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Receptor heteromerization and drug discovery

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Abstract

G-protein-coupled receptors (GPCRs) are membrane proteins that convert extracellular information into intra-cellular signals. They are involved in many biological processes and therefore represent powerful targets to modulate physiological and pathological states. Recent studies have demonstrated that GPCR activity is regulated by several mechanisms. Among these, protein–protein interactions (and in particular interactions with other receptors leading to heteromerization) has been shown to have an important role in modulating GPCR function. This has expanded their repertoire of signaling and added a new level of regulation to their physiological roles. Emerging studies provide evidence for tissue-specific and disease-specific receptor heteromerization. This suggests that heteromers represent novel drug targets for the identification of selective compounds with potentially fewer side-effects.

Introduction

G-protein-coupled receptors (GPCRs) are involved in most (if not all) physiological processes. As such, GPCRs represent important drug targets, accounting for 40–50% of current drugs on the market. Advances in the knowledge of GPCR biology suggests that, as a result of being expressed in more than one organ, a given GPCR is usually involved in more than one physiological process. As a consequence, drugs targeting one receptor type in a specific pathological context are likely to act on the same receptor type in other tissues that are not involved in the disease, leading to undesired effects of the drug. For instance, Mu opioid receptor (MOR) agonists are potent analgesic compounds, but also lead to constipation by acting on MOR in the myenteric plexus in the intestinal tract [1]. The type-1cannabinoid receptor (CB₁R) antagonist rimonabant was originally developed as an anti-obesity drug [2, 3]. However, rimonabant targets peripheral as well as central nervous system receptors, leading to side effects such as anxiety, mood changes and depressive disorders that prevent the therapeutic use of this compound [4,5].

In the last decade, several mechanisms that regulate the function and properties of GPCRs have been identified [6]. One such mechanism is crosstalk between distinct GPCRs. This often occurs as the result of protein–protein interaction between receptor types, or ‘heteromerization’ [7]. Heteromerization is emerging as an important process involved in the specialization of receptor function. Receptor heteromers exist in selected tissues and, in some instances, heteromerization has been reported to be regulated during pathological events [8]. These receptor complexes represent functional entities [9] that mediate distinct biological responses. These properties suggest that receptor heteromers can be used as novel drug targets because compounds that target specific heteromers are likely to have improved specificity and reduced undesirable effects.

In this review, we examine recent studies demonstrating that GPCR heteromerization alters the properties of individual receptors (i.e. modulation of receptor pharmacology by receptor–receptor association) [10–12]. We discuss examples of studies that demonstrate the contribution of receptor heteromers to the regulation of physiological processes, as well as studies that identify unique pathological states associated with alterations in receptor–complex formation. We also describe various current and emerging approaches and tools to study and target receptor heteromers.

GPCR heteromers: new receptor properties

An emerging concept in GPCR research is the promotion of heteromer-specific signaling cascades that enable the diversification of receptor-mediated effects in a context-dependent fashion (Figure 1).

Changes in receptor pharmacology

Modulation of the binding properties of GPCRs by heteromerization has been described for several receptor pairs [13–16]. This is thought to be the consequence of an alteration in the binding pockets, revealing that some ligands may prefer the receptors in their homomeric or heteromeric conformation. For example, a decrease in the affinity and potency of receptor-selective agonists was observed in the case of Delta-Kappa opioid receptors (DOR-KOR) [15], DOR-MOR [17] and somatostatin sst2A-sst3 receptors [18]. In contrast, an increase in the affinity of β 2-adrenergic receptor (AR) ligands was observed for β 1-AR- β 2-AR receptor pairs [19]. These studies indicate that heteromerization alters the ligand binding properties of the receptors, but the changes in receptor pharmacology do not seem to follow a common pattern because each receptor pair represents a unique alteration in receptor pharmacology. These changes may result from allosteric modulation of one protomer by ligand binding to the other protomer within the heteromer (positive or negative modulation depending on the receptor pair) [20].

Changes in receptor signaling

Studies have shown that receptor heteromerization leads to a change in G-protein coupling. In many cases, heteromerization leads to alterations in the extent of coupling to the same G-protein but recent findings show that, in some cases, there is a change in the nature of the coupled G-protein. A well-studied example of a change in the extent of G-protein coupling is the α 2a-AR-MOR heteromer: morphine binding inhibits norepinephrine-mediated signaling to $G_{\alpha i}$ and the downstream MAP kinase cascade [21]. Förster resonance energy transfer (FRET) analyses revealed a conformational change that propagates from one receptor to the other, causing the rapid inactivation of the second receptor with subsecond kinetics [22]. These findings suggest that heteromerization induces a change in receptor conformation that is responsible for the new properties of the receptors.

Heteromerization can also result in a shift in the type of the coupled G-protein.

Heteromerization between DOR and sensory neuron-specific receptor-4 was reported to lead to a switch from a $G_{\alpha i/o}$ - to a $G_{\alpha q}$ -mediated signaling pathway [23]. Heteromerization between D1-D2 dopamine receptors was found to lead to a switch from a $G_{\alpha s/olf}$ (D1R)- or $G_{\alpha i}$ (D2R)- to a $G_{\alpha q/11}$ -mediated pathway, and heteromerization between DOR and MOR was shown to lead to a switch from a $G_{\alpha i}$ - to a $G_{\alpha z}$ -mediated pathway [24–26].

Emerging studies also indicate that heteromer activation leads to a shift in the recruitment of distinct signaling molecules. In turn, this leads to specialized long-lasting downstream effects such as activation of distinct transcription factors and gene expression. Heteromerization of α 2a-AR with α 2c-AR reduces GRK-mediated receptor phosphorylation and β -arrestin

recruitment, affecting downstream Akt phosphorylation [27]. Heteromerization of MOR with DOR leads to a switch from a predominantly G-protein-mediated to a β -arrestin-mediated pathway, in turn resulting in differential activation of transcription factors [28]. The mechanism underlying the activation of new signaling cascades by heteromers is incompletely understood. One protomer may function as a scaffold to recruit signaling molecules that promote distinct signaling of the other protomer, as proposed in the case of $\alpha 2a$ -AR-MOR [21]. Such a mechanism has yet to be explored for other receptor pairs, but could represent a general means to diversify GPCR function.

GPCR heteromerization leads to qualitative as well as quantitative changes in coupling. This change can be from modulating the strength of an agonist-mediated response to switching to different G-protein coupling and/or associated signaling complexes. These novel properties highlight receptor heteromerization as a major mechanism that expands various GPCR-mediated effects, and contributes to the functional specification of receptors. This suggests that specific targeting of a receptor type within a heteromer could allow the activation of a discrete physiological pathway, with limited effects on the other processes involving the same receptor when it is not in a heteromeric complex.

GPCR heteromers in pathophysiology

In some cases, heteromers seem to constitutively exist in specific tissues where they participate to a given physiological process. In other cases, heteromers are disease-specific, making them attractive drug targets. Constitutive physiological heteromers have been reported to regulate cardiac contractility: angiotensin AT1 receptor (AT1R)- $\beta 2$ -AR heteromers regulate catecholamine-mediated control of heart rate [29]. $\beta 1$ -AR- $\beta 2$ -AR heteromers regulate sensitization to agonist stimulation and suppression of the spontaneous activity of $\beta 2$ -ARs in cardiac myocytes, optimizing β -adrenergic modulation of cardiac contractility [19]. Recent studies support a physiological role for post-synaptic cortical 5-HT2A-mGluR2 receptor heteromers because dysregulation of either component of the 5-HT2A-mGluR2 complex is thought to predispose schizophrenic patients to psychosis [30,31].

Disease-specific heteromers, through their novel signaling properties, can contribute to disease development. The best-described example is that of the AT1R-bradykinin B2 receptor (B2R) heteromer. The propensity of these two receptors to interact is controversial [32], but studies have demonstrated that upregulation of this receptor pair during pregnancy has a major role in Ang II-induced hypersensitivity in pre-eclampsia [8] and in the Ang II hyper-responsiveness of mesangial cells in experimental hypertension [33]. Another example is that of the prostaglandin EP1 receptor- $\beta 2$ -AR heteromer in airway smooth muscles; this heteromerization leads to reduced coupling of $\beta 2$ -AR to G α s, thereby reducing the bronchodilatory potential of the $\beta 2$ -AR agonist isoproterenol. This regulation of adrenergic signaling by heteromerization could represent an additional mechanism contributing to asthma [34]. Another heteromer that could exhibit unique disease-specific signaling is the apelin receptor (APJ)-AT1R heteromer; apelin has been shown to inhibit angiotensin-mediated development of atherosclerosis [35].

Taken together, these examples indicate that heteromers could represent new drug targets. If heteromers are disease-specific, these complexes are formed or upregulated in a specific tissue only during disease. Compounds acting at a receptor complex could modulate a specific function of a receptor in a particular tissue or physiological process. For example, targeting the $\beta 1$ -AR- $\beta 2$ -AR complex instead of individual adrenergic receptors is likely to specifically affect cardiomyocytes. Targeting a heteromer that exists only or is upregulated during a disease could increase drug specificity. For example, AT1R-B2R- or APJ-AT1R-selective compounds could be used to specifically target the angiotensin receptors involved in asthma and atherosclerosis, respectively, without affecting the homomeric receptors that do not play a part

in these diseases. Recent studies showing that a chaperone, RTP4, specifically increases the levels of DOR-MOR heteromers at the cell surface by protecting the complex from ubiquitination and degradation [36] suggest that factors regulating heteromer levels could serve as additional drug targets. Identification of proteins/factors involved in heteromer formation could open new avenues for the development of novel strategies to facilitate/prevent heteromer expression.

Targeting GPCR heteromers

Heteromer-specific ligands

As mentioned before, heteromerization is thought to lead to an alteration in ligand binding properties, suggesting that the altered binding pocket could accommodate compounds distinct from the corresponding protomer ligands. A proof of concept for such a drug is 6'-guanidinonaltrindole [37]. This acts as an opioid receptor agonist at the DOR-KOR heteromer but not at either homomer, suggesting that it represents a DOR-KOR heteromer-specific ligand. This drug has different effects according to administration site. Spinal (but not central) administration leads to analgesia, supporting the notion that selective tissue-specific targeting of GPCR heteromers can increase the local effects of drugs. Another example is that of SKF83959, a D1-like agonist [38] that reportedly binds the D1-D2 heteromer and activates the G α_q pathway [24] in the brain. These findings indicate that heteromer-specific ligands can also promote or block selected heteromer-mediated signaling pathways. This suggests that targeting heteromers allows for fine-tuned pharmacological intervention to modulate specific receptor function.

Bivalent ligands

Opioid receptor heteromers appear to play a part in the modulation of analgesic responses. Within DOR-MOR complexes isolated from spinalcord neurons [39], MOR responsiveness was increased by occupancy of the DOR-binding site by the DOR antagonist TIPPpsi, which was found to enhance morphine-induced analgesia [39], and to reduce development of tolerance to morphine [40]. This unique property of DOR-MOR heteromers suggests that targeting both receptors could yield increased analgesia while curbing the development of tolerance. MDAN-18, consisting of a MOR agonist (oxymorphone) and a DOR antagonist (NTI) tethered by a spacer, demonstrated improved anti-nociceptive activity compared with that achieved by co-administration of the two individual ligands [41], validating the bivalent ligand-based concept of targeting receptor heteromers.

Allosteric modulators

Compounds that work as allosteric modulators by acting at one receptor and causing an increased or decreased response at an associated receptor within a heteromer also represent potential new candidates to target heteromer-specific effects. Heteromerization of the adenosine 2A receptor (A2aR) and D2R leads to a reciprocal antagonistic effect between the two receptors [42]. As a consequence, A2aR antagonists potentiate the motor-activating effects of L-DOPA or D2 receptor agonists [43]; these represent promising allosteric modulators for the treatment of Parkinson's disease. As mentioned above, DOR antagonists found to enhance morphine-induced analgesia [39] act as allosteric modulators of the DOR-MOR heteromer, and potentiate the anti-nociceptive potential of endogenous opioid peptides or improve the action of exogenous MOR agonists such as morphine.

Future perspectives

Identification of receptor heteromers *in vivo* and their involvement in pathophysiological processes is critical for their recognition as novel drug targets. Only a few techniques allow

for the direct detection of GPCR complexes in native tissues (Figure 2). Fluorophore-coupled ligands have been developed [44,45] that could be used to carry out FRET analyses between interacting endogenous receptors. Antibodies specifically recognizing receptor heteromers [36,46] represent useful tools for the detection and analyses of GPCR complexes *in vivo*.

Elucidation of the role of a heteromer can be facilitated by the characterization and mutation of the residues or domains involved in receptor–receptor association. Several approaches, i.e. molecular modeling of receptor dimers [47,48], crosslinking, biophysical techniques [49] and transmembrane domain (TM) swapping [30] have been used to pinpoint such domains. These studies demonstrated the existence of multiple interaction interfaces, including TM1 and TM4 in the D2R homomer [49] and identified mGluR2 TM 4/5 as the dimerization interface with 5HT2aR in the 5HT2aR-mGluR2 complex [30]. Strategies aimed at preventing or disrupting receptor–receptor interaction using small molecules or peptides (such as a mGluR2 TM4 or mGluR2 TM5 homolog that would compete with the receptor for interacting with 5HT2aR) could yield alternative approaches to target heteromer activity.

Current and future challenges

Understanding the mechanisms governing GPCR heteromerization represent a outstanding challenge in all areas of biology, and is a prerequisite for the development of GPCR heteromer-targeted drugs. Design of simple and efficient high-throughput screening methods is hindered by the lack of basic knowledge about GPCR heteromer biology. For instance, the following questions must be answered:

- If two receptors that can interact are expressed in a cell to equal extents, will they preferentially form heteromers?
- How many receptors are present in an oligomer?
- Are receptors in a heteromer in a stoichiometric ratio? If not, in the context of a heterooligomer, what is the minimum proportion of one protomer required to change the other protomer's properties?
- Are there ways to 'force' receptors to associate that would increase the homogeneity (heteromeric form) of a sample?

Recent advances in GPCR research are starting to address the questions posed above. Studies analyzing receptor association using bioluminescence resonance energy transfer (BRET) determined the relative affinity of different receptors. The observation that $\beta 1$ -AR and $\beta 2$ -AR, or that DOR and KOR have the same propensity to form homo- or heteromers [50,51] suggest a binomial distribution of these receptors when they are co-expressed to the same extent. DOR or KOR have the same propensity to form homomers as compared with $\beta 2$ -AR-DOR, $\beta 2$ -AR-KOR or thyrotropin-releasing hormone receptor-KOR heteromers [51], suggesting that each receptor pair exhibits a distinct behavior. Other studies examining the multimeric nature of D2R provide evidence for an oligomeric rather than dimeric organization of GPCRs [49], in agreement with studies that estimate that the binding energy between only two protomers is too low to achieve stable interaction [52]. As mentioned before, emerging studies providing evidence for a chaperone-mediated increased formation of DOR-MOR heteromers [36] suggest that specific factors could facilitate heteromerization of specific receptor pairs. These and other recent studies help us to understand the basis of GPCR oligomerization, and are a prerequisite to efforts focusing on the development of screening of heteromer-based drugs.

Conclusion

Accumulating evidence showcases the critical role of GPCR heteromers in pathophysiological processes, and underscores the importance of these complexes as drug targets. Heteromers can

be targeted by three main approaches. To date, heteromer-specific ligands number only a few compounds, highlighting the technical and methodological difficulty of identifying and testing these molecules. However, they represent a conceptually exciting approach because of the inherent specificity and selectivity of such compounds for tissue-specific and disease-specific receptor pairs. Bivalent ligands, consisting of two connected molecules ligands, are usually large compounds with properties that are not 'drug-like' (not following Lipinski's rule of five [53]) and therefore are unlikely to be suitable in clinical settings. However, this approach is straightforward and has the advantage of using GPCR ligands that have already been identified and characterized. Allosteric modulation of one protomer by a ligand of the other protomer is an exciting approach, in particular those that target the allosteric site of the interacting partner. Identification of specific heteromers that are upregulated during pathological events and/or a heteromer-specific chaperone is likely to lead to novel strategies for pharmacological intervention.

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Figure 1. Modulation of receptor function by heteromerization (schematic)

(a) Heteromerization leads to the formation of an altered ligand binding site. Shown for DOR-KOR [15,37], DOR-MOR [14,17] and MOR-sst2A [54].

(b) Heteromerization leads to a decrease or increase in coupling to the G-protein. Decreased coupling was shown for the DOR-MOR heteromer [14]; increased coupling was shown for $\alpha 2$ -AR-MOR [21], and AT1R-B2R [13].

(c) Heteromerization leads to coupling to a new G-protein. Shown for MOR-DOR heteromer coupling to $G_{\alpha z}$ [26,55]; heteromerization of two differently coupled receptors leads to preferential coupling to one G-protein upon co-stimulation. Shown for DOR-SNSR-4 [23].

(d) Heteromerization leads to a switch in receptor coupling, from G-protein to β -arrestin, and subsequent differential signaling and transcription factor activation. Shown for the MOR-DOR heteromer [28].

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Figure 2. Emerging techniques to detect (a–c) and modulate (d–i) heteromers

GPCR heteromers can be detected using three main approaches.

(a) Fluorophore-coupled ligand-mediated FRET. Fluorophore-coupled ligands for two receptors can be analyzed using FRET only if their two cognate receptors are in close proximity in a heteromeric complex.

(b) Heteromer-selective antibodies that recognize only heteromers can be used to study the localization and levels of heteromers.

(c) Heteromer-specific ligands can bind only to the altered receptor binding pocket formed in a heteromer.

GPCR heteromers can be modulated using six main approaches.

- (d) Heteromer-specific agonists can activate a receptor only if in a heteromeric form.
- (e) Heteromer-specific antagonists can block a receptor only if in a heteromeric form.
- (f) Heteromer-specific antibodies can block receptor binding and/or signaling only if in a heteromeric form.
- (g) Activating heteromer-specific antibodies can activate receptors only if in a heteromeric form.
- (h) Bivalent ligands can bind and activate receptors only if their cognate receptors are in a heteromer.
- (i) The ligand of one receptor can act as a negative or positive allosteric modulator of the interacting receptor in a heteromer. The allosteric modulator can alter the binding and/or signaling properties of the interacting receptor.