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International Union of Basic and Clinical Pharmacology. LXVII. Recommendations for the Recognition and Nomenclature of G Protein-Coupled Receptor Heteromultimers

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***Abstract*——G protein-coupled receptors (GPCRs) have long been considered to be monomeric membrane proteins. Although numerous recent studies have indicated that GPCRs can form multimeric complexes, the functional and pharmacological consequences of this phenomenon have remained elusive. With the discovery that the functional $GABA_B$ receptor is an obligate heterodimer and with the use of energy transfer technologies, it is now accepted that GPCRs can form heteromultimers. In some cases, specific properties of such heteromers not shared by their respective homomers have been reported. Although in most cases these properties have only been observed in heterologous expression systems, there are a few reports describing data consistent with such heteromultimeric GPCR complexes also existing in native tissues. The present article illustrates well-documented examples of such native multimeric complexes, lists a number of recommendations for recognition and acceptance of such multimeric receptors, and gives recommendations for their nomenclature.**

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I. Introduction

G protein-coupled receptors were, for a long time, considered to be unique among integral membrane proteins, because they were monomeric (Klingenberg, 1981). Indeed, many biochemical and biophysical data are consistent with the ability of rhodopsin to activate transducin in a monomeric form (Chabre and le Maire, 2005). This finding is further supported by recent studies demonstrating that monomeric GPCRs[1] can activate G proteins (Jastrzebska et al., 2006; B. Kobilka and R. K. Sunahara, personal communication) as a result of conformational changes of the heptahelical domain (reviewed in Okada et al., 2001). However, more and more data indicate that GPCRs can form oligomers, either homo-oligomers or hetero-oligomers. For simplicity, only dimers will be considered in the present article although we recognize that higher multimers may also exist. Whereas in such GPCR dimers activation of a single protomer is sufficient for G protein activation (Hlavackova et al., 2005; Damian et al., 2006), specific signaling cascades, specific pharmacological properties, and specific internalization and recycling properties have been observed for GPCR heterodimers (Bouvier, 2001; Angers et al., 2002; George et al., 2002; Javitch, 2004; Milligan, 2004; Park et al., 2004; Terrillon and Bouvier, 2004; Bulenger et al., 2005; Filizola and Weinstein, 2005; Pfleger and Eidne, 2005; Prinster et al., 2005). Accordingly, it has been proposed that GPCR oligomerization may provide a way to increase the number of receptor entities with a limited number of genes (Park and Palczewski, 2005). Because GPCRs represent one of the largest gene families in mammalian genomes, such a proposal would make the GPCRs the most complex and diverse receptor system. However, before such a proposal can be accepted, a number of important issues need to be clarified. Three of these are the following: 1) Are the properties observed in transfected heterologous expression systems also valid in native tissue in vivo? 2) Do we have to consider such GPCR complexes as a receptor unit or just an association of receptor units? and 3) Are the pharmacological properties of homodimers or heterodimers substantially different from those of the monomeric receptor?

In the field of GPCRs, the aim of NC-IUPHAR is to provide a detailed, accurate, and comprehensive list of these receptors, including their pharmacological and functional properties, their in vivo function, and their localization. At present, NC-IUPHAR has a database that includes the most commonly studied GPCRs (http://www.iuphar-db.org/GPCR/index.html). NC-IUPHAR will now consider GPCR multimers after a clear demonstration that these multimers exist in native tissue and have properties (pharmacological or functional) that clearly distinguish them from existing well-defined receptors.

In the present article, we will first summarize the best characterized GPCR dimers, especially those of some class C GPCRs, and then we will examine some recent examples taken from the class A GPCR family with emphasis on the existing evidence for such dimers in vivo. These examples will then be used to define the requirements for such GPCR dimers to be accepted as a receptor entity by NC-IUPHAR, and finally we will propose a simple nomenclature for such dimeric receptors. Such recommendations for the recognition and nomenclature of GPCR multimers will be adopted in a Web-based information system, the G Protein-Coupled Receptor-Oligomerization Knowledge Base (GPCR-OKB) (http://www.gpcr-okb.org), in which all available information on GPCR multimers will be included.

II. The Class C G Protein-Coupled Receptors

Despite numerous observations that suggested the existence of GPCR multimers (reviewed in Bouvier, 2001), direct demonstration using biochemical approaches of GPCR dimers was first reported for the class A β_2 adrenergic (Hebert et al., 1996) and the class C mGlu5 receptors (Romano et al., 1996). Since then, as illustrated below, a number of studies demonstrated that class C GPCR dimerization is essential for function, with the association of two identical or two distinct subunits being required to produce a functional receptor. Class C includes the receptors for the main neurotransmitters glutamate (the mGlu receptors) and GABA (the $GABA_B$ receptor), as well as receptors for calcium (calcium-sensing receptor), for basic amino acids (GPRC6a), for sweet and umami taste compounds (T1 receptors), and for some pheromones, plus a few orphan receptors, including GPRC5A–D, GABABL (GPR156), GPR158, and GPR179 (GPR158L1) (Pin et al., 2003; Foord et al., 2005). Like any other GPCR, class C receptors possess a heptahelical domain; however, their ligand binding site is not located in this domain but rather in a venus flytrap domain (VFT) that is part of a large extracellular domain (except for the orphan class C receptors that all lack this domain).

In 1996, Romano et al. reported that the mGlu5 receptor is a homodimer, stabilized by a disulfide bridge. Such a covalent linkage between the subunits was then confirmed for most other class C GPCRs, and it was then firmly demonstrated that the disulfide bridge cross-links the two subunits at the level of their extracellular domain. Such a property allowed a straightforward demonstration that these receptors are also dimeric in native

[1] Abbreviations: GPCR, G protein-coupled receptor; NU-IUPHAR, International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification; GPCR-OKB, G Protein-Coupled Receptor-Oligomerization Knowledge Base; mGlu, metabotropic glutamate; VFT, Venus flytrap; KO, knockout; BRET, bioluminescence resonance energy transfer, DAMGO, [D-Ala^2,*N*-Me-Phe^4,Gly^5-ol]-enkephalin; 6-GNTI, 6′-guanidinoaltrindole; BMY 7378, 8-[2-(4-(2-methoxyphenyl)piperazin-1-yl)ethyl]-8-azaspiro[4,5]decane-7,9-dione; AR, adrenergic receptor.

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tissue, because a simple Western blot analysis only revealed dimeric proteins, and the monomeric form was only observed after reduction of the SS bonds.

Dimerization of these receptors is essential for allosteric coupling between the VFT and the heptahelical domain and thus between ligand binding and G protein activation. As nicely illustrated by the X-ray structure of the dimer of the mGlu1 VFTs, both in the presence and absence of agonist, glutamate binding results in a major change in the *relative orientation* of the VFTs within the dimer (Kunishima et al., 2000; Tsuchiya et al., 2002). This movement at the level of the VFT probably results in a *relative movement* of the heptahelical domains, leading to their activation, as nicely illustrated by recent fluorescence resonance energy transfer studies (Tateyama et al., 2004). This model has been further documented by a number of mutagenesis studies indicating that agonist binding in one VFT leads to the activation of either heptahelical domain in the dimer (Kniazeff et al., 2004). If a relative movement of one subunit compared with the other is the basis for signal transduction, then only a dimer can constitute a functional receptor entity, as already widely documented for many integral membrane proteins, such as guanylate cyclase (He et al., 2001; van den Akker, 2001) and the tyrosine kinase (Ferguson et al., 2003) receptors.

In the case of mGlu receptors, only homodimers have been described so far (Romano et al., 1996). However, much interest in GPCR dimerization arose when the class C $GABA_B$ receptor was shown to be an obligate heterodimer, composed of two similar but distinct subunits: $GABA_{B1}$ and $GABA_{B2}$ (Jones et al., 1998; Kaupmann et al., 1998; White et al., 1998). In that case, each subunit has a specific role, with agonists interacting with $GABA_{B1}$ (Galvez et al., 2001; Kniazeff et al., 2002) and $GABA_{B2}$ being responsible for G-protein activation (Galvez et al., 2001; Duthey et al., 2002). Moreover, a quality control system prevents $GABA_{B1}$ from reaching the plasma membrane in the absence of $GABA_{B2}$ (Margeta-Mitrovic et al., 2000). Not only is this true in heterologous expression systems, but it is also the case in vivo. First, $GABA_{B1}$ and $GABA_{B2}$ proteins are colocalized in the brain, even at the electron microscopic level (Kaupmann et al., 1998) and can be coimmunoprecipitated from native tissue. Second, deletion of either the $GABA_{B1}$ or $GABA_{B2}$ gene in mice suppressed all $GABA_B$-mediated responses and led to almost identical phenotypes (Prosser et al., 2001; Schuler et al., 2001; Gassmann et al., 2004). Although $GABA_{B1}$ is able to weakly couple to G proteins and to modulate atypically effector K^+ channels in vivo in the absence of $GABA_{B2}$ (Gassmann et al., 2004), it is currently unclear whether that observed coupling is physiologically relevant or not. Regardless, not only do these data support the current model for class C receptor activation but also, more importantly, they were the first to demonstrate that a G protein-coupled receptor entity could be a heterodimer.

Since then, two other class C GPCRs were shown to be heterodimeric, namely the sweet and umami taste receptors. Indeed, three genes, *T1R1*, *T1R2*, and *T1R3*, encoding these putative taste receptors were cloned but none of these generated a functional receptor when expressed alone (Nelson et al., 2001, 2002; Li et al., 2002). Indeed, a receptor with all known properties of the sweet receptor was obtained when both T1R2 and T1R3 were coexpressed, whereas a typical umami receptor was obtained with both T1R1 and T1R3. As observed with the $GABA_B$ receptor, each subunit in these receptor heterodimers has a specific role in terms of ligand recognition and G protein coupling (Xu et al., 2004). In that case also, the heterodimeric nature of these receptors is not a peculiarity of the heterologous expression system used, because both T1R2 and T1R3 mRNAs are found in sweet-sensitive cells, and both T1R1 and T1R3 are detected in umami-sensitive cells. Moreover, whereas sweet and umami detection are affected in *T1R1* and *T1R2* knockout (KO) mice, respectively, both sweet and umami taste are largely diminished in mice lacking the *T1R3* gene (Damak et al., 2003; Zhao et al., 2003). However, although these data are consistent with the notion of heterodimers, direct biochemical or biophysical evidence is still lacking.

Taken together, these data nicely demonstrate that a dimer composed of either two identical or two distinct subunits constitutes the receptor entity of class C GPCRs. This is not a consequence of the expression of these genes in a heterologous system as coimmunoprecipitation, colocalization, and KO studies confirm that at least some of these receptors are dimers in native tissue. Moreover, the allosteric interactions between the subunits observed in cell lines are consistent with the properties of the native receptors (Liu et al., 2004; Xu et al., 2004). Such a dimeric functioning of class C receptors may simply be the consequence of their multidomain structure, with their dimerization being required for the signal to be transferred from the VFT to the heptahelical domain. As such, class C GPCRs nicely illustrate the fact that dimers of heptahelical transmembrane proteins can, in some cases, be considered unique receptor entities, suggesting that this may also be the case for other GPCRs from the other classes.

III. The Class A G Protein-Coupled Receptors

Our aim in the present article is not to review all available information on class A GPCR dimers; such information can be found in a number of recent reviews (Bouvier, 2001; Angers et al., 2002; George et al., 2002; Javitch, 2004; Milligan, 2004; Park et al., 2004; Terrillon and Bouvier, 2004; Bulenger et al., 2005; Pfleger and Eidne, 2005; Prinster et al., 2005). Our aim here is to concentrate on some examples of class A GPCR dimers with specific properties that can be used or have been used to identify such dimers in native tissue.

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Recently, the existence of class A GPCR dimers has been challenged on the basis of quantitative bioluminescence resonance energy transfer (BRET) analysis (James et al., 2006). Although this article clearly highlights the need for proper controls in energy transfer-based approaches, the authors did not take into consideration the number of additional approaches that support the existence of class A GPCR dimers. Moreover, this study did not mention earlier work in which careful and rigorous BRET analyses were performed, leading to a different conclusion (Bouvier et al., 2006).

A. Rhodopsin

Although earlier reports (summarized by Chabre and le Maire, 2005) were consistent with rhodopsin as a monomeric membrane protein, recent data illustrate that rhodopsin, like many other GPCRs, may exist in the membrane as a dimer and higher order structures. Atomic force microscopy of native disc membranes from rod outer segments reveals that rhodopsin is arranged in large paracrystalline arrays, providing perhaps the most direct demonstration of GPCR oligomerization (Liang et al., 2003). Although this suggests that GPCRs can form large arrays, the functional consequences are less clear. Functional analysis of purified rhodopsin either as a monomer or a dimer indicates that both forms can activate transducin, although the activation process is much faster when rhodopsin dimers/oligomers are favored with specific detergents (Jastrzebska et al., 2006). This activation may result from the interaction of large heterotrimeric G proteins with interacting pairs of protomers in a rhodopsin oligomer, as proposed on the basis of the atomic force microscopy images and the solved structures of rhodopsin and transducin (Filipek et al., 2004). GPCR dimerization probably plays a role in receptor maturation during biosynthesis and translocation to the plasma membrane (or rod outer segment membranes for rhodopsin) (Overton et al., 2003). In this light, an abnormality in oligomerization may lead to human pathophysiology such as dominant-negative retinitis pigmentosa, as proposed previously (Liang et al., 2003), in which the mutated version of rhodopsin that is retained intracellularly also retains the wild-type protein. Although this important information on rhodopsin multimers did not reveal what could be considered as a new receptor entity, the approaches used to illustrate rhodopsin multimers may be applied to other GPCRs.

B. The Melatonin Receptor

One major difficulty in studying the specific pharmacological properties of GPCR heterodimers is that coexpression of two distinct GPCRs leads not only to the formation of heterodimers but also to formation of both types of homodimers (or monomers). This result, in addition to potential intracellular cross-talk between signaling cascades activated by each receptor subtype, makes it difficult to draw any clear conclusion regarding the specific properties of the heterodimer. To overcome this problem, Ayoub et al. (2004) took advantage of the changes in BRET signals observed upon activation of melatonin receptor dimers in which one subunit carries *Renilla* luciferase and the other yellow fluorescent protein. This protocol permits the analysis of activation of specific combinations of receptor dimers without contamination by signals generated by the monomers or homodimers. This system was used to examine the pharmacological properties of MT1-MT2 heterodimers, in comparison with those of either MT1 or MT2 receptors expressed alone (Ayoub et al., 2004). No correlation was observed between the potencies of various compounds on MT1-MT2 heterodimers and MT1 or MT2 receptors, clearly indicating that such GPCR heterodimers can have specific pharmacological properties, probably resulting from positive or negative allosteric interactions between the two binding sites, depending on the ligand. Such information provides a way of using classic binding experiments to examine whether such melatonin receptor dimers exist in native membranes, but this has not yet been accomplished.

C. The Glycoprotein Hormone Receptors

A few studies demonstrate that all three glycoprotein hormone receptors can form dimers in heterologous cells (Osuga et al., 1997; Horvat et al., 2001; Tao et al., 2004). A recent study by Vassart and colleagues used both biochemical and energy transfer technologies to demonstrate that thyroid-stimulating hormone and luteinizing hormone receptors can form homodimers and can also heterodimerize at the cell surface of transfected cells (Urizar et al., 2005). These authors also examined the pharmacological consequence of dimerization and found that dimerization is associated with strong negative cooperativity between the ligand binding sites within a dimer. Such a negative cooperativity is nicely illustrated by the acceleration of the dissociation kinetics of radiolabeled hormone by cold hormone. The authors not only produced compelling evidence that this results from receptor dimerization, but also showed that the same is true with native receptors. These data provide strong evidence for the existence of glycoprotein hormone receptor dimers in vivo and also provide an interesting possible approach to detect specific oligomeric entities in native membranes. However, more work is needed to determine whether heterodimers with specific pharmacological or functional properties that could be considered as new receptor entities do exist in native tissue.

D. The Opioid Receptors

Numerous studies reported both homo- and heterodimerization of the μ, δ, and κ opioid receptors, resulting in specific pharmacological, functional, or desensitization properties. These studies, performed in heterologous expression systems, have been reviewed extensively (Levac et al., 2002; Park and Palczewski,

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2005). However, only a few researchers have attempted to examine whether such dimeric entities are functionally relevant in vivo.

In one study, the authors took advantage of the differential internalization property of μ opioid receptors upon activation by morphine or DAMGO (Whistler et al., 1999). Indeed, in contrast with DAMGO, morphine does not induce μ receptor internalization, a property that may explain morphine tolerance, because internalization is thought to be required for dephosphorylation and reactivation of GPCRs. Accordingly, facilitating μ receptor internalization should limit tolerance. To test this possibility, He et al. (2002) hypothesized that low concentrations of DAMGO should be able to facilitate μ receptor internalization in the presence of high morphine concentrations, possibly through μ receptor dimers in which at least one protomer is occupied by DAMGO. They first demonstrated that this is indeed the case in heterologous cells expressing μ receptor dimers composed of a wild-type receptor and a mutant receptor that can internalize upon morphine activation. They then validated their proposal in vivo. They found that cotreatment of animals with morphine and low concentrations of DAMGO resulted in the maintenance of the analgesic property of morphine, without the appearance of tolerance. These data are consistent with the existence of μ receptor dimers in vivo, but other explanations exist such as indirect cross-talk between DAMGO- and morphine-activated μ opioid receptors.

Among the various opioid receptor heterodimers that have been described in heterologous cells (Wang et al., 2005), the μ-δ dimer displays binding and functional properties that can also be observed in native membranes (Gomes et al., 2004). It was observed that δ ligands (agonists, antagonists, and inverse agonists) can both potentiate the effect of μ agonists (i.e., increase the efficacy) and increase the specific binding of radiolabeled μ agonists. These effects are observed with native membranes from wild-type but not from δ knockout mice. Together with coimmunoprecipitation data, these observations are best explained by a direct allosteric interaction between μ and δ receptors associated in a dimeric unit.

Further evidence for the existence of μ-δ dimers in vivo has come with the use of bivalent ligands composed of a μ agonist linked to a δ antagonist (Daniels et al., 2005b). Of interest, the specific analgesic properties of such bivalent ligands in vivo depend on the length of the chain linking the two ligands, further supporting the proposal that such ligands specifically target μ-δ heterodimers.

Ligands with specific properties at the κ-δ opioid receptor dimer have also been reported. One of these compounds, 6-GNTI, is a potent δ antagonist, with κ agonist activity (Waldhoer et al., 2005). 6-GNTI was found to be more effective at activating the κ-δ dimer than the κ receptor expressed alone. Of interest, 6-GNTI displays specific analgesic in vivo properties that cannot be mimicked by the coinjection of a κ agonist and a δ antagonist. Bivalent ligands, such as KDN21 that contain both δ and κ antagonists (Xie et al., 2005) or KDAN-18 that contains a δ antagonist and a κ agonist (Daniels et al., 2005a), also seem to specifically target the κ-δ heterodimer.

Early pharmacological studies suggested the existence of more than three subtypes of opioid receptors, and, as such, the cloning of only three genes encoding such receptors was a surprise. However, evidence now suggests that some of the previously characterized receptors may indeed correspond to heteromeric entities of different opioid receptor subtypes.

E. The CCR2 and CCR5 Receptors

Several groups have reported that the two chemokine receptors CCR2 and CCR5 can form homo- and heterodimers (Springael et al., 2005). In a recent study, the group of Parmentier elegantly demonstrated that binding of the CCR2 radioligand ^{125}I-monocyte chemoattractant protein-1 could be partially inhibited by the selective CCR5 agonist macrophage inflammatory protein-1β, the maximal inhibition being correlated with the expected amount of CCR2-CCR5 heterodimers in the cell (El-Asmar et al., 2005). Conversely, binding of the CCR5 radioligand could also be partially inhibited by a CCR2 specific agonist, further demonstrating a negative cooperativity between the two binding sites in a CCR2-CCR5 heterodimer. Although these data were obtained in heterologous cells, the authors showed that this property could also be observed in native cells. Indeed, $CD4^+$ lymphoblasts are known to express both CCR5 and CCR2 receptors, although CCR5 is expressed at a higher level. In these cells, binding of the CCR2 radioligand could also be partly inhibited by macrophage inflammatory protein-1β, consistent with allosterically interacting CCR2 and CCR5 proteins and, thus, the existence of CCR2-CCR5 heterodimers in native tissue (El-Asmar et al., 2005). Consistent with the higher amount of CCR5 than CCR2 in these cells and, thus, the majority of CCR5 proteins not being part of such heterodimers, no inhibition of the binding of the CCR5 radioligand could be detected with a CCR2 agonist. More recently it was shown, as for glycoprotein hormone receptors, that the rate of radioligand dissociation from one unit of the heterodimer was strongly increased in the presence of an unlabeled chemokine ligand of the other unit (Springael et al., 2006). This allosteric interaction between CCR5 and CCR2 was observed both in recombinant systems and in native $CD4^+$ cells. Although these data nicely fit with a cross-talk between subunits within an oligomeric entity, one cannot so far rule out the possibility that such negative interaction may also result from indirect cross-talk (Springael et al., 2006).

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F. The AT1 and Mas Receptors

Another interesting observation is the heterodimerization between the *Mas* oncogene and the angiotensin II AT1 receptor (Kostenis et al., 2005). The heptahelical protein Mas was recently shown to be involved in angiotensin II signaling in vivo, even though it is not activated by this peptide (Ambroz et al., 1991). For example, angiotensin II function is affected in *Mas* oncogene-deficient mice (Von Bohlen und Halbach et al., 2000). The authors suspected that such a functional interaction between Mas and angiotensin II signaling may result from the direct interaction of Mas and AT1 receptors. Such a prediction was verified using fluorescence resonance energy transfer and BRET experiments, and, surprisingly, the heterodimerization was found to result in the inhibition of the AT1 receptor activity. Consistent with such an inhibitory action of Mas on AT1 signaling in vivo and, thus, the existence of Mas-AT1 heterodimers in native tissue, the authors showed that angiotensin II-mediated vasoconstriction in mesenteric microvessels that normally express both mRNAs is increased in mice lacking the *Mas* oncogene. This effect is specific to AT1 as no increase in endothelin-mediated contraction was observed in KO animals.

The AT1 receptor was also shown to heterodimerize with the bradykinin B1 receptor as well as with the AT2 receptor. The AT1-B1 heterodimer shows enhanced AT1 activity, and this has been proposed to underlie hypertension associated with pre-eclampsia in pregnant women (AbdAlla et al., 2000, 2001b). Similar to the Mas situation, AT2 association with AT1 inhibits AT1 activity (AbdAlla et al., 2001a).

G. The α_{1B} and α_{1D} Adrenoceptors

As observed with the $GABA_B$ receptor, association between α_{1D} and α_{1B} adrenergic receptors was shown to be required for efficient surface targeting of the α_{1D} receptor, which normally is mostly retained inside the cell in heterologous cells (Hague et al., 2004). Surprisingly, this α_1 receptor association was recently shown to result in the disappearance of the α_{1D} high-affinity binding site for the specific antagonist BMY 7378 (Hague et al., 2006). Such a finding provides a good explanation for the long-standing mystery in the α_1 adrenoceptor field: that α_{1D} receptors could not be detected in native tissue using high-affinity binding of BMY 7378 (Yang et al., 1997, 1998), despite a high level of mRNA expression. This proposal is also consistent with a number of data reported with KO animals, such as increased affinity of the α_{1D} selective antagonist BMY 7378 in α_{1B} knockout mice (Deighan et al., 2005) or the potent BMY 7378 antagonist activity in left ventricular phenylephrine-induced pressure observed in α_{1A}-α_{1B} double KO mice. Taken together, these different findings are consistent with the existence of α_{1B}-α_{1D} heterodimers in native tissue. If reproduced by others, these data will certainly be considered a convincing demonstration of α_{1B}-α_{1D} heterodimers as a new receptor entity.

H. The β_1 and β_2 Adrenoceptors

To follow-up on the observation, using a BRET approach, that β_1ARs and β_2ARs had the same propensity to form heterodimers and homodimers (Mercier et al., 2002) and the report that β_1-β_2AR dimers display specific functional properties (Lavoie et al., 2002), the group of Xiao used βAR KO mice lacking both subtypes to express either β_1AR, β_2AR, or both (Zhu et al., 2005). Through coimmunoprecipitation studies, colocalization, and functional studies, they showed that these two receptors could form functional heterodimers in cardiomyocytes. They concluded that "heterodimerization of β_1AR and β_2AR in intact cardiac myocytes creates a novel population of βARs with distinct functional and pharmacological properties, resulting in enhanced signaling efficiency in response to agonist stimulation while silencing ligand-independent receptor activation, thereby optimizing β-adrenergic modulation of cardiac contractility." In contrast, using a newly developed imaging technology (called near-field scanning optical microscopy), the β_1ARs and β_2ARs were observed in apparently two different populations of microdomains in cardiomyocytes (Ianoul et al., 2005), an observation inconsistent with the existence of β_1-β_2AR dimers. However, not all receptors are located in such microdomains, leaving the possibility that β_1-β_2AR dimers are not incorporated in these domains. Alternatively, only a small fraction of the receptor proteins may indeed be associated into such heteromeric entities.

IV. On the Nomenclature and Recognition of Multimeric G Protein-Coupled Receptors

The examples described above nicely illustrate the fact that GPCR heterodimers display specific pharmacological and functional properties that can be observed not only in heterologous cells but also in vivo. Of interest, these data clearly indicate that allosteric interactions occur between the two subunits of a GPCR dimer, as convincingly demonstrated with reconstituted BLT1 dimers (Mesnier and Baneres, 2004; Damian et al., 2006), further indicating that such dimers correspond to a functional protein complex rather than just two proteins contacting each other. Other data are consistent with a single heterotrimeric G protein being associated with such GPCR dimers. This finding was firmly documented with a reconstituted BLT1 dimer (Baneres and Parello, 2003) and is consistent with modeling studies (Filipek et al., 2004; Fotiadis et al., 2004), as well as with functional studies indicating that a single activated heptahelical domain of a GPCR dimer is sufficient for full activity (Goudet et al., 2005; Hlavackova et al., 2005; Damian et al., 2006). Taken together, such information indicates that a GPCR dimer can be considered, at least

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in some cases, to be a unique receptor functional unit, and as such should be given a specific name.

In some cases, the heterodimer corresponds to a well-characterized receptor, which was already given a name based on its pharmacological properties. This is the case, for example, for the $GABA_B$ receptor or for the sweet (T1R2-T1R3) and umami (T1R1-T1R3) receptors. In those cases, of course, the name of the heterodimer will remain the name originally given. In the case of receptor heterodimers not yet defined and to not overly complicate GPCR nomenclature, we propose use of the existing names of the two subunits separated by a hyphen "-" in alphabetical order or in a numerical order. As such, a receptor heterodimer composed of dopamine D1 and D2 subunits will be named D1-D2 receptor.

However, before such receptor heterodimers can be accepted by the scientific community, the existence of such heterodimeric receptors should be firmly demonstrated in native tissue. Accordingly, at least two of the following three criteria should be met:

1. Evidence for physical association in native tissue or primary cells.
   A. Both subunits that compose the receptor heterodimer must be identified in the same cell and, if possible, within the same subcellular compartment. Coimmunolocalization experiments using antibodies recognizing each of the subunits should be used, if possible, at the electron microscopic level. If the physical interaction is convincingly demonstrated in vivo (see B) the need for colocalization is less important. In contrast, a colocalization study without the physical evidence for interaction is more or less meaningless.
   B. The physical interaction between both subunits should be documented in native tissue. This can be achieved using coimmunoprecipitation experiments from native tissue. However, such an experiment would only demonstrate that both proteins are part of the same multimeric protein complex, but this result cannot be an argument for a direct interaction between the two partners. Alternatively, energy transfer technologies using labeled ligands and/or labeled antibodies or transgenic animals (knockin) expressing physiological levels of recombinant fluorescent proteins could be used to demonstrate close receptor proximity in native tissue. Alternatively, the use of antibodies selective for a specific receptor dimer may be useful (Wager-Miller et al., 2002).
2. A specific functional property for the heterodimeric receptor will be critical to identify such receptors in native tissue. This could include the identification of a specific pharmacological property such as a positive or negative allosteric interaction between the two binding sites or identification of a ligand specific for the heterodimer. Other properties could be the activation of a specific transduction cascade not activated by either receptor subunit alone or specific internalization or desensitization properties. These new functions should be shown to truly result from the dimerization and not merely from the coexpression.
3. The use of knockout animals or RNAi technology may also provide key information on the existence of heterodimeric GPCRs in vivo. Indeed, the response mediated by such a unique dimeric receptor should be greatly modified in the absence of either one of the subunits. These results can be meaningfully interpreted only if the dimer has been shown to occur in vivo or if the change in function has been shown to be related to the dimerization in a simpler heterologous expression system in which the dimerization can be more easily documented.

Evidence that meets at least two of the above three criteria is said to be compliant with the NC-IUPHAR recommendations for recognition and acceptance of GPCR multimers. As additional criteria emerge, they will be incorporated. The compliant multimers will be incorporated into the IUPHAR receptor database, and this will be indicated in the GPCR-OKB database.

In summary, evidence continues to mount that GPCRs can form multimers, both homo- and heterodimers. The increasing number of such dimers for which evidence of unique in vivo functions is being demonstrated requires that the field have both 1) a formal set of criteria to recognize a bona fide functional receptor dimer and 2) a consistent nomenclature to designate those dimers that meet the developed criteria. In this report, we provide both.

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