Published in final edited form as:

Comb Chem High Throughput Screen. 2008 July ; 11(6): 463–467.

Antibodies against G-protein coupled receptors: novel uses in screening and drug development

Achla Gupta, Andrea S. Heimann¹, Ivone Gomes, and Lakshmi A. Devi[@]

Department of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, One Gustave L. Levy Place, New York, NY 10029

¹ Proteimax Biotecnologia Ltda., Via das Margaridas 413, Cotia, São Paulo, Brazil

Abstract

Antibodies are components of the body's humoral immune system that are generated in response to foreign pathogens. Modern biomedical research has employed these very specific and efficient molecules designed by nature in the diagnosis of diseases, localization of gene products as well as in the rapid screening of targets for drug discovery and testing. In addition, the introduction of antibodies with fluorescent or enzymatic tags has significantly contributed to advances in imaging and microarray technology, which are revolutionizing disease research and the search for effective therapeutics. More recently antibodies have been used in the isolation of dimeric G protein-coupled receptor (GPCR) complexes. In this review we discuss antibodies as powerful research tools for studying GPCRs, and their potential to be developed as drugs themselves.

Antibodies have classically been used as analytical tools in the identification, localization and quantification of different antigens including hormones and pathogens. During the last 10 years they have also been used as therapeutic agents in experimental and clinical medicine. Antibodies provide significant advantages over conventional chemical drug-based therapies such as selectivity, potency and efficacy at various targets making them more effective in the treatment of a variety of conditions including cancer and immune disorders [1–3]. In addition antibodies have been shown to directly affect signaling pathways within the targeted cells by acting on their intracellular targets [4–7] or by binding to cell surface proteins [8].

GPCRs comprise the largest family of cell-surface molecules involved in signal transduction. They are involved in key physiological processes ranging from cell cycle control to metabolism and hormone action [9–12]. GPCR dysfunction has been implicated in a variety of pathological conditions including cancer and cardiovascular, metabolic, neurodegenerative & psychiatric disorders [13–15]. Today, GPCRs are the main focus for the development of drugs using novel approaches and comprise more than 50% of current drug targets [16]. Antibodies to GPCRs could be used as tools for receptor characterization, as reagents for their purification, tissue localization and as probes for mapping their functional domains.

Attempts to raise high affinity antibodies to GPCRs have been restricted by the limited availability of highly purified GPCR preparations, low titers due to high degree of receptor glycosylation or immunological tolerance because of a high degree of sequence homology that results in cross-reactivity to closely related receptors and to receptor subtypes. A more

[@]Corresponding Author: Lakshmi A. Devi, Ph.D., Department of Pharmacology and Systems Therapeutics, Mount Sinai School of Medicine, 19-84 Annenberg Building, One Gustave L. Levy Place, New York, NY 10029, Phone: (212) 241-8345, Fax: (212) 996-7214, lakshmi.devi@mssm.edu.

successful strategy has involved the generation of antibodies to short peptide sequences present in either the N- or C- terminal regions or the third intracellular loop since these regions exhibit a great degree of sequence diversity even among receptor subtypes. The antigenicity of these peptides is increased by coupling them to carrier proteins such as keyhole limpet hemocyanin or bovine serum albumin [17] or by synthesizing them in the form of multiple antigenic peptides (8–10 peptide sequence repeats) on an inert poly-L-lysine backbone [18]. The latter strategy was recently used to generate monoclonal antibodies against the N-terminal region of a variety of family A GPCRs including μ , δ , & κ opioid receptors [19,20]. These antibodies are highly receptor subtype selective and can recognize recombinant as well as native receptors in tissues as examined using enzyme-linked immunosorbent assay (ELISA), Western blotting or immunoprecipitation [19, 21]. These results support the notion that high affinity and highly selective anti-GPCR antibodies can be generated through a careful selection of an unique peptide sequence in its extracellular region and screening for hybridoma clones secreting high titer antibodies.

GPCR ANTIBODIES TO DETECT RECEPTOR ACTIVITY STATES

A remarkable effort has been put towards understanding the mechanism of activation of GPCRs at a molecular level as this would allow the design of new drugs that are effective in the treatment of diseases and exhibit fewer side effects. Because of the difficulties in obtaining large quantities of high quality receptor crystals only one GPCR, bovine rhodopsin, has been crystallized to date [22,23]. It is possible that monoclonal antibodies could aid in receptor crystallization by binding to a conformation-sensitive epitope [24]; Mancina *et al.*, [25] have recently reported the generation of conformationally sensitive antibodies to the 5HT_{2c} receptor with the intention of using them to facilitate the crystallization of this receptor. Antibodies have also been used to probe the mechanism of receptor activation. The use of antibodies to various portions of the receptor has helped in delineating the domains involved in activity-mediated conformational changes [26,27]. For example, a monoclonal antibody to the N-terminus of rhodopsin exhibited a higher degree of recognition for activated receptors than for inactive receptors. This suggests that photo-activation of rhodopsin induces a conformational change at the N-terminal region that exposes an epitope that is recognized by the monoclonal antibody [28,29]. Studies using monoclonal antibodies to peptide sequences in the carboxyl terminal, transmembrane loops and amino terminal regions of rhodopsin further support light-dependent conformational changes of this receptor [30]. A panel of monoclonal antibodies to different epitopes in the extracellular region was used to show that the chemokine CCR5 receptor exists in multiple conformational states [31]. Recently, we used antibodies to the N-terminal region of several GPCRs to show activity-mediated conformational changes. First, we targeted two different regions in the N-terminus of μ opioid receptors and showed that antibodies to the region proximal to the N-terminus exhibit enhanced recognition of activated receptors whereas antibodies to a region proximal to TM1 do not suggesting a local change in the conformation of the receptor upon activation [20]. This strategy of targeting the N-terminal region to generate conformation-sensitive antibodies was then applied to a number of family A GPCRs (irrespective of their coupling to G_{ai} , G_{as} or G_{aq}) [20]. We found that antibodies to the N-terminal region of α_2A and β_2 adrenergic receptors, CB₁ cannabinoid receptors and AT₁ angiotensin receptors were highly selective and exhibited enhanced recognition of the agonist-treated receptors [20]. This enhanced recognition was abrogated upon treatment with agents that uncouple G-proteins (such as GTP γ S, GPP(NH)p, AlF₃, NaF) indicating that the formation of a ternary agonist-receptor-G-protein complex was required to trigger the conformation recognized by these antibodies [20]. We also found that deglycosylation of the receptors abolished the increased recognition by the antibodies suggesting that glycosylated side-chains play a role in this recognition [20]. Studies have shown that deglycosylated receptors generally exhibit pharmacological profiles that are comparable to that of

glycosylated receptors since removal of oligosaccharide side-chains had no significant effect on the ligand binding pocket [32]. Thus it appears that these antibodies recognize a post-activation mediated conformational change. These conformation-sensitive antibodies were also used to investigate the extent and duration of activation of endogenous receptors. For example, we found that systemic drug administration leads to a time dependent increase in antibody binding to the receptors in the brain peaking at 30 min [20]. This shows that conformation sensitive antibodies can be used to probe the spatio-temporal dynamics of native receptors in the brain.

This is in contrast to bioluminescence resonance energy transfer (BRET) and fluorescence resonance energy transfer (FRET) techniques that have also been used to measure protein conformational changes in recombinant systems [33, 34]. This is because the use of BRET or FRET to investigate changes in conformation in GPCRs require that (i) the receptors be genetically engineered so as to contain a yellow fluorescent protein (YFP) tag and a luciferase tag (in the case of BRET) or a fluorescent donor such as cyan fluorescent protein (CFP) (in the case of FRET) and (ii) that these genetically engineered receptors be expressed in an heterologous expression system such as HEK-293 cells. The change in BRET or FRET signal upon treatment with ligand is then interpreted as a change in receptor conformation (although it is not possible to assess the contribution of the receptor tags themselves to these changes). In contrast to these methods, the use of conformational sensitive antibodies allows the detection of conformational changes in endogenous receptors in their native environment using a relatively inexpensive ELISA assay. Therefore antibodies that are sensitive to conformational changes are useful tools to probe the conformational state of native GPCRs.

We also examined if these antibodies can be used to characterize receptor-type specific ligands using a panel of compounds that represented full agonists, partial agonists and antagonists; these included peptides, peptidomimetic ligands, alkaloid and synthetic compounds. We found that the antibodies could accurately differentiate ligands with varying efficacies; for example, an antagonist did not affect the recognition by the antibody. Furthermore, a partial agonist with lower efficacy exhibited a lower level of recognition compared to a highly potent full agonist [20]. Taken together, these results suggest that these N-terminally directed antibodies to family A GPCRs can be used to characterize receptor selective ligands.

GPCR ANTIBODIES IN DRUG SCREENING

In recent years the pharmaceutical industry has employed cell-based assays in high-throughput screening (HTS) for drug discovery [35,36]. The ease with which a simple and rapid screening assay could be developed using conformation-sensitive antibodies makes them ideally suited for drug screening. As described above we used the ELISA assay to screen for a series of μ opioid receptor ligands that ranged from endogenous peptides to synthetic peptidic and non-peptidic agonists and antagonists [20]. There is a significant correlation between the extent of antibody recognition and the efficacies of the ligands indicating that these conformation-sensitive antibodies can be used to rapidly screen for receptor agonists or antagonists and novel molecules of therapeutic interest.

The specificity of these antibodies and their ability to recognize activity mediated changes in the conformation of the partner protomer (in the context of a heterodimer) makes them suitable for screening for allosteric modulators of GPCR heterodimers or for regulators of GPCRs. To test this idea, we targeted opioid-cannabinoid heterodimers; previously we had reported that μ and δ opioid receptors dimerize with CB₁ cannabinoid receptors and heterodimerization leads to a modulation of receptor function [37,38]. In order to see if occupancy of CB₁ receptors would affect the conformation of μ receptors that could be

detected by our antibodies, we used anti- μ opioid receptor antibodies and monitored the effect of CB₁ cannabinoid receptor agonists on the μ agonist mediated changes in recognition (Fig. 1). We find that the agonist (DAMGO)-mediated increase in the recognition of μ receptors was further enhanced by treatment with the CB₁ receptor agonists (Hu-210 and Win 55,212-2). Interestingly, Hu-210, a high efficacy agonist, potentiated DAMGO-mediated recognition at a lower concentration (10 nM) as compared to WIN55,212-2, a lower efficacy agonist, that potentiated antibody recognition at a substantially higher concentration (500 nM) (Fig. 1). In addition we find that this Hu-210 mediated potentiation of μ antibody recognition of DAMGO-treated receptors is biphasic in that the potentiation is seen with lower, but not at higher, doses of Hu-210. It is possible that a low dose of HU-210 induces a conformational change that is transmitted to the partner protomer (i.e. μ opioid receptor) leading to an increased exposure of the epitope recognized by the μ antibody. Higher doses of Hu-210 induce distinct conformational changes (different from those induced by lower doses of HU-201) that might not be transmitted to the partner protomer. The conformation change induced by low doses of HU-210 could be similar to that induced by high doses of WIN55,212-2 since both cases lead to changes in the conformation of the partner protomer. Taken together, these results support the idea that agonist-mediated changes in the conformation of a receptor can be transmitted to the partner receptor (within a heterodimer) and this can be probed using conformation-sensitive antibodies. A strategy based on this idea could be used to develop assays to examine protein-protein interactions that modulate GPCR function (including heterodimeric interactions). In addition, a screening assay could be developed to target the identification of allosteric modulators of a GPCR targeting the two protomers within the heterodimer.

GPCR ANTIBODIES CAN FUNCTION AS RECEPTOR AGONISTS/ANTAGONISTS

Although antibodies are widely used as probes for studying membrane proteins and receptors, their use as pharmacological agents has not been extensively explored. Since antibodies can be highly selective, they are ideal tools to serve as specific pharmacological agents. Monoclonal antibodies are more suitable candidates for pharmacological evaluation since they recognize a specific antigenic region (i.e. epitope) whereas polyclonal antibodies bind to more than one antigenic region (i.e. multiple epitopes). Furthermore, in some cases monoclonal antibodies have been shown to bind to an epitope in the ligand binding pocket of the receptor and thus function as agonists (inducing functional responses) or antagonists (blocking agonist-induced signaling). In the case of the δ opioid receptor, a monoclonal antibody to the N-terminal region was found to mimic the effects of δ receptor agonists [39]. Also like δ opioid receptor agonists, long-term treatment with these antibodies led to a compensatory increase in cAMP levels [39]. Similarly, a monoclonal antibody to the second extracellular loop of the human β_2 -adrenergic receptor was found to have an agonist-like effect on the spontaneous beating rate of neonatal rat cardiomyocytes [40]. This antibody was also able to activate L-type Ca²⁺ channels via β_2 -receptors through a cAMP dependent pathway. These agonist-like effects of the antibody were receptor specific since they could be blocked by a β_2 -receptor antagonist [40]. Similar agonist-like effects were observed with antibodies raised to peptides corresponding to the second extracellular loop of β_1 -adrenergic receptors [41], M₂ muscarinic receptors [42] and bradykinin B2 receptors [43]. Finally, autoantibodies to β_1 -adrenergic receptors (isolated from patients with idiopathic dilated cardiomyopathy) and to M2 muscarinic receptors (isolated from patients with Chagas' disease) were found to function as either agonists or partial agonists (44,45), thereby suggesting that the epitopes targeted by these antibodies are part of the agonist binding pocket or play a role in receptor activation and that they could be used as highly specific agents to target these receptors.

Antibodies have also been found to behave as antagonists/inverse agonists. A monoclonal antibody raised against a complex epitope of the thyrotropin receptor was found to exhibit an inverse agonist-like activity [46]. Similarly, polyclonal antibodies to the N-terminal region of the melanocortin-4-receptor [47] or to the second extracellular loop of 5-hydroxytryptamine (5-HT₄) receptors [48] were found to function as inverse agonists. Interestingly, antibodies to 5-HT₄ receptors were found to enhance agonist-mediated signaling at low (5 pM) agonist concentrations [48] suggesting that they could be a useful tool to sense the different conformations stabilized by ligands. In the case of β_2 receptors monomeric Fab fragments exhibited antagonist-like properties whereas the bivalent antibody exhibited agonist-like properties [49]. This suggested that the antibody functions as an agonist in the bivalent state and as an antagonist in the monovalent state. Finally, antibodies exhibiting both agonist- and antagonist-like behavior based on the functional assay have also been reported. In the case of type 1 sphingosine 1-phosphate receptor, the antibody was found to block T cell chemotactic responses to sphingosine 1-phosphate (i.e. an antagonist response) and to decrease the generation of γ -interferon in a manner similar to sphingosine 1-phosphate (i.e. an agonist-like response) [50]. These results suggest that the binding of the antibody puts the receptor in a conformation that allows coupling to one set of signaling molecules while blocking coupling to the other. These results further support the notion that antibodies are able to recognize and/or induce distinct conformations of the receptor enabling specific modulation of receptor function.

In an effort to generate antibodies that mimic highly specific ligands, the “anti-idiotypic” antibody approach has been used. In this case antibodies are first generated to the receptor ligand (either agonist or antagonist). These antibodies, called “idiotypic” antibodies, that are thought to mirror the ligand binding epitope in the receptor, are then used to generate another set of antibodies (anti-idiotypic antibodies) which should function in a manner similar to the immunogenic agonist/antagonist. Anti-idiotypic antibodies have been raised to a number of GPCRs including β -adrenergic receptors [51,52], platelet activating factor receptor [53] and opioid receptors [54] among others. In general it was observed that the anti-idiotypic receptor antibodies exhibited functional activities similar to those of the selective agonist/antagonist that were used as the initial immunogen. Interestingly, anti-idiotypic antibodies to the β -adrenergic receptor where the receptor antagonist, alprenolol, was the immunizing ligand exhibited agonistic activity [51]. Reciprocally, anti-idiotypic antibodies to opioid receptors where the agonist, β -endorphin, was the immunizing ligand functioned as a receptor antagonist [54]. These studies suggest that the anti-idiotypic antibodies probably mimic a conformation that is common to both agonists and antagonists: the receptor binding activity. The fact that antibodies against GPCRs can be generated so as to function either as agonists, inverse agonists or antagonists suggests that they represent valuable tools for studying the expression and localization of GPCRs and that they can be used to develop highly selective therapeutic agents for the treatment of variety of disorders.

GPCR ANTIBODIES AS DRUGS

The specificity of antibodies makes them ideally suited for use as drugs themselves. Over the past decade antibodies have been developed into effective therapeutic agents with broad applications in cancer, inflammation and infectious diseases. To date, there are more than a dozen unconjugated monoclonal antibodies approved for clinical use and more than a hundred monoclonal antibodies in different stages of clinical development or under regulatory review [for review, see 55]. Advances in recombinant DNA technology have enabled the production of recombinant proteins and the generation of partially or fully “humanized” monoclonal antibodies [56–62]. This has resulted in lowering allergic reactions due to hypersensitivity as well as reducing the costs involved in antibody generation. Monoclonal antibodies to GPCRs could be used in three main therapeutic

approaches, (i) for blocking the increased constitutive activity of receptors involved in a given pathological condition, (ii) for inducing the activation of the targeted GPCR, and (iii) for selectively delivering drugs/molecules to cells expressing the GPCR (targeted by the antibody). A case in point is with antibodies to the thyrotropin receptor [46, 63–65]. Monoclonal antibodies that function as inverse agonists of thyrotropin receptors have been generated with the intent of treating hyperthyroidism where the thyrotropin receptor exhibits higher than normal constitutive activity [46]. Alternatively, monoclonal antibodies that function as agonists of the thyrotrophin receptor have been generated that could be used in the treatment of hypothyroidism [63–65]. Finally, monoclonal antibodies to thyrotropin receptors that are designed to carry a payload of radioactive or cytotoxic material could be developed in order to treat thyroid carcinomas [66, 67]. Based on these ideas, it is conceivable that a new combination therapy could be developed that involves a small molecule drug exhibiting agonist-activity mixed with the conformation-selective antibody that recognizes the activated state of the receptor. Such a combination therapy would have the advantage of conferring high specificity in addition to potentially lowering the dose of the administered drug.

In conclusion antibodies, one of the “weapons” devised by the immune system to fight diseases, are currently being used by researchers and pharmaceutical companies as research tools, diagnostics, and drugs. The use of antibodies against GPCRs as drug targets is a currently underdeveloped area of research with great potential to become a selective and effective treatment in a wide variety of pathological conditions involving GPCRs.

Acknowledgments

This work was supported by NIH grant DA019521 to LAD) and FAPESP (grant 2004/14258-0 to ASH).

References

1. Bohen SP, Troyanskaya OG, Alter O, Warnke R, Botstein D, Brown PO, Levy R. *Proc Natl Acad Sci, USA*. 2003; 100:1926. [PubMed: 12571354]
2. Kornbluth A. *Inflamm Bowel Dis*. 1998; 4:328. [PubMed: 9836088]
3. Schuna AA, Megeff C. *Am J Health Syst Pharm*. 2000; 57:225. [PubMed: 10674776]
4. Arafat W, Gomez-Navarro J, Xiang J, Siegal GP, Alvarez RD, Curiel DT. *Cancer Gene Ther*. 2000; 7:1250. [PubMed: 11023197]
5. Cochet O, Kenigsberg M, Delumeau I, Virone-Oddos A, Multon MC, Fridman WH, Schweighoffer F, Teillaud JL, Tocque B. *Cancer Res*. 1998; 58:1170. [PubMed: 9515802]
6. Goncalves J, Silva F, Freitas-Vieira A, Santa-Marta M, Malho R, Yang X, Gabuzda D, Barbas C 3rd. *J Biol Chem*. 2002; 277:32036. [PubMed: 12039955]
7. Worn A, Auf der Maur A, Escher D, Honegger A, Barberis A, Pluckthun A. *J Biol Chem*. 2000; 275:2795. [PubMed: 10644744]
8. Neumann L, Wohland T, Whelan RJ, Zare RN, Kobilka BK. *Chembiochem*. 2002; 3:993. [PubMed: 12362365]
9. Kimple RJ, Willard FS, Siderovski DP. *Mol Interv*. 2002; 2:88. [PubMed: 14993354]
10. Devi, LA. *The G Protein-Coupled Receptors Handbook*. Humana Press; New Jersey: 2005.
11. Wettschureck N, Offermanns S. *Physiol Rev*. 2005; 85:1159. [PubMed: 16183910]
12. McCudden CR, Hains MD, Kimple RJ, Siderovski DP, Willard FS. *Cell Mol Life Sci*. 2005; 62:551. [PubMed: 15747061]
13. Rozec B, Gauthier C. *Pharmacol Ther*. 2006; 111:652. [PubMed: 16480771]
14. Escolar G, Diaz-Ricart M, Gomez-Gil E, Serra M, Gasto C, Bozzo J, Galan AM. *Drugs Today (Barc)*. 2005; 41:721. [PubMed: 16395413]
15. Volante M, Bozzalla-Cassione F, Papotti M. *Endocr Pathol*. 2004; 15:275. [PubMed: 15681851]
16. Simmons DL. *Drug Discov Today*. 2006; 11:210. [PubMed: 16580598]

17. Harlow, E.; Lane, D. *Antibodies: A laboratory manual*. Cold Spring Harbor Laboratory; New York: 1998.
18. Mason LJ, Timothy LM, Isenberg DA, Kalsi JK. *J Immunol*. 1999; 162:5099. [PubMed: 10227979]
19. Gupta A, Devi LA. *Mount Sinai J Med*. 2006; 73:673.
20. Gupta A, Decaillot FM, Gomes I, Tkalych O, Heimann AS, Ferro ES, Devi LA. *J Biol Chem*. 2007; 282:5116. [PubMed: 17148456]
21. Gomes I, Gupta A, Filipovska J, Szeto HH, Pintar JE, Devi LA. *Proc Natl Acad Sci, USA*. 2004; 101:5135. [PubMed: 15044695]
22. Salom D, Lodowski DT, Stenkamp RE, Le Trong I, Golczak M, Jastrzebska B, Harris T, Ballesteros JA, Palczewski K. *Proc Natl Acad Sci, USA*. 2006; 103:16123. [PubMed: 17060607]
23. Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M. *Science*. 2000; 289:739. [PubMed: 10926528]
24. Hunte C, Michel H. *Curr Opin Struc Biol*. 2002; 12:503.
25. Mancina F, Brenner-Morton S, Siegel R, Assur Z, Sun Y, Schieren I, Mendelsohn M, Axel R, Hendrickson W. *Proc Natl Acad Sci, USA*. 2007; 104:4303. [PubMed: 17360519]
26. Schmidt A, MacColl R, Lindau-Shepard B, Buckler DR, Dias JA. *J Biol Chem*. 2001; 276:23373. [PubMed: 11313343]
27. Vignal E, Blangy A, Martin M, Gauthier-Rouviere C, Fort P. *Mol Cell Biol*. 2001; 21:8022. [PubMed: 11689693]
28. Cha K, Reeves PJ, Khorana HG. *Proc Natl Acad Sci, USA*. 2000; 97:3016. [PubMed: 10737783]
29. Takao M, Iwasa T, Yamamoto H, Takeuchi T, Tokunaga F. *Zoology Sci*. 2002; 19:651.
30. Hicks D, Barnstable CJ. *J Histochem Cytochem*. 1987; 35:1317. [PubMed: 3655327]
31. Blanpain C, Vanderwinden JM, Cihak J, Wittamer V, Le Poul E, Issafras H, Stangassinger M, Vassart G, Marullo S, Schlondorff D, Parmentier M, Mack M. *Mol Biol Cell*. 2002; 13:723. [PubMed: 11854425]
32. Tansky MF, Pothoulakis C, Leeman SE. *Proc Natl Acad Sci, USA*. 2007; 104:10691. [PubMed: 17563389]
33. Ayoub MA, Couturier C, Lucas-Meunier E, Angers S, Fossier P, Bouvier M, Jockers R. *J Biol Chem*. 2002; 277:21522. [PubMed: 11940583]
34. Naksnishi J, Takarada T, Yunoki S, Kikuchi Y, Maeda M. *Biochem Biophys Res Commun*. 2006; 343:1191. [PubMed: 16580633]
35. Szekeres PG. *Receptors Channels*. 2002; 8:297. [PubMed: 12690957]
36. Digan ME, Pou C, Niu H, Zhang JH. *J Biomol Screen*. 2005; 10:615. [PubMed: 16103416]
37. Rios C, Gomes I, Devi LA. *Br J Pharmacol*. 2006; 148:387. [PubMed: 16682964]
38. Rios C, Gomes I, Devi LA. *Clin Exp Pharmacol Physiol*. 2004; 31:833. [PubMed: 15566403]
39. Gomes I, Gupta A, Singh SP, Sharma SK. *FEBS Lett*. 1999; 456:126. [PubMed: 10452543]
40. Lebesgue D, Wallukat G, Mijares A, Granier C, Argibay J, Hoebeke J. *Eur J Pharmacol*. 1998; 348:123. [PubMed: 9650839]
41. Mobini R, Fu M, Wallukat G, Magnusson Y, Hjalmarsen A, Hoebeke J. *Hybridoma*. 2000; 19:135. [PubMed: 10868793]
42. Elies R, Fu LX, Eftekhari P, Wallukat G, Schulze W, Granier C, Hjalmarsen A, Hoebeke J. *Eur J Biochem*. 1998; 251:659. [PubMed: 9490038]
43. Alla AS, Quitterer U, Grigoriev S, Maidhof A, Haasemann M, Jarnagin K, Muller-Ester W. *J Biol Chem*. 1996; 271:1748. [PubMed: 8576178]
44. Magnusson Y, Marullo S, Hoyer S, Waagstein F, Andersson B, Vahlne A, Guillet JG, Strosberg AD, Hjalmarsen A, Hoebeke J. *J Clin Invest*. 1990; 86:1658. [PubMed: 1700798]
45. Goin JC, Perez Leiros C, Borda E, Sterin-Borda L. *Neuroimmunodulation*. 1994; 1:284.
46. Chun-Rong C, McLachlan SM, Rapoport B. *Endocrinol*. 2007; 148:2375.
47. Peter JC, Nicholson JR, Heydet D, Lecourt AC, Hoebeke J, Hofbauer KG. *Am J Physiol Regul Integr Comp Physiol*. 2007; 292:R2151. [PubMed: 17322114]

48. Kamel R, Eftekhari P, Garcia S, Berthouze M, Berque-Bestel I, Peter JC, Lezoualc'h F, Hoebeke J. *Biochem Pharmacol.* 2005; 70:1009. [PubMed: 16102731]
49. Mijares A, Lebesgue D, Wallukat G, Hoebeke J. *Mol Pharmacol.* 2000; 58:373. [PubMed: 10908305]
50. Goetzi EJ, Dembrow D, Van Brocklyn JR, Graler M, Huang MC. *Immunol Lett.* 2004; 93:63. [PubMed: 15134901]
51. Schreiber AB, Couraud PO, Andre C, Vray B, Strosberg AD. *Proc Natl Acad Sci, USA.* 1980; 77:7385. [PubMed: 6261254]
52. Guillet JG, Kaveri SV, Durieu O, Delavier C, Hoebeke J, Strosberg AD. *Proc Natl Acad Sci, USA.* 1985; 82:1781. [PubMed: 2858851]
53. Wang CJ, Tai HH. *J Biol Chem.* 1991; 266:12372. [PubMed: 1648095]
54. Gramsch C, Schulz R, Kosin S, Herz A. *J Biol Chem.* 1988; 263:5853. [PubMed: 2833518]
55. Mascelli MA, Zhou H, Sweet R, Getsy J, Davis HM, Graham M, Abernethy D. *J Clin Pharmacol.* 2007; 47:533.
56. Hudson PJ, Souriau C. *Nat Med.* 2003; 9:129. [PubMed: 12514726]
57. Stockwin LH, Holmes S. *Biochem Soc Trans.* 2003; 31:433. [PubMed: 12653655]
58. Winter G, Griffiths AD, Hawkins RE, Hoogenboom HR. *Annu Rev Immunol.* 1994; 12:433. [PubMed: 8011287]
59. Marks JD, Hoogenboom HR, Bonnert TP, McCafferty J, Griffiths AD, Winter G. *J Mol Biol.* 1991; 222:581. [PubMed: 1748994]
60. Griffiths AD. *Curr Opin Immunol.* 1993; 5:263. [PubMed: 8507404]
61. Mendez MJ, Green LL, Corvalan JR, Jia XC, Maynard-Currie CE, Yang XD, Gallo ML, Louie DM, Lee DV, Erickson KL, Luna J, Roy CM, Abderrahim H, Kirschenbaum F, Noguchi M, Smith DH, Fukushima A, Hales JF, Klapholz S, Finer MH, Davis CG, Zsebo KM, Jakobovits A. *Nat Genet.* 1997; 15:146. [PubMed: 9020839]
62. Ishida I, Tomizuka K, Yoshida H, Tahara T, Takahashi N, Ohguma A, Tanaka S, Umehashi M, Maeda H, Nozaki C, Halk E, Lonberg N. *Cloning Stem Cells.* 2002; 4:91. [PubMed: 12006160]
63. Sanders J, Jeffreys J, Depraetere H, Richards T, Evans M, Kiddie A, Brereton K, Groenen M, Oda Y, Furmaniak J, Rees-Smith B. *Thyroid.* 2002; 12:1043. [PubMed: 12593717]
64. Ando T, Latif R, Pritsker A, Moran T, Nagayama Y, Davies TF. *J Clin Invest.* 2002; 110:1667. [PubMed: 12464672]
65. Costagliola S, Franssen JDF, Bonomi M, Urizar E, Willnich M, Bergmann A, Vassart G. *Biochem Biophys Res Commun.* 2002; 299:891. [PubMed: 12470663]
66. Kreitman RJ. *Curr Opin Investig Drugs.* 2001; 2:313.
67. Newton DL, Rybak SM. *Expert Opin Biol Ther.* 2001; 1:995. [PubMed: 11728231]

Fig. 1. An assay to study allosteric modulation of GPCRs using conformation-sensitive antibodies

The modulation of receptor conformation upon dual occupancy of protomers in a heterodimer was probed using SK-N-SH cells endogenously expressing μ and CB₁ receptors. Cells (2×10^5 cells/well) plated on poly-L-lysine treated 24-well plates, were treated with 1 μ M DAMGO (μ receptor agonist) in the absence or presence of indicated concentrations of Hu-210 or WIN515,212-2 (CB₁ receptor agonists), for 30 min at 37°C in 50 mM Tris-Cl, pH 7.5. Cells were quickly rinsed, fixed with ice-cold methanol and subjected to ELISA using conformation-sensitive antibodies to μ opioid receptors as described [14]. O.D. 490 nm values obtained with cells not exposed to any drugs were taken as 100%. Results are Mean $\pm$ SD of triplicate determinations from 3 independent experiments.