Building a new conceptual framework for receptor heteromers

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Receptor heteromers constitute a new area of research that is reshaping our thinking about biochemistry, cell biology, pharmacology and drug discovery. In this commentary, we recommend clear definitions that should facilitate both information exchange and research on this growing class of transmembrane signal transduction units and their complex properties. We also consider research questions underlying the proposed nomenclature, with recommendations for receptor heteromer identification in native tissues and their use as targets for drug development.

The 'receptor heteromer' concept, in which receptors of the same and different gene families can combine among themselves to generate dimers and possibly higher-order entities with uniquebiochemical and functional characteristics, is becoming widely accepted[1–3]. Although initially a matter of considerable debate, few researchers now dispute the presence of receptor heteromers in artificial systems (for example, transfected cell lines) in which biophysical and biochemical techniques such as resonance energy transfer (RET), bimolecular fluorescence complementation (BiFC) and cysteine crosslinking have been key to demonstrating very close proximity of two receptors, which is most likely indicative of direct intermolecular receptor-receptor interactions[4,5]. The controversy has now moved to the existence and functional significance of receptor heteromers in native tissues. As explained below, in order to address these questions, we must find evidence for the unique biochemical and functional signatures (different from those of its constituent receptors) that characterize the receptor heteromer.

As research in this field moves forward, however, trying to describe receptor heteromers is becoming a significant conceptual challenge. The literature presents a bewildering array of terms, and there is a need for standardization based on operationally clear definitions. Thus, we present a proposal for a consensus nomenclature, based on a classical definition of 'receptor' and designed to encompass not only G protein–coupled receptors (GPCRs) but also other known transmembrane receptors. Based on the proposed nomenclature, we also give recommendations for the identification of receptor heteromers in native tissues. Research on receptor heteromers is poised to revolutionize basic tenets of pharmacology and take rational drug development to a new level of specificity and efficacy. We envision that the adoption of the proposed nomenclature system and experimental criteria will advance communication (and thereby progress) in the field.

See end for affiliations.

Major definitions

A receptor is a signal transducing unit, a cellular macromolecule or an assembly of macromolecules that is concerned directly and specifically with chemical signaling between and within cells[6]. It is important to realize that implicit in this definition is the notion of a receptor as a 'minimal functional unit' capable of turning an input signal into an output functional signal. Furthermore, this receptor specifically recognizes and is activated by agonists and can be found in the plasma membrane, organelle membranes or nucleus[7]. The definitions put forward in the present commentary will be circumscribed to transmembrane receptors (**Box 1**).

It is well known that receptor proteins often have quaternary structures; namely, they represent an assembly of two or more different polypeptide chains, called subunits[6], that may or may not derive from the same gene. We propose that the term 'heteromeric receptor' be used to define a dimeric or oligomeric receptor for which the minimal functional unit is composed of two or more different subunits that are not functional on their own. This definition would apply to ligand-gated ion channels (ionotropic receptors) such as glutamate *N*-methyl-D-aspartate (NMDA) receptors (**Fig. 1a**) or most nicotinic acetylcholine receptors[8,9]. The term would also be used for some GPCRs and some tyrosine kinase receptors, such as receptors for glial cell line–derived neurotrophic factor (GDNF) family ligands, in which subunits are responsible either for the association with the ligand or for the catalytic response[10]. Similarly, the γ-aminobutyric acid B ($GABA_B$) receptor, a GPCR, is composed of two seven-transmembrane (7TM) proteins, $GABA_{B1}$ and $GABA_{B2}$, that are involved in ligand recognition and cell signaling, respectively[2]. According to the definition of receptor cited above, neither subunit of the $GABA_B$ receptor is a receptor because neither protomer is fully functional on its own. Hence, the $GABA_B$ receptor should be referred to as a heteromeric GPCR (**Fig. 1b**). Some taste receptors, for which genetic deletion of one of the subunits leads to suppression of the receptor function[2], would also be called heteromeric GPCRs. If the receptor subunits are identical, they would constitute a 'homomeric receptor'. This is the case for some ionotropic receptors, such as the α_7 nicotinic acetylcholine receptor[9], and also some tyrosine kinase receptors, such as those for neurotrophins, which require a ligand-induced dimerization or an alteration of a constitutive dimerization interface to become functional[11].

Box 1 Definitions of proposed receptor nomenclature

Receptor: A signal transducing unit, a cellular macromolecule or an assembly of macromolecules that is concerned directly and specifically with chemical signaling between and within cells.

Heteromeric receptor: Dimeric or oligomeric receptor for which the minimal functional unit is composed of two or more different subunits that are not functional on their own.

Homomeric receptor: As heteromeric receptor but composed of two or more identical subunits that are not functional on their own.

Receptor heteromer: Macromolecular complex composed of at least two (functional) receptor units with biochemical properties that are demonstrably different from those of its individual components.

Receptor homomer: As receptor heteromer but combining two or more identical (functional) receptor units.

Biochemical fingerprint of the receptor heteromer: Biochemical characteristic of a receptor heteromer, which can be used for its identification in a native tissue.

Allosteric interaction in the receptor heteromer: Intermolecular interaction by which binding of a ligand to one of the receptor units in the receptor heteromer changes the binding properties of another receptor unit.

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In contrast, we suggest that a 'receptor heteromer' be defined as a macromolecular complex, composed of at least two (functional) receptor units with biochemical properties that are demonstrably different from those of its individual components. These different receptor entities may or may not interact with the same ligand (**Fig. 1c**). By extension, a 'receptor homomer' refers to a complex molecule that combines two or more identical (functional) receptor units. It is worth noting that the definitions of receptor heteromer and receptor homomer allow for the possibility of receptor (hetero- or homo-) multimers, as recent evidence indicates the existence of complexes that engage more than two different receptors[12,13]. The term 'receptor heteromer' would also include macromolecular complexes that consist of a GPCR plus an ionotropic receptor, such as the dopamine D_1-NMDA (**Fig. 1d**) and the dopamine D_5-$GABA_A$ receptor heteromers[14]. These receptors would be good examples of receptor heteromers that contain a heteromeric receptor.

Refining receptor nomenclature

To make this receptor nomenclature operationally viable, we propose the use of an alphanumeric order system, similar to the one previously recommended by the International Union of Basic and Clinical Pharmacology (IUPHAR) for GPCR heterodimers[2]. Specifically, we would use the existing names of the two or more receptor units that are present in the heteromer, separated by a hyphen, in alphabetic and numerical order. For instance, the heteromer of dopamine D_1 and D_2 receptors should be named D_1-D_2 receptor heteromer; similarly, the proposed heteromer of adenosine A_{2A}, dopamine D_2 and cannabinoid CB_1 receptors should be named A_{2A}-CB_1-D_2 receptor heteromer. This alphanumeric order should also be used for receptors whose names contain Greek letters, such as a heteromer of opioid receptors—such a heteromer, for example, would be called an opioid δ-κ receptor heteromer. If needed, we suggest Greek letters before Latin letters (irrespective of the numbers).

As defined thus far, neither the term 'heteromeric receptor' nor the term 'receptor heteromer' would apply to a hetero-oligomeric species that in this context would be a protein complex composed of a receptor (as defined above) plus another membrane protein that modifies the biochemical properties of the receptor, such as, for example, some GPCRs associated with receptor activity–modifying proteins (RAMPs) or with 'orphan GPCRs'. According to the definition of receptor, true orphan GPCRs, whose activities are likely controlled by ligand-independent mechanisms, might be better referred to as 'orphan 7TM proteins' to distinguish them from those that are likely to be regulated by an as-yet unidentified ligand[15]. Obviously, an orphan 7TM protein would be reclassified as a GPCR if a ligand were identified, so the former term can only be applied provisionally. An example of this type of hetero-oligomer is the orphan 7TM protein GPR50 binding to the melatonin MT_1 receptor, thereby modifying its functional properties[15] (**Fig. 1e**). Additionally, three different RAMPs (RAMP1, RAMP2 and RAMP3) that are single transmembrane proteins have been identified so far[16]. The calcitonin (CT) receptor has high affinity for CT, and its association with any of the RAMPs results in a different receptor with high affinity for the CT-family neuropeptide amylin (AMY)[16] (**Fig. 1f**). We propose for those cases that do not fit the definitions of either heteromeric receptor or receptor heteromer that the name of the associated modifying protein be added to the name of the receptor (for example, MT_1-GPR50 receptor). When the associated modifying protein changes the ligands that are preferentially recognized by the complex, we propose to continue naming the complex based on the ligand that binds preferentially while specifying the identity of the proteins contributing to the complex (for example, the AMY_1 receptor, formed by CT receptor and RAMP1).

On the other hand, the so-called CT-like (CL) receptor is not functional when expressed alone and therefore is not a true receptor but rather a (nonfunctional) 7TM subunit found in three different heteromeric receptors that contain either RAMP1, RAMP2 or RAMP3 subunits, named $CGRP_1$, AM_1 and AM_2 receptors, respectively[16]. Because RAMPs are not receptors on their own, receptors formed by the assembly between CL receptor and RAMPs represent true heteromeric receptors that should continue to be named based on the identity of the ligands that they recognize while specifying the identity of the proteins forming the receptor complex. $CGRP_1$ is a high-affinity receptor for the neuropeptide CT gene–related peptide that is formed by CL receptor and RAMP1, whereas AM_1 and AM_2 selectively bind another peptide of the CT family, adrenomedullin, and are formed by CL receptor and RAMP2 or RAMP3, respectively[16].

GPCR homomers or homomeric GPCRs?

Growing evidence suggests that many GPCRs form functional homodimers in the native membrane[1,2,17], a process that may be essential for their biosynthetic quality control[18]. Rhodopsin and the adrenergic β_2 receptor signal efficiently through G proteins when reconstituted into lipid nanodiscs containing only a single receptor molecule, and thus after solubilization and reconstitution, these GPCRs can function without the need for oligomerization[19,20]. Nonetheless, in most cases, it is not yet known whether one GPCR molecule can constitute the minimal functional unit *in vivo*. Therefore, currently, we do not have sufficient knowledge to define most GPCRs as either homomeric receptors or receptor homomers. Knock-in animals co-expressing one mutant allele form of the receptor that cannot bind agonists and one that cannot transduce signals would allow determination of whether GPCRs can function as homomers but would not unambiguously prove that they normally require homo-oligomerization for their activity.

Identification of receptor heteromers in native tissues

As mentioned above, biophysical techniques (when using adequate controls) can provide

strong support for the existence of receptor heteromers in artificial cell systems[4,5], but these approaches are technically difficult to perform in native tissues. The general view is that receptor heteromers detected in transfected cells may occur in native tissues provided that the receptor units are expressed in the same cell and in the same subcellular compartment. However, their demonstration in native tissues remains a significant challenge because, to a large extent, the evidence we can gather has to be indirect.

Direct identification could be achieved by taking advantage of selective probes (for example, specific antibodies or labeled selective ligands) that could discriminate between the receptor heteromer and other configurations of the individual components. However, so far, specific antibodies have only been reported for cannabinoid CB_1 receptor homomers[21], and no specific receptor heteromer ligand has yet been found. The compound 6′-guanidinonaltrindole (6′-GNTI) has been shown to be a selective agonist for opioid δ-κ receptor heteromers, but (albeit with lower potency) it also acts as a δ receptor antagonist[22]. As a result, we must rely on indirect approaches for the identification of a receptor heteromer in native tissues by discovering characteristic biochemical signatures and elucidating the receptor domains or epitopes that determine the receptor heteromer's quaternary structure. For example, a biochemical characteristic could be first identified in an artificial cell system, which can then be used as a 'biochemical fingerprint' to demonstrate its presence in the native tissue. Importantly, detection of this fingerprint must be contingent upon true heteromerization and not the mere co-expression of the receptors.

A strong suggestion that a biochemical fingerprint is specific for a receptor heteromer can be obtained by showing that it is abolished or altered when the heteromerization is disrupted, or alternatively when the quaternary structure of the heteromer is significantly modified without disrupting heteromerization. This could be shown with biophysical techniques (for instance, a significant decrease in the RET signal). This strategy requires identification of the domains or epitopes (of at least one of the receptors) that form the interaction surface in the heteromer; this may allow the construction of appropriate mutant or chimeric receptors, or the design of peptides that can selectively occupy and disrupt the receptor heteromer interface. A better delineation of this interface may also allow for a more productive approach using transgenic animals. It might be possible, for example, to generate a knock-in animal expressing a mutated receptor that fails to heteromerize with the other units of the receptor heteromer in transfected cells. In this paradigm, a differential ability to co-immunoprecipitate the two receptors from wild-type but not from the knock-in animals would support the existence of the receptor heteromer in native tissue, as long as the distribution of the mutated receptor does not change relative to that of the wild-type receptor, and as long as the expression of the partner receptor also remains unaltered.

Allosteric interactions between receptor units have been considered a common biochemical characteristic of a number of receptor heteromers[1,3,23]. These interactions were initially called "intramembrane receptor-receptor interactions" because they were first observed in crude membrane preparations of brain tissue[1]. In the typical intramembrane receptor-receptor interaction, stimulation of one receptor leads to changes in the binding characteristics of an adjacent receptor, such as decreased or increased affinity for an agonist. Using extensively washed membrane preparations, this constitutes a strong indication that the ligand triggers an intermolecular change from which a new biochemical property, characteristic of the receptor heteromer, has now emerged. In many cases, the same kind of interaction has been shown in both cotransfected cells and native tissues; this could be interpreted as an indication of the existence of receptor heteromers *in vivo* (see refs. 24,25 for recent examples). However, the major challenge is to demonstrate that the direct physical interaction of the two receptors is necessary for the modification of their signaling. Thus, for a true allosteric interaction in the receptor heteromer, the biochemical signature should be characteristic of the receptor heteromer and not of, for instance, downstream cross-talk effects at the level of G proteins or other signaling effectors, as it has been recently shown for a receptor heteromer consisting of serotonin 5-HT_{2A} and glutamate metabotropic $mGlu_2$ receptors[25]. Nevertheless, an allosteric interaction in the receptor heteromer can in principle be identified by its particularly fast kinetics. For example, in the adrenergic α_{2A}-opioid μ receptor heteromer, allosteric effects took less than 500 ms, which is the time required for G protein activation by a receptor[26]. This makes indirect (G protein–mediated) effects very unlikely.

Ligand binding selectivity and signal switching induced by selective ligands have also been proposed as additional biochemical characteristics of receptor heteromers. Receptors can display different ligand binding properties depending on whether or not they are engaged in a receptor heteromer. The D_1-D_2 receptor heteromer provides an example of changes in ligand properties[27]. SKF83959 is an agonist at D_1 receptor, which usually signals through G_s proteins, thereby activating adenylyl cyclase.

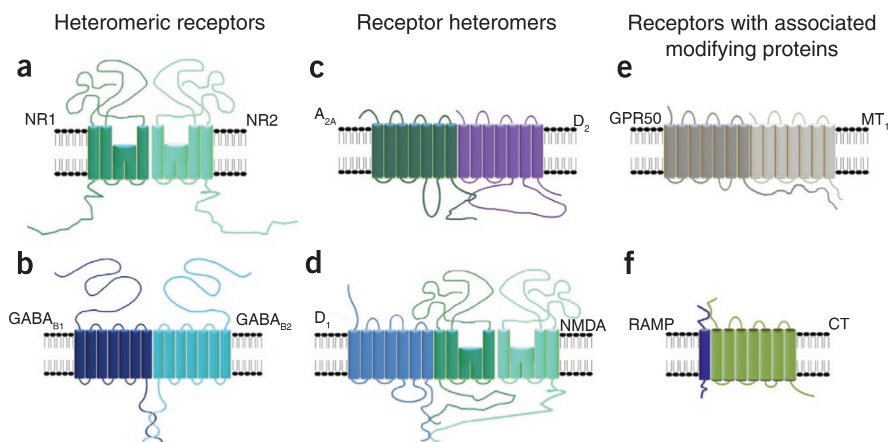

Figure 1 Examples of heteromeric receptors, receptor heteromers and receptors with associated modifying proteins. (**a**) The glutamate NMDA receptor as an example of a heteromeric ionotropic receptor. The NMDA receptor is a tetrameric complex formed by NR1 and NR2 subunits (only two subunits are shown) that bind glycine and glutamate, respectively[8]. (**b**) The $GABA_B$ receptor heteromeric receptor as an example of a heteromeric GPCR. $GABA_{B1}$ and $GABA_{B2}$ subunits establish coiled-coil interactions between their C termini[2]. (**c**) The A_{2A}-D_2 receptor heteromer. The C terminus of the adenosine A_{2A} receptor binds to the long third intracellular loop of the dopamine D_2 receptor[3]; whether this and other receptor heteromers are dimeric or higher order oligomeric species remains to be established. (**d**) The D_1-NMDA receptor heteromer, as an example of a receptor heteromer with a heteromeric receptor as one of the receptor units; the C termini of the NR1 and NR2 (NR2A) subunits bind to different epitopes of the C terminus of the dopamine D_1 receptor[14]. (**e**) The MT_1-GPR50 receptor, formed by the association of the melatonin MT_1 receptor and the orphan 7TM protein GPR50. TM domains are probably involved in the oligomerization, while the long C terminus of GPR50 is mostly involved in the modulation of MT_1 receptor function[15]. (**f**) The amylin (AMY) receptor, formed by the oligomerization of the calcitonin (CT) receptor and the single transmembrane protein RAMP; the two proteins bind through their N-terminal domains[16]. In **b**–**f**, two subunits are shown for schematic purposes, without ruling out multimerization.

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However, SKF83959 has a low affinity for the D_2 receptor, which signals through G_i proteins, thereby inhibiting adenylyl cyclase. Studies suggest that SKF83959 binds to both D_1 and D_2 receptors in the D_1-D_2 receptor heteromer, which selectively activates $G_{q/11}$ proteins and the phospholipase C cascade[27]. Thus, the presence of the same functional response to SKF83959 in brain tissue suggests that it depends on the existence of D_1-D_2 receptor heteromers in the brain. The regulation of receptor signaling efficacy has also been proposed to be affected by receptor heteromerization. For example, whereas the vasopressin V_2 receptor interacts stably with β-arrestin and undergoes a rapid endocytosis with little or no recycling upon vasopressin stimulation, the vasopressin V_{1a}-V_2 receptor heteromer only interacts transiently with β-arrestin and recycles quickly at the cell surface following endocytosis in response to vasopressin[28]. However, most of these studies have only been performed in transfected cells.

Future prospects: receptor heteromers and drug development

Receptor heteromers must be understood as dimeric or higher order molecular entities that are the result of combinatorial evolution and that are endowed with unique biochemical and functional properties that could be harnessed for therapeutic purposes. Consider adenosine A_{2A} receptor antagonists, for example, which are being evaluated as an adjuvant therapy to L-dopa or D_2 receptor agonists for Parkinson's disease, based on the evidence of allosteric interactions in the A_{2A}-D_2 receptor heteromers, which have been localized to a specific striatal neuronal population[1,3]. Another reason for considering receptor heteromers is their potential involvement in pathogenic processes. For instance (and also in the context of Parkinson's disease), preclinical studies support the possible involvement of D_1-D_3 receptor heteromers in the pathogenesis of L-dopa–induced dyskinesia[24].

Different approaches are being explored for the selective targeting of receptor heteromers. A current strategy is to screen for compounds that selectively target one of the receptors that constitute the receptor heteromer[22]. Another approach is to develop bivalent ligands that can interact simultaneously and specifically with both receptors in a receptor heteromer. In a recent study, an opioid μ receptor agonist–opioid δ receptor antagonist bivalent compound was developed by linking two moieties with a spacer[29]. Recent studies suggest that opioid δ-μ receptor heteromers modulate opioid μ receptor–mediated tolerance and dependence and that opioid δ-μ receptor bivalent ligands of precise spacer length exhibit a higher potency than morphine and the potential to achieve analgesia without tolerance and dependence[29]. However, such compounds, due to their large size, do not exhibit optimal drug-like properties[30], which could be overcome by using a combination of small molecules that selectively target each unit in the receptor heteromer.

In summary, we have laid out some specific recommendations on how to classify, identify and study the native properties of receptor heteromers. Following these recommendations will accelerate the discovery of additional functionally relevant receptor heteromers, which can then be evaluated as potential new targets for drug development.

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