The surfaces of heterotrimeric G proteins (αβγ) in contact with receptors and the molecular events at these sites, which lead to G protein activation, are largely unknown. We show here that a peptide from the C terminus of a G protein γ subunit blocks muscarinic receptor-stimulated G protein activation in a sequence-dependent fashion. A G protein mutated at the same site on the γ subunit shows enhanced receptor stimulated nucleotide exchange without affecting G protein heterotrimerization. Ineffective contact between the γ subunit and receptor increases the rate of receptor-stimulated nucleotide exchange. Specific interaction of the G protein γ subunit with the receptor thus helps the βγ complex to act at a distance and control guanine nucleotide exchange in the α subunit.

Although G protein signaling is central to a vast majority of pathways that control the physiology of mammalian cells, the coordinated changes in the conformations of the agonist bound receptor and the G protein subunits that trigger nucleotide release are not known. Peptide and mutant studies using biochemical assays that measure receptor-G protein coupling have implicated three protein domains, the N- and C-terminal domains of the α subunit and the C-terminal domain of the γ subunit in receptor interaction (1–3). Functional contact between the γ subunit and a receptor is one mechanism that can explain the universal requirement of the βγ complex for receptor-mediated nucleotide exchange in the α subunit. However, the particular role that the γ subunit plays at the receptor surface during G protein activation has not been clear. The crystal structure of the G protein heterotrimer indicates that the C termini of the γ and α subunits are a considerable distance from the nucleotide-binding site in the α subunit (4, 5). The mechanisms that help the receptor regulate nucleotide exchange by contacting these domains are therefore an outstanding puzzle.

Because a peptide specific to the C terminus of the γ1 subunit type stabilizes activated rhodopsin in a sequence-dependent manner (6) and a homologous γ5 peptide specifically inhibits muscarinic receptor modulation of a Ca2+ current in intact neurons (3), we examined the effect of a geranylgeranylated γ5 peptide on the M2 muscarinic receptor activation of a G protein. The peptide specific to the C-terminal 14 residues of the γ5 subunit was prenylated because the native γ5 subunit is modified with geranylgeranyl at the C-terminal Cys residue that is part of a CAAX motif (2). This peptide inhibited activation of G proteins by reconstituted M2 receptor. A peptide with the same sequence scrambled was inactive. These studies indicated that the γ subunit peptide interacts with the receptor in a sequence-specific fashion. To test the effect of mutations in this C-terminal domain of γ5 on G protein activation by M2, C-terminal residues of γ5 corresponding to the peptides were scrambled. The scrambled sequence was identical to the sequence of the scrambled peptide used in the earlier experiments. Wild type and mutant β1γ5 complexes were purified and bound to αo, and the ability of the M2 receptor to activate these heterotrimers was measured in a reconstituted system containing purified proteins. A significant difference in the receptor-stimulated GTPase activity between the wild type and mutant Goα was detected in this system. A phospholipase C enzyme-based assay indicated that efficacy of interaction with the α subunit was unaltered by the mutant γ subunit. Together these results indicate that the γ subunit interaction with the receptor regulates nucleotide exchange in the α subunit by affecting the positioning of the βγ complex with reference to the α subunit. The results thus identify a mechanism that allows a receptor to regulate nucleotide exchange at a distance in the G protein.

MATERIALS AND METHODS

Synthesis and Prelyation of Peptides—The amino acid sequences of the peptides were as follows: γ5pep-gg (wild type): VSSSTTNPRFQKVC and γ5pep-gg-scr (scrambled): PSRTVPNSQVSCK. Cys residues were retained at the C terminus for chemical geranylgeranylation using geranylgeranyl bromide. Prenylation and purification using fast protein liquid chromatography has been described (7). The integrity of the modified peptides were checked by mass spectrometry and chromatography, and peptide concentrations were estimated by amino acid analysis. Except where specified, all chemicals were from Sigma.

Purification and Reconstitution of M2 Receptors—Detailed description of M2 purification, reconstitution, and measurement of G protein stimulation is published elsewhere (7). Briefly, baculoviruses containing His-tagged M2 receptor cDNA (kind gift from Dr. E. M. Ross) were expressed in insect cells. M2 was purified according to a previously published protocol (8). Sf9 cell membranes containing M2 were solubilized in 50 mM Hepes, pH 7, 50 mM NaCl, digitonin (Calbiochem)/sodium cholate added to 10.5% final concentration. All procedures were performed at 4 °C. Solubilized receptor was bound to cobalt-chelate beads that were prepared using iminodiacetic acid beads and cobalt chloride. His-M2 eluted from these columns with 200 mM imidazole retain 50% of the N-[methyl-3H]scopolamine binding activity in Sf9 cell membranes. Using modifications of a previous method (8), purified M2 was reconstituted into brain lipids (Folch Fraction VII). The composition of the brain lipid mixture is sphingomyelin (20%), phosphatidylethanolamine (30%), phosphatidylserine (20%), and other lipids according to the distributor (Sigma). Typically, 100 μl (1 mg lipid) of the aqueous lipid suspension is added to 18 μl of 10% sodium deoxycholate and 4 μl of 10% sodium cholate. 100–500 μl of pure receptor is mixed with solubilized lipids (~122 μl from above). The mixture is applied to...
A 10–12-ml column of Sephadex G-50 (fine), equilibrated with solubilization buffer. Vesicle fractions were collected and concentrated. N-[methyl-3H]Scopolamine binding in a standard filter binding assay was used to estimate receptor concentration. The yields were 20–30% relative to purified solubilized receptors.

**M2 Stimulation of G Protein Activity**—αo subunit protein was expressed in bacteria with yeast myristoyl-transferase and purified as described before (9). β1 and β5 and β5scr complexes were produced in SF9 insect cells by triple infection of His-αo, β1 (kind gifts from Dr. T. Kozasa), and γ subunit viruses using a previously published procedure with minor modifications (10). The two βγ complexes were separately purified in complex with His-tagged αo subunit by binding the complex to nickel-nitrotriacetic acid resin and eluted the βγ complexes with aluminum fluoride. Yields of the β1γ and β5γscr were similar. The γ5Al and γ5A mutants were synthesized as His-tagged proteins, expressed in complex with β1 subunit in SF9 cells, and purified by directly binding to nickel-nitrotriacetic acid resin. As a control a wild type β1-His-tagged γ5 complex was expressed and purified. Although the yield of β1His-γ5 was comparable with or higher than that of β1γ, the yields of the β1Hisγ5Ala/Δ were relatively low. The final purity was examined by gel electrophoresis and Coomassie Blue staining where no significant levels of other proteins were detected. Protein concentration was determined by laser scanning densitometry using standards. G protein heterotrimer was formed by preincubating αo with βγ complex for 10 min at 4 °C. Lipid-reconstituted M2 was incubated with αo subunit with or without βγ complexes for 30 min at 4 °C in 20 mM Hepes buffer, pH 8, containing 2 mM MgCl₂, 100 mM NaCl, 10 mM dithiothreitol. Yields of the β1γ and β5γscr were similar. The γ5Al and γ5A mutants were synthesized as His-tagged proteins, expressed in complex with β1 subunit in SF9 cells, and purified by directly binding to nickel-nitrotriacetic acid resin. As a control a wild type β1-His-tagged γ5 complex was expressed and purified. Although the yield of β1His-γ5 was comparable with or higher than that of β1γ, the yields of the β1Hisγ5Ala/Δ were relatively low. The final purity was examined by gel electrophoresis and Coomassie Blue staining where no significant levels of other proteins were detected. Protein concentration was determined by laser scanning densitometry using standards. G protein heterotrimer was formed by preincubating αo with βγ complex for 10 min at 4 °C. Lipid-reconstituted M2 was incubated with αo subunit with or without βγ complexes for 30 min at 4 °C in 20 mM Hepes buffer, pH 8, containing 2 mM MgCl₂, 100 mM NaCl, 10 mM GDP, and 1 mM dithiothreitol. GTP-γS binding to αo was determined using previously published methods (11). In peptide assays, the incubation was performed by mixing with dried peptide or peptide vehicle (3 μM CHAPS) as a control. The final reaction mix contained 1 mM M2, 100 mM αo, and 0–10 mM β1γ in the buffer above and was equilibrated at 23 °C. The binding reaction was started by addition of 0.2 μM [35S]GTP-γS and 1 mM carboplatin, 1 mM atropine or vehicle. The reaction was stopped with ice-cold reaction buffer containing 200 μM GTP-γS and 1 mM atropine. [35S]GTP-γS bound to αo was measured in a filter binding assay. For GTPhase assays, the conditions were the same as above. αo GTPhase activity was measured essentially as described (11). The reaction was started with 0.2 μM γ[32P]GDP, 1 mM carboplatin, or vehicle and stopped with ice-cold 5% charcoal in 50 mM sodium phosphate, pH 7.0. The samples were centrifuged, and the radioactivity in the supernatant was estimated using scintillation counting. Purified RGS4 was kindly provided by Dr. Maurine Linder.

**Inhibition of βγ-stimulated PLC-β Activity by αo**—αo inhibition of βγ complex-stimulated PLC-β activity was measured as described before (12). αo and β1γ or β1γscr complexes were preincubated for 30 min at 4 °C. The reaction mixture contained G protein, PLC-β, and lipids. Freeze dried lipid mixtures were ultrasonicated before use. Final concentrations were 150 μM phosphatidyl ethanolamine, 50 μM [3H]phosphatidyl ethanolamine, 1.25 mM PLC-β, and 4 mM β1γ (wild type or mutant). Ca2+ was added to initiate the reaction. The reaction was performed at 30 °C for 30 min. The reaction was stopped with trichloroacetic acid with bovine serum albumin, and the radioactivity in the supernatant was measured. No more than 10–15% of the substrate, [3H]phosphatidyl ethanolamine, was used during the reactions. Purified recombinant PLC-β was a kind gift from Dr. A. Smrcka.

**Construction of γ5 Mutants**—The rat γ5 cDNA was mutated by replacing the 3′ end of the γ5 cDNA (beginning from a BsmI site at base 156) with a double-stranded DNA cassette encoding the scrambled sequence. Alanine substitutions and the deletion of 10 residues upstream of the Cys containing the pFastBac system (Invitrogen-Life Technologies, Inc.).

**RESULTS**

**Effect of γ5 Subunit Peptide on M2-dependent G Protein Activation**—A peptide specific to the C-terminal 14 residues of γ5 was geranylated (γ5pep-gg), and the ability of the peptide to compete with a G protein (αoβ1γ5) for interaction with M2 was examined. A reconstituted system containing purified M2 and G protein subunits was set up (described under “Materials and Methods” Fig. 1, A and B). Purified reconstituted M2 had a Kd for N-methyl-scopamine of 250 pm (12) and activated G0, in an agonist-(Fig. 1C) and βγ-dependent fashion (data not shown). M2-stimulated G0 activation was significantly inhibited by γ5pep-gg but not by a peptide with the same amino acids sequence scrambled, γ5pep-gg-scr (Fig. 1C). Because these peptides have no effect on the G protein heterotrimer (3), these results indicate that γ5pep-gg competes with the G protein for a site on the M2 receptor in a sequence-specific manner.

**Effect of Mutating the C-terminal Domain of the γ5 Subunit on Gγ Heterotrimer Activation by the M2 Receptor**—Competition of the γ5 peptide with the G protein indicated that the G protein γ5 C-terminal domain interacts with the M2 receptor. To obtain further evidence to support this mechanism, mutant forms of the γ subunit were synthesized with altered C-terminal sequences. If effective interaction of the γ5 subunit C terminus with the M2 receptor is a requirement for activation of a G protein, one or more of these mutant forms of γ5 were expected to alter the activation properties of the G protein. Three different mutants of the γ5 subunit were expressed and purified from insect cells in complex with the β1 subunit type (Fig. 2A). These mutants were as follows: (i) γ5sc: the last 13 residues upstream of the Cys in the CAAX box were scrambled identical to the sequence of γ5pep-gg-scr; (ii) γ5Δ: 10 residues upstream of the Cys residue were deleted; and (iii) γ5Ala: a short sequence of three residues, NPPR, which is conserved in all G protein γ subunits, was substituted with Ala residues.

His-tagged forms of γ5Ala and γ5Δ mutants were co-expressed with the β1 subunit and purified as β1γ5Δ and...
amino acid sequences at the C terminus of the mutant forms of the /H9252/H9253
activate PLC-
trations of /H9252/H9253
reaction is linear at this time point as in Fig. 1.

β1γ5Ala complexes. A wild type β1His-γ5 complex was also expressed and purified as a control. Gel exclusion chromatography indicated that the two mutants formed effective complexes with the β1 subunit. γ5WT, wild type; γ5scr, wild type amino acid sequence scrambled; γ5Ala, the wild type sequence NPFK has been substituted with Ala residues; γ5Δ, deletion of 10 residues upstream of the C-terminal Cys. B and C, M2-stimulated GTP-γS binding to αoβ1γ5 or αoβ1γ5scr. GTP-γS binding with 1 nm M2 and 100 nm αo was assayed for 2 min. The reaction is linear at this time point as in Fig. 1. B, at varying concentrations of βγ at 1 nm carbachol. C, at varying concentrations of carbachol at 7 mM βγ complex. The points represent the means ± S.E. (n = 3 in B and n = 2 in C).

β1γ5Ala mutants expressed as His and His tagged α2 subunits. The heterotrimer was bound to a nickel-nitrioltriacetic acid column. The β1γ5Ala complex was eluted using imidazole. As a control wild type β1γ5 was synthesized using a similar approach (Fig. 1B). The purified β1γ5scrs activated PLC-β3 and formed heterotrimers effectively similar to wild type β1γ5 (described below). All experiments were therefore performed with this mutant.

The mutant β1γ5scrs and wild type β1γ5 in complex with αo were first compared for their relative levels of activation by the M2 receptor. At a receptor to αo subunit ratio of (1:100), no significant difference in receptor-stimulated GTP-γS binding was detected between the wild type and mutant heterotrimers at various concentrations of the βγ complex (Fig. 2B). Assaying M2 activation of αoβ1γ5 and αoβ1γ5scrs at various concentrations of carbachol also did not indicate differential activation (Fig. 2C).

In contrast to M2-stimulated GTP-γS binding where each α subunit can bind utmost one molecule of GTP-γS, M2-stimulated GTPase activity can result in several cycles of activation of a G protein α subunit resulting in the formation of many molecules of P1 for each G protein. Because of this amplification, GTPase assays could be performed with significantly lower concentrations of the αo subunit compared with the GTP-γS binding assay, thus approaching a ratio of [receptor] to [G protein] of 1:1. Although the Kd for Gs binding to M2 is not known, the Kd for Gs binding to rhodopsin is 1 nM in the absence of nucleotide (13). The conditions in the GTPase assay were thus potentially closer to the Kd for Gs binding to M2 and likely to reveal differences between wild type and mutant β1γ5 complexes during M2 activation. To enhance sensitivity, we examined the receptor-stimulated GTPase activity in the presence of saturating concentrations of ROS protein, RGS4 (7). RGS4 acts as a GTPase-activating protein for the Gαi family (14). RGS4 potentiated M2-stimulated GTPase activity over 10-fold (7). When the time course of M2-stimulated GTPase activity was measured in the presence of RGS4, the M2 receptor activated αoβ1γ5scrs significantly more than the wild type (Fig. 3). In the absence of the agonist or the βγ complex, M2 did not effectively stimulate Gs GTPase activity (footnote a in Table 1). Gs containing the γ5scrs was also more active at several ratios of Gs/M2 (Table 1). Under these conditions the GTPase activity is a measure of the rate of receptor-stimulated nucleotide exchange (15). The results thus indicate that the mutant has a higher rate of receptor-stimulated nucleotide exchange compared with the wild type.

In the absence of the RGS protein, the mutant and wild type Gαis proteins still showed differential receptor-stimulated GTPase activities (Table 1). This result indicates that differential receptor-stimulated GTPase activities of αoβ1γ5scrs mutant and wild type are not due to differential effects of the RGS4 protein on receptor activation of the mutant and wild type.

Alternative explanations were excluded for this differential activity: (i) Both βγ complexes were not contaminated with α

![FIG. 2. Effect of γ subunit mutation on Gγ activation by M2. A, amino acid sequences at the C terminus of the mutant forms of the γ5 subunit. γ5WT, wild type; γ5scr, wild type amino acid sequence scrambled; γ5Ala, the wild type sequence NPFK has been substituted with Ala residues; γ5Δ, deletion of 10 residues upstream of the C-terminal Cys. B and C, M2-stimulated GTP-γS binding to αoβ1γ5 or αoβ1γ5scr. GTP-γS binding with 1 nm M2 and 100 nm αo was assayed for 2 min. The reaction is linear at this time point as in Fig. 1. B, at varying concentrations of βγ at 1 nm carbachol. C, at varying concentrations of carbachol at 7 mM βγ complex. The points represent the means ± S.E. (n = 3 in B and n = 2 in C).](image)

![FIG. 3. Time course of M2-stimulated GTPase activity with 1 nm M2 and different concentrations of αoβ1γ5 or αoβ1γ5scr. Activity in the presence of 100 nM RGS4 is shown. αo and βγ are equimolar. The points are the means ± S.E. from three independent experiments performed in duplicate. The differences in values are significant at p < 0.05. Moles of 32P produced during the reaction was 100–40% of total moles of GTP present. Nonspecific 32P was measured by the addition of 200 μM GTP to the reaction mix. This value was subtracted from the values obtained in experimental samples.](image)
subunit because M2 stimulated GTPase activity of the βγ complex alone was not detectable. (ii) The difference in activity was not due to variation in protein concentrations. When M2-stimulated GTPase activity was measured at various concentrations of β1γ5 (but constant concentrations of αo and M2), a 2–3-fold increase in βγ complex elicited the magnitude of increase in activity seen between the βγ mutant and wild type. Thus a 2–3-fold difference in concentration between β1γ5 and β1γ5scr must remain undetected. However, we could clearly detect 2-fold differences in the concentration of βγ subunits by densitometry of Coomassie Blue-stained proteins in SDS-PAGE gels (Fig. 1B). (iii) The difference was not due to differences in functional proportion of βγ complexes because in the GTPγS binding assay, a 2-fold difference in βγ concentration elicits an equivalent increase in GTPγS binding (Fig. 2A). (iv) Thin layer chromatography of βγ complex samples indicated that both samples contained the same concentrations of detergent. (v) Buffer components in the βγ complexes did not contribute to the differential activity because the addition of heat-denatured mutant sample to the wild type and vice versa had no effect. (vi) The differences in M2 activation of wild type and mutant αoβ1γ5 and αoβ1γ5scr also cannot be due to differences in the proportion of prenylated γ subunit because the βγ5 and βγ5scr proteins were purified using a His-tagged αi subunit. Prenylation is essential for βγ complex interaction with the α subunit. (vii) New stocks of βγ complexes that were independently expressed, purified, and assayed again showed the same difference in M2-stimulated αo GTPase activity.

Wild Type and Mutant β1γ5 Have Similar Affinities for αo—To examine the affinities of β1γ5 and β1γ5scr for αo in the 1–10 nM concentration range used in the GTPase assays, we used a recently developed assay (12) that relies on the overlap in binding sites for α subunits and PLC-β2/3 on the βγ complex. Thus binding of αo to the βγ complex inhibits PLC-β3 stimulation by the βγ complex. There was no significant difference in the inhibition of β1γ5 and β1γ5scr, indicating that αo affinity for both is the same (Fig. 4). This result also further confirmed that both the wild type and mutant γ subunits are prenylated to the same extent because prenylation is essential for βγ complex activation of PLC-β. The difference in GTPase activity between mutant and wild type thus arises from differential receptor interaction.

The results from the experiment above (Fig. 1C), where γ5 peptide interaction with M2 was tested, indicated that the γ5 scrambled peptide does not effectively interact with the receptor. An earlier report also indicated that in contrast to the wild type, the scrambled γ5 peptide had little effect on a muscarinic receptor modulation of a Ca2+ current or on a the muscarinic receptor modulation of an excitatory postsynaptic current (3). Viewed in this context, the higher M2-stimulated GTPase activity in the αoβ1γ5scr mutant indicates that weak interaction of the mutant γ subunit C terminus with M2 leads to a higher nucleotide exchange rate.

The receptor-G protein activation cycle involves three broad steps: (i) G protein binding to receptor; (ii) receptor-initiated nucleotide exchange in the G protein and dissociation of the ternary complex; and (iii) deactivation of Go by RGS protein and reassociation with the βγ complex. The difference in receptor-stimulated GTPase rates between the Go wild type and mutant must arise at one of these steps. We infer that differences between mutant and wild type proteins occurs during receptor-initiated nucleotide exchange in the G protein. Other steps in the receptor-G protein activation cycle cannot be affected because the results presented indicate that heterotrimer formation of the mutant is unaltered and that RGS4 has no influence on the differential activation of the mutant. The affected step cannot be initial binding of G protein with receptor because this would lead to lower GTPase rates in the mutant compared with wild type.

A model that explains the results here must take into account the effect of the γ subunit mutation on receptor-stimulated nucleotide exchange. It should also take into account (i) the inability of the scrambled γ5 peptide to interact with the receptor and (ii) the higher receptor-stimulated GTPase in mutant Go containing γ5scr.

The crystal structure of the G protein heterotrimer (G3) when compared with the structures of Got bound to GTP or GDP indicates that the domains on the α subunit that undergo the most significant changes in conformation during activation are the same domains that contact the β subunit (4). It was suggested based on this that the βγ complex occludes nucleotide release from the α subunit when the G protein is bound to the receptor (4). Receptor-stimulated nucleotide exchange will

### Table I

| Molar ratio of G protein/M2 | Turnover numbers  
|---------------------------|-------------------|-------------------|
|                           | +RGS4             | −RGS4             |
|                           | WT  
|                           | SCR  
|                           | WT  
|                           | SCR  
| 0.3                       | 0.44 ± 0.09 c    | ND                | ND                |
| 0.6                       | 0.67 ± 0.11      | 1.45 ± 0.34      | 0.13 ± 0.02      | 0.27 ± 0.02      |
| 1                         | 0.73 ± 0.09      | 1.50 ± 0.26      | 0.18 ± 0.02      | 0.31 ± 0.02      |
| 2                         | 1.34 ± 0.16      | 2.56 ± 0.20      | 0.18 ± 0.01      | 0.28 ± 0.02      |
| 4                         | 1.67 ± 0.19      | 2.88 ± 0.52      | 0.25 ± 0.02      | 0.33 ± 0.03      |

* M2-stimulated GTPase activity was measured at the following time points for various molar ratios: 0.3 and 0.6, 20 min; 1 and 2, 10 min; 3–5 min in the presence of 1 mM. In all cases reaction rates were linear at these time points. The turnover numbers in the absence of agonist were 0.03–0.06 at all molar ratios tested both in the presence and the absence of RGS4.

* WT, αoβ1γ5; SCR, αoβ1γ5scr.

* Means ± S.E. from at least four independent experiments performed in duplicate. All differences are significant at p < 0.05 except the reaction without RGS4 at a molar ratio of 4.
its muscarinic receptor-stimulated signaling (3), the M2 muscarinic receptor-stimulated signaling (3), the M2 muscarinic receptor (3) to propose that the M2 muscarinic receptor interacts with the G protein. Crystal structures of the heterotrimeric G protein and active a subunits indicate that guanine nucleotide release requires the βγ complex to move away from the α subunit (4). Any orientation of the βγ complex that results in exposing the bound GDP will therefore result in more rapid nucleotide release. If the γ subunit does not effectively interact with the receptor and anchor the βγ complex, the βγ complex may be inappropriately oriented with reference to the α subunit. The inappropriately oriented βγ complex will enhance nucleotide exchange by exposing the bound nucleotide in the α subunit. Fig. 5B shows one such potential orientation of a βγ complex where the γ subunit C terminus does not effectively anchor the βγ complex by interacting with the receptor. As shown in Fig. 5B, nucleotide exchange in the α subunit will be facilitated by this orientation of the βγ complex.

This model predicts that a mutant γ subunit that interacts weakly with a receptor will encourage higher nucleotide exchange in the associated α subunit compared with a wild type γ subunit that interacts strongly with the receptor. This prediction is borne out by the results presented here. Peptide evidence indicates that the γ5scr mutant interacts poorly with the receptor compared with the wild type. However, Gα containing the γ5scr mutant shows a higher rate of receptor-stimulated nucleotide exchange compared with the wild type. The model also predicts that a γ subunit type with a lower affinity for the receptor will allow higher rates of receptor-stimulated nucleotide exchange in the associated α subunit compared with a different γ subunit type with a higher affinity for the receptor. This prediction is also supported by previous results. Only the C-terminal peptide specific to γ5 but not γ7 disrupts muscarinic receptor regulation of Ca2+ current in neurons (3). This result indicated that the γ5 subunit type but not γ7 interacts with the M2/M4 receptor types. However, αoβ1γ7 shows significantly higher M2-stimulated nucleotide exchange compared with αoβ1γ5 (12). Thus, consistent with the results obtained with the γ5scr mutant, a G protein containing a γ subunit type that does not interact effectively with a receptor shows enhanced receptor-stimulated GTPase activity in comparison with the wild type.

Although αoβ1γ5sc contains a mutant γ subunit that does not effectively interact with the receptor, it still possesses a higher receptor-stimulated nucleotide exchange compared with wild type. This result does not imply that a model for receptor activation of a G protein that invokes receptor loops contacting the α and γ subunit C termini is correct. It does suggest that mutational approaches with purified proteins may not provide direct evidence for such a model because any mutant that disturbs the orientation of the βγ complex with reference to the α subunit can potentially encourage nucleotide exchange.

As mentioned before, there is evidence for the α subunit N and C termini interacting with the receptor. Inspection of the crystal structure for the heterotrimeric G protein indicates that the α subunit N terminus, C terminus and the γ subunit C terminus lie roughly along the same axis and can be oriented toward the plane of the membrane (Fig. 5). The distance between the C termini of α and γ subunits cannot be estimated precisely in the crystal structure because at least seven residues at the α subunit C terminus are not resolved, and the γ subunit is devoid of the prenyl moiety as well as three residues

![Fig. 5. Model of G protein interaction with receptor. A. The γ subunit C terminus (γ C terminus) interacts with the receptor (gray arrow) allows the βγ complex to orient itself appropriately with reference to the α subunit. Receptor-initiated nucleotide exchange from the α subunit (GDP and GTP arrows) is regulated by the appropriate orientation of the βγ complex with reference to the α subunit. α subunit C terminus (α C terminus) contact with the receptor is denoted with a gray arrow. The space-filling model of GDP bound to the α subunit is shown. B. Inefficient interaction of the γ subunit C terminus with the receptor affects orientation of the βγ complex with reference to the α subunit and increases the rate of nucleotide exchange during activation (gray arrows). G protein structure is from Lambright et al. (4).](http://www.jbc.org/)

Therefore, the peptide requires the receptor to shift the βγ complex away from the α subunit (Fig. 5A). This was later detailed as a model for receptor activation of a G protein (16). In this model it was proposed that two mechanisms account for the ability of the receptor to stimulate nucleotide exchange in the G protein α subunit: (i) the α subunit C terminus contacts a receptor, and receptor activation leads to conformational changes being triggered through the C-terminal domain to the β/a5 loop of the α subunit, which is in contact with GDP; (ii) receptor loop(s) enter the cavity between the α subunit and βγ complex, prising them apart and creating an opening through which GDP leaks out. It was proposed in this model that only the α subunit C terminus contacts the receptor and that the C terminus of the γ subunit interacts with membranes, despite evidence to the contrary (6, 17). Recently, this model has been modified to include the evidence indicating γ subunit interaction with a receptor (3, 6, 12, 17) to propose that the γ subunit C terminus also interacts with the receptor and that the receptor loops, instead of entering the cavity between the α subunit and βγ complex, actually prise the subunits apart through their interaction with the α and γ subunit C termini (18). This model is now similar to our earlier proposal that interaction with the α subunit and γ subunit C termini with a receptor is a requirement for G protein activation (2, 12). Such a model would predict that mutating the γ subunit C terminus would result in weaker activation of the G protein by the receptor. However, earlier results indicate that this may be a simplistic expectation. Although a γ5 but not a γ7 subunit-specific peptide inhibits muscarinic receptor-stimulated signaling (3), the M2 muscarinic receptor stimulates higher nucleotide exchange in Gα containing γ7 compared with γ5 (12). These results suggested that contrary to expectations, poor interaction of the γ subunit with a receptor can result in more robust G protein activation. This result is not surprising if potential mechanisms underlying nucleotide exchange are considered based on the crystal structures of a G protein. Crystal structures of the heterotrimeric G protein and active α subunits indicate that guanine nucleotide release requires the βγ complex to move away from the α subunit (4). Any orientation of the βγ complex that results in exposing the bound GDP will therefore result in more rapid nucleotide release. If the γ subunit does not effectively interact with the receptor and anchor the βγ complex, the βγ complex may be inappropriately oriented with reference to the α subunit. The inappropriately oriented βγ complex will enhance nucleotide exchange by exposing the bound nucleotide in the α subunit. Fig. 5B shows one such potential orientation of a βγ complex where the γ subunit C terminus does not effectively anchor the βγ complex by interacting with the receptor. As shown in Fig. 5B, nucleotide exchange in the α subunit will be facilitated by this orientation of the βγ complex.
The NMR structure of an 11-amino acid peptide specific to the α subunit C terminus indicates that this domain forms a constrained structure in the presence of a receptor (19). It is thus likely that the distance between the γ subunit C terminus that includes the prenyl group and the C terminus of the α subunit is less than 40 Å. The crystal structure of inactive rhodopsin that has been determined recently indicates that the intracellular portions of rhodopsin are folded such that the longest distance across the intracellular domains is more than 40 Å (20). This indicates that the exposed surface of the receptor will be sufficient for both the γ subunit C termini to contact the receptor simultaneously. However, this may not be a requirement if the two domains contact the receptor in a temporal sequence. We propose that by interacting with the receptor, the γ subunit appropriately positions the βγ complex with reference to the α subunit. This can allow the receptor to regulate nucleotide exchange at a site in the α subunit that does not contact the receptor directly.

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