include those between closely related GPCR pairs, such as κ and δ opioid receptors (Jordan and Devi, 1999) and μ and δ opioid receptors (George et al., 2000) but also more distantly related pairings, such as dopamine D_1 and adenosine A_1 receptors (Gines et al., 2000) and angiotensin II AT_1 and bradykinin B_2 receptors (AbdAlla et al., 2000). However, even in these studies there were several apparently contradictory observations. For example, although differentially epitope-tagged forms of the μ and δ opioid receptors could be co-immunoprecipitated, such hetero-interactions were not observed during simple immunoblotting of membranes co-expressing the two GPCRs, even though apparent homodimers could be detected in this way (George et al., 2000). Observations such as these have resulted in concern about the specificity and relevance of co-immunoprecipitation studies following high-level, transient co-expression of GPCRs in heterologous systems (Milligan, 2000; Milligan and Rees, 2000) and in a desire to study such potential interactions in intact cells (see below).

The $GABA_B$ receptor

Although for many years the molecular identity of the $GABA_B$ receptor was obscure, the capacity of GABA to stimulate high-affinity GTPase activity in brain membranes, and for this to be blocked by antagonists that inhibit pharmacological effects of the $GABA_B$ receptor, was central to the expectation that the receptor would be a GPCR. Use of a high-affinity radiolabelled $GABA_B$ antagonist allowed the identification and cloning of a seven-transmembrane polypeptide that has a large extracellular extension and shares sequence similarity with class III GPCRs, including the metabotropic glutamate receptors.

Although the cloned polypeptide ($GABA_BR1$) exhibits many of the expected properties of the endogenous $GABA_B$ receptor in terms of tissue distribution and antagonist ligand binding, when expressed in recombinant systems it bound agonist ligands poorly and did not couple effectively to signal transduction cascades (Kaupmann et al., 1997). In transfected mammalian cells this polypeptide was expressed as an immature glycoprotein and was restricted to intracellular membranes (White et al., 1998).

Since 1998, six groups have identified a second receptor, $GABA_BR2$, which, when co-expressed with $GABA_BR1$ in mammalian cells or *Xenopus* oocytes, reproduces the pharmacology of the authentic $GABA_B$ receptor (Jones et al., 1998; Kaupmann et al., 1998; Kuner et al., 1999; White et al., 1998; Ng et al., 1999; Martin et al., 1999). Like $GABA_BR1$, $GABA_BR2$ is a class III GPCR and shares 35% sequence identity with $GABA_BR1$.

These reports provided several lines of evidence that the functional form of the $GABA_B$ receptor is a heterodimer of $GABA_BR1$ and $GABA_BR2$. First, immunocytochemistry studies indicated that the two receptors co-localise at the cell surface in transfected cells, and tissue distribution studies in rat brain demonstrated that both receptors are expressed in the same neurones (Kaupmann et al., 1998; Jones et al., 1998). Second, when co-expressed in mammalian cells with $GABA_BR2$, FACS analysis indicated that the $GABA_BR1b$ splice variant is expressed as a mature glycoprotein and transported to the cell surface (White et al., 1998). This is accompanied by an increase in agonist potency in radioligand binding studies, and the receptor becomes able to regulate signal transduction pathways including the stimulation of $[^{35}S]GTP\gamma S$ binding and inhibition of forskolin-stimulated cAMP production (White et al., 1998). Third, in two-hybrid screens of a human brain cDNA library using the C-terminal domain of $GABA_BR1$ as bait, two groups identified $GABA_BR2$ as a major interacting protein (White et al., 1998; Kuner et al., 1999). Fourth, co-immunoprecipitation experiments in transfected cells and brain tissue showed that either receptor can be co-immunoprecipitated with the other (Kaupmann et al., 1998, Jones et al., 1998). A range of recent reviews have concentrated on the details of these interactions (for examples see, Marshall et al., 1999; Mohler and Fritschy, 1999).

Recent experiments have identified a C-terminal motif, RXR(R), responsible for ER retention of $GABA_BR1$, which is believed to be masked by interaction with $GABA_BR2$ (Margeta-Mitrovic et al., 2000) and thus control plasma membrane delivery of the receptor. Within the heterodimer the GABA-binding site is localised to $GABA_BR1$.

Methods to monitor GPCR oligomerisation in living cells

Extensive controls have often been performed in co-immunoprecipitation experiments to try to exclude contributions of non-specific interactions between the highly hydrophobic core transmembrane domains of GPCRs following detergent extraction. Such effects might be responsible for some reports of GPCR oligomerisation. Furthermore, investigators have used experiments in which crosslinking was performed with cell-impermeant reagents prior to immunoprecipitation to argue that GPCR oligomers are present preformed at the cell surface. Despite this, there have been determined efforts to develop methods to monitor GPCR oligomerisation in intact, living cells. These have generally involved resonance energy transfer techniques (see Pollock and Heim, 1999; Hovius et al., 2000 for reviews). Following attachment of fluoresence resonance energy transfer (FRET)-competent pairs of fluorescent proteins to the C-terminal tail of the α-factor receptor from *Saccharomyces cerevisiae*, co-expression of appropriately modified receptors resulted in efficient FRET between the two proteins (Overton and Blumer, 2000). Such results are consistent with the formation of a constitutive GPCR oligomer. Microaggregation of forms of the gonadotrophin-releasing hormone receptor in response to addition of an agonist has also recently been reported in a FRET-based approach (Cornea et al., 2001).

A modification of this approach, in which forms of the GPCR are tagged with either the luciferase from *Renilla reniformis* or with enhanced yellow fluorescent protein (eYFP), allows

bioluminescence resonance energy transfer (BRET) upon addition of coelenterazine, a cell-permeant luciferase substrate, if the GPCRs are closely associated. This approach has been used to provide further evidence for constitutive homo-oligomerisation of the human human β_2-adrenoceptor (Angers et al., 2000; McVey et al., 2001) and of the human δ opioid receptor (McVey et al., 2001). Key controls include expressing the two forms of the GPCRs in different cell populations, which are then combined before addition of coelenterazine. Given that the two forms of each GPCR cannot be in proximity in this situation, no BRET signal should be obtained.

A potential limitation of the BRET strategy is that it does not report on the cellular location of the GPCR oligomer. Because large fractions of expressed GPCRs can be observed either in transit to the plasma membrane or trapped within the Golgi (Milligan, 1998; Petaja-Repo, et al., 2000; Morello, et al., 2000), two energy-transfer-based approaches have been used to examine only those GPCR oligomers present at the cell surface. By co-expressing both N-terminally FLAG™- and Myc-tagged forms of the δ opioid receptor and then adding both an anti-Myc antibody labelled with Eu^{3+} as energy donor and an anti-FLAG™ antibody labelled with allophycocyanin as energy acceptor, McVey et al. used time-resolved FRET to monitor δ opioid receptor oligomers (McVey et al., 2001). In a similar manner, photobleaching of anti-receptor and anti-epitope-tag antibodies labelled with FRET-compatible partner dyes has been employed to detect both combinations of homo- and hetero-oligomers of somatostatin receptor subtypes (Rocheville et al., 2000a) and hetero-oligomeric complexes containing both the D_2 dopamine receptor and the sst_5 somatostatin receptor (Rocheville et al., 2000b). To date, all of these studies have been performed in cell lines transfected to express the GPCRs of interest. However, the D_2 dopamine and sst_5 somatostatin receptors are known to be co-expressed in medium aspiny striatal and pyramidal cortical neurons. The sensitivity of the photobleaching approach allows measurement of interactions in single cells, and if direct anti-receptor antibodies were available for each GPCR it would then be possible to perform such studies on single isolated neurones.

Mechanisms of GPCR oligomerisation

$GABA_B$ receptor heterodimerisation involves interactions between the coiled-coil domains within the C-terminal tails of the two $GABA_B$ receptors (White et al., 1998; Fig. 1). Coiled-coiled domains are present in many proteins and are well-characterised mediators of protein-protein interaction. However, this does not appear to be a general mechanism for GPCR oligomerisation. Although Cvejic and Devi report that a C-terminally truncated version of the δ opioid receptor that lacks 15 residues cannot dimerise (Cvejic and Devi, 1997), this receptor does not have an obvious coiled-coil domain in this region akin to those in the $GABA_B$ receptor partners.

The large extracellular extensions of class III GPCRs contain several cysteine residues, and disulphide cross-links can mediate (Romano et al., 1996) or certainly contribute to (Romano et al., 2001) dimerisation (Fig. 1). In the case of the metabotropic glutamate receptor 1, removal of the extracellular domain prevents dimerisation. Mutation of Cys140 indicates that this residue contributes to dimerisation but it is not the only contact point for dimer formation (Tsuji et al., 2000; Ray and Hauschild, 2000; Romano et al., 2001).

Only class III GPCRs possess the large extracellular domain. However, the N-terminus of the bradykinin B_2 receptor has been reported to be involved in dimerisation of this class I GPCR (AbdAlla et al., 1999). Computational studies (Goulsdon et al., 2000) and direct experimental observation have indicated that dimerisation of other GPCRs may involve interfaces produced by transmembrane helices V and VI. Two hypotheses exist for the mechanism of dimerisation of class 1 GPCRs (Fig. 1). The first of these is that domain swapping occurs between GPCR pairs. Transmembrane helices I-V and VI-VII form essentially independent folding domains. GPCR sequences can be split between transmembrane helices V and VI, and, when these fragments are co-expressed, functional receptors can be reconstituted (Maggio et al., 1996; Barbier et al., 1998; Scarselli et al., 2000). Co-expression of a reciprocal chimaera containing transmembrane regions I-V of the α_{2C} adrenoceptor and helices VI and VII of the muscarinic m_3 acetylcholine receptor, or vice versa, results in the reconstitution of binding sites for ligands for both of the native receptors. By contrast, neither chimaera expressed independently is able to identify either ligand (Maggio et al., 1993). This model does not appear to be consistent with studies of the co-expression of D_2 dopamine receptor mutants, which resulted in a loss of receptor-binding sites, whereas the domain swap model would have predicted an increase (Lee et al., 2000).

A second model simply envisages that a GPCR dimer is formed by lateral packing of the individual polypeptides (Fig. 1). This model is supported by studies of mutants of the vasopressin V_2 receptor (Schulz et al., 2000), a particularly widely examined GPCR because of the many distinct mutants known to be associated with the phenotype of diabetes insipidus. Interestingly, although unable to resolve these models, peptides corresponding to transmembrane regions V and VI of the β_2-adrenoceptor and the dopamine D_2 receptor interfere with dimerisation of their respective GPCRs (Hebert et al, 1996; Ng et al., 1996).

Most GPCRs, including the muscarinic m_3 receptor, possess a pair of cysteine residues in extracellular loops 1 and 2, which are believed to form an intramolecular disulphide bond. Using modified forms of this muscarinic receptor, Zeng and Wess (Zeng and Wess, 1999) observed receptor oligomers in non-reducing, but not reducing, conditions. Mutation of these two cysteines led to the conclusion that dimer formation precedes disulphide linkage but also that the disulphide links may be inter- rather than intra-molecular. Zhu and Wess suggest that this is also the case for the V_2 vasopressin receptor (Zhu and Wess, 1998).

GPCR dimers: roles as mutual chaperonins?

The requirement for interactions between $GABA_BR1$ and $GABA_BR2$ at the ER to allow plasma membrane delivery of the functional receptor, even though $GABA_BR2$ appears not to be directly involved in recognition of GABA, suggests that $GABA_BR2$ functions as a chaperonin for $GABA_BR1$. How is plasma membrane trafficking of other GPCRs altered by the co-expression of mutated or otherwise-altered receptors? When the wild-type D_3 dopamine receptor is expressed in HEK293 cells, it is effectively targeted to the plasma membrane. However, upon co-expression with a naturally occurring splice variant, D_3nf, which lacks transmembrane regions VI and VII, delivery of the wild-type receptor to the plasma membrane is blocked. Under these conditions the two forms of the receptor can form a

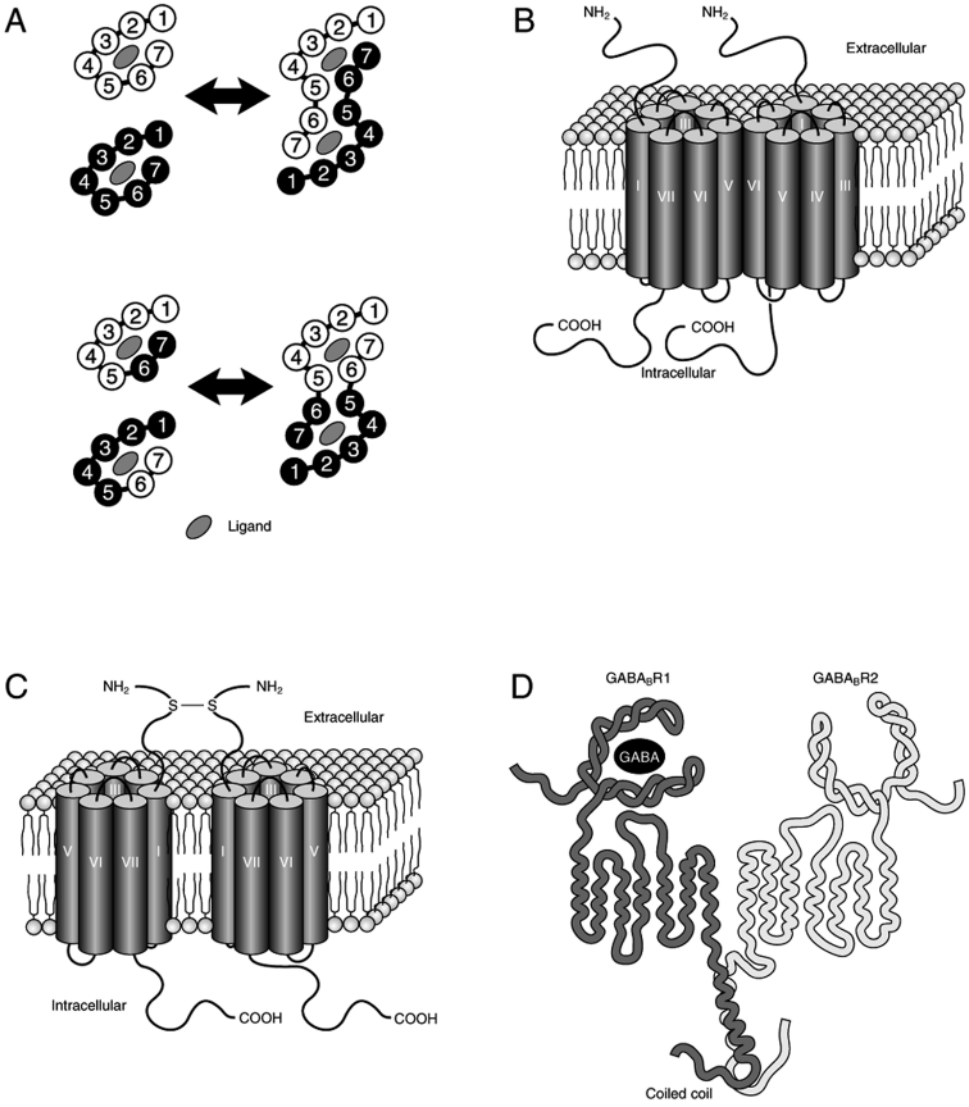

Fig. 1. Models of GPCR dimerisation (A) Domain swaps. Functional, ligand-binding GPCRs can be reconstituted following expression of separated fragments of a GPCR comprising transmembrane helices I-V and VI-VII. Because distinct ligand-binding properties can be produced when the two segments are derived from different GPCRs, various domain swapping models have been proposed. Two possibilities are illustrated (see Gouldson et al., 2000). (B) Lateral packing. For GPCRs that have ligands sufficiently large that all the binding determinants are unlikely to lie within the crevice formed by the architecture of the seven transmembrane helices, lateral packing and/or microaggregation may be required to initate ligand-induced signal transduction. Evidence from peptide inhibition studies suggest that contacts may occur between transmembrane helices V and VI (Ng et al., 1996; Herbert et al., 1996). (C) Intramolecular disulphide bonds. Class III GPCRs have long extracellular N-terminal extensions that bind the ligand (Kunishima et al., 2000). These regions also contain cysteine residues that provide intramolecular disulphide bonds that contribute to dimer stabilisation (Romano et al., 2001). (D) Coiled-coils. Heterodimerisation between the $GABA_BR1$ and $GABA_BR2$ to produce a functional $GABA_B$ receptor requires the C-terminal tails. These regions are predicted to produce a coiled-coil interaction that is vital for membrane delivery of the proteins (White et al., 1998). Panel D is modified from Marshall et al., 1999.

heterodimer (Karpa et al., 2000). The gonadotrophin-releasing-hormone receptor behaves similarly when expressed with a naturally occuring truncated mutant (Grosse et al., 1997). Co-expression of the wild-type D_2 dopamine receptor either with mutated forms of this GPCR or with individual N- or C-terminal domains of the receptor impairs function of the wild-type receptor (Lee et al., 2000). Lee et al. claim that this reflects prevention of effective membrane trafficking of the wild-type receptor due to its interaction with the receptor fragments (Lee et al., 2000), although the evidence provided is less than compelling. However, related studies of truncation mutants of the vasopressin V_2 receptor showed similar selective effects on the signalling function of the wild-type V_2 receptor (Zhu and Wess, 1998), and in this example data on the limiting effects of the truncation mutants on delivery of the full-length receptor to the cell surface were convincing. All of these studies are consistent with the view that receptor dimerisation can occur soon after protein synthesis and certainly prior to plasma membrane delivery.

Interestingly, direct protein-protein interactions between the wild-type vasopressin V_2 receptor and a series of truncation mutants did not require the presence of either transmembrane region V or VI. This raises further questions about the basis for these interactions. Furthermore, a series of mutants of the vasopressin V_2 receptor known to be improperly trafficked to the cell surface, and to be the molecular defect in individual families that suffer from diabetes insidipus, can be rescued by co-expression of a C-terminal segment of the receptor that encompasses the region of mutation (Schoneberg et al., 1996). Other groups have drawn similar conclusions on the lack of requirement of transmembrane regions V and VI in mutants of the vasopressin V_2 receptor for interactions with the wild-type receptor (Schulz et al., 2000). Hebert et al. however, offer a rather different view, having co-expressed wild-type β_2-adrenoceptor and a point mutant of this GPCR that cannot be post-translationally palmitoylated and has been described as being constitutively desensitised. In this case, rather than the mutant GPCR limiting the function of the wild-type receptor, the response to agonist is consistent with oligomerisation restoring function to the mutant (Hebert et al., 1998).

Do ligands alter GPCR oligomerisation?

a. Homo-oligomers

Although potentially very important, the question of whether

ligands affect GPCR oligomerisation is currently one of the most hotly debated. In early co-immunoprecipitation studies of the β_2-adrenoceptor, limitation of GPCR dimerisation was associated with reduced agonist-mediated signal transduction (Hebert et al., 1996). Using BRET, Angers et al. have now also observed, albeit limited, agonist stimulation of β_2-adrenoceptor oligomerisation in intact cells (Angers et al., 2000). Furthermore, Mijares et al. have recently described an anti–β_2-adrenoceptor antibody that functions as an agonist, although this is lost if Fab fragments are prepared (Mijares et al., 2000) and, as noted earlier, an agonist has been reported to promote microaggregation of the gonadotrophin-releasing-hormone receptor (Cornea et al., 2001). The requirement for antibody crosslinking and clustering of a GPCR to generate a signal transduction event is consistent with agonist-induced dimerisation, even though it does not address the issue directly. Interestingly, the dimer: monomer ratio of immunologically detected sstr5 somatostatin receptor has been reported to increase upon addition of agonist (Rocheville et al., 2000a). Cvejic and Devi, however, have reported that δ opioid receptor dimerisation is reduced by agonist ligands in a concentration- and efficacy-dependent manner (Cvejic and Devi, 1997), whereas, using both BRET and time-resolved FRET approaches, McVey et al. found that agonist and antagonist ligands have no effect on δ opioid receptor oligomerisation at the cell surface (McVey et al., 2001). FRET studies indicate that dimerisation of the α-factor receptor from *Saccharomyces cerevisiae* is constitutive and unaffected by ligand (Overton and Blumer, 2000), whereas, in CHO-K1 cells expressing relatively low levels of the sstr5 somatostatin receptor, FRET studies indicate that oligomers appear only upon addition of somatostatin (Rocheville et al., 2000a). Again in immunoprecipitation studies, dimers of the κ opioid receptor (Jordan and Devi, 1999) and of the muscarinic m_3 acetylcholine receptor (Zeng and Wess, 1999) have also been reported to be unaffected by agonist ligands. Given the overall structural similarity of class I GPCR family members, it is perhaps surprising that so many different forms of regulation have been observed. This complexity is both intriguing and worthy of further study and may suggest that GPCRs that have high mutual affinity form constitutive dimers whereas others require prior ligand binding.

At least within the chemokine receptor area, results have been consistent: interactions between monocyte chemoattractant protein-1 (MCP-1) and the CCR2b receptor (Rodriguez-Frade et al., 1999), between stromal-cell-derived factor-1α (SDF-1α) and the CXCR4 receptor (Vila-Coro et al., 1999) and between regulated upon activation, normal T-cell expressed and secreted (RANTES) protein and the CCR5 receptor (Vila-Coro et al., 2000) promote receptor dimerisation from essentially undetectable levels in the absence of the ligands. Furthermore, because an anti-CCR5-receptor monoclonal antibody that prevents HIV-1 infection is able to cause CCR5 dimerisation without producing a detectable rise in intracellular [Ca^{2+}], Vila-Coro et al. have argued that the ability of of chemokines to limit HIV-1 replication could reflect prevention of HIV-1 entry into cells by their producing receptor dimerisation (Vila-Coro et al., 2000).

Hetero-oligomers

Co-immunoprecipitation studies following co-expression of κ and δ opioid receptors have indicated that these receptors can interact (Jordan and Devi, 1999). Surprisingly, given that agonist ligands can reduce levels of δ but not κ opioid receptor homodimers, the effect of agonist ligand on heterodimerisation was not investigated. As in the case of homodimers of the sst_5 somatostatin receptor, heterodimerisation between this GPCR and the closely related sst_1 somatostatin receptor is ligand dependent (Rocheville et al., 2000a). Like the μ–κ opioid receptor hetero-oligomer (Jordan and Devi, 1999), a sst_4-sst_5 somatostatin receptor hetero-oligomer could not be detected when these receptors were co-expressed (Rocheville et al., 2000a), which further demonstrates that not all receptor subtypes that share a common ligand effectively form dimers. However, because Rocheville et al. used the same approach to detect sst_5-D_2 hetero-oligomers (Rocheville et al., 2000b), which are substantially less closely related, the basis for this selection remains unclear. Again this group detected little of this heterodimer unless an agonist for either GPCR was present (Rocheville et al., 2000b). Bio-informatic analysis directed at this burgeoning literature would provide an interesting means to address the basis for selectivity in GPCR oligomerisation.

Recent studies have provided evidence for stable heterodimerisation between the angiotensin AT_1 receptor and the bradykinin B_2 receptor, both following their co-expression in a heterologous system and in A10 smooth muscle cells that endogenously express both GPCRs (AdbAlla et al., 2000). Constitutive interactions between the D_1 dopamine and A_1 adenosine receptors have also been observed (Gines et al., 2000). In this case, however, the sustained presence of a D_1 agonist reduced co-immunoprecipitation, but this did not occur in the presence of an A_1 adenosine receptor agonist.

Very recent studies have used co-immunoprecipitation to detect interactions between transiently co-expressed δ opioid and β_2-adrenoceptors (Jordan et al., 2001; McVey et al, 2001), and limited interactions between this paring have also been detected in intact cells in BRET experiments (McVey et al., 2001). Interestingly, in cell co-expressing these two GPCRs, agonist ligands at either receptor are able to internalise both GPCRs, although the ligands do not produce this effect in the absence of their own GPCR (Jordan et al., 2001).

Functional consequences of GPCR oligomerisation

The literature on the functional significance of GPCR oligomerisation, particularly hetero-oligomerisation, is complex. Clearly, the interaction between $GABA_B$ receptors provides a mechanism to control efficient delivery of active GPCRs to the cell surface. Even if this is an unusual case, unravelling of the detailed mechanisms has been illuminating. Recently, however, another group of unusual GPCRs has provided insight into at least one other potential role for GPCR hetero-oligomerisation. Protease-activated receptors (PARs) are a small subfamily of GPCRs in which the ligand is encoded within the GPCR sequence. The N-terminal region of these GPCRs is the ligand, and a protease (e.g. thrombin) cleaves the receptor to release the ligand and induce activation. Recent studies have shown that PAR family GPCRs can induce transactivation of other family members (Nakanishi-Matsui et al., 2000; O'Brien et al., 2000). Although formally this does not require hetero-oligomerisation of GPCR subtypes, the capacity of one GPCR to present ligand to another would be likely to benefit from their close association.

Another key issue beginning to emerge from studies of

GPCR hetero-oligomerisation is that they might possess a quite distinct ligand pharmacology compared with homo-oligomers of either partner. This is too large and complex (and pharmacological) an issue to address here. As an example, however, it is instructive to consider opioid receptor pharmacology. There is an extremely large literature on opioid chemistry and pharmacology. This has resulted in characterisation in tissues of a much wider range of opioid responses than can be explained by single heterologous expression of the three molecularly defined GPCRs that are the known targets for opioids. Interestingly, co-expression of the δ and κ opioid receptors or the δ and μ receptors produces pharmacological characteristics quite distinct from those seen when either GPCR is expressed alone (Jordan and Devi, 1999; George et al., 2000). However, extrapolation from these observations needs to be treated with caution. Cell lines endogenously expressing multiple opioid receptor subtypes are well known and have contributed substantially to our definition of opioid receptor pharmacology. Presumably hetero-oligomeric interactions do not feature strongly in these systems, perhaps because expression levels of the individual GPCR are not very high or because they are differentially compartmentalised. Equally, different ratios of expression of individual GPCRs that can hetero-oligomerise might produce subtly different pharmacological profiles, and this has yet to be explored in any systematic manner. Furthermore, although not yet thoroughly examined, co-expression of two opioid receptor subtypes should produce homo-oligomers as well as hetero-oligomeric pairs. The properties of hetero-oligomers should thus reflect the relative levels of expression of the two gene products, as well as their affinities for each other.

Conclusion/perspectives

Regulated protein-protein interactions are a central feature of many aspects of biology. Recent studies have shown that GPCRs can form both homo- and hetero-oligomers. This literature is expanding rapidly as more potential GPCRs pairing are tested for interaction. Although the literature is exciting there are a number of surprises, and such dimerisation can clearly derive from a range of mechanisms. Key questions that must be addressed carefully include the regulation of such interactions and their functional significance in membrane delivery and signal transduction mechanisms. Studies that examine these processes will rapidly become dominated by approaches that are non-invasive or examine interactions in cells that express the GPCRs both endogenously and at modest levels. One of the most pressing and tantalising issues raised by the work to date is the significance of heterodimerisation for the pharmacology of GPCR function and whether this will result in the identification and development of novel ligands that interact selectively with such specific pairings.

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