Multiple regions in the extracellular domain of the glycine receptor determine receptor activity

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Glycine receptors (GlyRs) mediate fast synaptic inhibition in the brain stem and spinal cord. They are involved in the generation of motor rhythm, reflex circuit coordination, and sensory signal processing and therefore represent targets for therapeutic interventions. The extracellular domains (ECDs) of Cys-loop receptors typically contain many aromatic amino acids, but only those in the receptor binding pocket have been extensively studied. Here, we show that many Phe residues in the ECD that are not located in the binding pocket are also involved in GlyR function. We examined these Phe residues by creating several GlyR variants, characterizing these variants with the two-electrode voltage clamp technique in Xenopus oocytes, and interpreting changes in receptor parameters by using currently available structural information on the open and closed states of the GlyR. Substitution of six of the eight Phe residues in the ECD with Ala resulted in loss of maximal response to the partial GlyR agonist taurine compared with glycine, whereas taurine yielded maximal responses of 51% (Fig. 2).

Results

WT GlyRs

GlyRs have been previously well-studied using heterologous systems, and parameters we obtained from concentration–response curves following expression in Xenopus oocytes (EC50 = 49 μM, nH = 2.5) are consistent with previous studies (e.g. see Pless et al. (11)). In addition to examining responses with glycine, we also determined the maximal responses obtained with the partial agonists β-alanine and taurine; we observed that β-alanine was closed to a full agonist (80 ± 8%), whereas taurine yielded maximal responses of 51 ± 7% (Fig. 2).

Phe residues in or near the N-terminal α-helix

There are two Phe residues in or near the N-terminal α-helix; Phe-13 and Phe-32. Substitution of either of these with Ala...
resulted in large changes in the glycine EC$_{50}$, indicating that they are important for the correct function of the receptor (Table 1). Maximal responses from these (and all other mutant receptors that we created) had values that were not significantly different from those for WT receptors, indicating no expression problems (Fig. 3). To probe whether EC$_{50}$ changes might be due to alterations in binding affinity or channel gating, we determined maximal responses with taurine in these mutants. Both had changes to $I_{\text{max}}$ taurine/$I_{\text{max}}$ glycine (Table 1); taurine was a full agonist in F13A-containing mutant GlyR with maximal taurine responses of 97% compared with maximal glycine responses, whereas F32A-containing GlyR had significantly lower relative maximal taurine responses (14%) than WT receptors (51%). Examination of the structure of the receptor in the region of these residues shows that there are multiple possible interactions that could be made by Phe-13 with other residues in the subunit: a π–π interaction with Tyr-75 or Tyr-78, a cation–π interaction with Lys-16, an anion–π interaction with Glu-77, and hydrophobic interactions with Leu-17 and Leu-83. To explore these potential interactions, we made further substitutions: F13W, F13Y, F13R, F13E, K16A, and E77A. F13W-containing receptors had an EC$_{50}$ value similar to that of WT receptors, whereas there were large increases in EC$_{50}$ values in F13A-, F13Y-, F13R-, and F13E-containing GlyR (Fig. 4). The increase with a F13Y mutation is inconsistent with data from the F13W mutation, which suggests that an alternative aromatic can substitute effectively for Phe but in fact can be readily
The role of phenylalanines in the glycine receptor

Figure 2. Responses to glycine and taurine in WT GlyR. A, typical responses to the application of a range of concentrations of glycine to an oocyte expressing WT GlyR. B, typical maximal responses to glycine (1 mM) and taurine (red; 100 mM) in an oocyte expressing WT GlyR show that taurine is a partial agonist. Scale bars, 2 μA, 30 s. C, typical concentration–response curves for glycine and taurine in a single oocyte expressing WT GlyR. Data are typical of at least three oocytes.

Table 1
WT and Phe mutant GlyR parameters

| Mutant         | pEC50 | EC50   | Tau/100% Gly | nT |
|----------------|-------|--------|--------------|----|
| WT             | 4.31 ± 0.04 | 49     | 51 ± 7       | 2.5 ± 0.3 |
| F13A           | 2.98 ± 0.06* | 1050   | 97 ± 15*     | 3.2 ± 0.4 |
| F13W           | 4.17 ± 0.04 | 67     | 70 ± 5       | 2.0 ± 0.3 |
| F13Y           | 3.00 ± 0.03* | 998    | 53 ± 11      | 2.4 ± 0.2 |
| F13E           | 3.32 ± 0.06* | 478    | 88 ± 6*      | 2.8 ± 0.4 |
| F13R           | 3.10 ± 0.07* | 796    | 46 ± 9       | 2.1 ± 0.5 |
| F32A           | 2.79 ± 0.08* | 1600   | 14 ± 4*      | 2.4 ± 0.2 |
| F32Y           | 3.92 ± 0.04* | 120    | 89 ± 8*      | 2.2 ± 0.3 |
| F44A           | 2.20 ± 0.03* | 6250   | 10 ± 3*      | 2.0 ± 0.2 |
| F44Y           | 4.21 ± 0.05  | 62     | 80 ± 9*      | 2.3 ± 0.2 |
| F48A           | 3.95 ± 0.02* | 112    | 36 ± 3       |         |
| F63A (11)      | 89,000 ± 6000 |        |              |         |
| F63Y (11)      | 200 ± 400   |        |              |         |
| F99A (22)      | 82 ± 28     | ND     |              |         |
| F100A (22)     | 34 ± 14     | ND     |              |         |
| F108A          | 4.28 ± 0.01 | 52     | 70 ± 8       | 2.2 ± 0.4 |
| F145A (21)     | 775 ± 69    | 52 ± 3 |              |         |
| F159A (11)     | 8100 ± 200  | 86     |              |         |
| F159Y (20)     | 13.2 ± 1.0  | 95     |              |         |
| F168A          | 4.11 ± 0.06 | 78     | 67 ± 5       | 2.6 ± 0.2 |
| F187A          | 3.35 ± 0.04* | 443    | 16 ± 4*     | 2.8 ± 0.4 |
| F187Y          | 4.95 ± 0.05* | 11     | 66 ± 5*     | 1.7 ± 0.3 |
| F207A (11)     | NR          |        |              |         |
| F207Y (11)     | 161 ± 23    | ND     |              |         |
| F214A          | NR          |        |              |         |
| F214Y          | 4.15 ± 0.03 | 70     | 63 ± 5*      | 2.7 ± 0.2 |

Data are mean ± S.E., n = 4–8; *, significantly different from WT, p < 0.05 ANOVA with Dunnett’s post hoc test; NR, non-responsive; ND, not determined.

There are fewer possible potential interactions with Phe-32; one of these is a cation–π interaction with Lys-33, which the structure suggests is possible in the open (with a distance of 3.3 Å between these residues) but not the closed state (7.4 Å) of the receptor. We therefore also created and tested receptors containing K33A and K32R substitutions, but these had EC50 values similar to WT (K33A: pEC50 = 4.23 ± 0.1, EC50 = 54 μM; K32R: pEC50 = 4.46 ± 0.02, EC50 = 35 μM; data are mean ± S.E., n = 4). Another possible interaction of Phe-32 is with the adjacent subunit, where it could interact with Pro-10 (Fig. 5). A Pro–Phe interaction may seem unlikely, but Pro interacts well with aromatic residues due to its polarized C–H bond interacting with the π face (a CH–π interaction) (18). However, a P10A substitution resulted in no change in potency (pEC50 = 4.12 ± 0.02, EC50 = 76 μM; data are mean ± S.E., n = 4). The similar distances between Phe-32 and this Pro in the open and closed states (3.6 and 3.7 Å, respectively) also suggest that if such an interaction exists, it does not play a role in function.

Binding site Phe residues

The Phe residues that are located in the binding pocket (Phe-63 (loop D), Phe-99 (loop A), Phe-100 (loop A), Phe-159 explained by the structural data; substituting a Tyr for Phe here in silico results in a steric clash with Tyr-75 (Fig. 4C). The structure also reveals that the distances between Phe-13 and the residues mentioned above differ in the open and closed states of the receptor, with the most significant difference being the distance to Lys-16, suggesting the possibility of a cation–π interaction. However, a K16A substitution resulted in receptors with an EC50 similar to WT (pEC50 = 3.98 ± 0.05, EC50 = 106 μM; data are mean ± S.E., n = 4), and the relative orientation of this residue and Phe-13 is not optimal for such an interaction, so we consider it unlikely to occur. Similarly, a E77A substitution resulted in WT-like receptors (pEC50 = 4.17 ± 0.06, EC50 = 67 μM; data are mean ± S.E., n = 6), indicating that there is no anion–π interaction. Data from the Arg and Glu substitutions of Phe-13 also suggest that there are no charge interactions here, as they caused large increases in EC50 similar to those resulting from an Ala substitution.

Figure 3. Scatter plot showing maximal responses of WT and mutant GlyRs. Bars show mean ± S.E., n = 3–8. No values are significantly different from WT (ANOVA with Dunnett’s post hoc test).

Figure 4. Phe-13 substitutions. A, concentration–response curves of Phe-13 mutant GlyRs show that Phe can be replaced by Trp to give a WT-like curve, but replacement with Tyr, Ala, Glu, or Arg results in rightward shifts. Data = mean ± S.E., n = 4–8. Parameters derived from these curves are shown in Table 1. B, closed GlyR structure showing residues surrounding Phe-13. Note how far Lys-16 is from Phe-13. C, the open GlyR structure shows that Lys-16 is in a different location, and substitution of Phe with Tyr results in a steric clash with Tyr-75, whereas replacement with Trp does not (D).

J. Biol. Chem. (2018) 293(36) 13889–13896
The role of phenylalanines in the glycine receptor

(loop B), and Phe-207 (loop C)) have been investigated previously (9, 19–22); the data from substitutions to these residues are included in Table 1 for completeness. However, Phe-44, Phe-108, and Phe-168, which are located behind the binding pocket, have not been studied previously. Our data show that Ala substitutions to Phe-44 resulted in the largest change in EC₅₀ compared with WT receptors that we observed for any of our mutant receptors (Table 1) and also caused the largest reduction in the maximal taurine response. This residue is located below Tyr-202 and Phe-63 and is in a position to form a π–π interaction with either of these residues in the open state (Fig. 6). Further exploration of Phe-44 by substitution with Tyr revealed WT-like properties, confirming the importance of the aromatic ring. We note that Phe-63, which also causes a large increase in EC₅₀ when mutated to Ala (7, 11), could also form a π–π interaction with Phe-159, a residue that has long been known to be important for agonist binding (19), and therefore an interaction with Phe-44 may not occur. Thus, we propose that Phe-44 probably interacts with Tyr-202.

The EC₅₀ value for F108A-containing GlyR (52 μM) is similar to WT, and the mutation did not cause a change in the maximal response to taurine (Table 1). This residue is located 3.7 Å from Lys-116 in the closed state, giving it the potential to form a cation–π interaction here, with this distance increased to 5.3 Å in the open state (Fig. 7). However, the functional data suggest no effect of removing the aromatic, so we propose that such an interaction does not occur. The data are nevertheless not inconsistent with this Phe contributing to a hydrophobic region that includes Ile-130 and Leu-132.

The EC₅₀ value for F168A-containing GlyR (78 μM) is also similar to that of WT receptors, and again this substitution did not cause a change in the maximal response to taurine (Table 1). This residue is located at the end of loop B, and, although the structure shows that it has the potential to form a Pro–π interaction with Pro-96 (Fig. 8), the data do not support such an interaction, and we suggest that Phe-168 only contributes to hydrophobic interactions here.

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**Figure 5.** Phe-32 substitutions. A, concentration–response curves of Phe-32 mutant GlyR show that Phe can be replaced by Tyr to give a WT-like curve, but replacement with Ala results in a rightward shift (WT curve from Fig. 4A added for comparison). Data = mean ± S.E., n = 4–8. B, in the closed GlyR Phe-32 is close to Pro-10 on the adjacent subunit, as it is in the open structure (C). However, here it is also close enough to Lys-33 to form a cation–π interaction.

**Figure 6.** A, concentration–response curves of Phe-44 mutant GlyR show that Phe can be replaced by Tyr to give a WT-like curve, but replacement with Ala results in a rightward shift (WT curve from Fig. 4A added for comparison). Data = mean ± S.E., n = 4–8. B and C, aromatic residues in the GlyR binding pocket in the open (B) and closed (C) states.

**Figure 7.** A, concentration–response curves of Phe-108 mutant GlyR show that Phe can be replaced by Ala to give a WT-like curve. Data = mean ± S.E., n = 4–8. B, Phe-108 is located at the end of loop A, where it could have hydrophobic interactions with Ile-130 and Leu-132 (C) and perhaps a cation–π interaction with Lys-116. Distances between residues in the open (and closed) states are shown.
The largest number of Phe substitutions we probed were at or close to the ECD–TMD interface, and all Ala mutations caused changes in EC\textsubscript{50} values and/or maximal taurine responses. F48A-containing GlyR had a 2-fold increase in EC\textsubscript{50} compared with WT, with no change in the relative taurine response. This is interesting, as the structure reveals that Phe-48 on the \( \beta1 \) strand could form a \( \pi-\pi \) interaction with Phe-214, which is on the \( \beta10 \) strand, which links to M1. In addition, Phe-48 could interact with Tyr-58, which is located close to the binding pocket. However, the relatively small change in EC\textsubscript{50} with F48A suggests that the Phe here does not play an especially critical role in either binding or function. This is in contrast to Phe-214, which is sensitive to removal of the aromatic group; F214A-containing GlyR were nonfunctional despite being expressed (Fig. 9). Phe-214 is located in a part of the subunit where it could interact with many hydrophobic residues; substitution with Ala would mean it was too distant to allow most of these interactions. The structural data show there is also the potential for a \( \pi-\pi \) interaction with the sulfur on Met-154. However, the distance between Phe-214 and Met-154 does not differ between open and closed states, and the residues are too far apart for optimal interaction (<4.3 Å) (23), so we consider such an interaction unlikely, and if it does occur, it is probably not involved in receptor activation. Phe-145 and Phe-187 have been studied previously (10, 20). Data indicate that they provide a hydrophobic environment for an important salt bridge between Asp-148 and Arg-218. This hypothesis would explain our novel data from F187A-containing GlyR (~10-fold increase in EC\textsubscript{50}), and the structure provides an explanation for the gain of function we observed with F187Y-containing GlyR, a possible hydrogen bond with Glu-53 that could stabilize the structure (Fig. 10).

**Discussion**

Here, we show that a number of Phe residues in the ECD of the glycine receptor, which have not been previously identified as important, play a role in the function of the receptor. Some of these are located at or close to the ECD–TMD interface or close to the orthosteric binding site and thus could reasonably be expected to maybe affect ligand binding or receptor activation, but we also identify a novel region in the N terminus of the receptor. This region and the other Phe residues we identified as important are discussed in more detail below.
The role of phenylalanines in the glycine receptor

Figure 10. A, concentration–response curves of Phe-187 mutant GlyR show that replacement with Ala shifts the curve to the right, whereas replacement with Tyr shifts the curve to the left (WT curve from Fig. 7A added for comparison). Data = mean ± S.E., n = 4–8. B and C, Phe-145 and Phe-187 are located at the TMD–ECD interface on either side of the salt bridge formed by Asp-148 and Arg-218. D, substitution of Phe-187 with Tyr reveals a possible hydrogen bond with Glu-53.

Phe residues in or near the N-terminal α-helix

The large increases in EC_{50} caused by Ala substitutions of the two Phe residues in or close to the N terminus were unexpected, as these residues are some distance from the binding pocket and are not on the pathway between the binding site and the transmembrane domain and so would not be predicted to be an essential part of the conformational change needed to transduce agonist binding to channel opening. Nevertheless, changes in taurine efficacy are consistent with the latter (i.e., suggest that the mutations affect receptor gating). The N-terminal portion of Cys-loop receptors in the nACh receptor incorporates the main immunogenic region (MIR), which comprises the MIR loop (residues 60–81 in the α1 subunit) and the N-terminal α-helical region (residues 2–14). The MIR is the binding site for many antibodies that cause myasthenia gravis, an autoimmune disease targeting the neuromuscular junction of skeletal muscle (24). Such antibodies block the ACh-induced response, but the mechanism by which they achieve this may not be by a physical occlusion of the pore or binding pocket, as originally proposed, but instead may involve inhibition of the conformational change required for channel opening. Such a mechanism was proposed by Lou et al. (25) based on their data showing large changes in sensitivity to ACh following alterations to the MIR, combined with molecular dynamics simulation data from Henchman et al. (26) showing conformational changes in this region associated with receptor activation. Our data are consistent with an important functional role in this region of the protein, which may be essential for efficient channel gating. This may involve interactions between the N-terminal α-helix and the β1–β2 loop (which is located between loops D and A and thus could be directly influenced by agonist binding) and also between the N-terminal α-helix and the adjacent subunit. We propose these interactions are mediated at least in part via Phe residues in the GlyR. Similar interactions may occur in other Cys-loop receptors, but given the different lengths of the N-terminal α-helix and the regions between loops A and D in different receptors, the specific residues mediating these interactions may not be conserved.

In support of this hypothesis, we note that the GlyR positive allosteric modulator AM-3607 binds in this region (5). This compound, which increases glycine-binding affinity and potentiates channel activity, has been shown to bind to what has been designated a novel allosteric site. Binding of positive allosteric modulators to this site has not been observed previously for any Cys-loop receptor; therefore, these data, combined with our and other mutagenesis studies, suggest an exciting new potential therapeutic binding site not only for GlyR but also possibly other pLGICs.

Phe residues in the GlyR-binding site

Aromatic residues in the binding site (Phe-63, Phe-159, Tyr-202, and Tyr-207) form the “aromatic box,” which is a well-established feature of all Cys-loop receptors, and these residues in the GlyR have been extensively investigated by several groups. In particular, data have shown the importance of Phe-159, which forms a critical cation–π interaction with the natural agonist (9) and also with the partial agonists β-alanine and taurine (11). In the current study, we show that Phe-44, which is located just behind the binding pocket close to Tyr-202, also plays a role in receptor function and suggest that this is due to the formation of a π–π interaction with Tyr-202 (i.e., in effect tethering this aromatic box residue). Tyr-202 is in loop C, a region that undergoes significant change in conformation following agonist binding (loop C capping) and is likely the first step in the series of conformational changes that ultimately result in channel opening in Cys-loop receptors (3, 27). Thus, incorrect positioning of Tyr-202 could have a significant effect on this event, which would be consistent with our taurine data that indicate a role of this residue in the gating process.

Not all Phe residues located near the binding pocket, however, alter receptor function; our data from F108A- and F168A-containing Gly receptors revealed that aromatic residues are not essential at these locations.

Phe residues at or close to the ECD–TMD interface

The interface between the ECD and the TMD is a critical region for transducing agonist binding to channel gating. The most detailed work on this area has combined structural and functional studies in ELIC and GLIC (28), and this work, combined with data from many other studies, has shown that there is unlikely to be a range of conserved sets of critical pairwise ECD–TMD bonds, although it is clear that charged residue interactions are especially important (29–32). Aromatic residues can be involved in such interactions via their π rings, but they also can play other roles. A particular case is that of Phe-145 and Phe-187 in the GlyR, which have been extensively investigated by Pless et al. (10). These authors concluded that these residues provide a hydrophobic framework for a strong electrostatic interaction between Asp-148 in the Cys-loop and Arg-218 in M1. As altering either of these residues has a major
The role of phenylalanines in the glycine receptor

Receptor expression

cDNA was cloned into pGEMHE for oocyte expression and pcDNA3.1 (Invitrogen) for expression in HEK293 cells. Mutagenesis was performed using QuikChange (Agilent Technologies Inc.). cRNA was in vitro transcribed from linearized pGEMHE cDNA template using the mMessage mMACHINE T7 transcription kit (Ambion, Austin, TX). Oocytes were injected with 50 nl of ~400 ng μl⁻¹ cRNA, and currents were recorded 1–4 days postinjection.

Electrophysiology

Using a Robocyte voltage-clamp system (Multi Channel Systems, Reutlingen, Germany), Xenopus oocytes were clamped at ~60 mV. Currents were recorded at a frequency of 5 kHz and filtered at 1 kHz. Micro-electrodes were filled with 3 m KCl. Pipette resistances varied from 1.0 to 2.0 megohms. Oocytes were perfused with saline at a constant rate of 1 ml min⁻¹. Drug application was via a simple gravity-fed system calibrated to run at the same rate. Extracellular saline contained 96 mM NaCl, 2 mM KCl, 1.8 mM CaCl₂, 1 mM MgCl₂, and 5 mM HEPES, pH 7.4, with NaOH.

Analysis and curve fitting were performed using Prism (GraphPad Software, Inc., La Jolla, CA). Concentration–response data for each oocyte were normalized to the maximum current for that oocyte. Statistical significance was determined using an ANOVA with a Dunnett’s multiple-comparison post hoc test; p < 0.05 was taken as statistically significant.

Immunofluorescence

This was as described previously (33). Briefly, transiently transfected HEK293 cells were fixed (4% paraformaldehyde), washed in TBS (0.1 M Tris, pH 7.4, 0.9% NaCl), and incubated overnight at 4 °C in anti-glycine receptor α1 C-15 antiserum (Santa Cruz Biotechnology, Inc.) at 1:200. Following further washing, biotinylated anti-goat IgG (Vector Laboratories) and FITC avidin D (Vector Laboratories) were used to detect bound antibody. Coverslips were mounted in Vectashield mounting medium (Vector Laboratories). Immunofluorescence was observed using a Leica fluorescent microscope.

Structures

GlyR structures 5VDH (open) and 5CFB (closed) were downloaded from the Protein Data Bank and viewed using PyMOL or Swiss-PDBViewer. The GlyR structures are those of the homomeric α3 GlyR, but the majority of residues, and in particular all of the aromatic residues studied here, are identical to those in the α1 GlyR.

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References

1. Lynch, J. W., and Callister, R. J. (2006) Glycine receptors: a new therapeutic target in pain pathways. Curr. Opin. Investig. Drugs 7, 48–53
The role of phenylalanines in the glycine receptor

2. Thompson, A. J., Lester, H. A., and Lummis, S. C. (2010) The structural basis of function in Cys-loop receptors. Q Rev. Biophys. 43, 449–499 CrossRef Medline

3. Du, J., Liu, W., Wu, S., Cheng, Y., and Gouaux, E. (2015) Glycine receptor mechanism elucidated by electron cymro-microscopy. Nature 526, 224–229 CrossRef Medline

4. Huang, X., Chen, H., Michelsen, K., Schneider, S., and Shaffer, P. L. (2015) Crystal structure of human glycine receptor-a3 bound to antagonist strychnine. Nature 526, 277–280 CrossRef Medline

5. Huang, X., Shaffer, P. L., Ayube, S., Bregman, H., Chen, H., Lehto, S. G., Luther, J. A., Matson, D. J., McDonough, S. L., Michelsen, K., Plant, M. H., Schneider, S., Simard, J. R., Teffera, Y., Yi, S., et al. (2017) Crystal structures of human glycine receptor a3 bound to a novel class of analogic potentiators. Nat. Struct. Mol. Biol. 24, 108–113 CrossRef Medline

6. Braun, N., Lynagh, T., Yu, R., Biggin, P. C., and Pless, S. A. (2016) Role of an absolutely conserved tryptophan pair in the extracellular domain of Cys-loop receptors. ACS Chem. Neurosci. 7, 339–348 CrossRef Medline

7. Grudzinska, J., Schemm, R., Haege, S., Nicke, A., Schmalzing, G., Betz, H., and Laube, B. (2005) The β subunit determines the ligand binding properties of synaptic glycine receptors. Neuron 45, 727–739 CrossRef Medline

8. Grenningloh, G., Rienitz, A., Schmitt, B., Methfessel, C., Zensen, M., Beyreuther, K., Gundelfinger, E. D., and Betz, H. (1987) The strychnine-binding subunit of the glycine receptor shows homology with nicotinic acetylcholine receptors. Nature 328, 215–220 CrossRef Medline

9. Pless, S. A., Millen, K. S., Hanek, A. P., Lynch, J. W., Lester, H. A., Lummis, S. C., and Dougherty, D. A. (2008) A cation–π interaction in the binding site of the glycine receptor is mediated by a phenylalanine residue. J. Neurosci. 28, 10937–10942 CrossRef Medline

10. Pless, S. A., Leung, A. W., Galpin, J. D., and Ahern, C. A. (2011) Contributions of conserved residues at the gating interface of glycine receptors. J. Biol. Chem. 286, 35129–35136 CrossRef Medline

11. Pless, S. A., Hanek, A. P., Price, K. L., Lynch, J. W., Lester, H. A., Dougherty, D. A., and Lummis, S. C. (2011) A cation–π interaction at a phenylalanine residue in the glycine receptor binding site is conserved for different agonists. Mol. Pharmacol. 79, 742–748 CrossRef Medline

12. Beebe, D. L., Brandt, G. S., Zhong, W., Zacharias, N. M., Lester, H. A., and Dougherty, D. A. (2002) Cation–π interactions in ligand recognition by serotonicin (5-HT3A) and nicotinic acetylcholine receptors: the anomalous binding properties of nicotine. Biochemistry 41, 10262–10269 CrossRef Medline

13. Lummis, S. C., L. Beene, D., Harrison, N. J., Lester, H. A., and Dougherty, D. A. (2005) A cation–π interaction with a tyrosine in the binding site of the GABAA receptor. Chem. Biol. 12, 993–997 CrossRef Medline

14. Mu, T. W., Lester, H. A., and Dougherty, D. A. (2003) Different binding orientations for the same agonist at homologous receptors: a lock and key or a simple wedge?. J. Am. Chem. Soc. 125, 6850–6851 CrossRef Medline

15. Padgett, C. L., Hanek, A. P., Lester, H. A., Dougherty, D. A., and Lummis, S. C. (2007) Unnatural amino acid mutagenesis of the GABAB receptor binding site residues reveals a novel cation–π interaction between GABA and β2Tyr97. J. Neurosci. 27, 886–892 CrossRef Medline

16. Zhong, W., Gallivan, J. P., Zhang, Y., Li, L., Lester, H. A., and Dougherty, D. A. (1998) From ab initio quantum mechanics to molecular neurobiology: a cation–π binding site in the nicotinic receptor. Proc. Natl. Acad. Sci. U.S.A. 95, 12088–12093 CrossRef Medline

17. Brejc, K., van Dijk, W. J., Klaassen, R. V., Schuurmans, M., van Der Oost, J., Smit, A. B., and Sixma, T. K. (2001) Crystal structure of an ACh-binding protein reveals the ligand-binding domain of nicotinic receptors. Nature 411, 269–276 CrossRef Medline

18. Zondlo, N. J. (2013) Aromatic-proline interactions: electronically tunable CH–π interactions. Acc. Chem. Res. 46, 1039–1049 CrossRef Medline

19. Schmieden, V., Kuhse, J., and Betz, H. (1993) Mutation of glycine receptor subunit creates β-alanine receptor responsive to GABA. Science 262, 256–258 CrossRef Medline

20. Schofield, C. M., Trudell, J. R., and Harrison, N. L. (2004) Alanine-scanning mutagenesis in the signature disulfide loop of the glycine receptor α1 subunit: critical residues for activation and modulation. Biochemistry 43, 10058–10063 CrossRef Medline

21. Vafa, B., Lewis, T. M., Cunningham, A. M., Jacques, P., Lynch, J. W., and Schofield, P. R. (1999) Identification of a new ligand binding domain in the α1 subunit of the inhibitory glycine receptor. J. Neurochem. 73, 2158–2166 CrossRef Medline

22. Vandenberg, R. J., French, C. R., Barry, P. H., Shine, J., and Schofield, P. R. (1992) Antagonism of ligand-gated ion channel receptors: two domains of the glycine receptor α subunit form the strychnine-binding site. Proc. Natl. Acad. Sci. U.S.A. 89, 1765–1769 CrossRef Medline

23. Bhattacharyya, R., Pal, D., and Chakrabarti, P. (2004) Disulfide bonds, their stereospecific environment and conservation in protein structures. Protein Eng. Des. Sel. 17, 795–808 CrossRef Medline

24. Tzartos, S. J., Cung, M. T., Demange, P., Loutrari, H., Mamalaki, A., Marraud, M., Papadoul, I., Sakarellos, C., and Tsikaris, V. (1991) The main immunogenic region (MIR) of the nicotinic acetylcholine receptor and the anti-MIR antibodies. Mol. Neurobiol. 5, 1–29 CrossRef Medline

25. Luo, J., Taylor, P., Losen, M., de Baets, M. H., Shelton, G. D., and Lindstrom, J. (2009) Main immunogenic region structure promotes binding of conformation-dependent myasthenia gravis autoantibodies, nicotinic acetylcholine receptor conformation maturation, and agonist sensitivity. J. Neurosci. 29, 13988–13908 CrossRef Medline

26. Henschel, R. H., Wang, H. L., Sine, S. M., Taylor, P., and McCammon, J. A. (2003) Asymmetric structural motions of the homomeric α7 nicotinic receptor ligand binding domain revealed by molecular dynamics simulation. Biophys. J. 85, 3007–3018 CrossRef Medline

27. Purohit, P., and Auerbach, A. (2013) Loop C and the mechanism of acetylcholine receptor-channel gating. J. Gen. Physiol. 141, 467–478 CrossRef Medline

28. Hénault, C. M., and Baenzienger, J. E. (2017) Functional characterization of two prokaryotic pentameric ligand-gated ion channel chimeras: role of the GLIC transmembrane domain in proton sensing. Biochim. Biophys. Acta 1859, 218–227 CrossRef Medline

29. Mercado, J., and Czajkowski, C. (2006) Charged residues in the M2-M3 regions involved in GABAA receptor activation. J. Neurosci. 26, 2031–2040 CrossRef Medline

30. Hu, X. Q., Zhang, L., Stewart, R. R., and Weight, F. F. (2003) Arginine 222 in the pre-transmembrane domain 1 of 5-HT3A receptors links agonist binding to channel gating. J. Biol. Chem. 278, 46583–46589 CrossRef Medline

31. Lee, W. Y., Free, C. R., and Sine, S. M. (2009) Binding to gating transduction in nicotinic receptors: Cys-loop energetically couples to pre-M1 and M2-M3 regions. J. Neurosci. 29, 3189–3199 CrossRef Medline

32. Xiu, X., Hanek, A. P., Wang, J., Lester, H. A., and Dougherty, D. A. (2005) A unified view of the role of electrostatic interactions in modulating the gating of Cys loop receptors. J. Biol. Chem. 280, 41655–41666 CrossRef Medline

33. Reeves, D. C., and Lummis, S. C. R. (2006) Detection of human and rodent 5-HT3B receptor subunits by anti-peptide polyclonal antibodies. BMC Neurosci. 7, 27 CrossRef Medline