Genetic Analysis of Receptor-Gαq Coupling Selectivity*

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Many different G protein-linked receptors are preferentially coupled to G proteins of the Gαq/11 family. To elucidate the molecular basis underlying this selectivity, different Gαq/11-coupled receptors (m3 muscarinic, V1a vasopressin, and gastrin-releasing peptide receptor) were coexpressed (in COS-7 cells) with mutant αq subunits in which residues present at the C terminus of αq were replaced with the corresponding αq/11 residues. Remarkably, whereas none of the receptors was able to interact with wild type αq to a significant extent, all three receptors gained the ability to productively couple to a mutant αq subunit containing a single Glu → Asn point mutation at position -3. Moreover, the m3 muscarinic and the V1a vasopressin receptors but not the GRP receptor also gained the ability to interact with a mutant αq subunit containing a single Gln → Glu point mutation at position -5, indicating that the αq/11 residues present in these mutant G protein constructs play key roles in determining the selectivity of receptor recognition.

To identify the site(s) on Gαq/11-coupled receptors that can functionally interact with the C terminus of αq/11 subunits, we next analyzed the ability of a series of hybrid m2/m3 muscarinic receptors to interact with a mutant αq subunit (sq5) in which the last five amino acids of αq were replaced with the corresponding αq/11 sequence. Similar to the wild type m2 and m3 muscarinic receptors, none of the investigated hybrid receptors was able to efficiently interact with wild type αq. Interestingly, however, three mutant m2 receptors in which different segments of the second and third intracellular loops were replaced with the corresponding m3 receptor sequences were identified, which, in contrast to the Gαq-coupled wild type m2 receptor, gained the ability to efficiently activate the sq5 subunit. This observation suggests that multiple intracellular receptor domains form a binding pocket for the C terminus of G protein αq/11 subunits.

Upon binding of extracellular ligands, G protein-coupled receptors (GPCRs) undergo conformational changes that enable the receptor proteins to interact with specific classes of heterotrimeric G proteins consisting of α, β, and γ subunits (1–7). The activated G protein subunits (α-GTP and/or free βγ) are then able to bind to and modulate the activity of downstream effector enzymes and/or ion channels. Typically, an individual GPCR can interact with only a distinct subset of the many structurally similar G proteins that are expressed within a cell (1–7). How this selectivity is achieved at a molecular level is not well understood at present, particularly since the molecular architecture of the receptor-G protein complex has not been elucidated.

Numerous mutagenesis and biochemical studies (1–7) have shown that multiple intracellular GPCR domains, primarily including the second intracellular loop (i2) as well as the N- and C-terminal portions of the third intracellular loop (i3), play key roles in determining selective G protein recognition. Specific residues contained within these domains are predicted to form a binding pocket for the G protein heterotrimer (7), in analogy to the ligand binding domain present on the extracellular surface of GPCRs (1, 2, 4, 6).

Accumulating evidence (8–11) suggests that the C-terminal portion of the G protein α subunits, via binding to the intracellular receptor surface (12, 13), is of fundamental importance for dictating the specificity of receptor-G protein interactions. Several recent studies (8–11) have shown, for example, that substitution of αq or αo residues into the C-terminal segment of αq subunits results in mutant G proteins that can be activated by G1α- or G4α-coupled receptors, respectively. By taking advantage of this observation, the C-terminal amino acids in αo subunits that are critical for proper receptor recognition have been mapped in great detail (11). In contrast, the functional roles of individual amino acids present at the C termini of other classes of Gα subunits have not been studied systematically to date.

To identify the site(s) on GPCRs that can functionally interact with the C terminus of αo subunits, we have recently used a novel experimental strategy involving the coexpression of hybrid receptors with hybrid Gaα subunits. We initially showed that the wild type m2 muscarinic receptor, a prototypical Gαo-coupled receptor (14–16), does not efficiently interact with wild type αq but can productively couple to mutant αo subunits in which the last five amino acids of αo were replaced with the corresponding αq or αo sequences (9). We then demonstrated, by analyzing a large number of mutant m2 receptors, that the ability of the m2 receptor to interact with such hybrid αq subunits was critically dependent on the structural integrity of a four-amino acid motif (VTIL; Val186, Thr186, Ile186, and Leu186) predicted to be located at the C terminus of the i3 loop (9, 11). In addition, gain-of-function studies showed that substitution of the VTIL motif into mutant m3 muscarinic receptors (which were unable to couple to wild type αq) could confer onto these receptors the ability to efficiently couple to mutant αo containing αq or αo sequences at their C terminus (9). These data therefore suggested that the VTIL motif can functionally interact with the C terminus of αo subunits and that this contact is intimately involved in determining coupling selectivity and triggering G protein activation.

To investigate whether the results obtained with the Gαo−
couples m2 muscarinic receptor are generally applicable, we recently have extended these studies to other functional classes of GPCRs and Go subunits. In the present study, we first wanted to examine which specific amino acids present at the C terminus of αq/11 subunits are of particular importance for selective receptor recognition. Toward this goal, we systematically substituted distinct αq/11 residues into the C terminus of αs (a G protein subunit that mediates the activation of adenylyl cyclase) and studied whether the resulting mutant α subunits gained the ability to be activated by different G α/11-coupled receptors. Remarkably, two αq/11 residues were identified which, when substituted into wild type αs, resulted in αs single point mutants that, in contrast to wild type αs, could be recognized by Gq/11-coupled receptors.

The second goal of this study was to identify the site(s) on Gq/11-coupled receptors that can functionally interact with the C terminus of αq/11 subunits. To address this issue, we took advantage of the finding that the Gqα-coupled m2 muscarinic receptor, in contrast to the Gqα/11-coupled m3 muscarinic receptor (15, 16), cannot functionally interact with a mutant αs subunit containing αs sequence at its C terminus. Based on this observation, mutant m2 muscarinic receptors in which distinct intracellular domains were replaced with the corresponding m3 receptor sequences were studied for their ability to gain coupling to this mutant αs subunit. These coexpression experiments led to the novel finding that multiple m3 receptor regions, including residues within the i2 loop and the N- and C-terminal portions of the i3 domain, are critical for selective recognition of the C terminus of αq/11 subunits.

**EXPERIMENTAL PROCEDURES**

**DNA Constructs**—A rat αs cDNA (17) cloned into the pcDNA expression vector (10) was used as a template for PCR mutagenesis. All wild type and mutant Go/11 subunits contained an internal hemagglutinin epitope tag (DVPDYAS; Refs. 18 and 19). The presence of the epitope tag that replaced αs residues 76–82 did not affect the receptor and effector coupling properties of wild type αs (18, 19). The construction of sq5 (which codes for a mutant αs subunit in which the last five amino acids of αs were replaced with the corresponding αs sequence) has been described previously (10). To introduce mutations into the C-terminal segment of αs, a 42-base pair NsiI-XhoI fragment was removed from the wild type plasmid and replaced with PCR fragments containing the desired mutations. The correctness of all PCR-derived sequences was verified by dideoxy sequencing of the mutant plasmids (20).

The construction of the hybrid m2/m3 muscarinic receptors used in this study has been described previously (Refs. 21–23; for precise amino acid compositions see legend to Fig. 4).

**Cell Culture and Transfection Conditions**—COS-7 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum at 37 °C in a humidified 5% CO2 incubator. For transfections, 1 × 106 cells were seeded into 100-mm dishes. About 24 h later, COS-7 cells were cotransfected with the indicated G protein and receptor constructs (0.8 μg of DNA each) by using a DEAE-dextran procedure (24). To reduce the expression levels of the different receptor and G protein constructs, transfection mixtures were supplemented with 2.4 μg of pcDNA vector DNA. The following wild type receptor expression plasmids were used: human m2 muscarinic receptor in pcD (25), rat m3 muscarinic receptor in pcD (25), mouse gastrin-releasing peptide (GRP) receptor (26) in pcDNA3 (kindly provided by J. Battey, NIH), and rat V1a vasopressin receptor (27) in pcD-SP6/T7 (kindly provided by M. Brownstein, NIH). cAMP Assays—Approximately 20–24 h after transfections, cells were transferred into six-well plates, and 2 μCi/ml [3H]adenine (15 Ci/mmol; American Radiolabeled Chemicals) was added to the growth medium. After a 24–36 h labeling period, cells were preincubated in Hanks’ balanced salt solution containing 20 mM Hepes and 1 mM 3-isobutyl-1-methylxanthine for 20 min (room temperature) and then stimulated with the appropriate agonist ligands for 30 min at 37 °C. The reaction was terminated by aspiration of the medium and addition of 1 ml of ice-cold 5% trichloroacetic acid containing 1 mM ATP and 1 mM CAMP. Increases in intracellular [3H]CAMP levels were then determined by anion-exchange chromatography as described (28).

**Radioligand Binding Assays**—N-[3H]methylscopolamine (75.9 Ci/mmol; NEN Life Science Products) saturation binding experiments were carried out with membrane homogenates prepared from transfected COS-7 cells essentially as described (29). Nonspecific binding was determined in the presence of 1 μM atropine. Protein concentrations were determined by the method of Bradford (30). Binding data were analyzed by using the computer program Ligand (21).

**Western Blotting**—All wild type and mutant Goαq subunits were detected using the 12CA5 monoclonal antibody (Boehringer Mannheim) directed against the hemagglutinin epitope tag present in all G protein constructs. Samples containing 20 μg of membrane protein prepared from transfected COS-7 cells were resolved by SDS-polyacrylamide gel electrophoresis (10%), electroblotted onto nitrocellulose, and probed with the 12CA5 antibody as described (9). Immunoreactive proteins were detected by incubation with horseradish peroxidase-conjugated sheep anti-mouse antibody (Amersham Life Science, Inc.) and visualized using an enhanced chemiluminescence system (Amersham).

**Drugs**—[Arg5]Vasopressin was purchased from Sigma. All other ligands used in this study were obtained through Research Biochemicals Inc.

### RESULTS

**Functional Interaction of Gq/11-coupled Receptors with Mutant αs Subunits**—All studies were carried out with COS-7 cells cotransfected with different GPCRs and Go constructs. Initially, the ability of the rat m3 muscarinic receptor (25), a prototypical Gq/11-coupled receptor (15, 16), to interact with wild type αs (s(wt)) was examined. As expected, based on its known G protein coupling preference, the m3 muscarinic receptor, when cotransfected with s(wt) (or vector DNA as a control) and stimulated with the agonist carbachol (500 μM), did not efficiently stimulate cAMP production. The maximum increase in cAMP accumulation (above basal levels) mediated by the m3 receptor in the absence or presence of cotransfected s(wt) amounted to only 1.5–2-fold (see Fig. 2A). However, coexpression of the m3 muscarinic receptor with a mutant αs subunit in which the last five amino acids (QYELV) were replaced with the corresponding αs sequence (EYNLV; resulting in the hybrid α subunit, sq5) led to a pronounced stimulation (6–8-fold) of adenylyl cyclase activity (see Fig. 2A). Essentially similar results were obtained with two other Gq/11-coupled receptors, the rat V1a vasopressin (27) and the mouse GRP receptor (26). Like the m3 receptor, these two peptide receptors, when activated by the appropriate agonist ligands ([Arg5]vasopressin (1 μM) and bombesin (1 μM)), did not couple efficiently to s(wt) (see Figs. 2, B and C). In contrast, both peptide receptors were able to productively interact with sq5, mediating 4–8-fold increases in cAMP levels in the presence of this G protein subunit (see Figs. 2, B and C).

We next wanted to examine which specific amino acids within the C-terminal segment of αq/11 are of particular importance for proper receptor recognition. Toward this goal, specific residues at the C terminus of s(wt) were replaced either alone or in combination with the corresponding αq/11 residues (Fig. 1). The ability of different Gq/11-coupled receptors to gain coupling to these mutant αs subunits was then examined in cotransfected COS-7 cells.

As shown in Fig. 1, the C-terminal five amino acids of αs differ in only three residues from the corresponding αq/11 sequence (the leucine residue at position –2 is conserved among all mammalian α subunits, and a tyrosine residue is present at position –4 in both αs and αq/11). Therefore, three αs single point mutants, s(Q → E), s(E → N), and s(EL → NV), and all three possible αs double point mutants, s(QE → EN), s(EL → EV), and s(QL → EV), were prepared (Figs. 1 and 2). Western analysis using a monoclonal antibody directed against the hemagglutinin epitope tag present in all G protein constructs (18, 19) showed that all mutant αs subunits were expressed at similar levels as s(wt) and sq5 (Fig. 3).

Initially, the ability of the m3 muscarinic receptor to functionally interact with the different mutant αs subunits was...
examined (Fig. 2A). Remarkably, the m3 receptor gained the ability to activate two αi single point mutants, s(QE → EN), and s(QL → EV), were constructed and functionally analyzed. When coexpressed with the m3 muscarinic receptor, s(QE → EN) was able to mediate a marked increase in adenylyl cyclase activity (4-5-fold, as compared with an 6-8-fold increase observed with sq5) (Fig. 2A). In contrast, coexpression of the m3 muscarinic receptor with s(L → V) did not result in a CaMP response that was significantly different from background (as determined in cells transfected with either vector DNA or s(wt)) (Fig. 2A).

To examine whether the functional effects caused by the single amino acid substitutions were additive, three αi double point mutants, s(QE → EN), s(EL → NV), and s(QL → EV), were constructed and functionally analyzed. When coexpressed with the m3 muscarinic receptor, s(QE → EN) was able to mediate a CaMP response that was similar in magnitude to that observed with sq5 (Fig. 2A), indicating that the functional effects of the Q → E and E → N point mutations were additive. In contrast, the s(EL → NV) and s(QL → EV) double point mutants showed functional responses similar to those found with s(E → N) and s(Q → E), respectively, suggesting that the C-terminal amino acid of αq11 is not critical for receptor recognition.

To study whether the results obtained with the m3 muscarinic receptor are also applicable to other Gq11-coupled receptors, the different Gαq constructs were also coexpressed with the V1a vasopressin receptor. As shown in Fig. 2B, the V1a vasopressin receptor showed a functional profile that was very similar to that found with the m3 muscarinic receptor. In contrast, the activity pattern seen with the GRP peptide receptor differed somewhat from that found with the two other receptors. Similar to the m3 muscarinic and V1a vasopressin receptors, the GRP receptor gained the ability to activate s(E → N) (as well as the s(QE → EN) and s(EL → NV) double point mutants) and did not efficiently couple to s(L → V) (Fig. 2C). However, in contrast to the two other receptors examined in this study, the GRP receptor did not interact to a significant extent with the s(QE → E) single point and the s(QL → EV) double point mutants (Fig. 2C).

Identification of Receptor Sites Critical for the Recognition of the C-terminus of αq11—The second major goal of this study was to identify the site(s) on Gq11-coupled receptors that can functionally interact with the C terminus of αq11 subunits. To address this question, we employed a gain-of-function mutagenesis approach, analyzing the ability of a series of hybrid m2/m3 muscarinic receptors (Fig. 4) to interact with the mutant αq subunit, sq5, in which the last five amino acids of αq were replaced with the corresponding αq11 sequence. As shown in Fig. 5, the m2 muscarinic receptor, a prototypical Gq11-coupled receptor, did not activate either s(wt) or sq5 to a significant extent. We thus hypothesized that introduction into the m2 receptor of the m3 receptor domain(s) capable of recognizing the C terminus of αq should enable the resulting hybrid receptors to interact with the sq5 subunit. The m2-i2, m2-Ni3, m2-Y, and m2-AALS hybrid receptors (Fig. 4) were included in this analysis because previous studies had shown that the m3 receptor regions/residues contained in these constructs are critical for efficient recognition of Gq11 proteins (22, 23, 32, 33) (m2-tail was included as a negative control; see Refs. 21 and 23). N-[3H]Methylscopolamine saturation binding studies showed that all hybrid receptors were expressed at levels similar to those found with the m2 and m3 wild type receptors (for Bmax values, see legend to Fig. 5).

Interestingly, three of the investigated hybrid receptors, m2-i2, m2-Ni3, and m2-AALS, gained the ability to productively interact with the sq5 mutant subunit, mediating 4–6-fold increases in CaMP production (Fig. 5). In contrast, none of these hybrid receptors was able to efficiently interact with s(wt). In these constructs, the i2 loop, the first 21 amino acids of the i3 domain, and a four-amino acid motif (AALS; Ala488, Ala489, Leu492, and Ser493) at the C terminus of the i3 loop were derived from the m3 muscarinic receptor. The m2-Y mutant receptor (which contains a Ser493 → Tyr point mutation at the N terminus of the i3 loop) and the m2-tail construct (in which the last 66 amino acids of the m2 receptor were replaced with the corresponding m3 receptor sequence) showed a functional profile similar to that of the wild type m2 muscarinic receptor.

**DISCUSSION**

The α subunits of heterotrimeric G proteins are known to play central roles in receptor-G protein and G protein-effector interactions (3, 5). Whereas more centrally located loop and helical domains of Ga are known to be involved in effector binding, considerable evidence suggests that the C-terminal segment of Go can directly contact the receptor protein (3, 5). Consistent with this notion, high resolution crystal structures (34, 35) suggest that the C terminus of Ga subunits is surface-exposed and thus easily accessible for interactions with the receptor. In addition, several recent studies (8–11) show that the C-terminal portion of Ga subunits also plays a key role in dictating the specificity of receptor-G protein coupling. It could be demonstrated (8, 9) for example that mutant versions of αq a subunit that can activate various isoforms of phospholipase C-β in which the last five amino acids of αq replaced with the corresponding αq11 sequences allowed stimulation of phospholipase C-β by receptors (A1 adenosine, D2 dopamine, and m2 muscarinic receptors) that otherwise are exclusively coupled to G proteins of the Gq class which cannot activate phospholipase C-β efficiently. Subsequently, by studying the ability of the Gq11-coupled m2 muscarinic receptor to activate mutant αq subunits containing single C-terminal αq residues, the functional roles of individual amino acids present at the C terminus of αq subunits have been defined (11).

Whereas the structural characteristics of the C-terminal domain of subunits of the αq11 protein family (including α-transducin) have been analyzed in great detail (36–39), little is known about the functional roles of the corresponding amino acids present in other classes of Go subunits. One goal of the present study therefore was to examine which specific amino acids present at the extreme C terminus of G proteins of the
family are involved in proper receptor recognition. To address this question, we employed a gain-of-function mutagenesis approach involving the coexpression of different Gq/11-coupled receptors with mutant αs subunits containing distinct αq/11 residues at their C termini. Consistent with the results of a previous study (10), we initially demonstrated that the m3 muscarinic, the V1a vasopressin, and the GRP receptors (which are all prototypical Gq/11-coupled receptors) do not couple efficiently to wild type αs but can productively interact with a mutant αs subunit (sq5) in which the last five amino acids of αs were replaced with the corresponding αq/11 sequence.

Since the C-terminal five amino acids of αs differ in only three residues from the corresponding αq/11 sequence (Fig. 1), three αs single point mutants (s(Q3E), s(E3N), and s(L3V)) were prepared and functionally analyzed. Whereas none of the tested Gq/11-coupled receptors was able to activate the s(L3V) subunit, all of them gained the ability to productively interact with the s(E3N) single point mutant. Two of the receptors (m3 muscarinic and V1a vasopressin) were also capable of activating the s(Q3E) subunit. These two receptors were able to interact with the s(QE3EN) double point mutant with greater efficacy than observed with the s(Q3E) and s(E3N) single point mutants, mimicking quantitatively the functional activity of the sq5 subunit. Taken together, these gain-of-function experiments strongly suggest that two C-terminal αq/11 residues, an asparagine at position 23 and a glutamate at position 25, play key roles in determining the receptor selectivity of G protein αq/11 subunits. Consistent with this notion,
these two residues are found, with no exception, in all members of the αq/11 protein family (αq, α11, α14, α15, and α16; Fig. 1).

Interestingly, molecular genetic and biochemical studies (11, 36–38) show that the C-terminal residues of subunits of the αi10 protein family, which dictate the specificity of receptor recognition, are present at positions −4 (cysteine), −3 (glycine), and −1 (phenylalanine/tyrosine) (Fig. 1). Thus, the precise positions of the C-terminal amino acids that are critical for determining the specificity of receptor-G protein interactions differ between different functional classes of Ga subunits. However, in both αi10 and αq/11 subunits, the residue present at the −3 position is of critical importance for receptor-G protein coupling selectivity. As shown in Fig. 1, the residues present at this position are perfectly conserved within individual Ga subfamilies and can correctly predict the coupling profile of a given Ga subunit. It is therefore likely that the corresponding residues in αi and α12/13 (glutamate and methionine, respectively) have a similar functional importance as the glycine and asparagine residues present at −3 position of αi10 and αq/11 subunits, respectively. This, however, remains to be tested experimentally.

In the crystal structures of two G protein heterotrimeric, the C-terminal segment of Gaα was disordered and not visible (34, 35). However, NMR studies on a C-terminal α-transducin peptide suggest that the last four amino acids of αi10 subunits form a type II’ β-turn (which depends on the presence of the conserved glycine residue at −3 position) that is broken upon interaction with the ligand-occupied receptor (39). Dratz et al. (39) therefore speculated that different types of β-turn at the extreme C terminus of Gaα subunits are critical for determining the selectivity of receptor recognition. It is possible that the asparagine side chain present at −3 position in αi10 subunits is able to hydrogen bond back to the main chain, thus forming a type VIII β-turn characterized by a spatial arrangement of the C-terminal amino acids which differs from that found with αi10 subunits (39).

To gain deeper insight into the molecular mechanisms governing receptor-G protein interactions and G protein activation, the receptor site that interacts with the extreme C terminus of Gaα needs to be identified. By using a coexpression strategy analogous to that described here, we could previously show that the C terminus of αi10 subunits can contact a four-amino acid motif on the m2 muscarinic receptor (VTIL; Val385, Thr386, Ile387, and Leu388) predicted to be located at the C terminus of the i3 loop (9, 11). Since these residues are located in a region that is thought to be α-helically arranged (23, 40–42), the VTIL motif is predicted to form a hydrophilic surface that is critical for G protein recognition.

To examine whether this receptor-Gα contact site is also conserved among other functional classes of GPCRs and G proteins, the second major goal of the present study was to map the site(s) on Gα/i/o coupled receptors that is (are) recognized by the C terminus of αi10 subunits. We initially showed that neither the wild type m2 nor the wild type m3 muscarinic receptor, consistent with their known G protein coupling preference for Gaα and Gaq/11 subunits, respectively, can efficiently confer on the resulting hybrid receptor(s) the ability to activate sq5. Whereas the m3 muscarinic receptor of the m3 receptor region(s) (Figs. 4 and 5), in which the VTL motif was replaced with the corresponding m3 receptor residues (Ala388, Ala389, Leu392, and Ser393, respectively) gained the ability to functionally interact with the sq5 subunit. This observation suggests that the VTL motif and sq5 subunits should confer on the resulting hybrid receptor(s) the ability to activate sq5. Consistent with this concept, we found that a mutant m2 muscarinic receptor, m2-AALS (Figs. 4 and 5), in which the m2-AALS construct was not the only mutant receptor capable of activating sq5. Two additional m2 mutant receptors, m2-i2 and m2-Ni3 (Figs. 4 and 5), in which the residues present at the −3 position of m2-wt are replaced with alanine, glutamic acid, and serine, gained the ability to functionally interact with the sq5 subunit. These results are consistent with the idea that the VTL motif is critical for G protein selectivity.

To better understand the role of the VTL motif in G protein activation, we next sought to determine the contribution of the C-terminal amino acids of the m2 muscarinic receptor to G protein selectivity.

Fig. 4. Structure of chimeric m2/m3 muscarinic receptors used in this study. The following m2 receptor sequences (human) were replaced with the homologous segments of the m3 muscarinic receptor (rat): m2-i2 (m2303–329 → m3364–383), m2-Ni3 (m2308–322 → m3293–307), m2-Y (m2310–331 → m3324–345), m2-AALS (m2316–322 → m3324–336), and m2-tail (m2341–466 → m3350–369).
Fig. 6. Model of the intracellular m3 muscarinic receptor surface depicting residues critical for selective recognition of Gq11 proteins. The view is from the intracellular side of the membrane. The position and relative orientation of the seven transmembrane helices (I–VII) is based on the model proposed by Baldwin (43). The highlighted amino acids are predicted to determine the G protein coupling selectivity of the m3 muscarinic receptor (22, 23, 32, 33). Numbers refer to amino acid positions in the rat m3 muscarinic receptor sequence (25). For clarity, only the membrane-proximal portions of the i3 loop that are thought to form α-helical extensions of transmembrane helices V and VI (23, 40–42) are depicted. The highlighted residues at the N and C terminus of the i3 loop are predicted to form two hydrophobic patches, which, together with several hydrophilic residues in the i2 loop, are critical for proper G protein recognition (modified according to Ref. 7).

4, 5), were identified that showed a functional profile very similar to that of m2/AALS. In these two mutant receptors, the i2 loop and the N-terminal portion of the i3 loop, respectively, were replaced with the corresponding m3 receptor sequences. Previous studies have shown that these two domains, together with the AALS motif present at the C terminus of the i3 loop, are critical for selective recognition of Gq11 proteins by the m3 muscarinic receptor (23, 33).

One possible explanation for the observed ability of multiple m2/m3 hybrid receptors to activate the chimeric αq/α1 subunit, sq5, is that the C terminus of αq1/D subunits can be contacted by multiple m3 receptor regions. Based on detailed site-directed mutagenesis studies (33) and several theoretical considerations (43), we recently proposed a model of the intracellular m3 receptor surface in which the functionally critical receptor residues present in the i2 loop and at the N and C terminus of the i3 domain all project into the interior of the receptor protein, thus forming a well-defined G protein binding pocket (Fig. 6), analogous to the ligand binding site present on the extracellular receptor surface. It is therefore conceivable that the C-terminal portion of the Gq subunits, by binding to this intracellular receptor “cavity,” can interact with multiple receptor sites that lie adjacent to each other in the properly folded receptor protein.

As indicated above, we previously identified only one major receptor contact site for the C terminus of α1/D subunits by using a coexpression strategy analogous to that described here (9). One possible reason for the discrepancy between this finding and the results of the present study is that the relative functional importance of the i2 loop and the N- and C-terminal segments of the i3 domain may vary among different receptor-Gq pairs. Alternatively, it is possible that the assay system employed in this study was more sensitive than the one used previously. In the present study, neither of the two wild type nor the different m2/m3 hybrid receptors were able to couple to wild type αq, thus allowing for a straightforward interpretation of the coexpression experiments involving the chimeric Gα subunit, sq5. In contrast, the interpretation of our previous mutagenesis data was complicated by the fact that several of the analyzed m2/m3 hybrid receptors gained some degree of coupling to wild type αq (which served as template for introducing C-terminal α1 residues) and were therefore able to activate phospholipase C-β even in the absence of coexpressed hybrid αq/α1 subunits (9).

The conclusion drawn from these gain-of-function mutagenesis experiments that the C terminus of Gα subunits can be contacted by several different receptor sites is also supported by a recent loss-of-function study (44) examining the ability of an 11-amino acid peptide derived from the C terminus of α-transducin to bind to wild type and mutant versions of rhodopsin. This peptide, via direct binding to rhodopsin (12, 39), can stabilize the active metarhodopsin II state and can uncouple rhodopsin-transducin interactions. However, these effects were no longer observed when the peptide was incubated with mutant rhodopsins containing multiple mutations in the i2 loop and at the C terminus of the i3 domain, suggesting that the C terminus of α-transducin is recognized by at least two different receptor sites (44).

Although the present study again highlights the key role of the C terminus of Gα subunits in proper receptor recognition, considerable evidence suggests that other domains on the Gα subunits as well as the G protein βγ complex can also modulate the selectivity of receptor-G protein interactions, most likely by directly contacting the receptor protein (5, 45). The GPCR sites involved in these interactions remain to be identified.

In conclusion, we have shown for the first time that the coupling specificity of Gαq can be changed by single amino acid substitutions (α1 → αq), leading to the identification of two C-terminal αq1/D subunits that are critical for proper receptor recognition. Moreover, our gain-of-function mutagenesis data predict that multiple intracellular receptor domains form a binding pocket for the C-terminal segment of αq1/D subunits. By using a coexpression strategy similar to that described here, it should be possible to map other functionally relevant receptor-G protein contact sites. The information gathered from such studies should eventually lead to a refined model of the receptor-G protein interface.

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REFERENCES

1. Kobilka, B. (1992) Annu. Rev. Neurosci. 15, 87–114
2. Savarese, T. M., and Fraser, C. M. (1992) Biochem. J. 283, 1–19
3. Conklin, B. R., and Bourne, H. R. (1993) Mol. Pharmacol. 43, 1164–1168
4. Strader C. D., Fong, T. M., Tota, M. R., Underwood, D., and Dixon, R. A. F. (1994) Annu. Rev. Biochem. 63, 101–132
5. Rens-Domiano, S., and Hamm, H. (1995) FASEB J. 9, 1059–1066
6. Dohlman, H. G., Thorner, J., Caron, M. G., and Lefkowitz, R. J. (1991) Annu. Rev. Biochem. 60, 653–688
7. Wess, J. (1997) FASEB J. 11, 346–354
8. Conklin, B. R., Farfel, Z., Lustig, K. D., Julius, D., and Bourne, H. R. (1993) Nature 363, 274–276
9. Liu, J., Conklin, B. R., Blin, N., Yun, J., and Wess, J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 11643–11646
10. Conklin, B. R., Herzmark, P., Ishida, S., Vosnya-Yasenetskaya, T. A., Sun, Y., Farfel, Z., and Bourne, H. R. (1996) Mol. Pharmacol. 50, 885–890
11. Katzenis, E., Conklin, B. R., and Wess, J. (1997) Biochemistry 36, 1487–1495
12. Hamm, H. E., Deretic, D., Arendt, A., Hargrave, P. A., Koenig, B., and Hofmann, K. P. (1988) Science 241, 832–835
13. Rasenick, M. M., Watanabe, M., Lazarevic, M. B., Hatta, S., and Hamm, H. E. (1994) J. Biol. Chem. 269, 21519–21525
14. Parker, E. M., Kameyama, K., Higashijima, T., and Ross, E. M. (1991) J. Biol. Chem. 266, 519–527
15. Peraldi, E. G., Ashkenazi, A., Winslow, J. W., Ramachandran, J., and Capon, D. J. (1988) Nature 334, 434–437
16. Offermanns, S., Wieland, T., Homann, D., Sandmann, J., Bombien, E., Spiecher, K., Schulz, G., and Jakobs, K. H. (1994) Mol. Pharmacol. 45, 890–898
17. Jones, D. T., and Reed, R. (1987) J. Biol. Chem. 262, 14241–14249
18. Levits, M. J., and Bourne, H. R. (1992) J. Cell Biol. 119, 1297–1307
19. Wedegaertner, P. B., and Bourne, H. R. (1994) Cell 77, 1063–1070
20. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467
21. Wess, J., Bonner, T. I., and Brann, M. R. (1990) Mol. Pharmacol. 38, 872–877
22. Blu¨ml, K., Mutschler, E., and Wess, J. (1994) J. Biol. Chem. 269, 402–405
23. Blin, N., Yun, J., and Wess, J. (1995) J. Biol. Chem. 270, 17741–17748
24. Cullen, B. R. (1987) Methods Enzymol. 152, 684–704
25. Bonner, T. I., Buckley, N. J., Young, A. C., and Brann, M. R. (1987) Science 237, 527–532
26. Battey, J. F., Way, J. M., Corjay, M. H., Shapira, H., Kusano, K., Harkins, R., Wu, J. M., Slattery, T., Mann, E., and Feldman, R. I. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 395–399
27. Morel, A., O’Carroll, A.-M., Brownstein, M. J., and Lolait, S. J. (1992) Nature 356, 523–526
28. Salomon, Y., Londos, C., and Rodbell, M. (1974) Anal. Biochem. 58, 541–548
29. Dorje, F., Wess, J., Lambrecht, G., Tacke, R., Mutschler, E., and Brann, M. R. (1991) J. Pharmacol. Exp. Ther. 256, 727–733
30. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
31. Munson, P. J., and Rodbard, D. (1980) Anal. Biochem. 107, 220–239
32. Blu¨ml, K., Mutschler, E., and Wess, J. (1995) J. Biol. Chem. 270, 11537–11541
33. Wess, J. (1996) Crit. Rev. Neurobiol. 10, 69–89
34. Wall, M. A., Coleman, D. E., Lee, E., Inguez-Lluhi, J. A., Posner, B. A., Gilman, A. G., and Sprang, S. R. (1995) Cell 83, 1047–1058
35. Lambricht, D. G., Sondek, J., Bohm, A., Skiba, N. P., Hamm, H. E., and Sigler, P. B. (1996) Nature 379, 311–319
36. Garcia, P. D., Onrust, R., Bell, S. M., Sakmar, T. P., and Bourne, H. R. (1995) EMBO J. 14, 4460–4469
37. Martin, E. L., Rens-Domiano, S., Schatz, P. J., and Hamm, H. E. (1995) J. Biol. Chem. 271, 361–366
38. Osawa, S., and Weiss, E. R. (1995) J. Biol. Chem. 270, 31052–31058
39. Dratz, E. A., Furetenau, J. E., Lambert, C. G., Thireault, D. L., Rarick, H., Schepers, T., Pakhlevaranants, S., and Hamm, H. E. (1993) Nature 363, 276–281
40. Strader, C. D., Sigal, I. S., and Dixon, R. A. F. (1989) FASEB J. 3, 1825–1832
41. Burstein, E. S., Spalding, T. A., Hill-Eubanks, D., and Brann, M. R. (1995) J. Biol. Chem. 270, 3141–3146
42. Altenbach, C., Yang, K., Farrrens, D. L., Khorana, H. G., and Hubbell, W. L. (1996) Biochemistry 35, 12470–12478
43. Baldwin, J. M. (1993) EMBO J. 12, 1693–1703
44. Acharya, S., Saad, Y., and Karnik, S. S. (1997) J. Biol. Chem. 272, 6519–6524
45. Bourne, H. R. (1987) Curr. Opin. Cell Biol. 9, 134–142
46. Watson, S., and Arkininstall, S. (1994) in The G Protein-linked Receptor Facts Book (Watson, S., and Arkininstall, S., eds) pp. 1–291, Academic Press, Ltd., London
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