Extreme C Terminus of G Protein α-Subunits Contains a Site That Discriminates between G\textsubscript{i}-coupled Metabotropic Glutamate Receptors*

(Received for publication, May 12, 1998, and in revised form, July 9, 1998)

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Metabotropic glutamate receptors (mGlu receptors), the Ca\textsuperscript{2+}-sensing receptor, γ-aminobutyric acid type B receptors, and one group of pheromone receptors constitute a unique family (also called family 3) of heptahelical receptors. This original family shares no sequence similarity with any other G protein-coupled receptors. The identification and comparison of the molecular determinants of receptor/G protein coupling within the different receptor families may help identify general rules involved in this protein/protein interaction. In order to detect possible contact sites important for coupling selectivity between family 3 receptors and the G protein α-subunits, we examined the coupling of the cyclase-inhibiting mGlu2 and mGlu4 receptors to chimeric α\textsubscript{q}-subunits bearing the 5 extreme C-terminal amino acid residues of either G\textsubscript{ai}, G\textsubscript{ao}, or G\textsubscript{aq}. Whereas mGlu4 receptor activated all three chimeric G proteins, mGlu2 receptor activated G\textsubscript{ai} and G\textsubscript{aq} but not G\textsubscript{ao}. The mutation of isoleucine −4 of G\textsubscript{ao} into cysteine was sufficient to recover coupling of the mutant G protein to mGlu2 receptor. Moreover, the mutation of cysteine −4 of G\textsubscript{ao} into isoleucine was sufficient to suppress the coupling to mGlu2 receptor. Mutations at positions −5 and −1 had an effect on coupling efficiency, but not selectivity. Our results emphasize the importance of the residue −4 of the α-subunits in their specific interaction to heptahelical receptors by extending this finding on the third family of G protein-coupled receptors.

Transduction of extracellular signals to intracellular responses via G protein-coupled receptors (GPCRs)\textsuperscript{1} includes activation of the receptor and subsequent regulation of effectors via trimeric G proteins. A variety of heterotrimeric G proteins have different target proteins (1). Accordingly, the intracellular response depends on the G protein subtypes (among those located in the proximity of the receptor) that can be activated by the receptor (selectivity). Identification of the molecular basis of receptor/G protein coupling selectivity is therefore of interest since this determines the transduction mechanism of these receptors.

Three major families of GPCRs can be defined, and the members of each family share no sequence similarity with receptors from the other families. Receptors homologous to rhodopsin (receptors for catecholamines, acetylcholine, certain peptides, and glycoproteins) constitute the first family (family 1) which is to date the best characterized one. Family 2 receptors are those homologous to the vasoactive intestinal peptide and the glucagon receptors. Family 3 receptors comprise the metabotropic glutamate receptors (mGlu receptors) (2), the γ-aminobutyric acid, type B receptors (3), the Ca\textsuperscript{2+}-sensing receptor (4), and a recently discovered new family of putative pheromone receptors (5, 6). The unique feature of family 3 GPCRs is a large extracellular N terminus that forms the ligand binding site (7, 8). Common to all three families are some structural elements including seven transmembrane domains so that all GPCRs are also called heptahelical receptors.

Molecular determinants involved in the coupling between family 1 GPCRs and G proteins have been studied (9–11). Specific motifs in the third intracellular loop (in most cases) and the second intracellular loop (in some cases) of these receptors have been shown to determine which G protein subtypes will be activated. Although the receptors probably contact both the α-subunit, and the βy dimer of the G protein (12–14), the direct interaction with the α subunit is presumed to play a critical role in the coupling specificity. On the G protein α-subunit several elements have been shown to determine specificity of coupling to family 1 receptors (9, 10, 15–20). Among these, the extreme C terminus was shown to play a critical role by interacting with certain motifs within a cavity formed by the second and third intracellular loops of the receptor (9, 11, 20–23).

Comparison of the molecular determinants of receptor/G protein coupling between members of the different GPCR families should help to establish some general principles for this interaction. Accordingly, we recently started to identify the structural determinants of G protein coupling in mGlu receptors that are used here as representatives of the family 3 GPCRs. Three groups of mGlu receptors have been defined based on their sequence similarity and G protein-coupling selectivity (2, 24). In these receptors, the first and third intracellular loops (i1 and i3) are short with low variability. Major sequence differences are found in the second intracellular loop (i2). The highly variable intracellular C termini play a role in G protein interaction but probably not in a discriminatory manner (25–28).
Was it shown that the C terminus of the G protein α-subunit plays a critical role in the recognition of the Gq/G11-coupled groups II and III mGlu receptors (29, 30). The aim of the present study was to identify the important residues in the C-terminal end of the G protein α-subunit controlling the selectivity.

**EXPERIMENTAL PROCEDURES**

**Materials—**Chemicals including glutamate were obtained from Sigma (L’Isle d’Abeau, France) unless otherwise indicated. Serum, culture media, and other solutions used for cell culture were from Life Technologies, Inc. (Cergy Pontoise, France). The following mGlu receptors and G proteins were detected using primary monoclonal antibodies against MRGS-His6 epitope (Qiagen, Paris, France) or hemagglutinin epitope (generous gift of Dr. B. Mouillac, Montpellier, France), respectively. ECL chemiluminescence system was used to stain the secondary antibodies (Amersham Life Sciences, Paris, France).

**RESULTS**

**Differential Coupling of Groups II and III mGlu Receptors to Phospholipase C via Chimeric Ga Proteins**—Group II (mGlu2 and mGlu3 receptors) and group III mGlu receptors (mGlu4, 6, 7, and 8 receptors) inhibit adenyl cyclase activity in transfected cells via pertussis toxin-sensitive G proteins and are not capable of stimulating IP formation (2, 29, 30). Their coupling to chimeric G protein α-subunits, that correspond to Goq with the 5 C-terminal residues of Gαq (Gαq5), Gαz (Gαqz), or Gαz (Gαqz) (34), can therefore be easily determined by measuring their capability of stimulating IP formation upon activation with glutamate (29). We previously reported that both group II and group III mGlu receptors coupled to Gαq and Gαz but that Gαq was activated by group II receptors only (29, 30). By using hemagglutinin-tagged G protein α-subunits (21, 35) and receptors tagged with the MRGS-His6 sequence at their C terminus, similar data were obtained (Fig. 1, a and b, Table 1). The group II mGlu2 receptor stimulated phospholipase C when co-expressed with Gαq or Gαz but not when expressed alone or with Gαz or Gαq, even though these G proteins were expressed at very similar levels (Fig. 2). Like mGlu2 receptor, the group III mGlu4 receptor activated Gαz and Gαqz, but not Gαq (Fig. 1, c and d). Only mGlu4, but not mGlu2, activated Gαz (Fig. 1, c and d), even though these two receptors were expressed at comparable levels (Fig. 2). These data indicate that the differential coupling of mGlu2 and mGlu4 receptors to Gαqz is not due to a difference in the level of expression of either the receptor or the G protein. This raises the question of the sequence element and amino acid residues responsible for this coupling selectivity.

**Isoleucine -4 in the C Terminus of Gaq Prevents Coupling to the mGlu2 Receptor**—As shown in Fig. 3, the Ga chimera used in our study vary in a span of 5–extreme C-terminal amino acid residues, among which three differ between Gαq and Gαz (at positions –1, –4, and –5). All the mutant G proteins were expressed at a similar level in transfected HEK 293 cells (Fig. 2).

Reciprocal exchange of the residue at position –5 or –1 between Gαq and Gαqz did not change their coupling specificity toward mGlu2 and mGlu4 receptors (Fig. 4, a and c). In contrast, swapping the residue at position –4 between Gαq and Gαqz mutants Gaqz-Cl and GαqzIC where isoleucine replaces cysteine and vice versa, respectively) resulted in the exchange of coupling properties of the G proteins toward the mGlu2 receptor (Fig. 4, a and b). The mGlu2 receptor activated GαqzIC upon activation with glutamate in a concentration-dependent manner, but not GαqzCl (Fig. 4b). As expected, both are stimulated by mGlu4 receptor (Fig. 4, c and d). The potency of glutamate in stimulating IP formation was determined in cells expressing mGlu2 or mGlu4 receptors with any of the mutated
null
in independent experiments performed in triplicate.

Values correspond to the glutamate-induced IP formation expressed as the percentage of the maximum response measured in cells expressing mGlu2 receptor and Goq,IC. In b, values correspond to the glutamate-induced IP formation expressed as the percentage of the maximum. Values are means ± S.E. of four to eight (a and b) and four (c and d) independent experiments performed in triplicate.

**FIG. 4.** Isoleucine –4 in Goq,a prevents coupling to mGlu2 receptor. Basal (2) and 1 mM glutamate-induced (2) IP formations were determined in HEK 293 cells co-expressing the mGlu2 (a) or mGlu4 (c) receptors with the G protein α,β subunit chimeras Goq,a, Goq,c, and their mutants. In b, cells co-expressing mGlu2 receptors with reciprocal mutants at position –4 GoqCI (O) or GoqIC (■) were stimulated with increasing concentrations of glutamate. d, same as in b, with the mGlu4 receptor. In a and c, values correspond to the radioactivity in the IP fraction divided by the total radioactivity in the membranes. In b, values correspond to the glutamate-induced IP formation expressed as the percentage of the maximum response measured in cells expressing mGlu2 receptor and Goq,IC. In d, values correspond to the glutamate-induced IP formation expressed as the percentage of the maximum. Values are means ± S.E. of four to eight (a and b) and four (c and d) independent experiments performed in triplicate.

The present data extend our knowledge on the importance of the last few C-terminal amino acid residues of the G protein α-subunit in the specific interaction with heptahelical receptors, including the metabotropic glutamate receptors from family 3 GPCRs. Usage of chimeric G protein α-subunits that do not differ in other portions of the proteins than the extreme C terminus allowed us to study specifically the relevance of this region. We show that a single residue (at position –4) on the extreme C terminus is crucial for chimeras of Goq,a proteins to distinguish group II from group III mGlu receptors. Residues at position –5 and at position –1 do not control specificity, but still play a role in coupling efficiency to mGlu receptors.

Several studies using different approaches indicated that the extreme C-terminal part of the G protein α-subunit has a critical role in specifying interactions with family 1 GPCRs (for reviews, see Refs. 9–11). These include the coupling of chimeric Ga-proteins and receptors (15, 21, 34, 36, 37), alamine scanning mutagenesis (20), functional competition with the α-subunit C-terminal-derived peptides (23, 38), direct interaction of such C-terminal peptides with receptors (16, 39), or functional inhibition with antibodies directed against the C terminus of the α-subunit (for reviews, see Refs. 1 and 10). The present study extends our knowledge of the role of this portion of the α-subunit in the specific interaction with family 1 GPCRs. Moreover, our data indicate that a single amino acid residue within this sequence is sufficient to cause discrimination between group II and group III mGlu receptors. Cysteine at position –4 is compatible with a coupling of Goq,a and Goq,c to either mGlu2 or mGlu4 receptors, whereas in Goq4_iso,oleucine at position –4 is compatible with a coupling to mGlu4 receptor, but prevents coupling to the mGlu2 receptor. In agreement with our observation, post-translational modifications of residue –4 has been shown to play an important role in the interaction with family 1 GPCRs. In Goq,a and Goq,c, this position corresponds to the cysteine residue that is ADP-ribosylated by pertussis toxin, which prevents coupling of these G proteins to their receptors (for reviews, see Refs. 1 and 10). In Goq,a and Goq,c, tyrosine –4 has to be phosphorylated to allow coupling to family 1 as well as to the mGlu1a receptor from family 3 GPCRs (40). Residues at positions –4 and –3 in Goq have been reported to play an important role in discriminating between Goq,a and Goq,c-coupled receptors (15). Moreover, residues at positions –3 and –5 in Goq,a chimeras play a key role in coupling to muscarinic and vasopressin receptors and that a residue at position –5 discriminates between these two receptors from family 1 GPCRs (37). The three-dimensional structure of a peptide corresponding to the C-terminal end of transducin bound to rhodopsin was determined by nuclear magnetic resonance (16). This study revealed the glycine residue at position –3 which is conserved in Goq,a, Goq,c, and Goq,a isoleucine at position –1 which favors the contact between the receptor and the side chain of the –4 residue.

It is interesting to note that in Goq15, which is activated by both group II and group III mGlu receptors (29, 30), there is an isoleucine at position –4, as in Goq4. However, the residue at position –3 in Goq15 is not a glycine, but an asparagine. This possibly changes the flexibility in this area and thus neutralizes the decisive discriminatory effect of the –4 residue. Taken together, these observations are consistent with the same sequence and/or structural element of the G protein α-subunit C-terminal end for the specific interaction with either family 1 or family 3 receptors.

Such similarity in the molecular determinants of the G protein α-subunit involved in the specific recognition of family 1 and family 3 GPCRs was not expected. The C terminus of the G protein α-subunit is likely to fit into a cavity formed by the second and the third intracellular loops of family 1 GPCRs (9, 11). The third intracellular loop of these family 1 receptors is the longest and often plays a discriminatory role toward the Ga protein, while the second contains a highly conserved sequence (DRY) important for G protein activation (41). In contrast, the third intracellular loop of mGlu receptors is very short, highly conserved (10 conserved residues out of 13), and involved in G protein activation (42), whereas the second is longer (24–26 residues), more variable (only 5 are conserved in all mGlu receptors), and involved in G protein recognition (26, 28, 42). It would therefore be of interest to examine how family 1 and 3 GPCRs could recognize the same features of the C terminus of the G protein α-subunits with such different intracellular loops.

The C-terminal end of the α-subunit is only one of several areas that is likely to contact the receptor directly, these also include the N-terminal end and the o4–o6 loop (9, 10, 15–20). Accordingly, Goq5 and Goq5 which share identical C-terminal ends, but mostly differ in their o4–o6 loop, coupled differently to group II and group III mGlu receptors (29, 30). One may therefore propose that all portions of heterotrimeric G proteins that are in contact with the receptor influence the receptor interaction with (and activation of) Ga, and therefore the coupling has to be seen as an outcome of network coordination. Moreover the βγ dimer interaction with various α-subunits might play a role in the coupling efficiency as well (12–14). When interactions at certain contact points are weak, absent, or negative, coupling between the receptor and G protein can still be possible if this is overcome by a strong interaction at some other points, or when such regions that weaken coupling are removed either from the receptor or from the G protein (see, for example Refs. 27, 43, and 44).
Acknowledgments—We thank Drs. Annie Varault and Thierry Galvez for critical reading of the manuscript. Drs. B. Conklin (The Gladstone Institute, San-Francisco, CA) and S. Nakanishi (Kyoto University, Japan) are greatly acknowledged for the generous gift of the monoclonal anti-hemagglutinin epitope cDNAs, respectively. We thank Dr. B. Mouillac (CClPE, Montpellier, France) for the gift of the monoclonal anti-hemagglutinin epitope antibody.

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