Structure and Mechanism of Glycine Receptor Elucidated by Cryo-Electron Microscopy

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Glycine receptors (GlyRs) are pentameric ion channels that mediate fast inhibitory neurotransmission. GlyRs are found in the central nervous system including the spinal cord, brain stem, and cerebellum, as well as in the retina, sperm, macrophages, hippocampus, cochlea, and liver. Due to their crucial roles in counter-balancing excitatory signals and pain signal transmission, GlyR dysfunction can lead to severe diseases, and as a result, compounds that modify GlyR activity may have tremendous therapeutic potential. Despite this potential, the development of GlyR-specific small-molecule ligands is lacking. Over the past few years, high-resolution structures of both homomeric and heteromeric GlyRs structures in various conformations have provided unprecedented details defining the pharmacology of ligand binding, subunit composition, and mechanisms of channel gating. These high-quality structures will undoubtedly help with the development of GlyR-targeted therapies.

Keywords: glycine receptor, cryo-EM, agonist, inhibitory receptor, antagonist, partial agonist, potentiator, gating mechanism

INTRODUCTION

Glycine receptors (GlyRs) are ligand-gated ion channel that are members of the Cys-loop superfamily which also includes the GABA_A receptor (GABA_A R), nicotinic acetylcholine receptor (nAChR), serotonin type-3 receptor, and zinc-activated ion channel (Lester et al., 2004). GlyRs can be activated by a variety of endogenous ligands including the full agonist glycine as well as the partial agonists taurine, β-alanine, and GABA (Lynch, 2004). The activation of GlyRs caused by agonist binding results in a Cl⁻ flow across the membrane that is regulated by the Cl⁻ equilibrium potential and induces the membrane hyperpolarization, which in turn inhibits neuronal activity (Legendre, 2001; Lynch, 2004).

There are four known GlyR α subunits (α1–α4) and one β subunit identified by molecular cloning (Grenningloh et al., 1987; Grenningloh et al., 1990; Akagi et al., 1991; Kuhse et al., 1991; Matzenbach et al., 1994). Each GlyR subunit is composed of an extracellular domain (ECD), a transmembrane domain (TMD), and a long intracellular loop connecting transmembrane domains M3 and M4. The binding pockets are formed by two adjacent subunits located in the ECD (Lynch, 2009). All of the GlyR α subunits have substantial sequence similarity, exceeding 90%, whereas the GlyR β subunit has a considerable sequence variation when compared with GlyR α subunits (Lynch, 2009). Functional GlyRs include homomeric α GlyRs and heteromeric α-β GlyRs. In the prenatal stage, the predominant type of GlyR is homomorphic α2, whereas the adult GlyR types are mainly heteromeric α1-β GlyRs (Becker et al., 1988; Lynch, 2009). The GlyR subunit α3 is involved in nociceptive signaling pathways and function as a novel analgesic candidate (Huang et al., 2017b;
In 1982, GlyRs were isolated for the first time via strychnine affinity chromatography (Pfeiffer et al., 1982). The structural investigations of GlyR, on the other hand, are behind. Significant progresses have been achieved in studying the structures of GlyR as a result of the development of cryo-EM (Du et al., 2015; Kumar et al., 2020; Yu H. et al., 2021a; Yu J. et al., 2021b; Zhu and Gouaux, 2021). At present, high resolution GlyR structures bound with different ligands have been reported (Table 1). Also, these structures have revealed crucial information about the ligand binding and gating mechanism. In this minireview, I have discussed the recent progress in elucidating the structures of GlyR–ligand complexes and progress in elaborating the gating mechanism in GlyRs using single particle cryo-electron microscopy (cryo-EM).

### Full Agonist: Glycine

Glycine, the full agonist to GlyR that is co-released with GABA from presynaptic vesicles (Jonas et al., 1998), can efficiently activate GlyRs. The single channel recordings demonstrated that glycine elicits a maximum open probability ($P_{\text{open}}$) of 0.97, much higher than other agonists (Yu J. et al., 2021b). A
FIGURE 1 | GlyR-ligand interactions and the ion channel permeation pathway. (A) Side view of isolated homomeric GlyR dimer in cartoon representation. The principle (+) and complementary (−) subunit are colored in pink and green, respectively. The boxed area is enlarged in (B–E). (B–E) Views of binding pockets of homomeric GlyR bound with glycine (B), GABA (C), taurine (D), and strychnine (E). The ligand molecules are shown in stick representations with oxygen in red, nitrogen in blue, and carbon in cyan. The possible hydrogen bonds are shown as dashed lines. (F,G) Views of ivermectin (F) and picrotoxin (G) binding to homomeric GlyR. (H–K) Shape and size of the homomeric GlyR ion permeation pathway for apo (H), closed (I), open (J), and desensitized (K) state. M2 helices are shown as cartoons and the side chains of pore-lining residues are in ball and stick representation. Purple, green, and red spheres define radii of >3.3 Å, 1.8–3.3 Å and <1.8 Å, respectively. (L–O) (Continued)
The side chain of 9′L pointing to the channel axis, creating a constriction less than 3 Å. Compared with glycine, more interactions were observed for taurine and GABA. A potential hydrogen bond was found between the carbonyl group of S174 and the amino group of taurine, as well as an interaction between E173 and GABA (Figures 1C,D). These additional interactions for GABA and taurine are possibly due to the ligand’s greater length. A comparison of the GlyR structures bound with full and partial agonists shows that the binding of partial agonists induces a lesser extended binding pocket than the full agonist. Moreover, the efficiency of the agonists has a close relationship with the volume of the ligands, with the more efficient of the ligand having a lower ligand volume.

Upon the binding of partial agonists, the gating cycle of GlyRs involves transitions of multiple states (Lape et al., 2008; Lape et al., 2012). At present, three physiological GlyR states bound with partial agonists have been captured: closed, open, and desensitized. The researchers hypothesized that the partial agonists bound closed state is a pre-open state between the apo and open state (Yu J. et al., 2021b). Based on the available structures, the partial agonist gating mechanism was established. During the transition from the apo to the closed state, after the ligand accesses the binding site, a contraction of the binding pocket occurs. But no conformational changes were observed for the TMD, with 9′L functioning as the constriction point (Figures 1H,I). From the closed to the open state, the binding pockets shrink more. The conformational changes that happen at the binding pockets travels down to the ECD-TMD interface, which further triggers the tilt of the M2 helices. The tilt of the M2 helices prompts the rotation of the side chain of 9′L and causes the channel to open (Figure 1J). After opening, the lower part of the M2 helices will undergo a further tilt, creating a constriction point at −2′P (Figure 1K) and blocking the ion permeation (Yu J. et al., 2021b).

Antagonist: Strychnine

Strychnine, an alkaloid that can bind and antagonize GlyR, has been widely employed in radioligand binding and affinity purification experiments (Lynch, 2004, 2009; Breitinger and Breitinger, 2020; Cerdan et al., 2020). According to the human GlyR α3 X-ray crystal and GlyR_{EM} (Du et al., 2015; Huang et al., 2015; Yu H. et al., 2021b), strychnine shares the same binding pocket as glycine (Figure 1E). One state was captured for GlyR_{EM} bound with strychnine (Du et al., 2015), which features parallel M2 helices with 9′L pointing to the channel axis, resulting in a diameter of 3 Å and blocking the permeation pathway. In total, two states were obtained for human heteromeric GlyR α2-β bound with strychnine (Yu H. et al., 2021a), and both of the two states exhibit a constriction point at 9′L, but the conformation of their TMD are in markedly different (Figures 1N,O,R,S).
Potassium: Ivermectin

The ivermectin functions as the potentiator to GlyR, which can enhance the glycine sensitivity and increase the $P_{\text{open}}$ (Shan et al., 2001a; Breitinger and Breitinger, 2020; Cerdan et al., 2020). There are several ivermectin-bound homomeric GlyR structures available (Table 1). The structures demonstrate that ivermectin is bound at the interface of M3 and M1 and forms a polar interaction with M2 (Figure 1F). Compared with the glycine-bound GlyRmm open state (Du et al., 2015), when ivermectin binds with GlyR, the M2 helices undergo movement toward the pore lumen and contract the intracellular opening the ion channel at $-2^\circ$ and enabling chloride ions to pass through. The cryo-EM structures also prove that the binding of ivermectin traps the zebra fish homomeric GlyR α1 at a partially-open state (Du et al., 2015), with the narrowest point at $-2^\circ$ comparable to glycine bound open state in SMA (Yu H. et al., 2021b).

Channel Blocker: Picrotoxin

Homomeric GlyR is more sensitive than heteromeric GlyR to the inhibition of the channel blocker picrotoxin (Pribilla et al., 1992). The picrotoxin IC50 values for homomorphic and heteromeric GlyR are 18±1 and 259±44 μM, respectively (Shan et al., 2001b). The zebra fish homomeric GlyR α3 structure bound with picrotoxin (Kumar et al., 2020) shows that the picrotoxin is nestled between 2’G and 9’L and forms hydrogen bonds with 6’T (Figure 1G). The structural basis for heteromeric GlyR’s resistance to picrotoxin has been recently revealed (Zhu and Gouaux, 2021). The pig heteromeric GlyR structure demonstrates that the side chain bulk of 6’F on GlyR β subunit, the corresponding residue to 6’T in homomeric GlyR, on the one hand, provides a steric hindrance for picrotoxin accessing the binding site, while, on the other hand, it pushes the M2 helices away from each other and prevents picrotoxin binding.

Heteromeric Glycine Receptors

The predominant type GlyR in adult is heteromeric GlyR (Becker et al., 1988; Lynch, 2009), which is composed of α and β subunits. Since the ligand-binding pockets are located at the interface of the subunits, appropriate knowledge of the subunit stoichiometry of heteromeric GlyRs is thus important to understand the molecular pharmacology. Several subunit stoichiometries have been investigated including 3α:2β, 2α:3β, and 4α:1β obtained by different methods (Langosch et al., 1988; Burzomato et al., 2003; Grudzinska et al., 2005; Durisić et al., 2012; Yang et al., 2012). Because α and β subunits show high similarity in the secondary and tertiary structures (Dutertre et al., 2012), the inconsistent results on the subunit stoichiometry reflect the difficulties in distinguishing these two subunits. The fundamental factor to solve the subunit stoichiometry problem is to precisely tag one of the subunits. In 2021, both the Gouaux group and the Wang group published the near-atomic structures of heteromeric GlyR using tissue-isolated and recombinant pig α1-β GlyR and recombinant human α2-β GlyR, respectively (Yu H. et al., 2021a; Zhu and Gouaux, 2021). A same subunit stoichiometry, which is 4α:1β, was achieved for both groups using different methods, and no other subunit composition was described. In the Gouaux group’s research, the native GlyRs were purified from pig’s spinal cord and the brain stem by strychnine affinity resin (Graham et al., 1985). A monoclonal antibody specific to GlyR α subunit was prepared to enable differentiation of α and β subunits. Data has suggested that N terminal–fused GFP is tolerated by GlyR (David-Watine et al., 1999). Interestingly, the Wang group employed an EGFP which was inserted between M3 and M4 helices to identify the GlyR β subunit. The 4α:1β stoichiometry provides important implications associated with heteromeric GlyR function and pharmacology, such as the clustering of heteromeric GlyR and drug development specific to heteromeric GlyRs. There are current two states reported for heteromeric GlyR bound with glycine: one is desensitized state and the other is semi-open (Table 1). In contrast to the semi-open state, the desensitized state shows a five-fold quasi-symmetrical TMD (Figures 1L,M,P,Q), which is similar to the homomeric desensitized state in SMA (Figures 1K,L).

Assembly Pathway of Glycine Receptors

Members of the heteromeric Cys-loop family are composed of at least two different types of subunits. The investigation of the assembly intermediates can provide insights into the oligomerization process. Though research on the assembly process of nAChRs (Green and Claudio, 1993) and GABA$_A$Rs (Klausberger et al., 2001) have been reported, little is known regarding the assembly pathway of heteromeric GlyR. Compared with nAChRs and GABA$_A$Rs, the assembly process of heteromeric GlyR is comparatively simple due to the involvement of two types of subunits. By using strychnine affinity resin (Pfeiffer et al., 1982), the GlyR assembly intermediates were successfully isolated from the native materials (Zhu and Gouaux, 2021). A total of two assembly intermediates including a homomeric α tetramer and a homomeric α trimer were captured. However, the homomeric α dimer and β subunit containing assembly intermediates were missing, which might be due to non-functional binding pockets that needs further investigation. Given that GlyR assembly intermediates were captured by strychnine affinity resin, which demonstrates that the functional binding pockets are forming during receptor assembly. The findings reveal that the GlyR oligomerization occurs in steps, with one subunit added at each step. Because all of the assembly intermediates are α homomers, there will be insufficient supply of free α subunit. As a result, in the final step, the chance of homomeric α tetramer assembly with a β subunit to produce a heteromeric pentamer is larger than the likelihood of homomeric pentamer formation. The findings may be relevant for future drug development focusing on the GlyR assembly intermediates.

DISCUSSION

This review summarizes the recent progress of elucidating the structures of GlyR in a complex with different ligands at
different conformations by single particle cryo-EM. These structures shed light on the gating mechanism and assembly pathway of GlyR and may provide important details for subsequent GlyR-specific drug design and screening of the authorized drugs. Despite this, little is known about the structural basis of GlyR clustering, GlyR–metal interaction, as well as other GlyR bound ligands (Cerdan et al., 2020), all of which are important goals in future.

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AUTHOR CONTRIBUTIONS

HZ designed and wrote the manuscript.

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