Molecular Determinants for G Protein βγ Modulation of Ionotropic Glycine Receptors*

Gonzalo E. Yevenes, Gustavo Moraga-Cid, Leonardo Guzmán, Svenja Haeger, Laerte Oliveira, Juan Olate, Günther Schmalzing, and Luis G. Aguayo

From the 1Laboratory of Neurophysiology, Department of Physiology, University of Concepción, Concepción, Chile, the 2Department of Molecular Pharmacology, Medical School of the Technical University of Aachen, Aachen, Germany, the 3Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, Brazil, and the 4Laboratory of Molecular Genetics, Department of Biochemistry and Molecular Biology, University of Concepción, Concepción, Chile

The ligand-gated ion channel superfamily plays a critical role in neuronal excitability. The functions of glycine receptor (GlyR) and nicotinic acetylcholine receptor are modulated by G protein βγ subunits. The molecular determinants for this functional modulation, however, are still unknown. Studying mutant receptors, we identified two basic amino acid motifs within the large intracellular loop of the GlyR α3 subunit that are critical for binding and functional modulation by Gβγ. Mutations within these sequences demonstrated that all of the residues detected are important for Gβγ modulation, although both motifs are necessary for full binding. Molecular modeling predicts that these sites are α-helices near transmembrane domains 3 and 4, near to the lipid bilayer and highly electropositive. Our results demonstrate for the first time the sites for G protein βγ subunit modulation on GlyRs and provide a new framework regarding the ligand-gated ion channel superfamily regulation by intracellular signaling.

The ionotropic glycine receptors (GlyRs) are members of the ligand-gated ion receptor superfamily, which includes inhibitory γ-aminobutyric acid type A receptors and excitatory nAChRs and 5-HT3 receptors. These homologous receptors mediate fast synaptic transmission in the central nervous system (1, 2). Inhibitory GlyRs are critical for the control of excitability in the mammalian spinal cord and brain stem. Binding of glycine to the extracellular region induces a rapid increase in Cl− ion conductance, generating a hyperpolarization of the cell membrane (3–5). In neurons, the inhibitory action of GlyRs regulate several important physiological functions, like pain transmission, respiratory rhythms, motor coordination, and development (3–7). Like all members of the LGIC superfamily, GlyRs are pentamers composed of five subunits in which each subunit possesses four transmembrane domains arranged to form the ion pore. In this structure, the individual subunits provide extracellular and intracellular domains that play roles in ligand binding and intracellular modulation, respectively (1–3, 5).

The function of GlyRs can be effectively modulated by extracellularly acting compounds like strychnine, picrotoxin, zinc ions, and ethanol (3–5, 8, 9). Furthermore, the receptor can also be modulated by intracellular signaling. One of the most studied and recognized pathways involved in regulation of ligand-gated ion channel function are phosphorylation processes through protein kinases. Indeed, GlyR and other members of the LGIC superfamily are modulated by activation of cAMP-dependent kinases and protein kinase C (10). This involves specific serine residues in the loop between transmembrane domains 3 and 4 (6, 10–14). On the other hand, recent reports have shown that the activity of GlyRs and nAChRs can be modulated by G protein βγ subunits in a phosphorylation-independent manner (15, 16). In both cases, activation of G proteins, using nonhydrolyzable GTP analogs or by application of purified Gβγ dimers, generates a strong enhancement in the agonist-evoked current, related to a shift to the left in the agonist concentration-response curve and an increased open channel probability (15, 16). However, the molecular determinants involved for this modulation are unresolved.

On the other hand, some molecular characteristics for binding and modulation of Gβγ to other effector proteins are better understood (17–19). In fact, previous studies implicated the presence of basic amino acids, such as lysine and arginine, in the modulation of voltage-gated calcium channels, phospholipase Cβ, and β-adrenergic receptor kinases (20–23). For example, mutations in basic residues reduced Gβγ binding to β-adrenergic receptor kinase 1 and phospholipase Cβ3 (21, 22). Furthermore, similar results were obtained with mutations in the N-terminal region and in the cytoplasmic linker between the first and second transmembrane repeats of voltage-gated calcium channels (19, 20, 23).

In the present study, we have examined the molecular determinants for G protein modulation of the human GlyR α3 subunit. The present study shows that most of the large intracellular loop is not involved in the modulation. We identified two basic amino acid motifs within the loop of the GlyR as critical for binding and functional modulation by Gβγ dimers. Acidic
residues, on the other hand, are not important for this regulation. These results provide novel information about the Gβγ modulatory sites in the GlyR, a member of the LGIC superfamily, and also demonstrate a new role for specific residues in the intracellular loop for the GlyR function.

**EXPERIMENTAL PROCEDURES**

**cDNA Constructs**—The cDNA construct encoding the human glycine receptor α1 subunit with a C-terminal hexahistidinyl tag has been described previously (25). This construct was first subcloned in a pCI vector (Promega) for expression in HEK 293 cells. Mutations were inserted using the QuikChange™site-directed mutagenesis kit (Stratagene). All of the constructions were confirmed by sequencing. The glycine receptor amino acids were numbered according to their position in the mature protein sequence. pEFYFP-Gββ, and pECFP-Gγ2, expression vectors were kindly provided by Stephen R. Ikeda (National Institutes of Health) (27). G protein β1-FLAG and G protein γ2 were purchased from UMR cDNA resource.

**Cell Culture and Transfection**—HEK 293 cells were cultured using standard methodologies. HEK 293 cells were transfected using Lipofectamine 2000 (Invitrogen) with 2 μg of DNA for each plasmid studied per well. Expression of GFP was used as a marker of positively transfected cells, and recordings were made after 18–36 h.

**Electrophysiology**—Whole cell recordings were performed as previously described (16, 38). A holding potential of −60 mV was used. Patch electrodes were filled with 140 mM CsCl, 10 mM 1,2-bis-(2-aminophenoxy)-ethane-N,N,N,N-tetraacetic acid, 10 mM HEPES (pH 7.4), 4 mM MgCl2, 2 mM ATP, and 0.5 mM GTP. The external solution contained 150 mM NaCl, 10 mM KCl, 2 mM CaCl2, 1.0 mM MgCl2, 10 mM HEPES (pH 7.4), and 10 mM glucose. For G protein activation experiments, GTPγS (0.5 mM) was added directly to the internal solution, replacing GTP. The amplitude of the glycine current was assayed using a short (1–2 s) pulse of 30–40 μM glycine every 60 s as previously described (16). Strychnine (1 μM) blocked all of the current elicited by wild type and mutant glycine receptors (not shown).

**Construction of Glutathione S-Transferase Fusion Proteins and GST Pull-down Assays**—DNA fragments encoding wild type and mutant GlyR intracellular loops were subcloned in the GST fusion vector pGEX-5X3 (GE Healthcare). GST fusion proteins were generated in Escherichia coli BL21 using 50 μM isopropyl β-D-thiogalactopyranoside. After 6 h, the cells were collected and sonicated in lysis buffer (1 X phosphate buffer, 1% Triton X-100, and protease inhibitor mixture set II (Calbiochem)). Subsequently, the proteins were purified using a glutathione resin (Novagen). Normalized amounts of GST fusion proteins were incubated with purified Gβγ protein (10 ng; Calbiochem). Incubations were done in 800 μl of binding buffer (200 mM NaCl, 10 mM EDTA, 10 mM Tris, pH 7.4, 0.1% Triton X-100, and protease inhibitor mixture set II) at 4 °C for 1 h. The beads were washed five times in binding buffer, and bound proteins were separated on 12% SDS-polyacrylamide gels. Bound Gβγ was detected using a Gβ antibody (1:1000; Santa Cruz Biotechnology) and a chemiluminescence kit (PerkinElmer Life Sciences). Finally, the relative amounts of Gβγ were quantified by densitometry. Similar conditions have been used to study the interaction between Gβγ and other effector proteins (15, 20–22, 35, 36).

**Immunofluorescence, Visualization, and Analysis**—HEK 293 cells were first fixed for 30 min with 4% paraformaldehyde (0.1 M phosphate buffer, pH 7.4) and were then permeabilized (0.3% Triton X-100, 30 min) and blocked (10% normal horse serum, 60 min). Subsequently, all night incubation with a monoclonal FLAG (Stratagene) and polyclonal hexahistidine antibodies (His-Tag; U.S. Biological) was carried out. Epitope visualization was performed incubating the sample with two secondary antibodies conjugated to fluorescein isothiocyanate and Cy3 (1:600; Jackson ImmunoResearch Laboratories). Finally, the cells were coverslipped using fluorescence mounting medium (Dako Cytomation; Dako). For quantitative analysis, the cells were chosen randomly for imaging using a Nikon confocal microscope. Stacks of optical sections in the z axis were acquired, and dual color immunofluorescent images were captured in simultaneous two-channel mode. Colocalization was studied by superimposing both color channels. The cross-correlation coefficient (r) between both fluorescence channels was measured using computer software (Metamorph; Universal Imaging Corp.) starting from separate immunoreactivity to GlyR-His and Gβ1-FLAG in the same cell (39). The theoretical maximum for (r) is 1 for identical images, and a value close to 0 implies a complete different localization of the labels. Subsequently, the obtained data were compiled, analyzed, and plotted.

**Molecular Modeling**—The GlyR model was constructed by homology using coordinates from the Torpedo nAChR at 4 A° resolution (28, 29) (Protein Data Bank code 2BG9) using the program WHAT IF (40). The ligand-binding segment of the GlyR was modeled from the acetylcholine-binding protein structure (30) (Protein Data Bank code 1UV6). Electrostatic surface potentials were calculated using APBS (41). The individual charges were assigned using pdb2pqr software (42) with the AMBER force field (43). The final images were generated with Pymol (44).

**Data Analysis**—Statistical analyses were performed using ANOVA and are expressed as the arithmetic means ± S.E.; values of p < 0.05 were considered statistically significant. For all of the statistical analysis and plots, the Origin 6.0 (MicroCal) software was used. The normalized values were obtained by dividing the current amplitude obtained with time of dialysis by the current at minute one. The control GTPγS percentage potentiation is the average for all of the single experiments.

**RESULTS**

**Effects of G Protein Activation on Wild Type and Truncated Human α1, Glycine Receptor Subunits**—The currently accepted topology of the LGIC superfamily predicts that the large intracellular loop between transmembrane domains 3 and 4 is the region for interaction and regulation by intracellular pathways, including receptor phosphorylation (10–14). Noteworthy, Ser391 was the first residue in this sequence reported to be modulated by phosphorylation by protein kinase C (13). The cytosolic loop polypeptide contains ~84 amino acids (Fig. 1A), which can present alternative splicing. Therefore, we examined the sensitivity of a recently identified α1 splicing variant, in which the sequence between residues Glu326 and Lys455 was...
deleted (24), to Gβγ modulation (Fig. 1A). In addition, we constructed a truncated GlyR, in which the whole sequence between residues Glu\textsuperscript{326} and Gln\textsuperscript{382} was deleted (Fig. 1A). As previously reported (16), the amplitude of the glycine-activated current in human wild type GlyRs was strongly enhanced above control (80 ± 10%, \(n = 18\)) after 15 min of intracellular dialysis with GTPγS (Fig. 1, B and C). Interestingly, similar effects were found on \(\Delta 326-355\) and \(\Delta 326-382\) truncated GlyRs (Fig. 1, B and C), and no statistical differences were detected (Fig. 1C). Thus, these data indicate that the whole deleted sequence between residues 326 and 382 is not a critical determinant for Gβγ modulation and directs further analysis toward other regions in the intracellular loop.

**Two Basic Motifs Are Critical for G Protein βγ Functional Modulation of Glycine Receptors**—Studies on the molecular determinants involved in the binding and modulation by G protein βγ to several proteins have shown the existence of key basic amino acids (19–23). Interestingly, the large intracellular loop of the GlyR has a high density of residues, such as lysine and arginine (23 of 84), with several in a cluster near the transmem-
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The graph illustrates the percentage of tonic modulation obtained following 15 min of dialysis with GTP γS. The asterisks denote a significance of *p < 0.05*, **p < 0.01**, or ***p < 0.001***; ANOVA. The enhancement of the glycine current in the 326–328A mutant was not different from wild type GlyRs (3647 pA, 394 pA, 13) for the 377–378, 385–386A mutant, which was not significantly different from wild type GlyRs (3647 ± 394 pA, n = 23). On the other hand, although the truncated GlyRs showed lower I_{max} relative to wild type (3326–382, 1584 ± 210 pA, n = 11), the G protein modulation was unchanged. These data suggest that the elimination of G protein βγ modulation is independent of changes on cell surface expression. Thus, all of the above shows the importance of the basic residues in the functional G protein βγ modulation of GlyRs.

To identify critical amino acids involved in the G protein modulation, we carried out a mutagenesis scanning in these two regions found to be important for modulation. For the 316RFRRKRR cluster, all single point mutations showed marked
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attenuations in the potentiating effect produced by the GTP analog, compared with the wild type GlyRs (Fig. 4A). For example, the potentiation for R316A was 40 ± 17% (n = 5), whereas the effect for K320A was only 29 ± 13% (n = 8). Interestingly, the mutation F317Q also altered the modulation (29 ± 14%, n = 5) implicating hydrophobic residues, in addition to basic ones, in the modulatory effects. Double mutants RK319–320A and RR316,318A were also less affected than the wild receptor (27 ± 8%, n = 6 and 33 ± 14%, n = 7, respectively) but were no different from the point mutants (Fig. 4A and B). On the other hand, for the basic residues detected near the C-terminal region, no potentiation was detected in the double mutant 385–386A (2 ± 6%, n = 9), suggesting a critical role for the 385KK residues (Fig. 4A–D). Subsequent studies with F317Q and K386A showed significant attenuations (3 ± 4%, n = 6 and 32 ± 16%, n = 5, respectively), confirming an important role for the 316RFRRK and 385KK motifs in the TM3-TM4 loop. These results imply that the wild type TM3-TM4 loop was able to bind Gβγ in comparison with GST alone (Fig. 5, A and B), confirming the direct interaction. The GST-GlyR fusion proteins that contained the 316–320A and 385–386A mutations were also able to bind Gβγ, although to a lower degree as compared with the wild type GlyR sequence (Fig. 5, A–C). Therefore, we also tested the capacity of a GST-GlyR construct containing mutations in both functional regions to bind purified Gβγ. Interestingly, when both motifs were mutated, the Gβγ binding to the GlyR intracellular sequence was significantly reduced (Fig. 5, A–C). These results indicate that the 316RFRRK and 385KK motifs in the TM3-TM4 loop are directly involved in the Gβγ binding and suggest that each site can independently bind Gβγ in the GST pull-down assay. To further study the functional and biochemical data in a more intact cellular context, we performed a set of double immunofluorescent experiments in HEK 293 cells transfected with mutant and wild type GlyRs with Gβγ2 subunits using hexahistidine and FLAG epitopes to identify the expressed GlyRs and Gβγ subunits, respectively. In agreement with the biochemical and functional tonic modulation data, the cellular distribution of wild type GlyRs and Gβγ dimers displayed a significant overlap in their expression patterns (Fig. 5D). Subsequently, we made similar experiments using mutant GlyRs functionally insensitive to G protein modulation. For the 316–320A and 385–386A mutants, for example, the near cellular association found between wild type GlyRs and Gβγ immunoreactivities was attenuated, showing a reduced degree of colocalization (Fig. 5D). To quantify this data, a correlation analysis for the colocalization of both proteins was performed, and the correlation coefficient (r), a quantitative measure of colocalization, was calculated from each cell analyzed. For the wild type GlyRs, correlated analysis yielded a high correlation coefficient value (r = 0.73 ± 0.02, n = 23) (Fig. 5E), providing quantitative support for a high level of colocalization between wild GlyRs and Gβγ. On the other hand, data with the 316–320A and 377–378,385–386A mutants, for example, the near cellular association found between wild type GlyRs and Gβγ immunoreactivities was attenuated, showing a reduced degree of colocalization (Fig. 5D). To quantify this data, a correlation analysis for the colocalization of both proteins was performed, and the correlation coefficient (r), a quantitative measure of colocalization, was calculated from each cell analyzed. For the wild type GlyRs, corre

Basic Motifs Are Involved in the Protein Interaction between Gβγ and Glycine Receptor—To determine whether the intracellular motifs identified are necessary for the binding of Gβγ to the GlyR, we constructed GST fusion proteins encoding wild type and mutated forms of the GlyR intracellular loop in which the functional G protein modulation was abolished. GST fusion proteins were first expressed and purified (Fig. 5A), and then in vitro binding assays were performed using purified Gβγ, under conditions that warrant the native conformation of the heterodimer (15, 17, 18, 20–22, 36). In agreement with the functional data and previous reports for other effectors (15, 19–22, 35, 36), we found that the wild type TM3-TM4 loop was able to bind Gβγ in comparison with GST alone (Fig. 5, A and B), confirming the direct interaction. The GST-GlyR fusion proteins that contained the 316–320A and 385–386A mutations were also able to bind Gβγ, although to a lower degree as compared with the wild type GlyR sequence (Fig. 5, A–C). Therefore, we also tested the capacity of a GST-GlyR construct containing mutations in both functional regions to bind purified Gβγ. Interestingly, when both motifs were mutated, the Gβγ binding to the GlyR intracellular sequence was significantly reduced (Fig. 5, A–C). These results indicate that the 316RFRRK and 385KK motifs in the TM3-TM4 loop are directly involved in the Gβγ binding and suggest that each site can independently bind Gβγ in the GST pull-down assay. To further study the functional and biochemical data in a more intact cellular context, we performed a set of double immunofluorescent experiments in HEK 293 cells transfected with mutant and wild type GlyRs with Gβγ2 subunits using hexahistidine and FLAG epitopes to identify the expressed GlyRs and Gβγ subunits, respectively. In agreement with the biochemical and functional tonic modulation data, the cellular distribution of wild type GlyRs and Gβγ dimers displayed a significant overlap in their expression patterns (Fig. 5D). Subsequently, we made similar experiments using mutant GlyRs functionally insensitive to G protein modulation. For the 316–320A and 377–378,385–386A mutants, for example, the near cellular association found between wild type GlyRs and Gβγ immunoreactivities was attenuated, showing a reduced degree of colocalization (Fig. 5D). To quantify this data, a correlation analysis for the colocalization of both proteins was performed, and the correlation coefficient (r), a quantitative measure of colocalization, was calculated from each cell analyzed. For the wild type GlyRs, correlation analysis yielded a high correlation coefficient value (r = 0.73 ± 0.02, n = 23) (Fig. 5E), providing quantitative support for a high level of colocalization between wild GlyRs and Gβγ. On the other hand, data with the 316–320A and 377–378,385–386A mutants, for example, the near cellular association found between wild type GlyRs and Gβγ immunoreactivities was attenuated, showing a reduced degree of colocalization (Fig. 5D). To quantify this data, a correlation analysis for the colocalization of both proteins was performed, and the correlation coefficient (r), a quantitative measure of colocalization, was calculated from each cell analyzed. For the wild type GlyRs, correlation analysis yielded a high correlation coefficient value (r = 0.73 ± 0.02, n = 23) (Fig. 5E), providing quantitative support for a high level of colocalization between wild GlyRs and Gβγ.
the intracellular loop are involved in the protein interaction and functional modulation between GlyRs and Gβγ.

Localization and Characterization of G Protein βγ Modulatory Sites within the LGIC Member Structure—To study the possible conformation and localization of these motifs in the ion channel, we generated a GlyR α1 subunit model based on the acetylcholine-binding protein and Torpedo nAChR structure coordinates (28–30). In this model, the motifs 316RFRRKK and 385KK are cytosolic α-helices near transmembrane regions 3 and 4, in close proximity to the lipid bilayer and display a high density of positive charges (Fig. 6A, shown in blue). Additionally, the model also predicts close proximity of these regions in the whole GlyR pentameric structure (Fig. 6B). Thus, in the quaternary receptor structure, the Gβγ-binding site can be shaped by positive residue motifs in neighboring subunits in close relationship to the membrane and the receptor vestibule. Additional computer simulations indicated that the regions identified are potentially favorable to interact with the effector-interacting face of Gβγ, in agreement with previous reports (31) (data not shown). Thus, in line with experimental evidence in voltage-gated calcium channels (20), the data allow proposal of a model in which the two regions identified, composed mainly by basic residues at both sides of the cytoplasmic loop of GlyRs, are key regions for appropriate Gβγ binding and functional modulation.

FIGURE 5. Role of the basic motifs in the protein interaction between Gβγ and GlyRs. A, Coomassie Blue stain for the GST fusion proteins used. B, Gβγ binding to wild type and mutant GlyRs. An arrow indicates the Gβγ detected with a polyclonal anti-Gβ antibody. C, relative amounts of bound Gβγ compared with the wild type intracellular loop (n = 4). The differences were significant (*, p < 0.05; ***, p < 0.001; ANOVA). D, transfected HEK 293 cells were stained with antibodies against the hexahistidine (green) and FLAG epitopes (red) that recognize tagged GlyRs and Gβγ, respectively. The images were merged to visualize colocalization (yellow). E, the graph summarizes the mean correlation coefficients (r) between the wild type and mutant GlyRs with Gβγ subunits. The differences were significant (***, p < 0.001; ANOVA).

DISCUSSION

The GlyR is a member of the ligand-gated ion receptor superfamily, which also includes γ-aminobutyric acid type A receptors, nAChRs, and 5-HT3 receptors. These homologous ionotropic receptors mediate fast synaptic transmission in the central nervous system. Interestingly, all of the members of the superfamily share common features. For example, all have the signature Cys-loop, a conserved disulfide loop in their extracellular ligand-binding domain, and all are composed of five subunits in a heteropentameric quaternary structure arranged around a central pore. Each subunit possesses a large extracellular domain and four transmembrane α-helices per subunit with a large intracellular loop between the third and fourth TM. This model was first suggested by hydrophobicity plots of the ligand-gated channels and was recently confirmed by biochemical and crystallographic data from studies with nAChR (2, 28, 29, 32). Coincidentally, several recent studies showed similar channel gating mechanisms for the LGIC members; involving the ligand-binding domain and the extracellular loop between TM2 and TM3 (32–34).

Because of these common receptor properties, it was not surprising to find that the regulation of the LGIC family by intracellular signal transduction pathways is also conserved. Several studies, for example, have demonstrated functional regulation of the LGIC family by protein kinases, involving the phosphorylation of specific residues inside the intracellular loop between TM3 and TM4 (6, 10–14). Besides protein kinases, recent evidence have shown that the activity of two members of the LGIC family (GlyRs and nAChRs) was directly modulated by G protein βγ subunits, enhancing the activity of both channels (15, 16).

For several Gβγ effectors, a large variety of G protein modulatory sites were characterized. Biochemical and functional data studies determined multiple binding and modulatory sites in voltage-gated calcium channels and GIRK channels, indicating a high degree of complexity (19, 20, 23, 35, 36). Despite this, the presence of basic residues inside the critical regions for binding and modulation appears to be a common feature. For example, intracellular arginine residues in different domains of voltage-gated calcium channels have been shown to be critical for binding and regulation by Gβγ (19, 20, 23). Moreover, mutations in basic amino acids decreased the Gβγ binding to β-adrenergic receptor kinase 1 and phos-
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For several Gβγ effectors, a large variety of molecular determinants for binding and modulation have been characterized (17–22, 31, 35, 36). In this study, we provide novel information for the identification of Gβγ modulatory sites in the GlyR, a member of the LGIC superfamily. To our knowledge, we demonstrate a new role for the specific residues in the intracellular loop, in addition to the growing number of critical functions of the LGIC main intracellular loops for correct channel structure, localization, function, and regulation (6, 13, 25, 37).

In our study, using truncated and mutated GlyRs in their intracellular loop, we identify two critical motifs for the Gβγ modulation of α1 GlyRs. Interestingly, almost all of the amino acids involved were basic, in agreement with most of the data for other Gβγ effectors. The sites 316RFRRK and 388KK were located near transmembrane domains 3 and 4, respectively, suggesting a close relationship with the cell membrane. Mutations on either of these sites abolished the functional modulation of GlyRs by G protein activation, supporting critical but independent roles of these regions for functional Gβγ regulation. The GST pull-down assays confirmed a direct interaction between Gβγ and the intracellular loop of α1 GlyR and indicate that only their concurrent alteration is able to extensively reduce the Gβγ binding. On the other hand, confocal microscopy analysis shows a lower degree of colocalization between Gβγ dimers and mutant forms of GlyRs that includes only one altered motif compared with wild type receptors. This evidence is in agreement with the functional data obtained indicating that both motifs are necessary for a full functional modulation. Besides these observations, our GlyR homology model shows that the motifs identified are cytosolic α-helices near transmembrane regions 3 and 4, in close proximity to the lipid bilayer and highly electropositive. Interestingly, in a whole GlyR pentameric model both motifs are in close proximity, generating an electropositive surface composed of all of the basic residues of neighboring subunits. Therefore, in line with the experimental evidence, we can postulate that region shapes the membrane-bound Gβγ interaction surface necessary for the functional modulation of GlyRs. Thus, based on this proposal, the alteration of a single basic motif can change this critical interaction surface between pentameric GlyRs and membrane-associated Gβγ.

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FIGURE 6. Localization and characterization of the G protein βγ modulatory sites within the GlyR structure. A, ribbon diagram and electrostatic potential surface representations of a single GlyR α1 subunit derived from the nAChR template. The right panel shows a detailed view of the motifs important for Gβγ modulation. Negative and positive charges are in red and blue, respectively. B, ribbon and electrostatic potential surface models of homopentameric GlyRs. In this conformation, the Gβγ modulatory sites of neighboring subunits shape an electropositive surface that serves for binding and functional modulation of channel function.

phospholipase Cβ3, affecting the functional modulatory effects (21, 22). However, for GIRK channels no basic residues have been found to be important for Gβγ regulation, indicating vast types of molecular determinants in the heterodimer effects (35, 36).

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