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ROLE OF ANTIBODIES IN DEVELOPING DRUGS THAT TARGET G PROTEIN-COUPLED RECEPTOR DIMERS

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Abstract

G protein-coupled receptors (GPCRs) are important molecular targets in drug discovery. These receptors play a pivotal role in physiological signaling pathways and are targeted by nearly 50% of currently available drugs. Mounting evidence suggests that GPCRs form dimers and various studies have shown that dimerization is necessary for receptor maturation, signaling and trafficking. However, the physiological implications of dimerization *in vivo* have not been well explored since detection of GPCR dimers in endogenous systems has been a challenging task. One exciting new approach to this challenge is the generation of antibodies against specific GPCR dimers. Such antibodies could be used as tools for characterization of heteromer-specific function, as reagents for their purification, tissue localization and regulation *in vivo* and as probes for mapping their functional domains. In addition, such antibodies could serve as alternative ligands for GPCR heteromers. Thus, heteromer-specific antibodies represent novel tools for the exploration and manipulation of GPCR dimer pharmacology.

Keywords

G protein-coupled receptors; GPCRs; dimers; heteromers; antibodies

G protein-coupled receptors (GPCRs) are a family of integral membrane proteins located on the cell surface, whose activation induces second messenger amplification. These receptors are drug targets in a wide range of major diseases such as asthma, cancer, diabetes, hypertension, obesity, chronic pain, and other neurological disorders.

[Call-out] Located on the cell surface, G protein-coupled receptors (GPCRs) are a family of integral membrane proteins that are drug targets in a wide range of major diseases such as asthma, cancer, diabetes, hypertension, obesity, chronic pain, and other neurological disorders.

For a number of years it was thought that individual GPCRs functioned as a monomeric unit. However, in recent years several studies showed that GPCRs can form dimers such as homo- and heteromers as well as more complex oligomers (1–3). There is also growing evidence suggesting that dimerization substantially modulates receptor function (4). Studies have shown that receptor heteromers have important roles in receptor maturation, signaling and trafficking (5–8). Most of these studies were carried out in heterologous cell systems over-expressing different epitope tagged GPCRs using a variety of assays including ligand binding, coimmunoprecipitation, (9–10), Bioluminescence Resonance Energy Transfer (BRET) (11–13) or Fluorescence Resonance Energy Transfer (FRET) (14–16). However, detection and characterization of specific GPCR dimers in an endogenous system has been

challenging due to the lack of selective tools. We propose the generation of GPCR dimer-specific antibodies that could be used as tools for the characterization of heteromer pharmacology and signaling, and as reagents for the study of their *in vivo* localization and regulation. In addition, such antibodies could be developed as alternative highly selective ligands targeting GPCR heteromers (Figure 1); the functional and physiological diversity of GPCR dimers provides the necessary targets for enabling novel drug discovery.

In this review, we focus our attention on the potential implications of antibodies in the development of selective reagents targeting GPCR dimers.

G PROTEIN-COUPLED RECEPTOR DIMERIZATION AND ALLOSTERISM

It is now well established that GPCRs exist and function as dimers/oligomers. Interactions between identical protomers (a single GPCR) are referred to as homomers and interactions between nonidentical protomers are referred to as heteromers. Additionally, there is growing evidence that heteromerization can generate receptors with novel characteristics, leading to altered pharmacological properties (1,17–18).

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The most convincing evidence for receptor heteromerization has come from studies with metabotropic GABA_B receptors in heterologous systems where the functional receptor requires the co-expression of both the GABA_BR1 and GABA_BR2 subunits (19). In the absence of GABA_BR2, the GABA_BR1 subunit is retained in the endoplasmic reticulum while in the absence of GABA_BR1, the GABA_BR2 subunit is expressed at the cell surface but is non-functional; only the co-expression of the two subunits leads to plasma membrane expression of a functional receptor. Heteromerization leads to the masking of an ER retention sequence in GABA_BR1 by GABA_BR2, allowing the heterodimers to be efficiently expressed at the cell surface (20,21). This kind of association is found to occur very shortly after biosynthesis, meaning that dimerization could be key to receptor maturation in the case of this and other GPCRs (7,21–22).

The concept that GPCR heteromerization could have a role in pharmacological diversity was first observed in studies on the δ - and κ -opioid receptors (17). These studies show that the δ - κ complex exhibits ligand binding, functional and trafficking properties that are distinct from those of each individual receptor (17). Studies also show that μ and δ receptors, once thought to only function individually, can dimerize and form a complex exhibiting distinct functional properties (23) that have profound effects on morphine mediated analgesia. It is probable that additional pairings involving the μ -opioid receptor and other GPCRs could play crucial roles in regulating analgesia and addiction (24). In another compelling case, the mammalian ability to perceive sweet tastes depends on the heteromerization of T1R2 and T1R3 taste receptors whereas the umami taste relies on a T1R1/T1R3 dimer, indicating that heterodimerization between different subtypes of the taste receptor leads to modulation of ligand binding properties resulting in the recognition of very distinctive tastes (25). Studies also show that receptors thought to be normally involved in completely different physiological systems may form heterodimers (26) thus allowing for a new level of specificity for drug targeting.

In some cases, GPCR dimerization/heterodimerization is associated with changes in ligand binding affinity for the complex compared to the affinity for the homomer implying heteromer-mediated modulation of the ligand binding pocket. Several studies have shown positive or negative co-operativity in ligand binding following receptor co-expression likely resulting from receptor heterodimerization. Examples include GPCR dimer pairs such as muscarinic m2/m3 (27), somatostatin SSTR5/dopamine D2 (15), adenosine A2A/dopamine D1 (28) and the opioid δ/μ (23,29) receptors. These results suggest that heteromerization between pharmacologically different receptors provides a level of diversity that could generate new opportunities for the development of more selective compounds that would target specific heterodimers without affecting the activity of individual protomers.

In an attempt to develop drugs that target GPCR heterodimers bivalent ligands have been developed (30). Bivalent ligands are composed of two functional pharmacophores linked by a spacer that bind to the orthosteric sites of the protomers within a heteromer. In principle, bivalent ligands would preferentially bind to the heteromer, the latter is likely to be selectively expressed only in tissues co-expressing both of the interacting receptors, and this would lead to improved drug targeting and tissue selectivity. Some bivalent ligands were found to be effective in increasing the specificity of drug effects i.e. increased analgesia with a reduction in side-effects such as development of tolerance suggesting that bivalent ligands could be developed as potential novel analgesics (31–32). A set of such ligands targeted both μ and δ receptors and consisted of a μ agonist pharmacophore (oxymorphone) and a δ antagonist pharmacophore (NTI) separated by spacers of variable lengths (33). The antinociceptive activity of these bivalent ligands was reported to be better than that achieved by co-administration of the individual pharmacophores and could be titrated by manipulating the distance between the pharmacophores (33). Another group of bivalent ligands targeting δ - κ heteromers were recently synthesized consisting of δ and κ antagonists (NTI and 5'-GNTI, respectively) linked by variable length spacers (34). One these compounds (KDN-21) exhibited a much higher affinity and selectivity for δ - κ heterodimers relative to δ or κ receptors alone (35). KDN-21 was found to have antinociceptive activity when administered directly into the spinal cord, but not into the brain supporting the notion that the relative levels of a heterodimer vary in different regions and that selective targeting a heterodimer could increase the specificity of a drug with probable reduction of associated side-effects. Taken together, these studies show that bivalent ligands to opioid receptors could be developed as novel analgesics with greater tissue selectivity and lesser degree of side-effects normally associated with chronic opiate administration.

The possibility of allosterically modulating the function of individual protomers within a GPCR dimer makes the heteromer a new type of drug target. Classically, the majority of drugs target the receptor's orthosteric site that is often the binding site for the endogenous agonist. Recent studies have begun to explore the possibility of targeting allosteric sites and have identified synthetic allosteric modulators (activator or inhibitors of GPCRs) (36,37). These alternate sites can bind small molecule ligands leading to the modulation of agonist binding to the orthosteric site. In the case of a GPCR dimer, a ligand bound to the orthosteric site of one protomer could function as an allosteric modulator of the other protomer within the pair, which could potentially alter the conformation and functional outcome of either protomer, receptor heteromer and/or the whole complex. The design of such drugs would help us understand the unique pharmacology and signaling mechanisms activated by GPCR dimers given the mounting evidence suggesting that every GPCR dimer displays unique functional properties with different pharmacological characteristics (15,27–29). It is likely that heteromerization produces a unique binding pocket that binds ligands with distinct affinity and display unique ligand structure-activity relationships compared to receptor homomers. Thus it would be advantageous to begin to find smaller, drug-like synthetic compounds that would bind to allosteric sites in either protomer and alter the conformation

of a dimer's orthosteric binding pocket. These smaller, single-target drugs might also be made to target specific sites in the novel binding site created in the pocket between the two interacting receptor proteins. In this context, dimer-selective antibodies would be useful agents for the exploration of dimerization within *in vitro* as well as *in vivo* systems.

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For example, it has been reported that preeclamptic hypertensive women exhibit a significant increase in heteromers between the AT₁ angiotensin receptor (that responds to the vasopressor angiotensin II), and the B₂ bradykinin receptor (that responds to the vasodepressor bradykinin). AT₁ and B₂ bradykinin receptors normally regulate blood pressure by controlling vasoconstriction and smooth muscle relaxation, respectively. During preeclampsia the dimeric AT₁-B₂ receptor complex becomes hypersensitive to angiotensin II and exhibits reduced affinity for the B₂ receptor agonist, bradykinin (38). This example of *in vivo* alteration of GPCR pharmacology by dimerization indicates that dimers could be useful for controlling blood pressure. Disease-specific GPCR dimers are also thought to play roles in the regulation of cardiac muscle cell function, asthma, schizophrenia, drug-related analgesia and tolerance, and other pathologies (23,39–42). However, the lack of suitable tools to study the distribution and regulation of heteromers *in vivo* has made such studies difficult. Recent advances in antibody technology have begun to help address some of these difficult questions; these are described below.

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ANTIBODIES AS G PROTEIN-COUPLED RECEPTOR DRUG TARGETS

Antibodies have been used as tools for receptor characterization, purification, localization and as probes for mapping their functional domains. Antibodies are now becoming integral tools in drug research and are even being developed as drugs. Their unique design makes them especially suited for attaining a high level of specificity for a large variety of organic, pharmacologically significant molecules and epitopes on larger molecules. In particular, monoclonal antibodies which by definition recognize a single epitope are quite useful in these applications, unlike polyclonal antibodies which target multiple epitopes.

Antibodies are excellent diagnostic screening tools as they can detect the domains involved in activity-mediated conformational changes of signaling proteins, including receptors (43–51).

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For example, a monoclonal antibody to the N-terminal region of rhodopsin exhibited a higher degree of recognition for activated receptors than for inactivated receptors even after detergent treatment, suggesting that photoactivation of rhodopsin induces a conformational change at the N-terminus that exposes an epitope that is recognized by the monoclonal antibody (52). Recently, Gupta et al. (53,54) showed that antibodies targeting the N-termini

of family A GPCR homomers can discriminate between activation states of the receptors. Since the extent to which a certain antibody binds to a receptor can depend on whether the latter is activated by a functional ligand, an assay using antibodies could be used to effectively screen for novel GPCR ligands. This method recently led to the identification of hemopressin as an interesting new peptide antagonist of the CB1 cannabinoid receptor (55). In addition, it will be important to develop heteromer-specific antibodies, given the critical role of receptor heteromers in certain cell signaling and disease processes.

Several studies have shown that antibodies directed against GPCRs can act as allosteric receptor agonists or antagonists (56–59).

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In the case of δ opioid receptor, an antibody directed to the N-terminal region behaves like a classic agonist and activates the receptor (58). For the β -adrenergic receptor, a monoclonal antibody to the second extracellular loop had agonist-like activity, while a monovalent (Fab) fragment acted as an antagonist (59). Antibodies are usually very effective when they target the extracellular N- and C-termini or the third intracellular loop of GPCRs (53,60). Because these regions are highly diverse, the problem of antibodies binding to similarly structured molecules, which plagues synthetic drugs, is practically eliminated.

Antibodies can also be useful in examining the conformational changes of a particular protomer that arise upon binding of a ligand to the partner protomer (57).

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For example, an antibody binding to an allosteric site on one protomer could differentially detect whether one or both protomers are bound by selective orthosteric/allosteric ligands (57). This could be used as a screening strategy for the detection of novel allosteric ligands targeting heteromers. Allosteric drugs could be useful because they leave the main active site free, only modulating its behavior. The side-effects associated with modern orthosteric drugs are often due to the replacement of the receptor's endogenous ligand, altering homeostasis far more than is necessary. Thus, using antibodies as allosteric drugs could circumvent this problem.

Antibodies, including humanized antibodies, are currently being used to detect receptors, their activity states, and their location and density in the treatment of immune system diseases and cancers (61).

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As already mentioned, antibodies can act as functional modulators, direct agonists, inverse agonists and antagonists. In addition, antibodies can be designed to carry marker molecules that bind to a selective secondary drug, or they may simply be designed to carry a toxin, for example, to kill target cancer cells (62). This way, only cells expressing certain receptors or specific receptor dimers would be targeted and altered or destroyed. Currently, 22 monoclonal antibody drugs have been approved by the FDA for therapeutic use, while hundreds more are undergoing clinical trials. Other than vaccines, antibodies are the fastest growing drug category and have the potential to bring under control a vast array of disorders (62).

CONCLUSION

Mounting evidence suggests that GPCR dimerization is an important physiological method of restoring equilibrium. It is easy to see that dimer regulation is uniquely useful in the body and that understanding dimer pharmacology could lead to the development of far more selective drugs and to more effective treatments for diseases. Thus, antibodies against G-protein-coupled receptor dimers could be useful tools in detection of receptor dimers, in assessment of dimer pharmacology, in screening during drug development. In the near future, antibodies could be generated and optimized to serve as drugs by directly targeting tissues that exhibit altered levels of GPCR dimers and selectively block or activate heteromer function.

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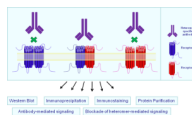


Figure 1. A monoclonal antibody can be generated that specifically recognizes receptor heteromers, but not homomers. It can be used as a tool to detect receptor heteromers in vivo and to characterize heteromer-specific signaling.