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Two Amino Acids within the α4 Helix of Ga11 Mediate Coupling with 5-Hydroxytryptamine1B Receptors*

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We previously reported that residues 299–318 in Ga11 participate in the selective interaction between Ga11 and the 5-hydroxytryptamine1B (5-HT1B) receptor (Bae, H., Anderson, K., Flood, L. A., Skiba, N. P., Hamm, H. E., and Graber, S. G. (1997) J. Biol. Chem. 272, 32071–32077). The present study more precisely defines which residues within this domain are critical for 5-HT1B Receptor-mediated G protein activation. A series of Ga11/Ga11 chimeras and point mutations were reconstituted with Gβγ and Sf9 cell membranes containing the 5-HT1B receptor. Functional coupling to 5-HT1B receptors was assessed by 1) [35S]GTPγS binding and 2) agonist affinity shift assays. Replacement of the α4 helix of Ga11 (residues 299–308) with the corresponding sequence from Ga11 produced a chimera (Chi22) that only weakly coupled to the 5-HT1B receptor. In contrast, substitution of residues within the α4-β6 loop region of Ga11 (residues 309–318) with the corresponding sequence in Ga11, either permitted full 5-HT1B receptor coupling to the chimera (Chi24) or only minimally reduced coupling to the chimeric protein (Chi25). Two mutations within the α4 helix of Ga11 (Q304K and E308L) reduced agonist-stimulated [35S]GTPγS binding, and the effects of these mutations were additive. The opposite substitutions within Chi22 (K300Q and L304E) restored 5-HT1B receptor coupling, and again the effects of the two mutations were additive. Mutations of other residues within the α4 helix of Ga11 had minimal to no effect on 5-HT1B coupling behavior. These data provide evidence that α4 helix residues in Ga11 participate in directing specific receptor interactions and suggest that Gln304 and Glu308 of Ga11 act in concert to mediate the ability of the 5-HT1B receptor to couple specifically to inhibitory G proteins.

The interaction of heptahelical receptors with their cognate heterotrimeric guanine nucleotide-binding proteins (G proteins) represents an initial step in the transmission of extracellular signals across the plasma membrane (2–4). The receptor-G protein interaction modulates specific second messenger systems that result in a unique physiologic response to the extracellular signal. The particular downstream effect of G protein activation is not the result of an explicit interaction between each heptahelical receptor and a unique heterotrimeric G protein. On the contrary, G protein-coupled receptors have repeatedly been demonstrated to couple to several related members within the same family of G protein α subunits, albeit with differing levels of efficiency (5–11). Clawges et al. (12) demonstrated that the serotonin (5-HT)1B receptor couples to heterotrimers containing either Ga11, Ga21, Ga31, or Ga11. Nevertheless, this receptor does not couple to heterotrimers containing another member of this same family, the Ga subunit (12). Therefore, the 5-HT1B receptor represents one receptor system that can be exploited to investigate the precise molecular determinants governing selective receptor-G protein interactions.

Numerous biochemical studies have suggested that several subregions of Ga (13–21) in addition to regions on Gβ (20, 21) and Gγ (22–25) may act in concert to determine selective receptor-G protein interactions. The carboxyl-terminal domain of Ga subunits, in particular, has been demonstrated to play a key role in eliciting several specific receptor-G protein interactions. However, the carboxyl-terminal regions of Ga11, Ga21, Ga31, and Ga11 are highly homologous, and therefore, the carboxyl-terminal domain is not likely to be the primary determinant of 5-HT1B receptor-G protein selectivity between Ga11 and Ga11. The selectivity profile of the 5-HT1B receptor has facilitated the use of Ga11/Ga11 chimeras to map the residues that play a role in determining the specific interaction of the 5-HT1B receptor to inhibitory G proteins.

By using this approach, we previously demonstrated that substitution of the α4 helix and α4–β6 loop (amino acids 299–318) regions of Ga11 with the respective sequence from Ga11 markedly reduced the ability of this chimera to couple to the 5-HT1B receptor (1). These studies determined that the region corresponding to amino acids 299–318 in Ga11 plays a key role in determining the selective interaction between Ga11 and the 5-HT1B receptor (1). The intent of the present study was to define more precisely which amino acids within this domain are critical for selective 5-HT1B receptor coupling to inhibitory G proteins.

In addition to providing a useful model for receptor-G protein selectivity, the 5-HT1B receptor plays an important modulatory role in the central nervous system. 5-HT1B receptors are the primary terminal autoreceptors within the brain serotonin system (26). Activation of these receptors inhibits the release of

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1 The abbreviations used are: 5-HT, 5-hydroxytryptamine; GTPγS, guanosine 5′-3-O-(thio)triphosphate; AMP-PNP, adenosine 5′-(β,γ iminonitriphosphate; ANOVA, analysis of variance; CHAPS, 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonic acid.
5-HT into the synaptic cleft (27, 28). In addition, drugs that selectively interact with 5-HT<sub>1B</sub> receptors have proven to be clinically useful for the treatment of migraine headache (29). Thus, deducing the molecular events that are essential to 5-HT<sub>1B</sub> receptor-catalyzed G protein activation may aid our understanding of both normal and pathologic processes in brain serotonin systems.

By utilizing a series of Ga<sub>0</sub>/Ga<sub>a</sub> chimeras coupled with site-directed mutagenesis, the present study reveals that α4 helical residues Gln<sup>304</sup> and Glu<sup>308</sup> of Ga<sub>a</sub> are critical determinants of 5-HT<sub>1B</sub> receptor coupling to inhibitory G proteins. This conclusion is supported by the observed marked reduction in receptor-catalyzed GDP/GTP exchange on Ga<sub>a</sub> Q304K, E308L, and Q304K-E308L mutants. Moreover, whereas Gln<sup>304</sup> and Glu<sup>308</sup> are absolutely conserved among all Ga<sub>α</sub> subfamilies, they are divergent between Ga<sub>a</sub><sub>α</sub> and Ga<sub>α</sub> subunits. The crystal structure of Ga<sub>a</sub><sub>α</sub> reveals that Gln<sup>304</sup> and Glu<sup>308</sup> are surface-exposed (30, 31), and mutation of these residues (Q304K and E308L) may alter the surface potential of the α subunit. Hence, these residues may interact directly with the 5-HT<sub>1B</sub> receptor to mediate receptor coupling. Mutation of these residues may also indirectly influence the secondary structure of neighboring domains resulting in an inability of the 5-HT<sub>1B</sub> receptor to couple to the mutant inhibitory α subunits.

**EXPERIMENTAL PROCEDURES**

**Materials**—Nucleotides and enzymes were purchased either from Boehringer Mannheim or from Amersham Pharmacia Biotech. Serotonin was obtained from Sigma. [35S]GTP<sup>S</sup> (1250 Ci/mmol) and [3H]5-HT (22–30 Ci/mmol) were purchased from NEN Life Science Products. Boehringer Mannheim or from Amersham Pharmacia Biotech. Serotonin was obtained from Sigma. [35S]GTP<sup>S</sup> (1250 Ci/mmol) and [3H]5-HT (22–30 Ci/mmol) were purchased from NEN Life Science Products. By utilizing a series of G<sub>α</sub> chimera mutants, we previously demonstrated that G<sub>α</sub><sub>i1</sub> and G<sub>α</sub><sub>i3</sub> are selectively coupled with 5-HT<sub>1B</sub> receptors to a coupled, high affinity agonist state (12). Binding values were estimated by nonlinear regression analysis with GraphPad PRISM of the specific binding data using a fixed value (0.62 nM) for the high affinity <i>K<sub>i</sub></i> (12). For affinity shift assays, high affinity binding to 5-HT<sub>1B</sub> receptors was determined using a single concentration of [3H]5-HT near the <i>K<sub>i</sub></i> value for the radioligand (0.8–1.3 nM).

**RESULTS**

The ability of 5-HT<sub>1B</sub> receptors to couple selectively to heterotrimers containing members of the Ga<sub>α</sub><sub>α</sub> family of G proteins (12) facilitated the use of a series of Ga<sub>α</sub>/Ga<sub>α</sub> chimera mutants to determine which amino acids within G<sub>α</sub><sub>α</sub><sub>α</sub> subunits are critical for the activation of the 5-HT<sub>1B</sub> receptor. Using this approach, we previously demonstrated that Ga<sub>α2</sub> amino acid residues 290–318 (corresponding to the α4 helix and α4–α6 loop regions of Ga<sub>α2</sub>) play a major role in determining the selective interaction with the 5-HT<sub>1B</sub> receptor (1). Additional residues in the amino-terminal domain of Ga<sub>α1</sub>...
were also determined to play a secondary role in 5-HT₁B receptor coupling (1).

The α Helical Domain of Ga₁½ Mediates the Ability of the 5-HT₁B Receptor to Couple to Inhibitory G Proteins—The present study initially used the same approach described above to identify which subdomain within this stretch of amino acids (Ga₁ 299–318) is critical for functional 5-HT₁B receptor coupling to inhibitory G proteins. As shown schematically in Fig. 1, three chimeric Ga₅/Ga₁ proteins were generated in which either the α₁ helix (Chi22) or portions of the α₁–β₁ loop region (Chi24 and Chi25) of Ga₁ were replaced with the corresponding sequence from Ga₅. Prior to the assessment of coupling activity, the ability of each chimeric Ga subunit to bind GDP and undergo conformational change upon binding to GTP was tested by determining if the proteins undergo an AlF₄⁻ dependent conformational change (Table I). The increase in tryptophan fluorescence indicates that all constructs were capable of undergoing conformational change in the presence of AlF₄⁻. As full-length Ga₅, is not easily expressed or purified, this Ga₅/Ga₅ chimera was generated previously, crystallized, and shown to exhibit α₁-like coupling activity (32, 51).

Functional coupling between the 5-HT₁B receptor and the chimeric proteins was assessed by examining both the ability of the 5-HT receptor agonist, serotonin, to stimulate receptor-catalyzed GDP/GTP exchange on Ga₁ and Ga₅/Ga₁ chimeras. Membranes expressing the 5-HT₁B receptor were reconstituted with either 40 nM Ga₅ (A), Chi22 (B), Chi24 (C), or Chi25 (D) in the presence of 40 nM 5-HT. Data are expressed as the percentage of maximal GDP/GTP exchange binding obtained in the presence of excess (500 nM) light-activated rhodopsin. Squares indicate GTPγS binding in the presence of 1 µM 5-HT (added at the 8.5-min time point), and triangles indicate the binding in the absence of agonist. Data represent the mean ± S.E. from three independent experiments.

![Fig. 1. Secondary structure and AlF₄⁻-dependent tryptophan fluorescence change of Ga subunits. Numbers above the wild type forms of Ga₅ and Ga represent the corresponding residues in each respective α subunit. Numbers above chimeric structures indicate the junction points of Ga₅ and Ga₁ sequences and refer to amino acid positions in Ga₁. Diagram of secondary structural domains common to Ga subunits. SI, SII, and SIII refer to the switch regions of Ga. Percent increase in tryptophan fluorescence in the presence of 10 mM NaF and 20 µM AlCl₃ compared with basal (see “Experimental Procedures” for detail). The increase in tryptophan fluorescence indicates that all constructs were capable of undergoing conformational change in the presence of AlF₄⁻. As full-length Ga₅ is not easily expressed or purified, this Ga₅/Ga₅ chimera was generated previously, crystallized, and shown to exhibit α₁-like coupling activity (32, 51).

![Fig. 2. Time-dependent 5-HT₁B receptor-catalyzed GDP/GTP exchange on Ga₅ and Ga₅/Ga₁ chimeras. Membranes expressing the 5-HT₁B receptor were reconstituted with either 40 nM Ga₅ (A), Chi22 (B), Chi24 (C), or Chi25 (D) in the presence of 40 nM 5-HT. Data are expressed as the percentage of maximal GDP/GTP exchange binding obtained in the presence of excess (500 nM) light-activated rhodopsin. Squares indicate GTPγS binding in the presence of 1 µM 5-HT (added at the 8.5-min time point), and triangles indicate the binding in the absence of agonist. Data represent the mean ± S.E. from three independent experiments.](https://example.com/fig2.png)
Affinity shift data represent the means ± S.E. from 3 to 18 independent experiments. Initial rate data represent the means ± S.E. from 3 independent experiments. Affinity shift and initial rate data were analyzed separately using a one-way analysis of variance by followed by Newman Keuls' post hoc test.

**TABLE I**

| Gαi1 mutants | Affinity shifta | Initial rateb | Chimeras and Chii22 mutants | Affinity shiftc | Initial rated |
|---------------|----------------|--------------|-----------------------------|----------------|--------------|
| Gαi1          | 3.95 ± 0.20    | 870 ± 74.6   | Chi24                       | 3.63 ± 0.43    | 756 ± 53.5   |
| Gαi1-A301N    | 4.90 ± 0.32    | 977 ± 40.6   | Chi25                       | 3.06 ± 0.44    | 629 ± 41.5   |
| Gαi1-C305V    | 4.88 ± 0.14    | 846 ± 49.8   | Chi22                       | 1.86 ± 0.14    | 88 ± 7.3    |
| Gαi1-Q304K    | 4.51 ± 0.13    | 463 ± 32.7   | Chi22-N297A                 | 1.97 ± 0.16    | 40 ± 26.8   |
| Gαi1-Q304K-C305V | 4.03 ± 0.22 | 465 ± 22.4  | Chi22-V301C                 | 2.71 ± 0.01    | 67 ± 6.5    |
| Gαi1-E308L    | 3.45 ± 0.43    | 261 ± 18.8   | Chi22-K300Q                 | 2.65 ± 0.16    | 215 ± 17.6   |
| Gαi1-Q304K-E308L | 3.73 ± 0.43 | 261 ± 23.8   | Chi22-L304E                 | 3.19 ± 0.25    | 382 ± 33.5   |
| Gαi1-Q304K-E308L | 3.18 ± 0.38 | 140 ± 13.9   | Chi22-K300Q-L304E           | 4.51 ± 0.29    | 799 ± 35.2   |

*a* Affinity shift activities refer to the -fold enhancement above buffer controls of high affinity [3H]5-HT binding to 5-HT1B receptors reconstituted with G protein heterotrimers containing the indicated α subunits.

*b* Initial rates of GDP/GTP exchange were calculated from the binding curves (Fig. 5A and 6A) and expressed as pmol of GTPγS bound per mole of protein per s.

*c* Significantly different than corresponding Gαi1 value (p < 0.05).

*d* Chi22 mutants which are significantly greater than corresponding Chi22 value (p < 0.05).

*e* Significantly lower than Gαi1-Q304K initial rate value (p < 0.05).

*f* Significantly greater than Chi22-K300Q and Chi22-L304E (p < 0.05).

*g* Chi22 mutant with an affinity shift value which is significantly different than Chi22-K300Q and Chi22-V301C (p < 0.05).



**FIG. 3.** Affinity shift activity of Gαi1/Gαi2 chimeras with 5-HT1B receptors. Affinity shift activities refer to the -fold enhancement above buffer controls of high affinity [3H]5-HT binding to 5-HT1B receptors reconstituted with G protein heterotrimers containing the indicated α subunits. Data represent the mean ± S.E. from 5 to 9 independent determinations using three separate membrane preparations where 5-HT1B receptors were expressed between 5.2 and 11.7 pmol/mg membrane protein. Exogenous G proteins were 1.3-3.3 μM during reconstitution and 45-112 nM during the binding assays which was a 35-50-fold molar excess over receptors. The concentration of [3H]5-HT was 0.8-1.3 nM in all experiments. Data were analyzed using a one-way ANOVA followed by a Newman Keuls' post hoc test as described under "Experimental Procedures." *b*, significantly lower than Gαi1 (p < 0.05).



**FIG. 4.** Primary sequence alignment of the α4–α4/β6 loop region of bovine Gαi1 (residues 296-318) and Gαi6 (residues 292-314). Numbers immediately above the primary sequence of Gαi1 correspond to residues in Gαi6. Numbers immediately below the primary sequence of Gαi1 correspond to residues in Gαi6. The box indicates the region of Gαi1 that was substituted with the corresponding sequence from Gαi6 to generate Chii22. Boldface letters indicate residues within Gαi1 which diverge from Gαi6 residues. Single or double mutations of Gαi1 or Chii22 are listed below the sequence alignment.

and Chii22-K300Q-L304E) demonstrated varying degrees of receptor-catalyzed GDP/GTP exchange (Fig. 5A and Table I) and affinity shift activity (Fig. 5B and Table I). Similar to Chii22, Chii22-N297A exhibited only very weak coupling to the 5-HT1B receptor as exemplified both by the low levels of agonist-stimulated GTPγS binding (Fig. 5A, Table I) and by the low affinity shift activity of the mutant chimeras (Fig. 5B). Mutation of valine 301 to cysteine in Chii22 clearly failed to elevate the initial rate of GTPγS binding above Chii22 (Table I and Fig. 5A). Likewise, the affinity shift activity of this mutant did not significantly differ from Chii22. Mutants Chii22-K300Q and Chii22-L304E exhibited significant (p < 0.05) 2-4-fold increases in agonist-stimulated GDP/GTP exchange in comparison to the parent Chii22 (Table I). Consistent with these data, enhanced 5-HT1B receptor coupling was also observed with Chii22-L304E, as measured by a significant 72% increase in the affinity shift activity over Chii22 (Fig. 5B). Although Chii22-K300Q caused a 42% increase in affinity shift activity over that observed for Chii22, this increase did not reach statistical significance. The critical nature of residues Gln304 and Glu308 is supported by the observation that the double mutant Chii22-K300Q-L304E completely restored GDP/GTP exchange and affinity shift activity to wild type Gαi1 levels (Fig. 5 and Table I).

To confirm and further support the contention that Gαi1 residues Gln304 and Glu308 are particularly important determinants of 5-HT1B receptor coupling, single or double mutations were constructed in Gαi1 (A301N, Q304K, C305V, and E308L). The aim of these experiments was to determine whether coupling to the 5-HT1B receptor could be reduced or eliminated by replacing these specific Gαi1 residues with the corresponding residues from Gαi6. As illustrated in Fig. 6A and Table I, the single amino acid substitution Gαi1-E308L markedly (−70%) and significantly (p < 0.05) reduced agonist-mediated stimulation of GTPγS binding to the mutant α subunit. Likewise, mutation Gαi1-Q304K resulted in a moderate (−40%; p < 0.05) reduction in agonist-mediated GTPγS binding in comparison to recombinant Gαi1. Consistent with these data, the double mutant Gαi1-Q304K-E308L exhibited an even greater reduction in agonist-mediated GTPγS binding than either single mutation alone (Fig. 6A and Table I). Gαi1-C305V did not significantly alter coupling to the 5-HT1B receptor as evidenced by the lack of effect on agonist-stimulated GDP/GTP exchange with this mutant (Table I). Quite unexpectedly, Gαi1-A301N resulted in a small (+12%) but statistically significant (p < 0.05) increase in the initial rate of GDP/GTP exchange in comparison to Gαi1 (Table I). In contrast to the marked reductions in [35S]GTPγS binding (Fig. 5A and Table I). Gαi1 and Gαi6 mutants Affinity shift values which are significantly different than Gαi1-K300Q and Gαi1-V301C (p < 0.05).
binding observed with Gα11 mutants E308L, Q304K, and Q304K-E308L, no significant differences in affinity shift activities were observed between these mutants and Gα1 (Table I and Fig. 6B). Affinity shift activity also did not significantly vary between Gα11 and Gα11-A301N, C305V, or C305V/E308L (Table I).

This lack of correspondence between affinity shift activity and [35S]GTPγS binding data for the Gα11 mutants is likely to result from differences in the sensitivity of these assays stemming from technical aspects involved in these measures. For example, the initial exchange rates (Table I) are determined from linear regression analysis of the [35S]GTPγS binding data generated over the course of 10 min following the introduction of agonist to the assay (see Figs. 5A and 6A) with saturation of [35S]GTPγS binding occurring by 30 min (Fig. 2A). In contrast, as is required for radioligand binding assays, the affinity of [3H]5-HT for the 5-HT1B receptor is determined only after equilibrium is reached (i.e. following a 1.5-h incubation with the agonist) and in the absence of GTP. Therefore, if GDP release is impaired in the Gα11 mutants (as would be suggested by alterations in [35S]GTPγS binding) but not prevented, sufficient GDP could be released over the course of the experiment (1.5 h) such that at equilibrium the amount of high affinity receptors present in the preparation is similar for both Gα11 and the Gα11 mutants. Alternatively, the divergence between affinity shift activity and [35S]GTPγS binding for the Gα11 mutants may be indicative of a change in GTPγS binding in the absence of a change in GDP release. This situation could only arise if GTPγS binding (rather than GDP release) has become the rate-limiting step as a result of the Gα11 mutants and their Gα11 subunits.

Nonetheless, despite the variations between the affinity shift data and the GDP/GTP exchange rates for the Gα11 mutants, the affinity shift activity appeared to show overall trends in the same direction as the [35S]GTPγS binding data. We, therefore, determined whether there was a correlation between affinity shift activity and the initial rates of GDP/GTP exchange as measured by [35S]GTPγS binding. Fig. 7 illustrates the comparative comparison between these two data sets. Correlation analysis yielded a Pearson correlation coefficient of 0.80, indicating a significant correlation between the GDP/GTP exchange rate and the affinity shift activity.
forms a hydrogen bond with both the side chain carboxyl groups of Glu308 and with the y hydroxyl group of Thr321. In contrast, G0 residue Lys300 forms a van der Waals interaction with the δ carbon on Leu304. Substitution of Ga residues Glu304 and Glu308 with the corresponding amino acids from G8 (Lys300 and Leu304) results in the loss of strong side chain interactions (compare Ga and G0 contacts in Fig. 10B). This suggests that these mutations might weaken the interaction of the a helical domain with the b6 strand resulting in an a subunit structure that is less responsive to 5-HT1B receptor-mediated conformational change.

DISCUSSION

By using Ga/Ga chimeras and site-directed mutagenesis, the present study determined that two residues (Gln304 and Glu308) within the a helical domain of Ga1 are required for 5-HT1B receptor coupling to Ga1. These results are consistent with our previous work implicating the a helix and a4—b6 loop region of Ga1 in 5-HT1B receptor coupling to inhibitory G proteins. Taken together, these studies provide evidence for a previously unappreciated role for the a helix of a subunits in directing G protein-coupled receptor interactions.

Previously published work has shown that there are several receptor-binding regions present in heterotrimeric G proteins. The primary receptor recognition region is believed to be localized to the carboxyl-terminal domain of Ga subunits (13–18), although at least three other regions in Ga are involved in receptor interaction: the amino-terminal domain (18, 20, 41); the a2 helix and a2-b4 loop regions (16, 19); and the a helix and a4-b6 loop domain (1, 16, 42). In addition, segments of the b and y subunits may contribute to the receptor interacting surface of heterotrimers (20–25). Whether individual G protein-coupled receptors interact simultaneously with several regions on heterotrimers and/or whether the profile of physical contacts for a particular receptor may direct more subtle features of specificity such as the efficiency of receptor coupling remains to be determined.

Other studies implicating the a helix and/or a4—b6 loop regions of Ga in receptor interactions include studies from alanine scanning mutagenesis on Ga1 (16), patterns of evolutionary conservation (43), and tryptic digestion of Ga bound to rhodopsin (42). Interestingly, the recently resolved crystal structure of Ga by Sunahara et al. (44) indicates that although the overall structures of Ga and Ga1 are quite similar, the a helix and a4-b6 loop region varies between these subunits both in the length of the helical domain and the positioning of this region within the molecule itself (44). Thus the sequence divergence and structural differences between these Gα’s is consistent with an important role in specific receptor recognition.

According to the crystal structure of Ga1 (30), in three-dimen-
sional space Glu304 and Glu308 are situated on the same molecular surface as the carboxyl-terminal tail of the Ga subunit which has been well established as a receptor-binding site (Fig. 8). The a4 helix is connected to the carboxyl-terminal domain via the a4—b6 loop, followed by the b6 strand and the b6—a5 loop which contains a conserved guanine nucleotide binding motif TCAT (Fig. 10A). Several biochemical studies have reported that mutations within this TCAT motif dramatically decrease the affinity of the a subunit for GDP (16, 45, 46). Therefore, one could hypothesize that the 5-HT1B receptor interaction with both the a helical domain and the carboxyl-terminal region might trigger changes in the b6—a5 loop. Upon agonist activation of the receptor, both domains may translate a conformational change to the TCAT motif resulting in a lowered affinity of GDP for the nucleotide binding pocket. Key mutations within either one of these domains (i.e. the a4 helix or carboxyl terminus) may alter the transmission of the recep-

**FIG. 7.** Correlation between GDP/GTP exchange and affinity shift activity. The data plotted represent the means of each data set as reported in Table I. Filled circle represents the Ga1 control value; open circle represents Chi22; open squares represent Chi22 mutants; filled squares represent Ga1 mutants. Chi24 is represented by x, and Chi25 is represented by an asterisk. The Pearson correlation coefficient was 0.80, representing a significant correlation between both data sets.
tor-induced conformational signal to the TCAT domain and result in an inability to release GDP upon agonist binding.

Consistent with this idea, it has been suggested that the agonist-activated receptor interacts with the GDP-bound form of the G protein, and the release of GDP from the Ga subunit is the rate-limiting step in G protein activation (47–49). This guanine nucleotide-free form of the G protein exists in a highly stable complex with the agonist-bound receptor in the absence of guanine nucleotides in the medium (50).

Therefore, mutations of Ga that generate defects in agonist-activated receptor-catalyzed GDP release from the Ga subunit should also result in a failure to establish the high affinity ternary complex of agonist, receptor, and G protein. In fact, as shown in Fig. 7, a good overall correlation does exist between the two measurements of GTPγS binding and affinity shift activity.

Sequence alignment and comparison of the primary structure between Ga family members reveals that a4 helical residues Glu304 and Glu308 are absolutely conserved among the members of the Ga family of subunits shown in Fig. 11. In contrast, residues in the homologous position on Gt are different. The conservation of these critical residues across Ga, Gao, Gai, and Ga members of the Ga family is consistent with the ability of the 5-HT1b receptor to couple selectively to heterotrimeric containing any one of these members within this family of subunits (12). These data are also consistent with published studies indicating that the 5-HT1b receptor is incapable of coupling to heterotrimers containing Ga (1, 12).
In summary, 2 amino acids within the α4 helix of the Ga subunit play a key role in directing the specificity of 5-HT1b receptor coupling. These residues are essential for ensuring both the formation of the high affinity state of the receptor in the presence of agonist and receptor-catalyzed GDP/GTP exchange. Conservation of these residues across several members of the Gi/o family of α subunits strengthens the importance of these residues in agonist G protein activation and suggests that other receptors that distinguish between Ga and Gb may utilize these residues as well. It remains to be determined whether these residues interact directly with the receptor or act indirectly by affecting the secondary structure of Ga or the transmission of conformational changes to the GDP-binding pocket. Future work on the generalizability of these results to other Gt-coupled receptors will contribute to the understanding of the mechanism of receptor-catalyzed G protein activation and the nature of selectivity governing various cellular responses elicited by different biological stimuli.

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FIG. 11. Primary sequence alignment of α4 helical region of Ga subunits. Numbers above the sequences refer to amino acid positions in the context of Ga1. Boldface letters represent identical amino acid residues among Ga1 subunits. The circled amino acid residues in Ga1 are those that were determined to be critical for 5-HT1b receptor coupling. Notice that these amino acids are conserved among several members of the Ga1 family of α subunits but diverge from the corresponding residues in Ga, bov., bovine; hum., human; caup., guinea pig.

FIG. 10. Secondary structure and residue contact sites in Ga1 and Ga2. Superimposed Ca traces of α4 helix through carboxyl-terminal residues (A) of free Ga1-GTP (green) and Ga2-GTP (blue). This overlay demonstrates that there is little difference between the secondary structure of Ga1 and Ga2 within this domain. In addition, the TCAT motif is “linked” to the carboxyl-terminal domain via the α5 helix and to the α4 helix via the β6 strand followed by the α4–β6 loop. Ribbon representation of portions of the α4 helix and β6 strand of free Ga1-GTP (B) and Ga2-GTP (C). The side chains from Ga1 residues Glu308, Glu321, and Thr321 in addition to Ga2 residues Lys304, Lys317, and Ser317 are drawn as stick models to illustrate physical contacts of critical residues. Fuchia residues represent oxygen atoms, and green residues represent nitrogen atoms. Dashed lines indicate intramolecular contacts between residues. Amino acids are labeled based on their respective sequence positions (30, 52). The GTP-bound α subunits are illustrated because the GDP-bound form of Ga contains a microdomain that changes the conformation of the carboxyl terminus.

Clawges et al. (12) demonstrated that whereas Ga1, Ga2, Ga3, or Gs can couple to the 5-HT1b receptor, these subunits exhibited a rank order profile of coupling efficiency (Ga1 ∼ Ga2 > Ga3 > Gs) to the receptor. Together, the current data and previously published work suggest that residues other than those identified within the α4 helical domain may mediate more subtle differences in coupling efficiency between G proteins within the same subfamily.
α4 Helical Residues of Ga1i Mediate 5-HT1B Receptor Coupling

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