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MOLECULAR PHARMACOLOGY

PERSPECTIVE

Bring Your Own G-Protein

By

John D. Hildebrandt

Department of Cell and Molecular Pharmacology, Medical University of South Carolina, Charleston, SC, 29425.

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Running Title: GPCR-G Protein Fusion Constructs

Corresponding author:

John D. Hildebrandt
Department of Cell and Molecular Pharmacology
Medical University of South Carolina
173 Ashley Ave, 303BSB
Charleston, SC 29425

Abbreviations:

GPCR, G-protein-coupled receptor;

TPα, Thromboxane A2 receptor α;

Rho-GEF, Rho Guanine Nucleotide Exchange Factor;

PTA$_2$, pinane thromboxane A2;

8-iso-PGF$_{2\alpha}$, 8-iso-prostaglandin F$_{2\alpha}$;

GTPγS, guanosine 5'-(3-O-thio)triphosphate;

β2-AR, β2-adrenergic receptor;

G protein, GTP-binding regulatory protein.

ABSTRACT

G Protein-coupled receptor (GPCR)-Gα fusion proteins were first characterized more than 10 years ago as a strategy for studying receptor-G protein signaling. A large number of studies have used this approach to characterize receptor coupling to members of the G_s, G_i and G_q families of Gα subunits, but this strategy has not been widely used to study $G\alpha_{12}$ and $G\alpha_{13}$. As described in the article by Zhang et al. in this issue of *Molecular Pharmacology(Mol Pharmacol   )*, characterization of the signaling properties of Thromboxane A2 receptor (TPα) -$G\alpha_{12}$ and -$G\alpha_{13}$ fusion constructs demonstrates the applicability of this strategy to members of this unique family of Gα subunits, and how this strategy can be used to resolve otherwise difficult problems of receptor pharmacology associated with these proteins. The general strategy of making receptor-Gα fusion constructs has wide applicability to a number of research problems, but there are perhaps also "hidden messages" in how different receptor-Gα subunit fusion pairs differ from one another.

$G\alpha_{12}$ and $G\alpha_{13}$ are the least understood of the larger family of heterotrimeric G proteins

that mediate the effects of a multitude of endogenous and exogenous regulators of cellular

function (Riobo and Manning, 2005). From the beginning, $G\alpha_{12}$ and $G\alpha_{13}$ were pursued by a

different tack than their better-characterized cousins that are members of the G_s, G_i and G_q

subfamilies of $G\alpha$ subunits. These latter three families were initially described by following the

biology of signaling pathways - for example by looking for the transducers of the regulation of

cAMP or phosphatidylinositol turnover. In contrast, $G\alpha_{12}$ and $G\alpha_{13}$ were "discovered" by

cloning studies designed to look for homologues of already-identified proteins (Strathmann et al.,

1989). Hence, they were accorded numbers instead of the earlier names that were used to denote

primary downstream signaling targets, such as "s" for stimulation of adenylyl cyclase, "i" for

inhibition of adenylyl cyclase and, whimsically, "q" for stimulation of phospholipase C (p

already having been claimed). The $G\alpha_{12}/G\alpha_{13}$ proteins, however, segregate into a distinct arm of

the G protein α subunit family (Strathmann and Simon, 1991) and were, from the beginning,

orphan proteins in search of an intracellular function. One such function, at least for $G\alpha_{13}$,

turned out to be regulation of a Rho-GEF (Hart et al., 1998), i.e., a guanine nucleotide exchange

factor for a member of the small G protein family of GTP binding proteins. Numerous variants

of this protein have been identified as $G\alpha_{12}/ G\alpha_{13}$ targets, as well as several other interacting

proteins (Riobo and Manning, 2005).

Several features of the biology of $G\alpha_{12}$ and $G\alpha_{13}$ has made them difficult to study: They

have fairly slow nucleotide exchange rates and are hard to express (Kozasa and Gilman, 1995;

Singer et al., 1994); they regulate cellular processes that have coincident regulation through

multiple other G protein-related processes - as by $G\alpha_q$ and $G\beta\gamma$; and they do not appear to cause

generation of a specific small molecule mediator, such as cAMP or IP3, that would lead to easily

assayed downstream effects (Riobo and Manning, 2005). Consequently, it has not been easy to

evaluate whether a receptor signals through this family of proteins nor to study the pharmacology

of their interactions with receptors. To circumvent these limitations Zhang et al. report in this

issue (Zhang et al., 2006) the characterization of fusion proteins between a Thromboxane A2

receptor (TPα) and Gα_{12} or Gα_{13}, characterizing direct responses of these constructs expressed

in Sf9 cells by [^{35}S]GTPγS binding in response to known and suspected TPα receptor agonists

and antagonists. Using this assay the authors show that isoprostanes related to 8-iso-

prostaglandin F$_{2\alpha}$ (8-iso-PGF$_{2\alpha}$) target Gα_{12} and Gα_{13} through TPα receptor activation. These

compounds are generated nonenzymatically from arachidonic acid in response to oxidative stress

and may play a role in multiple human diseases (Montuschi et al., 2004). Previous studies have

ambiguously associated these compounds with multiple receptors, and uncertainly with TPα.

(reviewed in (Zhang et al., 2006)). Zhang et al. also characterize the response of TPα-Gα_{12} and

TPα-Gα_{13} to other agonists (U46619 and U44069) and antagonists (pinane thromboxane A2

(PTA$_2$) and SQ29548) of TPα. Their studies indicate that all ligands tested, except SQ29548,

have agonist activity for TPα-Gα_{13}, including the purported antagonist PTA$_2$, and that SQ29548

decreases activity of TPα-Gα_{13}, compatible with the idea (but as admitted by the authors, not

definitive proof) that it is an inverse agonist. In contrast to TPα-Gα_{13}, TPα-Gα_{12} did not

respond to PTA$_2$, and had a substantially decreased potency for 8-iso-PGF$_{2\alpha}$ that precluded

evaluation of its efficacy. To validate the conclusions from the fusion constructs, Zhang et al

showed that PTA$_2$ and 8-iso-PGF$_{2\alpha}$ were also agonists for Gα_{13} in HEK293 cells through both

expressed and endogenous TPα receptors that are not fusion constructs. These cells do not

express Gα_{12}, which precluded validation of those results.

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The work reported by Zhang et al. is the latest in a series of reports to use receptor G-protein fusion constructs to study the biology and pharmacology of signaling through specific receptor/G protein interactions (Milligan, 2000; Milligan et al., 2004; Seifert et al., 1999; Wurch and Pauwels, 2001). These constructs express $G\alpha$ subunits as a C-terminal extension of the receptor protein so that their expression is linked in a 1:1 stoichiometry, with the expressed proteins in obligatory close proximity. This strategy was first used more than ten years ago to characterize a β2-adrenergic receptor-$G\alpha_s$ fusion construct (β2-AR-$G\alpha_s$); showing, first and foremost, that it was expressed as a functional protein, and that it had increased sensitivity to agonists (Bertin et al., 1994). Receptor-G protein fusion proteins have now been used extensively to characterize a large number of receptors, notably those coupled to members of the G_s, G_i and G_q family of proteins (TABLE I). (An extended version of TABLE I is available as an online supplement.) Only one previous report has characterized a receptor construct fused to a member of the $G\alpha_{12}$/$G\alpha_{13}$ subunit family (Sugimoto et al., 2003). That study used a similar fusion construct to show that sphingosine-1-phosphate activation of SIP_2/Edg5 receptors can use either $G\alpha_{12}$ or $G\alpha_{13}$ to stimulate Rho and inhibit Rac and cell motility, but the authors of that study did not evaluate the utility of the constructs for studies of receptor pharmacology. A primary goal of the work reported here was to establish the $G\alpha_{12}$/$G\alpha_{13}$ family of proteins as targets of this strategy for studying receptor-G protein signaling (Zhang et al., 2006). Thus, this work opens up the possibility of using such constructs both for characterizing responses to additional receptors and, perhaps, for studying the unique signaling properties associated with this particular family of $G\alpha$ proteins.

The future utility of the constructs characterized by Zhang et al. relates in part to the utility of these receptor-fusion constructs in general. The advantages of the 1:1 receptor:G-protein stoichiometry of these constructs has led to a large number of studies evaluating specific receptor-G protein pairs (TABLE I); there are theoretical reasons for using such constructs to study (GPCR) receptor theory (Colquhoun, 1998). These constructs have been widely used for characterizing mutations and modifications of receptors (Barclay et al., 2005; Loisel et al., 1999; McLean et al., 2002; Moon et al., 2001; Pauwels and Colpaert, 2000; Stevens et al., 2001; Ward and Milligan, 1999; Ward and Milligan, 2002) and G proteins (Barclay et al., 2005; Dupuis et al., 1999; Kellett et al., 1999; Liu et al., 2002; Loisel et al., 1999; Moon et al., 2001; Stevens et al., 2001; Ugur et al., 2003; Wang et al., 1999; Wise and Milligan, 1997). Such constructs are similarly useful for characterizing receptor and G protein polymorphisms (essentially mutant constructs) (Milligan, 2002). Receptor-G-protein fusion constructs have also been implemented as a successful means for characterizing orphan receptors, so as to identify exogenous (Takeda et al., 2003), as well as endogenous (Hosoi et al., 2002), regulators of pharmacological significance. They have even been targeted for developing gene therapy reagents (Small et al., 2001). The work of Zhang et al. indicates the likelihood that members of the $G\alpha_{12}$ family, and possibly all G proteins, will be amenable targets for this research strategy.

Some of the interesting results reported by Zhang et al. are differences in the responses of the $G\alpha_{12}$ **and** $G\alpha_{13}$ constructs. TPα-$G\alpha_{13}$ responded to PTA$_2$ as a partial agonist with relatively high potency, whereas TPα-$G\alpha_{12}$ did not respond to PTA$_2$ and this compound functioned as an antagonist. Nevertheless, both constructs responded with similar potency to the full agonist U46619. This may indicate ligand-dependent conformations of TPα that differentially interact with G proteins - i.e., some form of agonist-directed trafficking (Kenakin, 2003; Leff et al.,

1997; Perez and Karnik, 2005). There was, however, also a more subtle difference between the two constructs that may or may not relate to the same phenomenon. Zhang et al. measured activation by agonist-induced GTPγS binding. Whereas TPα-Gα$_{12}$ responded to agonists with slow GTPγS binding, the TPα-Gα$_{13}$ response was rapid and had to be assayed at very short time points to obtain valid estimates of potency. Previous studies of purified proteins do not suggest differences in these two G proteins for GDP/GTPγS binding kinetics, and both of them have slow binding kinetics relative to other Gα proteins (Kozasa and Gilman, 1995; Singer et al., 1994). Thus, results with the receptor fusion contrasts could be due to receptor-Gα specific interactions indicative of important biological properties or perhaps to differences in the constructs. Interestingly, the recent report of crystals of Gα$_{12}$ and Gα$_{13}$ as chimeric proteins containing the N-terminal helix of Gα$_{i1}$ found that they crystallized in opposite (active versus inactive, respectively) conformations under otherwise similar conditions in the presence of aluminum fluoride and GDP (Kreutz et al., 2006). Such results argue for Gα-specific preferences of these proteins that have inversely-related interactions with guanine nucleotides, on the one hand, as in the crystallization studies, and receptors on the other, as in fusion constructs.

In the most general sense, the report of Zhang et al. focuses attention on the utility of receptor-Gα fusion constructs for studying GPCR signaling mechanisms. These constructs have been very successful for a number of applications, but as artificial constructs, they have both hidden caveats and potential important surprises for otherwise unapparent biological processes. In general, fusion constructs are perceived to have increased sensitivity to activation, which can include increased constitutive activity. Such observations make sense based upon the proximity and theoretical interactions of the two components of the fusion protein (Milligan, 2000; Milligan et al., 2004; Seifert et al., 1999; Wurch and Pauwels, 2001). These results are not

universal, however, and there are substantial differences in the properties of various constructs formed of different receptor-G protein fusion pairs. For example, some, but not all, constructs activate endogenous G proteins as well as their tethered protégé (Burt et al., 1998; Fong and Milligan, 1999; Massotte et al., 2002; Molinari et al., 2003; Vorobiov et al., 2000). Although some of these results may be due to high level over expression of the fusion constructs (Carrillo et al., 2003), it is not clear that this accounts for all of them, or that such signaling is necessarily seen only at high levels of expression. Colquhoun has analyzed the utility of the use of fusion constructs of defined 1:1 stoichiometry for evaluating receptor signaling mechanisms for systems that are otherwise designed to be catalytic with spare receptor and other phenomenon that circumvent an easy analysis of efficacy and potency (Colquhoun, 1998). The activation of endogenous G-proteins by fusion constructs seems to minimize these advantages, along with one of the potential uses of the constructs, i.e., evaluation of mechanisms of receptor theory. But is this really true, or are these proteins and this strategy telling us something (important) by disclosing otherwise unsuspected events?

Another emerging concept in GPCR action is the role of receptor dimerization in their synthesis, trafficking and action (Javitch, 2004; Milligan, 2004; Terrillon and Bouvier, 2004). The functional existence of GPCR dimers took years to establish, but is now well accepted, particularly for their role in GPCR biosynthesis and maturation (Bulenger et al., 2005), and particularly for the Class C receptors (Javitch, 2004; Milligan, 2004; Terrillon and Bouvier, 2004). The general functional role of receptor dimerization in signaling, particularly for the rhodopsin-related Class A GPCRs, is still being established (Javitch, 2004; Milligan, 2004; Terrillon and Bouvier, 2004). Is it possible that signaling by fusion constructs is in part mediated through dimer complexes either with themselves or with endogenous proteins? Dimerization of

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such fusion proteins has in fact been demonstrated, along with the ability of such dimers to

cross-regulate one another (Carrillo et al., 2003). Could the dimerization of fusion constructs

play a major role in the divergent phenotypes of different receptor-G protein pairs? For example,

might the constructs characterized by Zhang et al. have different properties due to differences in

their ability to form dimers? Do associated G proteins play a role in receptor dimerization?

According to the concepts of agonist-directed trafficking, agonists select different G proteins by

inducing agonist-specific conformations of the receptor compatible with that G protein (Kenakin,

2003; Leff et al., 1997; Perez and Karnik, 2005). According to the principle of microscopic

reversibility, if agonist induces a conformation of the receptor specific for a G protein, binding of

that G protein should also induce a conformation of the receptor specific for that agonist, i.e., G

protein-specific receptor conformations. If this interaction is stable, perhaps indicative of

precoupling, then some of these G protein-specific receptor conformations may have a tendency

to dimerize, whereas other might not. Might such dimerization also explain other observations

about fusion constructs that have remained elusive? For example, these constructs often exhibit

high and low affinity binding states that are not easily explained theoretically or experimentally

(Hoare, 2000; Seifert et al., 1999; Seifert et al., 2000). Although the functional role of dimers in

the actions of these constructs is speculative, access to the full repertoire of Gα isoforms capable

of serving as donors for fusion constructs, in part resulting from the work reported by Zhang et

al., provides a mechanism to address this and other questions regarding the signal transduction

mechanisms of these proteins and GPCRs in general.

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REFERENCES

Barclay E, O'Reilly M and Milligan G (2005) Activation of an alpha-2A adrenoceptor-G alpha-o1 fusion protein dynamically regulates the palmitoylation status of the G protein but not the receptor. *Biochem J* **385**:197-206.

Bertin B, Freissmuth M, Jockers R, Strosberg AD and Marullo S (1994) Cellular signaling by an agonist-activated receptor/Gs alpha fusion protein. *Proc Natl Acad Sci USA* **91**:8827-8831.

Bulenger S, Marullo S and Bouvier M (2005) Emerging role of homo- and heterodimerization in G-protein-coupled receptor biosynthesis and maturation. *Trends Pharmacol Sci* **26**:131-137.

Burt AR, Sautel M, Wilson MA, Rees S, Wise A and Milligan G (1998) Agonist occupation of an alpha-2A adrenoreceptor-Gi1 alpha fusion protein results in activation of both receptor-linked and endogenous Gi proteins. *J Biol Chem* **273**:10367-10375.

Carrillo JJ, Pediani J and Milligan G (2003) Dimers of class A G protein coupled receptors function via agonist-mediated trans-activation of associated G proteins. *J Biol Chem* **278**:42578-42587.

Colquhoun D (1998) Binding, gating, affinity and efficacy: The interpretation of structure-activity relationships for agonists and of the effects of mutating receptors. *Br J Pharmacol* **125**:923-947.

Dupuis DS, Tardif S, Wurch T, Colpaert FC and Pauwels PJ (1999) Modulation of 5-HT1A receptor signalling by point-mutation of cystein 351 in the rat G alpha-o protein. *Neuropharmacology* **38**:1035-1041.

Fong CW and Milligan G (1999) Analysis of agonist funciton at fusion proteins between the IP prostanoid receptor and cognate, unnatural and chimaeric G proteins. *Biochem J* **342**:457-463.

Hart MJ, Jiang X, Kozasa T, Roscoe W, Singer WD, Gilman AG, Sternweis PC and Bollag G (1998) Direct stimulation of the guanine nucleotide exchange activity of p115 RhoGEF by G alpha-13. *Science* **280**:2112-2114.

Hoare SRJ (2000) G protein coupled receptos: what limits high-affinity agonist binding? *Trends Pharmacol Sci* **21**:82-83.

Hosoi T, Koguchi Y, Sugikawa E, Chikada A, Ogawa K, Tsuda N, Suto N, Tsunoda S, Taniguchi T and Ohnuki T (2002) Identification of a novel human eicosanoid receptor. *J Biol Chem* **277**:31459-31465.

Javitch JA (2004) The ants go marching two by two: Oligomeric structure of G protein coupled receptors. *Mol Pharmacol* **66**:1077-1082.

Kellett E, Carr C and Milligan G (1999) Regulation of G protein activation and effector modulation by fusion proteins between the human 5-hydroxytryptamine-1A receptor and the alpha subunit of Gi1 alpha: Differences in receptor-constitutive activity imparted by single amino acid substitutions in Gi1 alpha. *Mol Pharmacol* **56**:684-692.

Kenakin T (2003) Ligand-selective receptor comformations revisited: the promise and the problem. *Trends Pharmacol Sci* **24**:346-354.

Kozasa T and Gilman AG (1995) Purification of recombinant G proteins from Sf9 cells by hexahistidine tagging of associated subunits. *J Biol Chem* **270**:1734-1741.

Kreutz B, Yau DM, Nance MR, Tanabe S, Tesmer JJG and Kozasa T (2006) A new approach to producing functional G alpha subunits yields the activated and deacted structures of G alpha 12/13 proteins. *Biochemistry* **45**:167-174.

Leff P, Scaramellini C, Law C and McKechnie K (1997) A three-state receptor model of agonist action. *Trends Pharmacol Sci* **18**:355-362.

Liu S, Carrillo JJ, Pediani JD and Milligan G (2002) Effective information transfer from teh alpha-1b-adrenoceptor to G alpha-11 requuires both betagamma interactions and an aromatic group four amino acids from the C terminus of the G protein. *J Biol Chem* **277**:25707-25714.

Loisel TP, Ansanay H, Adams L, Marullo S, Seifert R, Lagace M and Bouvier M (1999) Activation of the beta-2 adrenergic receptor-G alpha-s complex leads to rapid depalmitoylation and inhibition of repalmitoylation of both the receptor and G alpha-s. *J Biol Chem* **274**:31014-31019.

Massotte D, Brillet K, Kieffer BL and Milligan G (2002) Agonists activate Gil alpha or Gi2 alpha fused to the human mu opioid receptor differently. *J Neurochem* **81**:1372-1382.

McLean AJ, Zeng F-Y, Behan D, Chalmers D and Milligan G (2002) generation and analysis of constitutively active and physically destabilized mutants of the human beta-1 adrenoceptor. *Mol Pharmacol* **62**:747-755.

Milligan G (2000) Insights into ligand pharmacology using receptor-G-protein fusion proteins. *Trends Pharmacol Sci* **21**:24-28.

Milligan G (2002) The use of receptor G protein fusion proteins for he study of ligand activity. *Recept Channels* **8**:309-317.

Milligan G (2004) G proein-coupled receptor dimerization: function and ligand pharmacology. *Mol Pharmacol* **66**:1-7.

Milligan G, Feng G-J, Ward RJ, Sartania N, Ramsay D, McLean AJ and Carrillo JJ (2004) G protein-coupled receptor fusion proteins in drug discovery. *Curr Pharmaceutical Design* **10**:1989-2001.

Molinari P, Ambrosio C, Riitano D, Sbraccia M, Gro MC and Costa T (2003) Promiscuous coupling at receptor-G alpha fusion proteins. *J Biol Chem* **278**:15778-15788.

Montuschi P, Barnes PJ and Roberts LJ (2004) Isoprostanes: markers and mediators of oxidative stress. *FASEB J* **18**:1791-1800.

Moon H-E, Bahia DS, Cavalli A, Hoffmann M and Milligan G (2001) Control of the efficiency of agonist-induced information transfer and stability of the ternary complex containing the delta opioid receptor and the alpha subunit of the Gi1 by mutation of a receptor/G protein contact interface. *Neuropharmacology* **41**:321-330.

Pauwels PJ and Colpaert FC (2000) Disparate ligand-mediated Ca2+ responses by wild-type, mutant Ser200Ala and Ser204Ala alpha-2A-adrenoceptor: G15 fusion proteins: evidence for multiple ligand-activation binding sitess. *Br J Pharmacol* **130**:1505-15112.

Perez DM and Karnik SS (2005) Multiple signaling states of G protein-coupled receptors. *Pharmacol Rev* **57**:147-161.

Riobo NA and Manning DR (2005) Receptors coupled to heterotrimeric G proteins of the G12 family. *Trends Pharmacol Sci* **26**:146-154.

Seifert R, Wenzel-Seifert K and Kobilka B (1999) GPCR-G alpha fusion proteins: molecular analysis of receptor-G protein coupling. *Trends Pharmacol Sci* **20**:383-389.

Seifert R, Wenzel-Seifert K and Kobilka B (2000) Relpy: G protein coupled receptos: what limits high-affinity agonist binding? *Trends Pharmacol Sci* **21**:83-84.

Singer WD, Miller RT and Sternweis PC (1994) Purification and characterization of the alpha subunit of G13. *J Biol Chem* **269**:19796-19802.

Small KM, Brown KM, Forbes SL and Liggett SB (2001) Modification of the beta-2 adrenergic receptor to engineer a receptor effector complex for gene therapy. *J Biol Chem* **276**:31596-31601.

Stevens PA, Pediani JD, Carrillo JJ and Milligan G (2001) Coordinated agonist regulation of receptor and G protein palmitoylation and functional rescue of palmitoylation-deficient mutants of the G protein G11 alpha following fusion to the alpha-1b-adrenoceptor. *J Biol Chem* **276**:35883-35890.

Strathmann M and Simon MI (1991) G alpha-12 and G alpha-13 subunits define a fourth class of G protein alpha subunits. *Proc Natl Acad Sci USA* **86**:7407-7409.

Strathmann M, Wilkie TM and Simon MI (1989) Diversity of the G protein family: Sequences from five additional alpha subunits in the mouse. *Proc Natl Acad Sci USA* **86**:7404-7409.

Sugimoto N, Takuwa N, Okamoto H, Sakurada S and Takuwa Y (2003) Inhibitory and stimulatory regulation of Rac and cell motility by the G12/13-Ro and Gi pathway integrated downstream of a single G protein -coupled sphingosine-1-phosphate receptor isoform. *Mol Cell Biol* **23**:1534-1545.

Takeda S, Yamamoto A, Okada T, Matsumura E, Nose E, Kogure K, Kojima S and Haga T (2003) Identification of surrogate ligands for orphan G protein-coupled receptors. *Life Sci* **74**:367-377.

Terrillon S and Bouvier M (2004) Roles of G-protein-coupled receptor dimerization. From ontogeny to signalling regulation. *EMBO Reports* **5**:30-34.

Ugur O, Onaran HO and Jones TLZ (2003) Partial rescue of functional interactions of a nonpalmitoylated mutant of the G protein G alpha-s by fusion to the beta-adrenergic receptor. *Biochemistry* **42**:2607-2615.

Vorobiov D, Bera AK, Keren-Raifman T, Barzilai R and Dascal N (2000) Coupling of the muscarinic m2 receptor to G protein-activated K+ channels via G alpha-z and a receptor-G alpha-z fusion protein. *J Biol Chem* **275**:4166-4170.

Wang Y, Windh RT, Chen CA and Manning DR (1999) N-myristoylation and betagamma play roles beyond anchorage in the parlitoylation of the G protein alpha-o subunit. *J Biol Chem* **274**:37435-37442.

Ward RJ and Milligan G (1999) An Asp79Asn mutation of the alpha-2A adrenoceptor interferes equally with agonist activation of individual Fa alpha-family G protein subtypes. *FEBS Lett* **462**:459-463.

Ward RJ and Milligan G (2002) Reciprocal mutations of highly conserved residues in transmembrane helices 2 and 7 of the alpha-2A adrenoceptor restore agonist activation of Gi1 alpha. *Cell Signal* **14**:139-144.

Wise A and Milligan G (1997) Rescue of functional interactions between the alpha-2A-adrenoceptor and acylation-resistant forms of Gi1 alpha by expressing the proteins from chimeric open reading frames. *J Biol Chem* **272**:24673-24678.

Wurch T and Pauwels PJ (2001) Analyatical pharmacology of G protein-coupoled receptors by stoichiometric expression of the receptor and G alpha protein subunits. *J Pharmacol Therapeut Meth* **45**:3-16.

Zhang L, Dilizio C, Kim D, Smyth E and Manning DR (2006) The G12 family of G proteins as a reporter of thromboxane A2 receptor activity. *Mol Pharmacol*:in press.

TABLE I

Gs Family

GsαL	GsαS	Golfα
β1-AR	β1-AR	β2-AR
β2-AR	β2-AR	
D1	GR	
GR	H2	
H2		
IP		
NK1		
δOR		
V2		

Gi Family

Gi1α	Gi2α	Gi3α	Go1α	Gzα	Gq/i1α chimera
α2A-AR	α2A-AR	α2A-AR	α2A-AR	m2	α2A-AR
A1	A1	A1	A1		α2B-AR
5HT1A			5HT1A		α2C-AR
β2-AR	β2-AR	β2-AR			
m2	CXCR1	FPR	D2short		
δOR	FPR		δOR		
Edg2	μOR		μOR		
FPR	NR		NR		
IP					
mOR					
NTS-1					
TG1019					
V2					

Gq Family

Gqα	G11α	G15α	G16α
β2-AR	α1B-AR	α2A-AR	β2-AR
Edg5			CX3C
NK1			m1
NTS-1			m2
			UR-II

G12 Family

G12α	G13α
Edg5	Edg5

Receptors Expressed as Fusion Proteins with C-terminal G protein α Subunits. Summary
of receptors that have been reported on in the literature as fusion constructs with different G
protein α subunits. The table is arranged according to Gα subunit family, and the specific
isoforms of each family for which constructs have been made. A complete list of references for
these constructs is available as an online supplement to this article.

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Receptors referred to in the Table: 5HT1A, 5-hydroxytryptamie-1A receptor; A1, Adenosine A1 receptor; α1B-AR, α1B-adrenergic receptor; α2A-AR, α2A-adrenergic receptor; α2B-AR, α2B-adrenergic receptor; α2C-AR, α2C-adrenergic receptor; β1-AR, β1-adrenergic receptor; β2-AR, β2-adrenergic receptor; CX3C, CX3C chemokine receptor 1; CXCR1, CXCR1 chemokine receptor; D1, Dopamine D1 receptor; D2short, Dopamine D2short receptor; δOR, Delat opioid receptor; Edg2, Edg2 receptor; Edg5, Edg5/S1P2 receptor; FPR, formyl peptide receptor; GR, Glucagon receptor; H2, Histamine H2 receptor; IP, IP prostanoid receptor; m1, Muscarinic m1 receptor; m2, Muscarinic m2 receptor; µOR, mu opioid receptor; NK1, tachykinin NK1 receptor; NR, nociceptin receptor; NTS-1, NTS-1 (neurotensin receptor); TG1019, orphan (eicosanoid) receptor; UR-II, Urotensin II receptor; V2, Vasopressin V2 receptor