A Cation-π Interaction at a Phenylalanine Residue in the Glycine Receptor Binding Site Is Conserved for Different Agonists

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ABSTRACT

Cation-π interactions have been demonstrated to play a major role in agonist-binding in Cys-loop receptors. However, neither the aromatic amino acid contributing to this interaction nor its location is conserved among Cys-loop receptors. Likewise, it is not clear how many different agonists of a given receptor form a cation-π interaction or, if they do, whether it is with the same aromatic amino acid as the major physiological agonist. We demonstrated previously that Phe159 in the glycine receptor (GlyR) α1 subunit forms a strong cation-π interaction with the principal agonist, glycine. In the current study, we investigated whether the lower efficacy agonists of the human GlyR β-alanine and taurine also form cation-π interactions with Phe159. By incorporating a series of unnatural amino acids, we found cation-π interactions between Phe159 and the amino groups of β-alanine and taurine. The strengths of these interactions were significantly weaker than for glycine. Modeling studies suggest that β-alanine and taurine are orientated subtly differently in the binding pocket, with their amino groups further from Phe159 than that of glycine. These data therefore show that similar agonists can have similar but not identical orientations and interactions in the binding pocket and provide a possible explanation for the lower potencies of β-alanine and taurine.

Introduction

Glycine receptors (GlyRs) are ligand-gated chloride channels expressed mainly at inhibitory synapses in brain stem and spinal cord (Lynch, 2004). In these tissues, GlyRs contribute to generation of motor rhythm, coordination of reflex circuits, and processing of sensory signals (Legendre, 2001; Harvey et al., 2004). Along with GABA_A receptors, 5-hydroxytryptamine_3 (5-HT_3) receptors, and nicotinic acetylcholine receptors (nAChRs), GlyRs belong to the Cys-loop receptor family (Le Novere and Changeux, 2001). All Cys-loop members are composed of five subunits that are pseudosymmetrically arranged around a central ion-conducting pore. Individual subunits consist of a large N-terminal ligand-binding domain and four transmembrane helices that are connected by loops of varying size (Unwin, 1995; Miyazawa et al., 2003). Structural studies on acetylcholine-binding protein (AChBP) (Brejc et al., 2001) and prokaryotic Cys-loop receptor homologs (Hilf and Dutzler, 2008; Bocquet et al., 2009; Hilf and Dutzler, 2009) have confirmed the location of the agonist binding site at the interface of adjacent subunits. Three loops from one subunit (A–C) and three β strands from the adjacent subunit (D–F) create the binding pocket. Like all Cys-loop receptors, the GlyR binding site is lined with aromatic residues, and we have shown previously that a cation-π interaction between the positively charged amine of glycine and Phe159 in loop B makes a substantial contribution to agonist binding (Pless et al., 2008). Likewise, other studies have shown that aromatic side chains form cation-π interactions with the principal agonists of other Cys-loop receptors, such as nACh, 5-HT_3, MOD-1 (modulation of locomotor defect 1), and GABA_A receptors (Zhong et al., 1998; Beene et al., 2002; Mu et al., 2003; Lummis et al., 2005;
Padgett et al., 2007; Dougherty, 2008). However, the nature and location of the aromatic residue that interacts with the cation is not conserved among Cys-loop receptors or among different agonists for a given receptor (Beene et al., 2002; Mu et al., 2003; Dougherty, 2008; Xiu et al., 2009). Recent kinetic studies on the mechanism of partial agonism (Lape et al., 2008) have sparked a renewed interest in the question how partial agonists differ in their mechanism of action. Although a number of studies have established how glycine binds to the GlyR (Grudzinska et al., 2005; Pless et al., 2008), little is known about the binding mode of other GlyR agonists, such as β-alanine and taurine, which are both endogenous amino acid agonists of the GlyR (Schmieden et al., 1992; Lewis et al., 2003). β-Alanine and taurine are structurally similar to glycine (Fig. 1A), and all agonists compete for the same binding site (Schmieden et al., 1992; Schmieden and Betz, 1995) According to a single-channel kinetic analysis, dissociation rates for β-alanine and taurine are increased 2.5- and 4-fold, respectively (compared with glycine), and efficacy by 2- and 5-fold, respectively (Lewis et al., 2003). To explore the molecular mechanism(s) involved in these differences, we used the nonsense suppression method (Dougherty, 2000; Beene et al., 2003) to incorporate unnatural amino acids into the GlyR binding site to determine whether β-alanine and taurine, like glycine, form a cation-π interaction with a phenylalanine residue at position 159.

### Materials and Methods

#### Mutagenesis and Preparation of mRNA and Oocytes.

For optimal expression in oocytes, the human GlyR α1 subunit cDNA was subcloned into the pGEMHE vector. The QuikChange mutagenesis kit (Stratagene, La Jolla, CA) was used for site-directed mutagenesis. Successful incorporation of mutations was confirmed by automated sequencing. Capped mRNA for oocyte injection was generated using the mMessage mMachine kit (Ambion, Austin, TX). *Xenopus laevis* (Nasco, Fort Atkinson, WI) oocytes were prepared as described previously (Pless et al., 2007) and injected with mRNA, either alone or together with tRNA. After injection, oocytes were incubated for 18 to 36 h at 18°C.

#### Synthesis of tRNA and dCA Amino Acids.

The procedure used here has been described previously (Beene et al., 2004). In short, unnatural amino acids (as shown in Fig. 1) were chemically synthesized as nitroveratryloxycarbonyl-protected cyanomethyl esters and coupled to the dinucleotide dCA. The resulting product was subsequently ligated enzymatically to a 74-mer THG73 tRNA\textsubscript{Ala} as described previously (Nowak et al., 1998). The aminoacyl tRNA was deprotected by photolysis (Kearney et al., 1996) directly before coinjection with the mRNA. Typically, 10 to 15 ng of mRNA were coinjected with 20 to 30 ng of tRNA-amino acid in a total volume of 50 nl. In control experiments, mRNA was injected alone or together with tRNA-dCA (with no amino acid attached). Under these conditions, we never observed measurable currents, even at high (5 mM) concentrations of agonist (n = 6). This result confirmed that the constructs containing the TAG stop codon generated truncated and hence nonfunctional receptors. It also suggests that no endogenous amino acids from the oocyte are incorporated at these sites and, finally, that the tRNAs are not reacylated with naturally occurring amino acids by endogenous synthetases.

#### Characterization of Mutant Receptors.

The OpusXpress voltage-clamp system (Molecular Devices, Union City, CA) was used to record peak currents from individual oocytes induced by agonist. All recordings were performed at 22–25°C. Glycine, β-alanine, and taurine (Sigma, St. Louis, MO) were stored at −20°C as 1 M and 500 mM aliquots, respectively. They were diluted in ND96 (96 mM NaCl, 2 mM KCl, 1 mM MgCl\textsubscript{2}, 1.8 mM CaCl\textsubscript{2}, and 5 mM HEPES, pH 7.4) directly before the experiments. Delivery to the cells was achieved using the computer-controlled perfusion system of the OpusXpress. Glass microelectrodes had resistances between 0.5 and 3 MΩ and were backfilled with 3 M KCl. All recordings were performed at a holding potential of −60 mV. The empirical Hill equation, fitted with a nonlinear least-squares algorithm, was used to obtain half-maximal concentrations (EC\textsubscript{50}) and Hill coefficient (n\textsubscript{H}) values for ligand-induced activation (SigmaPlot 9.0; Systat Software, Point Richmond, CA). Data are presented as mean ± S.E.M.

#### Modeling.

This was performed as described previously (Reeves et al., 2003; Thompson et al., 2005). Three-dimensional models of the extracellular region of the glycine receptor were built using MODELLER 6, version 2 (http://salilab.org/modeller/) based on the crystal structure of acetylcholine-binding protein in the agonist-bound state (carbamylcholine; Protein Data Bank ID 1UV6). The model was energy-minimized in SYBYL version 6.8 (Tripos Inc., St. Louis, MO) using the AMBER force field by moving side chains alone (Weiner et al., 1984). Models and predicted hydrogen bonds were viewed using ViewerLite version 5.0 (Accelrys, San Diego, CA). Docking was performed using GOLD 3.0 (The Cambridge Crystallographic Data Centre, Cambridge, UK) as described previously (Thompson et al., 2007) with the ligands constrained to dock within 4.5 Å of Arg65, because a previous study has convincingly demonstrated that Arg65 is a common anchor point for the carboxyl termini of different agonists (e.g., glycine and taurine) (Grudzinska et al., 2005).

### Results

#### Locating the Residues of Interest Using Conventional Mutagenesis.

In this study, we chose to focus on

![Fig. 1. Structures of the agonists used in this study (A), as well as Phe (B) and unnatural Phe analogs (C) incorporated at positions 63, 159, and 207.](image)
Phe63, Phe159, and Phe207. Mutations at these sites markedly affect receptor function, and aligning residues in other receptors formed cation-π interactions in previous studies (Rajendra et al., 1995b; Grudzinska et al., 2005; Pless et al., 2008). F63A substitution caused increases in EC$_{50}$ values of 760-, 280-, and 360-fold for glycine, β-alanine, and taurine, whereas F63Y substitution had smaller effects on glycine and β-alanine responses (17- and 57-fold increases in EC$_{50}$, respectively), but no responses were detected with taurine, perhaps indicating that size here is critical for taurine binding/activation (Table 1).

F159Y substitution decreased EC$_{50}$ values for all three agonists as reported previously (Schmieden et al., 1993), whereas F159A substitution caused large increases (70–260-fold) (Table 1). F207A substitution eliminated all agonist responses. F207Y had no significant effect on EC$_{50}$ values. This mutation did, however, decrease $R_{\text{max}}$ (Table 1). Other aromatic residues near the GlyR binding pocket, such as Phe99, Phe100, and Tyr202 could also potentially contribute to ligand binding, but previous studies using conventional mutagenesis have ruled out cation-π interactions at these sites (see Discussion for details).

Characterization of the Phe63TAG Construct. Incorporating a Phe at position 63 with the nonsense suppression method resulted in functional receptors with EC$_{50}$ values similar to those of wild-type (WT) receptors (De Saint Jan et al., 2001; Pless et al., 2008). We thus conclude that the WT phenotype was successfully “rescued” by coinjection of Phe63TAG mRNA and Phe tRNA. A single fluorination (4-F-Phe) at position 63 resulted in a small decrease in EC$_{50}$ for β-alanine or taurine, respectively (Tables 2 and 3). In contrast, introduction of Phe derivatives with two or three fluorines (3,4-F$_2$-Phe and 3,4,5-F$_3$-Phe) resulted in 10- and 5-fold increases in the β-alanine EC$_{50}$, respectively (compared with WT). Because the EC$_{50}$ values for 4-F-Phe are lower than those for Phe for both β-alanine and taurine, and there is no pattern in the EC$_{50}$ values for 3,4-F$_2$-Phe and 3,4,5-F$_3$-Phe with β-alanine, we conclude that neither agonist forms a cation-π interaction with Phe63.

Phe159 Forms a Cation-π Interaction with β-Alanine. Successive addition of fluorine atoms to Phe derivatives at position 159 results in a stepwise increase in the β-alanine EC$_{50}$ (Fig. 2, A, C; and E; Table 2). These results strongly suggest a cation-π interaction between β-alanine and Phe159. The data include not only fluorinated analogs, but also a Phe derivative with a cyano group (CN) in the 4 position. 4-CN-Phe is slightly larger in size than the fluorinated Phe derivatives but is well tolerated in Cys-loop receptor binding sites (Padgett et al., 2007; Pless et al., 2008). This substitution yielded a β-alanine EC$_{50}$ between those for 3,4-F$_2$-Phe and 3,4,5-F$_3$-Phe, consistent with the cation-π binding ability of 4-CN-Phe.

A plot of the data reveal a strong linear correlation between the relative log EC$_{50}$ value (scaled to WT) and the cation-π binding ability of the incorporated Phe derivative (Fig. 3A), consistent with a cation-π interaction between β-alanine and Phe159 (Mecozzi et al., 1996; Zhong et al., 1998). The slope of this linear fit was significantly lower than that of glycine (Fig. 3C).

Comparing glycine-induced currents (Fig. 2, B and D) with β-alanine-induced currents (Fig. 2, A and C) reveals that 3,4,5-F$_3$-Phe, but not Phe, markedly reduces receptor desensitization for β-alanine-induced currents. This may suggest that a strong cation-π binding ability at position 159 is necessary for WT-like ligand-binding and gating properties.

Phe159 Also Forms a Cation-π Interaction with Taurine. Introduction of Phe derivatives with a range of cation-π interaction energies revealed that Phe159 also forms such an interaction between taurine and Phe159. The slope of this linear fit was significantly lower than that of glycine (Fig. 3C).

### TABLE 1

<table>
<thead>
<tr>
<th>Glycine</th>
<th>β-Alanine</th>
<th>Taurine</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT EC$_{50}$</td>
<td>100 ± 13</td>
<td>303 ± 61</td>
</tr>
<tr>
<td>$R_{\text{max}}$</td>
<td>0.9 ± 0.03</td>
<td>0.9 ± 0.04</td>
</tr>
<tr>
<td>F63A EC$_{50}$</td>
<td>89,000 ± 6000</td>
<td>115,000 ± 8,000</td>
</tr>
<tr>
<td>$R_{\text{max}}$</td>
<td>0.4 ± 0.05</td>
<td>1.2 ± 0.08</td>
</tr>
<tr>
<td>F63Y EC$_{50}$</td>
<td>2,000 ± 400</td>
<td>23,000 ± 3,000</td>
</tr>
<tr>
<td>$R_{\text{max}}$</td>
<td>1.0 ± 0.08</td>
<td>1.0 ± 0.08</td>
</tr>
<tr>
<td>F159A EC$_{50}$</td>
<td>8,100 ± 200</td>
<td>104,000 ± 17,000</td>
</tr>
<tr>
<td>$R_{\text{max}}$</td>
<td>1.1 ± 0.05</td>
<td>0.9 ± 0.07</td>
</tr>
<tr>
<td>F159Y EC$_{50}$</td>
<td>13.2 ± 1.0</td>
<td>9.3 ± 0.7</td>
</tr>
<tr>
<td>$R_{\text{max}}$</td>
<td>1.4 ± 0.04</td>
<td>1.0 ± 0.11</td>
</tr>
<tr>
<td>F207A EC$_{50}$</td>
<td>N.R.</td>
<td>N.R.</td>
</tr>
<tr>
<td>$R_{\text{max}}$</td>
<td>N.R.</td>
<td>N.R.</td>
</tr>
<tr>
<td>F207Y EC$_{50}$</td>
<td>161 ± 23</td>
<td>520 ± 88</td>
</tr>
<tr>
<td>$R_{\text{max}}$</td>
<td>0.6 ± 0.06</td>
<td>0.7 ± 0.04</td>
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</tbody>
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$R_{\text{max}}$, ratio of maximal currents induced by β-alanine or taurine versus glycine; N.R., no response at 100 mM.

### TABLE 2

<table>
<thead>
<tr>
<th>Glycine</th>
<th>β-Alanine</th>
<th>Taurine</th>
</tr>
</thead>
<tbody>
<tr>
<td>63-Phe (WT)</td>
<td>315 ± 16</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>63-F-Phe</td>
<td>123 ± 4</td>
<td>1.1 ± 0.4</td>
</tr>
<tr>
<td>63-F$_2$-Phe</td>
<td>3,890 ± 150*</td>
<td>1.6 ± 0.5</td>
</tr>
<tr>
<td>63-F$_3$-Phe</td>
<td>1,590 ± 210*</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>159-Phe (WT)</td>
<td>3,73 ± 8</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>159-F-Phe</td>
<td>470 ± 33*</td>
<td>0.9 ± 0.3</td>
</tr>
<tr>
<td>159-F$_2$-Phe</td>
<td>3,290 ± 120*</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>159-CN-Phe</td>
<td>11,506 ± 500*</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>159-F$_3$-Phe</td>
<td>20,600 ± 210*</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>207-Phe (WT)</td>
<td>559 ± 30</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>207-F-Phe</td>
<td>615 ± 54</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>207-F$_2$-Phe</td>
<td>3,230 ± 150*</td>
<td>0.2 ± 0.03</td>
</tr>
<tr>
<td>207-CN-Phe</td>
<td>5,080 ± 140*</td>
<td>0.1 ± 0.04</td>
</tr>
<tr>
<td>207-F$_3$-Phe</td>
<td>4,790 ± 210*</td>
<td>0.1 ± 0.03</td>
</tr>
</tbody>
</table>

* $P < 0.05$, significantly different from WT for EC$_{50}$ values (Student’s t test).

### TABLE 3

<table>
<thead>
<tr>
<th>Glycine</th>
<th>β-Alanine</th>
<th>Taurine</th>
</tr>
</thead>
<tbody>
<tr>
<td>159-Phe (WT)</td>
<td>507 ± 29</td>
<td>2.1 ± 0.3</td>
</tr>
<tr>
<td>159-F-Phe</td>
<td>681 ± 56</td>
<td>0.9 ± 0.04</td>
</tr>
<tr>
<td>159-CN-Phe</td>
<td>5,100 ± 458*</td>
<td>0.1 ± 0.04</td>
</tr>
<tr>
<td>159-F$_3$-Phe</td>
<td>9,700 ± 2400*</td>
<td>0.3 ± 0.005</td>
</tr>
<tr>
<td>207-F-Phe</td>
<td>720 ± 89*</td>
<td>0.4 ± 0.10</td>
</tr>
<tr>
<td>207-F$_2$-Phe</td>
<td>5,660 ± 430*</td>
<td>0.2 ± 0.03</td>
</tr>
<tr>
<td>207-F$_3$-Phe</td>
<td>3,600 ± 230*</td>
<td>0.4 ± 0.004</td>
</tr>
<tr>
<td>63-F-Phe</td>
<td>389 ± 41</td>
<td>0.6 ± 0.03</td>
</tr>
<tr>
<td>63-F$_2$-Phe</td>
<td>N.R.</td>
<td>0.7 ± 0.04</td>
</tr>
<tr>
<td>161-F-Phe</td>
<td>2,500 ± 200*</td>
<td>0.2 ± 0.03</td>
</tr>
<tr>
<td>161-CN-Phe</td>
<td>424 ± 14*</td>
<td>0.7 ± 0.04</td>
</tr>
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</table>

N.R., no response at 100 mM.

* $P < 0.05$, significantly different from WT for EC$_{50}$ values (Student’s t test).
interaction with taurine. Thus, 4-F-Phe, 4-CN, and 3,4,5-F3-Phe caused ~1.3-, 10-, and ~19-fold increases in EC50, respectively (Table 3; Fig. 2F). A plot of relative log EC50 values and the cation-π binding ability of the incorporated Phe derivative indicate a cation-π interaction between taurine and Phe159 (Fig. 3B). The slope of the linear fit for taurine is less than that for β-alanine (Fig. 3C), suggesting a weaker interaction here.

**Taurine Effects at Tyr161 Mutant Receptors.** Previous data suggest that Tyr161 may participate in agonist binding at the GlyR: its substitution with Phe, combined with an F159Y mutation, resulted in a receptor that was ~100-

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**Fig. 2.** Successive fluorination leads to monotonic decreases in β-alanine and taurine sensitivity at position 159. β-Alanine-induced current traces recorded from oocytes injected with GlyR Phe159TAG mRNA and Phe tRNA (A) or GlyR Phe159TAG mRNA and 3,4,5-F3-Phe tRNA (C). Glycine-induced current traces in B and D are shown for comparison. Horizontal bars indicate duration of agonist application. E and F, concentration-response curves for β-alanine- and taurine-induced currents, respectively.

**Fig. 3.** GlyR responses from Phe159 mutant receptors yield linear cation-π plots for both β-alanine and taurine. A and B, the log EC50 (based on data in Tables 2 and 3) normalized to the WT log EC50 was used to generate a plot of the GlyR Phe159 mutants for β-alanine (A) and taurine (B). The linear fits were \( y = 3.34 - 0.13x \) (\( r = 0.96 \)) for β-alanine and \( y = 2.41 - 0.09x \) (\( r = 0.97 \)) for taurine. C, cation-π plots comparing the cation-π interactions for glycine, β-alanine, and taurine at the Phe159 GlyR (glycine data from Pless et al., 2008). For better comparison, the x values are offset so all linear fits converge at zero. Note that the value for CN-Phe is omitted in the gradient for β-alanine and taurine. This did not change the slope of the linear fit significantly [for comparison, the linear fit for glycine (without CN-Phe) is \( y = 5.58 - 0.22x \) (\( r = 0.99 \))].
fold more highly sensitive to β-alanine and ~30-fold more sensitive to taurine (Schmieden et al., 1993). The β-alanine effect has been shown to be indirect (De Saint Jan et al., 2001), but the increase in taurine EC₅₀ (~5 fold) with 4-F-Phe at this position suggests a possible cation-π interaction. Substitution with 4-CN-Phe, however, resulted in a taurine EC₅₀ similar to that in WT receptors, indicating there is no cation-π interaction here (Table 3).

**Characterization of the Phe207TAG Construct.** 4-F-Phe substitution at position 207 did not change the β-alanine EC₅₀ compared with WT (Table 2). Incorporation of 3,4-F₂-Phe and 3,4,5-F₃-Phe resulted in large increases in the β-alanine EC₅₀ (60- and 90-fold, respectively), although substitution with 4-CN-Phe produced only a 9-fold increase in EC₅₀; these data are inconsistent with a cation-π interaction. The pattern was broadly similar for taurine, with no change in the EC₅₀ with 4-F-Phe, but some increases when 3,4-F₂-Phe and 3,4,5-F₃-Phe were incorporated (11- and 7-fold, respectively; Table 3). These data also do not support a cation-π interaction at position 207.

Compared with the maximum currents observed with Phe207 mutants for glycine (similar to wild type), Rₘₐₓ values were much reduced when these mutants were activated by β-alanine or taurine (Tables 2 and 3). Changes in Rₘₐₓ values may indicate an effect on receptor gating, so Phe207 may play a different role in gating for these partial agonists than for the full agonist glycine.

**Locating β-Alanine in the Binding Pocket.** We have previously created a model of the glycine receptor binding pocket, and docking of glycine revealed the potential for a strong cation-π interaction with Phe159 (Pless et al., 2008) (Fig. 4, B and C). Here we have docked β-alanine into the same model, and the data show that it is located in a broadly similar orientation to glycine, but the amino group is further from the center of Phe159 than the amino group of glycine (Fig. 4, D and E).

**Locating Taurine in the Binding Pocket.** We also docked taurine into the ligand binding pocket, and the results reveal that its amino group, like that of β-alanine, is further from Phe159 than the amino group of glycine. However, the major difference compared with glycine is the location of the sulfonate group, which is close to Phe63 (Fig. 4, F and G). This location is supported by the mutagenesis data, which suggest that taurine is more sensitive than glycine and β-alanine to changes in this residue (Tables 1 and 3).

**Discussion**

In this study, we demonstrate that the GlyR partial agonists β-alanine and taurine can form cation-π interactions with Phe159 in the binding pocket of the glycine receptor. For both ligands, there is a clear correlation between the EC₅₀ and the cation-π binding ability of phenylalanine derivatives incorporated at position 159 in the GlyR (Fig. 3), making a strong argument for cation-π interactions between the amino groups of β-alanine and taurine and this loop B phenylalanine. The data do not support a cation-π interaction between either taurine or β-alanine and Phe63 or Phe207. The identification of single cation-π interactions at Phe159 with both these agonists is consistent with an earlier study that also demonstrated a single cation-π interaction between the amino group of the principal GlyR agonist glycine and Phe159 (Pless et al., 2008). The data also conform to the observations that the most common cation-π interactions in Cys-loop receptors are mediated through aromatic amino acids in loop B (Trp149 of the nAChR, Trp183 of the 5-HT₃ receptor, Tyr198 of the GABA₃ receptor, and Phe159 of the GlyR), although cation-π interactions with residues on loop A

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**Fig. 4.** Docking of β-alanine and taurine into the GlyR binding pocket. A, location of an agonist in the binding pocket. Model of the extracellular domain of the GlyR showing two of the five subunits and some of the residues (in stick form) that surround the agonist (Corey/Pauling/Koltun coloring) in the binding pocket: Phe159 and Phe207 are located on the principal (red) subunit, whereas Phe63 and Arg65 are located on the complementary (blue) subunit. B and C, two views of glycine docked into the binding site from different perspectives showing the amino group located between Phe159 and Phe207 and the carboxyl group some distance from Phe63. D and E, two views of β-alanine docked into the binding site showing that the amino group is further from Phe159 compared with the amino group of glycine, although the carboxyl group is similar distance from Phe63. F and G, two views of taurine docked into the binding pocket showing that the amino group is again further from Phe159 compared with glycine, and the sulfonate group is closer to Phe63 than the carboxyl of glycine.
site. The only structural difference between glycine and
sible locations of the agonists in a model of the GlyR binding
and Dougherty, 1999).

An intriguing aspect of our new data is the fact that the
slopes of the fluorination plots for β-alanine and taurine are
significantly reduced compared with that for glycine (Fig.
3C). This suggests that the relative strength of the cation-π
interactions is glycine > β-alanine > taurine, as weakening
of the cation-π interaction by successive fluorination has the
most dramatic effect on activation by glycine, then β-alanine,
and finally taurine. The slope for glycine (Pless et al., 2008)
resembles that of other agonists containing a primary amine,
such as GABA (Padgett et al., 2007), whereas the slopes for
β-alanine and taurine are more similar to that of the quater-
ary ammonium ion of acetylcholine (β-alanine = −0.13;
taurine = −0.09; acetylcholine = −0.10) (Zhong et al., 1998).

It has been suggested previously that the less focused charge
of the quaternary ammonium ion of acetylcholine leads to a
weaker cation-π interaction compared with agonists with a
primary amine, such as glycine, serotonin, and GABA (Beene
et al., 2002; Pless et al., 2008). Here, however, we show that
agonists with primary amines (β-alanine and taurine) can
also form cation-π interactions that are similar to those
formed by agonists with a quaternary ammonium. This
seems to suggest that not only the nature of the cationic
moiety but also the exact orientation determines the strength
of a cation-π interaction at a given site. Such an idea is
consistent with a computational study, which demonstrated
that the exact orientation of the aromatic residue and the
cation are crucial for a strong cation-π interaction (Gallivan
and Dougherty, 1999).

To further explore this hypothesis, we examined the pos-
ible locations of the agonists in a model of the GlyR binding
site. The only structural difference between glycine and β-
alanine is the slightly elongated carbonyl backbone of β-
alanine (Fig. 1A). Docking studies locate the amino group of
β-alanine further from the center of the aromatic ring of
Phe159, which would result in a weaker cation-π interaction
(Fig. 4).

Like β-alanine, taurine also has an elongated carbonyl
backbone and a sulfonate group in place of the carboxyl group
(Fig. 1A). Ligand docking shows that this molecule is also
oriented with the amino group further displaced from Phe159
than it is for glycine, and it is in a different orientation to
β-alanine, providing an explanation for the weaker cation-π
interaction. It is possible, however, that the model is not
quite accurate for partial agonists, resulting in inaccuracies
in docking; a recent crystallographic study revealed that
partial agonists bind to a more open binding pocket in ace-
tylcholine binding protein because of the reduced closure of
the C loop (Hibbs et al., 2009). It is noteworthy, therefore,
that substitutions of Phe207 in loop C had severe effects on
β-alanine- and taurine-induced currents (taurine > β-alani-
nine) but little effect on glycine-induced currents. Taken
gether, these results suggest that glycine, β-alanine, and
taurine may each induce distinct conformational changes in
and around the GlyR binding site (taurine < β-alanine <
glycine), an idea that is also supported by a recent study
using fluorescent reporter groups (Pless and Lynch, 2009).

These different conformational changes could possibly be
the cause of the different magnitudes of the responses pro-
duced by these different agonists (i.e., their efficacy). Intrin-
sic efficacy is related to agonist concentration, agonist affin-
ity, and receptor number (Furchgott, 1966) but also depends
on other factors, including the receptor state (e.g., open,
closed, desensitized) and the kinetics of the interactions be-
tween these states (Colquhoun and Hawkes, 1987). In addi-
tion, there are contributions from more “external” factors,
such as the expression system or brain region; taurine, for
example, has different efficacies in oocytes and human em-
brionic kidney cells, and also in brain and spinal cord neu-
rons (Killcross et al., 1997; Farroni and McCool, 2004).
Therefore, it is not surprising that a quantitative under-
standing of the link between the receptor-ligand interactions
and efficacy is still in its infancy. We are tempted to speculate
that the different strengths of the different cation-π interac-
tions with the different agonists are related to their different
efficacies. A lower efficacy could be the result of reduced loop
C closure, as a result of a decrease in cation-π binding en-
ergy, which could result in less efficient receptor activation.
This may be reflected in—or caused by—the decrease in
desensitization rates that we observed, which indicates a
change in the kinetics of the interactions between the differ-
ent states. Considerable work is needed to prove or disprove
this speculative hypothesis.

In principle, aromatic amino acids other than Phe159 could
also contribute to a cation-π interaction in the GlyR binding
site. Phe99, Phe100, Tyr161, and Tyr202 are located close to
the binding site, but previous studies have suggested that
they are unlikely to contribute to a cation-π interaction.
Substituting Phe99 or Phe100 with a small and nonaromatic
amino acid, alanine, resulted in only a minor shift in the
EC50 values (Vafa et al., 1999). A pioneering study suggested
that Tyr161 was important for β-alanine binding (Schmieden
et al., 1993), but more recent work convincingly demon-
strated that this residue has only indirect effects (De Saint
Jan et al., 2001). The present study also indicates that there
is no cation-π interaction between this residue and taurine.
Finally, two studies have demonstrated that the hydroxyl
group of Tyr202, rather than its aromatic character, is cru-
ial for agonist binding (Rajendra et al., 1995a; Grudzinska
et al., 2005).

It is also conceivable that the cation originates from a
positively charged amino acid near the binding site, rather
than the amino group of the ligand: Arg65, Arg131, Lys200,
and Lys206 are all in close physical proximity to the binding
site. However, neutralizing Arg131, Lys200, or Lys206 re-
results in only minor shifts in agonist EC50 values (Vandenberg
et al., 1992; Yang et al., 2007), whereas Arg65 interacts with
the negatively charged head group of both full and partial
agonists (Grudzinska et al., 2005). We thus conclude that the
amino groups of β-alanine and taurine form cation-π inter-
actions with Phe159.

GlyRs are emerging as pharmacological targets, and thus
the exact binding mechanism of full agonists, partial ago-
nistsb and even antagonists in GlyRs is of particular interest
(Laube et al., 2002). The results presented here will aid
future efforts to delineate the exact binding mode of these
compounds and may be useful to predict (and control) agonist
efficacy in Cys-loop receptors.
References


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