

Heterodimerization of G Protein-Coupled Receptors: Specificity and Functional Significance

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Abstract—G protein-coupled receptors (GPCRs) are cell surface receptors that mediate physiological responses to a diverse array of stimuli. GPCRs have traditionally been thought to act as monomers, but recent evidence suggests that GPCRs may form dimers (or higher-order oligomers) as part of their normal trafficking and function. In fact, certain GPCRs seem to have a strict requirement for heterodimerization to attain proper surface expression and functional activ-

ity. Even those GPCRs that do not absolutely require heterodimerization may still specifically associate with other GPCR subtypes, sometimes resulting in dramatic effects on receptor pharmacology, signaling, and/or internalization. Understanding the specificity and functional significance of GPCR heterodimerization is of tremendous clinical importance since GPCRs are the molecular targets for numerous therapeutic drugs.

I. Introduction

The largest class of cell surface receptors in mammalian genomes is the superfamily of G protein-coupled receptors (GPCRs¹). Of the approximately 1000 genes thought to encode GPCRs in humans, about 300 to 400 mediate the effects of endogenous ligands, with the remainder being sensory receptors. GPCRs mediate nu-

merous physiological processes and are the targets of many clinically important drugs, with approximately half of all current prescription drugs acting through GPCRs (Drews, 1996).

GPCRs are characterized structurally by an amino-terminal extracellular domain, a carboxyl-terminal intracellular domain, and seven hydrophobic transmembrane domains. The interaction of an agonist with a GPCR binding pocket elicits or stabilizes a conformational change in the receptor's transmembrane domains. This conformational change allows the receptor to associate with heterotrimeric G proteins and initiate a signaling cascade inside the cell leading to a physiological response (Lefkowitz et al., 1993). GPCRs have traditionally been thought to act as monomers, but this idea has been challenged over the past few years by accumulating pharmacological and biochemical data. The emerging view in the field is that the primary functional GPCR signaling unit may actually consist of dimers or oli-

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Article, publication date, and citation information can be found at <http://pharmrev.aspetjournals.org>.

doi:10.1124/pr.57.3.1.

¹Abbreviations: GPCR, G protein-coupled receptor; mGluR, metabotropic glutamate receptor; ER, endoplasmic reticulum; AR, adrenergic receptor; OPR, opioid peptide receptor; SSTR, somatostatin type receptor; OR, olfactory receptor; OTR, oxytocin receptor; 5-HT, serotonin; TRH, thyrotropin-releasing hormone; HIV, human immunodeficiency virus; Fz, frizzled; FEVR, familial exudative vitreoretinopathy.

gomers of receptors (Jordan et al., 2000; Marshall, 2001; George et al., 2002; Kroeger et al., 2003; Milligan, 2004; Terrillon and Bouvier, 2004b).

Many transmembrane receptors are known to dimerize as part of their normal function, with receptor tyrosine kinases being a particularly well studied example. The idea that GPCRs might also dimerize was first proposed in 1982 (Agnati et al., 1982), although the idea did not gain wide acceptance until more than a decade later. Early evidence for GPCR dimerization came from unexplained cooperativity observed in ligand binding assays and unexpectedly large estimates of the size of receptor complexes on gel filtration columns. Detailed descriptions of these early studies are available in several previous reviews (Angers et al., 2002; Agnati et al., 2003).

Multimerization of GPCRs was originally proposed based on studies investigating the propensity of certain GPCRs to dimerize with themselves ("homodimerization"). There is now a growing list of receptors that have been found to associate with other receptors ("heterodimerization"). One concern in the field has been that GPCR heterodimerization might be an artifact of receptor over-expression and/or a result of the techniques used to study receptor associations. Such concerns are best addressed by considering the evidence that these receptor-receptor interactions are specific versus non-specific and that they are functionally important versus physiologically irrelevant. This review will focus on the current state of research on GPCR heterodimerization, with an emphasis on the specificity of these interactions and their functional significance.

A. GABA_B Receptors

Although the concept of GPCR heterodimerization was initially proposed in the early 1980's (Agnati et al., 1982), the first widely accepted demonstration of functional consequences for GPCR heterodimerization came from the GABA_B receptors. γ -Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the mammalian brain, and the physiological actions of GABA are mediated via activation of three distinct types of cell surface receptors: GABA_A and GABA_C receptors, which are ligand-gated chloride channels, and GABA_B receptors, which are GPCRs. In 1997, the first GABA_B receptor cDNA was cloned (Kaupmann et al., 1997). This receptor was termed GABA_BR1 and found to bind to a number of GABA_B receptor ligands. However, this receptor was also found to be largely nonfunctional when expressed alone in most cell types, due to its inability to be efficiently trafficked to the cell surface (Couve et al., 1998). One year later, a receptor with extensive homology to GABA_BR1 was cloned by six independent groups and termed GABA_BR2 (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Kuner et al., 1999; Martin et al., 1999; Ng et al., 1999). This new receptor did not bind GABA_B receptor ligands when expressed alone.

However, it was found to physically assemble with GABA_BR1 when the two receptors were coexpressed in cells, resulting in the formation of functional GABA_B receptors that trafficked efficiently to the cell surface and possessed many of the properties ascribed to neuronal GABA_B receptors (Marshall et al., 1999). These studies provided strong evidence that heterodimerization is essential for GABA_B receptor function.

The interaction between GABA_BR1 and GABA_BR2 appears to be quite specific in nature. However, GABA_BR1 is found in many brain regions that do not detectably express GABA_BR2 (Jones et al., 1998; Kaupmann et al., 1998; Durkin et al., 1999; Kuner et al., 1999; Margeta-Mitrovic et al., 1999; Ng et al., 1999; Calver et al., 2000; Clark et al., 2000; Ige et al., 2000; Charara et al., 2004), and therefore, several groups have explored the possibility that GABA_BR1 might be able to assemble with other GPCRs to form functional receptors in these regions of the brain. The eight members of the metabotropic glutamate receptor (mGluR) family are the closest relatives of the GABA_B receptors in terms of primary sequence (Kaupmann et al., 1997); thus, the various mGluR subtypes have received special attention as potential partners for GABA_BR1 (Ng et al., 1999; Pagano et al., 2001; Balasubramanian et al., 2004). One group found no evidence for association of any of the mGluR subtypes with GABA_BR1 (Pagano et al., 2001), whereas a second group reported an interaction with mGluR4 that did not appear to be of functional significance (Sullivan et al., 2000). A third group performed a much wider screen for the ability of other GPCRs to promote GABA_BR1 surface expression and found no evidence that any of the mGluR subtypes or 30 other GPCRs examined could enhance the targeting of GABA_BR1 to the cell surface. These studies did reveal, although, a surprising interaction between GABA_BR1 and GABA_A receptors (Balasubramanian et al., 2004). However, in terms of heterodimerization with other GPCRs, the current evidence suggests a high degree of specificity in the interaction between GABA_BR1 and GABA_BR2.

The structural determinants of the GABA_BR1/GABA_BR2 interaction have been examined in some detail. Both receptors have coiled-coil regions on their carboxyl-termini that physically interact with each other in yeast two-hybrid and fusion protein pull-down assays (White et al., 1998; Kuner et al., 1999). However, it has clearly been shown that the carboxyl-termini of GABA_BR1 and GABA_BR2 are not required for heterodimerization (Margeta-Mitrovic et al., 1999; Calver et al., 2001; Pagano et al., 2001). Thus, it seems likely that interactions between the transmembrane and/or amino-terminal domains of the receptors are the keys to the GABA_BR1/GABA_BR2 association. Despite the fact that the carboxyl-terminus of GABA_BR1 is not required for its interaction with GABA_BR2, this region of the receptor does contain a key motif involved in the retention of GABA_BR1 in the endoplasmic reticulum (ER)

(Margeta-Mitrovic et al., 1999; Pagano et al., 2001). The association of the GABA_BR1 coiled-coil domain with that of GABA_BR2 probably serves to mask the GABA_BR1 ER retention signal and allow trafficking of the heterodimer to the cell surface.

B. Taste Receptors

For many years, it was believed that sweet taste sensation was likely to be mediated by specialized GPCRs found in the taste buds. Two candidate taste receptors, called T1R1 and T1R2, were cloned and found to be selectively expressed in distinct taste bud regions (Hoon et al., 1999), but neither of these receptors was found to be responsive to sweet stimuli when expressed in heterologous cells. In parallel studies, a number of labs studied the genetics of *Sac* mice, a strain that exhibits reduced sensitivity to sweet stimuli such as saccharin and sucrose. In 2001, six independent groups reported that a gene in the *Sac* locus encodes a GPCR with extensive homology to T1R1 and T1R2 (Bachmanov et al., 2001; Kitagawa et al., 2001; Max et al., 2001; Montmayeur et al., 2001; Nelson et al., 2001; Sainz et al., 2001). As with T1R1 and T1R2, this new receptor (termed T1R3) also was not found to be activated by sweet stimuli when expressed by itself in heterologous cells. However, when T1R3 was coexpressed with T1R2, this resulted in the formation of receptors that were robustly activated by saccharin, sucrose, and other sweet tastants (Nelson et al., 2001). These findings suggest that heterodimerization of T1R2 and T1R3 is required for the formation of functional sweet taste receptors, much as heterodimerization of GABA_BR1 and GABA_BR2 seems to be required for the formation of functional GABA_B receptors.

Heterodimerization of T1R family taste receptors appears to have dramatic effects not only on receptor functionality but also on the pharmacology of the receptors. If T1R3 is coexpressed with T1R1 instead of T1R2, this results in the formation of receptors preferentially responsive to amino acids instead of sweet stimuli (Nelson et al., 2001). Interestingly, monosodium glutamate and other amino acids are known to give rise to a distinct taste known as "umami", and studies with knockout mice suggest that the T1R1/T1R3 heterodimer is required for umami sensation (Zhao et al., 2003). The structural determinants of the T1R1/T1R3 and T1R2/T1R3 interactions have not yet been explored in detail, and at present, no other GPCRs outside the T1R family have been reported to interact with any of the T1R subtypes. Thus, T1R taste receptor heterodimerization, like the heterodimerization of GABA_BR1 and GABA_BR2, seems to possess a high degree of specificity.

C. Adrenergic Receptors

Adrenergic receptors (ARs) mediate the physiological effects of epinephrine and norepinephrine. There are nine AR subtypes, with three subfamilies (α_1 , α_2 , and β) comprised of three subtypes each. These receptors are

Family A (rhodopsin-like) GPCRs, in contrast to the GABA_B and T1R taste receptors, which belong to GPCR Family C. β_2 AR is the most widely-expressed AR subtype and was the focus of a number of early studies that provided key evidence in support of the notion of GPCR dimerization. β_2 AR homodimerization has been demonstrated via 1) coimmunoprecipitation of differentially tagged versions of the receptor (Hebert et al., 1996), 2) functional studies demonstrating rescue of a constitutively desensitized form of the receptor (Hebert et al., 1998), and 3) bioluminescence resonance energy transfer between versions of the receptor with distinct fluorescent proteins fused to the cytoplasmic carboxyl-termini (Angers et al., 2000). More recent work has demonstrated that mutation of a putative dimerization motif in the β_2 AR sixth transmembrane domain results in ER retention of the receptor, suggesting an important role for homodimerization in the targeting of β_2 AR to the cell surface (Salahpour et al., 2004).

β_2 AR is distinct from the aforementioned examples of GABA_BR1 and T1R3 in that it is functional when expressed by itself in heterologous cells. Thus, it seems clear that heterodimerization is not required for β_2 AR activity. Nonetheless, β_2 AR has been found to heterodimerize with several different GPCRs with distinct consequences depending on the GPCR involved. For example, β_2 AR has been found to associate with the closely related β_1 AR (Lavoie et al., 2002; Mercier et al., 2002; Lavoie and Hebert, 2003) and β_3 AR (Breit et al., 2004), with the primary functional consequences of these interactions being a reduction in the rate of agonist-induced internalization of β_2 AR and a reduced ability of the receptor to stimulate extracellular signal-regulated kinase phosphorylation (Lavoie et al., 2002; Breit et al., 2004). Both β_1 AR and β_2 AR have been found to heterodimerize with α_{2A} AR, resulting in cross-internalization of the receptors following agonist stimulation of one of the subtypes (Xu et al., 2003). β_1 AR and β_2 AR have also both been found to associate with the AT1 angiotensin receptor, which may underlie cross-inhibition of receptor signaling by β -adrenergic and angiotensin receptor antagonists (Barki-Harrington et al., 2003). Furthermore, β_2 AR has been found to associate with opioid receptors (Jordan et al., 2001; McVey et al., 2001; Ramsay et al., 2002), resulting in significant cross-internalization of the receptor complex by adrenergic and opioid ligands (Jordan et al., 2001). This cross-internalization phenomenon may be a very general and important functional consequence of GPCR heterodimerization, since agonist-induced receptor endocytosis is known to be a key mechanism regulating the desensitization and resensitization of GPCR-mediated responses (Claing et al., 2002).

Like the aforementioned cases of GABA_BR1 and T1R3, α_{1D} AR demonstrates little or no functional activity when expressed alone in most heterologous cells (Theroux et al., 1996; Hirasawa et al., 1997; Chalothorn

et al., 2002). The reason for this lack of α_{1D} AR functionality is that the receptor is not trafficked efficiently to the cell surface and is mostly retained in the ER complex (Chalothorn et al., 2002; Uberti et al., 2003; Hague et al., 2004b). Interestingly, it has been found that α_{1D} AR exhibits robust heterodimerization with α_{1B} AR (Uberti et al., 2003; Hague et al., 2004b) and β_2 AR (Uberti et al., 2005), resulting in markedly enhanced cell surface expression and functional activity of α_{1D} AR. In contrast, α_{1D} AR does not display any detectable physical association with α_{1A} AR (Uberti et al., 2003) or β_1 AR (Uberti et al., 2005), or any increase in surface expression upon cotransfection with α_{1A} AR (Hague et al., 2004b; Uberti et al., 2003) or any one of 25 other GPCRs that were examined in a systematic screen (Uberti et al., 2005). These findings suggest that α_{1D} AR may primarily function in cells as a heterodimer and that its preferences for heterodimerization are quite specific. Physical interactions have also been reported between α_{1A} AR and α_{1B} AR (Stanasila et al., 2003; Uberti et al., 2003) as well as α_{1B} AR and the H₁ histamine receptor (Carrillo et al., 2003), with the functional consequences of these heterodimeric associations being receptor cross-internalization (Stanasila et al., 2003) and cross-activation of G proteins (Carrillo et al., 2003). These interactions are quite specific, as α_{1B} AR does not significantly associate with β_2 AR, the NK1 substance P receptor, or the CCR5 chemokine receptor (Stanasila et al., 2003). Thus, heterodimerization seems to be a key mechanism involved in the regulation of α_1 AR activity, with specific heterodimerization of α_{1D} AR being especially critical for the surface expression and subsequent functionality of this receptor subtype.

D. Opioid Receptors

The opioid peptide receptor (OPR) family is comprised of three cloned subtypes: δ , κ , and μ . These subtypes exhibit 65 to 70% sequence homology and all couple to G_i/G_o proteins to inhibit adenylyl cyclase and regulate the activity of various ion channels. OPRs are activated by endogenous peptides, such as enkephalins and endorphins, and are also activated by drugs of abuse such as morphine and heroin. These receptors are extremely important clinical targets in the treatment of pain and additionally have profound effects on the neuroendocrine system and immune response.

OPRs are known to homodimerize (Cvejic and Devi, 1997; Jordan and Devi, 1999; George et al., 2000; He et al., 2002; Ramsay et al., 2002) and also heterodimerize within the OPR family. Heterodimerization has been reported between δ -OPR and κ -OPR (Jordan and Devi, 1999) and between δ -OPR and μ -OPR (George et al., 2000; Gomes et al., 2000, 2004). However, no interactions have been reported between κ -OPR and μ -OPR, revealing a significant degree of specificity in opioid receptor heterodimerization. The interaction between δ -OPR and κ -OPR results in alterations in the pharma-

cological properties of the receptors (Jordan and Devi, 1999), which may account for the existence of unexplained OPR subtypes from native tissues that were defined pharmacologically in the precloning era but which do not correspond to any of the individual OPR cDNAs. Heterodimerization between δ -OPR and μ -OPR also influences receptor pharmacology and furthermore results in synergistic signaling (George et al., 2000; Gomes et al., 2000, 2004).

In addition to heterodimerization within the OPR family, OPRs have also been found to exhibit specific and functionally relevant dimerization with other types of GPCRs. For example, both δ -OPR and κ -OPR have been found to associate with β_2 AR (Jordan et al., 2001; McVey et al., 2001; Ramsay et al., 2002), with an important functional consequence being cross-internalization between β_2 AR and δ -OPR (Jordan et al., 2001). Such heterodimerization between OPRs and ARs may help to explain reports of unusual cross talk between opioid peptides and β -adrenergic agonists in various tissues (Pepe et al., 1997, 2004; Xiao et al., 1997). The μ -OPR subtype does not demonstrate any associations with β -adrenergic receptors but has been shown to interact with the α_{2A} -adrenergic receptor (Jordan et al., 2003), the SSTR2A somatostatin receptor (Pfeiffer et al., 2002), and the NK1 substance P receptor (Pfeiffer et al., 2003). The μ -OPR/SSTR2A and μ -OPR/NK1 interactions facilitate cross-internalization of the receptors following agonist stimulation, resulting in the cross-modulation of receptor desensitization (Pfeiffer et al., 2002, 2003). Conversely, coexpression of α_{2A} AR with μ -OPR results in enhanced μ -OPR signaling in the absence of α_{2A} AR agonist stimulation, with this effect being abolished following treatment of α_2 AR agonists (Jordan et al., 2003). Finally, all three OPR subtypes have been found to interact in immune cells with the chemokine receptor CCR5 (Suzuki et al., 2002; Chen et al., 2004), with these interactions contributing to cross-desensitization between opioids and chemokines (Chen et al., 2004).

E. Somatostatin Receptors

The somatostatin receptor family contains five subtypes (SSTR1–5) that are activated by the ligands SST-14, SST-28, and cortistatin (Moller et al., 2003). These receptors are found in a number of different tissues and play key roles in regulating hormone release, endocrine function, sleep, and cognition (Lahlou et al., 2004). The five SSTR subtypes are known to form homodimers (Rocheville et al., 2000b; Pfeiffer et al., 2001; Grant et al., 2004a) and heterodimers in specific combinations. For example, SSTR5 associates robustly with SSTR1 but not with SSTR4 (Rocheville et al., 2000b; Patel et al., 2002; Grant et al., 2004b). The SSTR1/SSTR5 interaction has modest effects on the ligand binding (Rocheville et al., 2000b; Grant et al., 2004b) and internalization properties of the receptors; SSTR1 does not undergo efficient agonist-promoted internalization when ex-

pressed alone, but does undergo significant endocytosis when coexpressed with SSTR5 (Rocheville et al., 2000b). SSTR2 and SSTR3 specifically heterodimerize with each other, but have not been shown to interact with other somatostatin receptor subtypes (Pfeiffer et al., 2001). An important functional consequence of the SSTR2/SSTR3 interaction is a marked alteration in the desensitization profile of the heterodimer relative to that of the individually expressed receptors (Pfeiffer et al., 2001).

The SSTR subtypes interact not only with each other but also with other GPCRs. As mentioned earlier, SSTR2 has been found to interact with the μ -OR (Pfeiffer et al., 2002). This association has no evident effect on the ligand binding properties of the receptors, but does allow for significant cointernalization and cross-desensitization (Pfeiffer et al., 2002). Additionally, SSTR5 exhibits heterodimerization with the D2 dopamine receptor (Rocheville et al., 2000a). The SSTR5/D2R heterodimer displays enhanced signaling and altered pharmacological properties relative to the individually expressed receptors (Rocheville et al., 2000a) and may help to account for well known examples of synergistic interactions between somatostatin and dopamine in the central nervous system (Havlicek et al., 1976; Chneiweiss et al., 1985; Izquierdo-Claros et al., 1997; Marzullo et al., 1999).

F. Purinergic Receptors

Purines such as adenosine and ATP are neurotransmitters with key actions in the central nervous system, cardiovascular system, immune system, and other tissues (Ralevic and Burnstock, 1998). Adenosine activates a family of four GPCRs (A_1 , A_{2A} , A_{2b} , and A_3), whereas ATP activates P2X receptors, which are ion channels, as well as a family of seven GPCRs known as P2Y receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, and $P2Y_{13}$). Specific heterodimerization between purinergic receptor subtypes has been reported for A_1 R and $P2Y_1$ R in both transfected cells (Yoshioka et al., 2001, 2002b) and native brain tissue (Yoshioka et al., 2002a). The A_1 R/ $P2Y_1$ R heterodimer exhibits a pharmacological profile that is distinct from either receptor expressed alone, which may contribute to the diversity of purinergic receptor binding sites that have been described in native tissues (Yoshioka et al., 2001).

A number of specific interactions between purinergic receptors and other types of GPCRs have been described. For example, A_1 R undergoes heterodimerization with D_1 dopamine receptors (Gines et al., 2000). The A_1 R/ D_1 R interaction is quite specific, since three independent groups have reported that A_1 R does not detectably interact with D_2 dopamine receptors (Gines et al., 2000; Yoshioka et al., 2001; Kamiya et al., 2003). In contrast to A_1 R, the closely related A_{2A} R specifically heterodimerizes with D_2 R (Hillion et al., 2002; Canals et al., 2003; Ferre et al., 2004). Both the A_1 R/ D_1 R and A_{2A} R/ D_2 R associations allow for substantial cointernal-

ization and cross-desensitization between the interacting receptors (Gines et al., 2000; Hillion et al., 2002). Such receptor-receptor cross talk probably represents a key molecular mechanism underlying the well known functional antagonism that is observed between adenosine and dopamine in a variety of cell types (Franco et al., 2000).

A_1 R and A_{2A} R not only specifically interact with different dopamine receptor subtypes, they also differentially associate with mGluR subtypes; A_1 R interacts with mGluR1 α (Ciruela et al., 2001), whereas A_{2A} R undergoes heterodimerization with mGluR5 but not mGluR1 (Ferre et al., 2002). Unlike the functionally antagonistic interactions between adenosine and dopamine receptors, the A_1 R/mGluR1 and A_{2A} R/mGluR5 interactions facilitate synergistic signaling between adenosine receptor and glutamate receptor agonists (Ciruela et al., 2001; Ferre et al., 2002).

G. Olfactory Receptors

Olfaction in mammals begins in the nasal epithelium, where inhaled odorant molecules bind to specific cell surface olfactory receptors (ORs) to activate olfactory sensory neurons (Buck and Axel, 1991). ORs represent the largest family of GPCRs, with more than 300 members in humans and more than 1000 members in rodents, but the functional activity and pharmacology of these receptors have been very difficult to study because they are poorly trafficked to the cell surface and largely nonfunctional when expressed heterologously in most cell types (McClintock et al., 1997; Lu et al., 2003; Mombaerts, 2004). As in the cases of poorly trafficking receptors such as $GABA_B$ R1, T1R3, and α_{1D} AR discussed above, it has recently been shown that receptor heterodimerization can facilitate OR surface expression and functional activity (Fig. 1). The OR known as M71 has been found to heterodimerize with β_2 AR, with this interaction resulting in a striking enhancement in the surface trafficking of M71 in heterologous cells (Hague et al., 2004a). The M71/ β_2 AR interaction exhibits at least some degree of specificity, since M71 surface expression is not affected at all by coexpression with any of the eight other adrenergic receptor subtypes (Hague et al., 2004a). Heterodimerization with β_2 AR results not only in enhanced surface expression of M71, but also in enhanced M71 functional activity as well as M71/ β_2 AR cointernalization upon agonist stimulation of either receptor (Hague et al., 2004a). Interestingly, parallel genetic experiments in *Drosophila* have suggested that heterodimerization with a specialized chaperone GPCR termed OR83b may be required for the functional activity of most if not all *Drosophila* ORs (Larsson et al., 2004; Neuhaus et al., 2005). Given the large number of ORs in mammals, the specificity and functional importance of heterodimerization for the vast majority of receptors in this family remain to be determined.

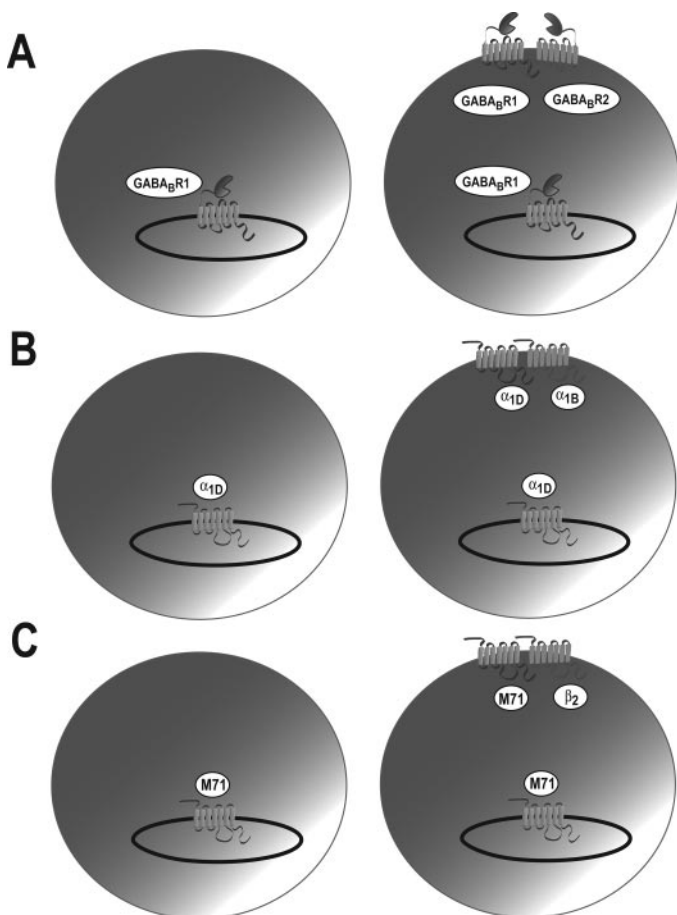


FIG. 1. Heterodimerization plays a key role in the surface trafficking of certain GPCRs. GABA_BR1, α_{1D} AR, and M71-OR do not efficiently traffic to the cell surface when expressed alone in heterologous cells, but rather are retained in the ER/Golgi complex (indicated by the black circles in the figure). However, coexpression with specific partners results in the formation of receptor heterodimers and enhanced trafficking to the cell surface.

H. Vasopressin, Oxytocin, and Other Receptors

The closely related neurohypophysial hormones arginine vasopressin and oxytocin activate a family of GPCRs that include the vasopressin V1a, V1b, and V2 receptors, as well as the oxytocin receptor (OTR). Several heterodimer combinations have been reported to occur within this receptor family: V1aR/V2R (Terrillon et al., 2003, 2004), V1aR/OTR, and V2R/OTR (Terrillon et al., 2003; Devost and Zingg, 2004). The specificity of these interactions is highlighted by the fact that no heterodimerization has been detected between V1aR and GABA_BR1 (Terrillon et al., 2003) or between OTR and the bradykinin B2 receptor (Devost and Zingg, 2004). The V1aR/V2R interaction is functionally important in that it has been shown to result in substantial cointernalization of the two receptors (Terrillon et al., 2004), whereas the physiological relevance of the OTR associations with both V1aR and V2R remains to be determined.

In addition to the examples discussed above, GPCR heterodimerization has been reported for a number of

other receptor combinations. These other examples are listed in Table 1 and include muscarinic acetylcholine M2 and M3 (Maggio et al., 1999), muscarinic M3 and adrenergic α_{2C} (Maggio et al., 1993), dopamine D1 and D2 (Lee et al., 2004), dopamine D2 and D3 (Scarselli et al., 2001; Maggio et al., 2003), serotonin 5-HT1 (Xie et al., 1999; Salim et al., 2002), sphingosine-1-phosphate (Van Brocklyn et al., 2002), calcium-sensing receptor and mGluR1 α (Gama et al., 2001), angiotensin AT1 and bradykinin B2 (AbdAlla et al., 2000, 2001b), angiotensin AT1 and AT2 (AbdAlla et al., 2001a), melatonin MT1 and MT2 (Ayoub et al., 2002, 2004), thyrotropin-releasing hormone TRHR1 and TRHR2 (Hanyaloglu et al., 2002), cholecystinin type A and B (Cheng et al., 2003), and endothelin type A and B receptors (Gregan et al., 2004).

II. Clinical Significance of GPCR Heterodimerization

As mentioned earlier, GPCRs are very common targets for therapeutic pharmaceuticals. Thus, it is natural to ask: is there any evidence that GPCR heterodimerization has clinical significance? One line of such evidence comes from unusual cross talk between different classes of drugs. For example, beta blockers can in some cases block signaling by both β -adrenergic and angiotensin receptors, perhaps due to heterodimerization of β ARs and AT1 angiotensin receptors, as described above (Barki-Harrington et al., 2003). Furthermore, the aforementioned interaction between δ -OPR and μ -OPR has been postulated to account for the well known effects of δ -OPR ligands on μ -OPR-mediated analgesia (Heyman et al., 1989; Abdelhamid et al., 1991; Horan et al., 1992; Malmberg and Yaksh, 1992; Porreca et al., 1992; Sanchez-Blazquez et al., 1997; He and Lee, 1998; Gomes et al., 2004). Synergistic and antagonistic interactions between drugs are extremely important to consider in a clinical setting, and heterodimerization between receptors represents a specific mechanism that may potentially underlie certain drug-drug interactions at the molecular level.

Evidence for the clinical significance of GPCR heterodimerization has also come from work on chemokine receptors. Chemokines are a family of cytokines that are involved in the attraction and activation of leukocytes through stimulation of GPCRs. Interestingly, two subtypes of chemokine receptor, CCR5 and CXCR4, are known to act as coreceptors for HIV entry into cells (Cairns and D'Souza, 1998), and a polymorphism (V64I) in the chemokine receptor CCR2 has been found to correlate with a markedly decreased rate of AIDS progression (Smith et al., 1997; Lee et al., 1998). The V64I CCR2 polymorphism has also been shown to enhance heterodimerization between CCR2/CCR5 and CCR2/CXCR4 (Mellado et al., 1999). Considered together with data revealing that anti-CCR2

TABLE 1

Summary of GPCRs that have been found to heterodimerize. This list is not comprehensive, but does contain a number of key examples from the literature. The receptors listed as "negative controls" were examined for heterodimerization with the receptors listed in the first column and not found to detectably interact. The reported functional significance for each positive interaction is briefly summarized in the fourth column. The methods used in all of these studies were coimmunoprecipitation, fluorescence resonance energy transfer, and/or bioluminescence resonance energy transfer. These techniques have been described in detail in a number of previous reviews (Jordan et al., 2000; Marshall, 2001; George et al., 2002; Kroeger et al., 2003; Milligan, 2004; Terrillon and Bouvier, 2004b).

Receptor	Heterodimer Partner	Negative Controls	Effect of Heterodimerization	Reference
5-HT _{1B}	5-HT _{1D}		n.d.	Xie et al., 1999
Adenosine A1	Dopamine D1	Dopamine D2	Signaling	Gines et al., 2000
Adenosine A1	P2Y ₁		Pharmacology	Yoshioka et al., 2001, 2002a, 2002b
Adenosine A1	mGluR1 α	mGluR1 β	Signaling	Ciruela et al., 2001
Adenosine A2A	Dopamine D2	Dopamine D1	Internalization, signaling	Hillion et al., 2002; Canals et al., 2003; Ferre et al., 2004
Adenosine A2A	mGluR5	mGluR1 β	Signaling	Ferre et al., 2002
Angiotensin 1A	Angiotensin 2		Signaling	AbdAlla et al., 2001a
Angiotensin 1A	β_2 AR		Signaling, trafficking	Barki-Harrington et al., 2003
Angiotensin 1A	Bradykinin B2		Internalization, pharmacology, signaling	AbdAlla et al., 2000, 2001b
Calcium sensing receptor	mGluR1 and mGluR5		Trafficking	Gama et al., 2001
CCR2	CXCR4 and CCR5		Signaling, reduction of HIV infection	Mellado et al., 2001; Rodriguez-Frade et al., 2004
CCR5	δ -, κ -, and μ -OPR		Desensitization	Suzuki et al., 2002; Chen et al., 2004
Cholecystokinin A	Cholecystokinin B	β_2 AR	Signaling, internalization	Cheng et al., 2003
Dopamine D1	Dopamine D2		Signaling	Lee et al., 2004
Dopamine D2	SSTR5		Pharmacology, signaling	Rocheville et al., 2000a
Dopamine D2	Dopamine D3		Pharmacology	Scarselli et al., 2001; Maggio et al., 2003
Endothelin A	Endothelin B		Internalization	Gregan et al., 2004
GABA _B R1	GABA _B R2		Gain of function, pharmacology	Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998; Kuner et al., 1999; Martin et al., 1999; Ng et al., 1999
Melatonin MT1	Melatonin MT2	β_2 AR	n.d.	Ayoub et al., 2002, 2004
Muscarinic M2	Muscarinic M3		Pharmacology	Maggio et al., 1999
Oxytocin	Vasopressin V1a and V2	GABA _B R2	n.d.	Terrillon et al., 2003; Devost and Zingg, 2004
S1P1	S1P2 and S1P3	LPA1	n.d.	Van Brocklyn et al., 2002
SSTR1	SSTR5	SSTR4	Internalization, pharmacology, expression	Rocheville et al., 2000b; Patel et al., 2002; Grant et al., 2004a
SSTR2A	SSTR3		Desensitization	Pfeiffer et al., 2001
SSTR2A	μ -OPR		Desensitization	Pfeiffer et al., 2002
Substance P (NK1)	μ -OPR		Internalization, desensitization	Pfeiffer et al., 2003
TRHR1	TRHR2	GnRHR	Internalization	Hanyaloglu et al., 2002
Vasopressin V1a	Vasopressin V2		Internalization	Terrillon and Bouvier, 2004a; Terrillon et al., 2003, 2004
α_{1B} AR	α_{1A} AR	β_2 AR, NK1, CCR5	Internalization	Stanasila et al., 2003; Uberti et al., 2003
α_{1B} AR	Histamine H ₁		n.d.	Carrillo et al., 2003
α_{1D} AR	α_{1B} AR	α_{1A} AR	Gain of function, internalization	Uberti et al., 2003; Hague et al., 2004b
α_{1D} AR	β_2 AR	β_1 AR	Gain of function, internalization	Uberti et al., 2005
α_{2A} AR	β_1 AR		Internalization, pharmacology	Xu et al., 2003
α_{2A} AR	μ -OPR		Signaling	Jordan et al., 2003
β_1 AR	β_2 AR		Internalization, pharmacology, signaling	Lavoie et al., 2002; Mercier et al., 2002; Lavoie and Hebert, 2003
β_2 AR	δ -, κ -OPR		Internalization, signaling	Jordan et al., 2001; McVey et al., 2001; Ramsay et al., 2002
β_2 AR	β_3 AR	GABA _B R2	Internalization, signaling	Breit et al., 2004
β_2 AR	M71-OR		Gain of function, internalization	Hague et al., 2004a
κ -OPR	δ -OPR	μ -OPR	Internalization, pharmacology, signaling	Jordan and Devi, 1999
μ -OPR	δ -OPR		Pharmacology, signaling	George et al., 2000; Gomes et al., 2000, 2004

n.d., not determined.

antibodies that enhance CCR2/CCR5 and CCR2/CXCR4 heterodimerization also lead to markedly decreased HIV infectivity (Rodriguez-Frade et al., 2004), these findings suggest that heterodimerization of chemokine receptors is a key determinant in the ability of

HIV to use these receptors to gain entry into cells. Heterodimerization of CCR2 and CCR5 has also been shown to result in synergistic signaling between the two receptors (Mellado et al., 2001), revealing that the interaction of these receptors has physiological rele-

vance for normal leukocyte function in addition to pathophysiological relevance for the regulation of HIV entry.

Further evidence for the clinical significance of GPCR heterodimerization has also come from recent studies on Wnt receptors. Wnts are secreted glycoproteins that play diverse roles in regulating cell fate and proliferation via activation of GPCRs belonging to the frizzled (Fz) family. Mutations to the Fz4 subtype have been found to underlie an autosomal dominant form of a disease known as familial exudative vitreoretinopathy (FEVR), which is characterized by impaired growth of retinal capillaries leading to eventual retinal degeneration (Robitaille et al., 2002). The mutant Fz4 receptor in FEVR patients is truncated and retained in the ER. Moreover, it has been shown that the mutant Fz4 can form homodimers with wild-type Fz4 as well as heterodimers with other Fz subtypes, leading to ER retention of these receptors (Kaykas et al., 2004). Thus, the capacity of Fz receptor subtypes to heterodimerize in vivo may explain the genetic dominance of the mutant Fz4 allele in causing the pathology associated with FEVR.

III. Conclusions

Numerous reports of GPCR heterodimerization have appeared in the literature over the past few years. In cases where the question of specificity has been examined, these interactions between receptors often seem to be quite specific. These interactions may be regulated in various ways, such as via agonist stimulation, and the issue of regulation has been covered in detail in several previous reviews (Jordan et al., 2000; Marshall, 2001; George et al., 2002; Kroeger et al., 2003; Milligan, 2004; Terrillon and Bouvier, 2004b). Regarding the functional significance of these interactions, there are at least three distinct ways that GPCR heterodimerization may be physiologically relevant. First, some GPCRs are completely nonfunctional when expressed alone and clearly require assembly with a specific partner to achieve surface expression and functional activity. Second, even GPCRs that do not absolutely require heterodimerization may still associate with other receptors, allowing for cross talk and mutual regulation between specific receptor subtypes. Third, GPCR heterodimerization can in some cases alter the pharmacological properties of the associated receptors, such that novel pharmacological entities are created. Since GPCRs are important drug targets in the treatment of many different diseases, the further elucidation of the specificity and physiological significance of GPCR heterodimerization may lead to insights that will fundamentally impact the development of future therapeutics.

Acknowledgments. S.C.P. and R.A.H. are supported by grants from the National Institutes of Health and W. M. Keck Foundation. C.H. is supported by a grant from the American Heart Association.

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