Minireview

Structure and Function of the Glycine Receptor and Related Nicotinicoid Receptors*

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The inhibitory glycine receptor (GlyR)1 is a member of the nicotinicoid receptor superfamily that includes the inhibitory γ-aminobutyric acid, type A, receptor (GABAAR) and the excitatory nicotinic acetylcholine receptor (nAChR) and serotonin, type 3, receptor (5-hydroxytryptamine, type 3, receptor; PDB, Protein Data Bank). This paper is available online at http://www.jbc.org

1 GlyR subunits in insect cells provides 1 chain of the GlyR is sufficient to reconstitute native-like activity when expressed in heterologous cells (6), and thus expression of α1 GlyR subunits in insect cells provides relatively large quantities of functional homomeric receptors (7).

Receptor Topology

The GlyR, as well as all members of the nicotinicoid superfamily of neurotransceptors, has been modeled as having a large extracellular globular domain and four transmembrane (4 TM) α-helices per subunit with a large intracellular loop between the third and fourth TM domain (Fig. 1). This “historical” 4 TM helix model was suggested by hydrophobicity plots of the sequences of the ligand-gated channels (8). This 4 TM helix model is consistent with most of the accumulated biochemical data, principally from studies on nAChR. However, critical evaluation of this model in the absence of high resolution structural data is problematic. Electron diffraction studies conducted on two-dimensional crystalline arrays of the nAChR have progressively refined the 4 helix model of the pentameric αβββγ nAChR isolated from Torpedo (9) (Fig. 1C). These studies have provided the first glimpses of a vertebrate ion channel, elegantly defining the funnel-shaped structure of the nicotinicoid receptors and changes in receptor structure upon channel activation. These channels are 120 Å long and have an outer diameter of 70–80 Å, a central pore, and a funnel-shaped extracellular domain protruding 60 Å above the bilayer. At a resolution of 4 Å, the most recent nAChR model contains a four-helix bundle as the transmembrane domains, but at this resolution the molecular details at the level of individual amino acids are not readily apparent.

The ligand-binding sites of the nicotinicoid receptors are in the interface of the large N-terminal extracellular domains of neighboring subunits. The loops defining these binding sites have been extensively characterized by cross-linking and mutagenic studies (3, 10). Recently, the crystal structure of a soluble pentameric acetylcholine-binding protein (AChBP) secreted from snail gial cells was determined (11). The sequence of this protein is highly homologous to the N-terminal domain of the nicotinicoid receptors. The crystal structure of the AChBP suggests that the N-terminal domain of the nicotinicoid superfamily is a sandwich of antiparallel β-sheets and contains very little α-helix (Fig. 1B). The binding site for ligand was observed to be located in the interface between neighboring subunits, and the residues lining the binding site in AChBP correlated well with residues implicated in ligand binding in the receptor (12). Sine et al. (13) systematically mutated the extracellular domain of the ε subunit of the nAChR and unequivocally showed that this ligand-binding domain does, indeed, share structural homology with the AChBP. Thus it appears that the extracellular domain of the nicotinicoid receptors consists primarily of antiparallel β-strands arranged approximately perpendicular to the membrane surface, with connecting membrane-proximal loops contacting protein at the membrane surface.

The topology of the membrane-spanning domains of the GlyR and other members of the nicotinicoid receptor superfamily has been partially characterized by identifying sites of covalent modification subsequent to addition of hydrophilic or lipophilic reagents. Most commonly, the structures of ion channels have been probed via the substituted cysteine accessibility method (SCAM) (14). Covalent modification of Cys located in the channel with bulky charged methanethiosulfonate reagents will reduce or block channel activity. The periodicity of channel inactivation of systematic Cys mutants provides information regarding local structure. This method has been used extensively to examine proposed pore-lining segments in the homologous nAChR (15), serotonin receptor (16, 17), and GABAAR (18). These studies confirmed that, as originally shown in studies of nAChR (19), the M2 membrane-spanning segment of these receptors has a periodicity consistent with an α-hel-
tirely helical, as well as a significant portion of the M3 domain that the M2 and M4 regions of the nicotinicoid receptors are expressed as described above, SCAM and lipophilic labeling studies indicate estimate the helical content of the transmembrane domains of the nAChR. Coordinates were from Protein Data Bank numbers 1I9B and 1OED, respectively. All ribbon figures were prepared with MIDAS and surface representations by VMD.

In the absence of structural data, many integral membrane proteins have been typically modeled to contain membrane-spanning α-helices (with intrachain H-bonding) or barrels of β-strands (with interchain H-bonding) given the tremendous energetic costs of burying any non-hydrogen-bonded backbone carbonyls or amides (33). The potential pitfall of mapping transmembrane topology via sequence analysis has been illustrated in investigations of the glutamate receptor in which, contrary to previous models, the putative second transmembrane domain does not span the bilayer but rather forms a re-entrant loop (34). The recent determination of the high resolution structures of the KcsA channel (35), the glyc erol-conducting aquaporin (GlpF) (36), the CIC channel (37), the voltage-gated K+ channel (38), and the large mechanosensitive (MscL) channel (39) have challenged long-held suppositions of permitted structures in the membrane-spanning domains of ion channels. Surprisingly, the crystal structures of all of these channels have significant amounts of membrane-embedded residues that are neither helical nor β-sheet structures but contain helices that only penetrate partially through the membrane (Fig. 2). The portions of the non-helical regions that do not penetrate through the membrane are not in contact with the acyl chains of the lipid but rather are sequestered and effectively shielded from the lipids by surrounding protein. This scenario was originally posited by Lodish (40) and more recently described by Hamasaki et al. (41). As described in this latter publication, freed from the energetic constraints thought to limit single-pass TM proteins to being a rod-like rigid helix, the membrane-embedded portions of membrane proteins with multiple TM domains may be shorter and more flexible, providing the allosteric mechanisms essential for activity (e.g. channel gating). Clearly additional information is needed to more rigorously define a new topological model that reflects the structure of the GlyR and other nicotinicoid receptors.

**Channel Gating**

Models of the ligand-binding domain of nicotinicoid receptors are fairly easy to generate given the AChBP structure (15–24% homology) as a template (13, 42, 43). In addition, differential accessibilities to labeling reagents in SCAM studies have illustrated many of the structural changes in the resting, open, and desensitized state of the receptor (15, 44). Photo labeling studies of the nAChR also showed differential accessibility of residues in the α1 subunit in open, closed, and desensitized states, particularly in the signature Cys-loop (45). What is not apparent is how the extracellular domain interacts with the transmembrane domain and its surface loops to provide for channel gating. Hints at this linkage have been sup-

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**Minireview: GlyR Structure and Function**

![Fig. 1. Nicotinicoid receptor structures. A, schematic representation of the topology of the α1 subunit of GlyR. B, ribbon model of a single subunit of the heteropentameric AChBP. C, surface representation of the top and side view of the transmembrane domains of the homopentameric nAChR. Coordinates used in panels B and C were from Protein Data Bank numbers 1I9B and 1OED, respectively. All ribbon figures were prepared with MIDAS and surface representations by VMD.](image_url)

![Fig. 2. The structures of ion channels contain significant non-helical structure in the plane of the bilayer. Non-helical structure is shown in red. The edges of the ~30 Å acyl portion of the bilayer are represented by horizontal lines. All proteins are presented as monomers except where noted: KcsA (PDB 1R3J) (A), GlpF (PDB 1FX8) (B), and CIC dimer (PDB 1KR3) (C).](image_url)
plied by inherited mutations in the GlyR M2-M3 linker domain, that are associated with hyperkplexia and starele syndrome in animals, as well as mutations in this linker in nACHR associated with a form of congenital myasthenic syndrome and GABAAR mutations associated with epilepsy (3). In the GlyR, residues in the M2-M3 loop, as well as the M1-M2 loop, were shown to be involved in gating (46). In addition, changes in the accessibility of residues in the M2-M3 linker in GlyR and GABAAR as a function of the activation state of the receptor suggests a movement of M2-M3 loop in gating (44, 47). Specific residues in the ligand-binding domain that interact with distinct residues in the M2-M3 loop of GABAAR, coupling agonist binding to channel gating, have been identified, and the interaction of the ligand-binding domain with the transmembrane domains has been elegantly modeled (48). Analogous studies on the GlyR have shown that the negative charge on the conserved Asp residue in the Cys-loop (Asp^468) is required for efficient channel gating (49). Recent studies have implicated ionic interactions between membrane-proximal loops of the extracellular domain (Glu^133, Asp^57, and Asp^148) and residues in the M2-M3 linker (Lys^276) (50). These studies are also consistent with single-channel kinetics studies of nACHR that have also shown that the M2-M3 linker (51) as well as other membrane-proximal loops in the extracellular domain (52) are involved in channel gating. Thus it appears that ligand binding is coupled with channel activation via interactions of membrane-proximal loops of the extracellular domain with surface loops linking transmembrane segments of these receptors.

The dynamics of the nicotinicoid superfamily of receptors are also affected by the binding of other ligands. For example, the functions of these receptors are also allosterically modulated by a wide variety of anesthetics (53). Many of these volatile anesthetics and x-alcohols appear to bind in a volume-dependent manner to an interface between M1, M2, and the M3 transmembrane segments in the region thought to reside in the extracellular leaflet of the bilayer. In the GlyR and GABAAR, these interactions act to potentiate receptor activity. The mechanism of this potentiation is unknown, but it is hypothesized that anesthetic binding to this pocket in the TM domain alters receptor dynamics and, thus, function. As described above, movements of M3 residues in the nicotinicoid receptors have been inferred by differential accessibility in SCAM studies. Similarly, systematic cryptophan mutagenesis in M3 of the nACHR subunits has shown that movement of M3 contributes to the gating machinery of these receptors (54). The periodicity of the observed effects suggest a mixture of helical structures in M3 (55). In living cells, real-time allosteric rearrangements of the GABAAR upon channel activation and antagonism have been visualized by site-specific fluorescence labeling of this receptor (56). Although the molecular details involved in channel dynamics are still not well understood, it appears that the multiple allosteric states of the receptor provide for fine physiological control of receptor function.

**Effects of the Bilayer on Receptor Structure and Function**

Because proteins are affected by local environment, the dynamics and function of any membrane protein are necessarily dictated by the interdependence of its structure with that of the bilayer. The structures of the limited number of membrane proteins that have been determined at high resolution show evidence of specific binding of some anular lipids (57). The effects of these interactions on the function of ion channels (58) has perhaps been most dramatized in structural studies of the MscL channel, where the choice of lipids used in reconstitution can stabilize this pressure-sensitive channel in closed, partially open, or open states (39). Spin-label electron paramagnetic studies have shown that many integral membrane proteins bind an annulus of lipids that are motionally restricted (59). In addition to the direct effect of lipid binding on protein structure and function, differential lipid affinities (60), and in particular, the selective binding of a lipid shell by a membrane protein (61) may act to target that protein to particular lipid domains because of the compatibility of the bound lipid to selected microdomains (e.g. lipid rafts).

More specifically, the lipid composition affects nACHR function, and the presence of neutral lipids such as cholesterol and negatively charged phospholipids such as phosphatidylserine or phosphatidic acid are essential for activity (for review see Ref. 62). Delipidation studies of nACHR indicated that a lipid/protein mole ratio below ~45 causes irreversible inactivation of the receptor, consistent with the requirement of an annular shell of lipids around the periphery of the hydrophobic region (63). This requirement for a lipid annulus of 40–50 lipid molecules has been supported by a variety of spectroscopic and paramagnetic studies, with the receptor showing a marked preference for neutral and negatively charged lipids and cardiolipin (64). Only those membranes that contain cholesterol or anionic phospholipids have been shown to support agonist-induced conformational changes in the nACHR (65). Membranes lacking these substituents stabilize a receptor conformation similar to the agonist-induced desensitized state, although measurements of agonist affinity suggest that the receptor may reside in an alternate conformation (66, 67). Cholesterol-binding studies indicate that the sites of interaction appear to be near the interface between the nACHR and its lipid environment in the interfacial region of the bilayer (31, 68, 69).

Recent data manipulating the cholesterol content in whole-cell patch clamp measurements of the GABAAR indicated a role for cholesterol in maintaining optimal GABA potency (70) and in modulating the effects of steroidal and non-steroidal potentiators of the GABA receptor (71). Similar experiments with the serotonin receptor revealed a specific requirement for cholesterol for maximal receptor activity (72). Fewer studies have been conducted on the GlyR, but preliminary studies in which the cholesterol content of the cell is reduced by treatment with b-methylcyclodextran indicate that GlyR function is modulated by cholesterol content (73). In addition, changes in the receptor superfacedependent functional change to receptor structure, CD studies of GlyR revealed no net changes in the secondary structure of the GlyR as a function of cholesterol content. Nevertheless, a picture emerges of multiple lipid-binding sites on nicotinicoid receptors with a concomitant modulation channel structure and function, providing yet another way for fine cellular control of the ion channel conductance properties in discrete areas of postsynaptic cleft.

**Large Intracellular Loop and Protein-Protein Interactions**

Correct neuronal functioning is based on the generation, propagation, and coordinated integration of signals among billions of neurons communicating across their synapses. The specialized presynaptic active zones required for efficient synaptic signaling requires the dynamic establishment and maintenance of high local concentrations of specific membrane proteins. The complexity of these assemblies results from the specific assembly, trafficking, targeting, clustering, and allosteric modulation of a diverse array of macromolecular assemblies (74). This is especially important for postsynaptic receptor function because a single neuron may receive >100,000 synaptic inputs on its dendritic plasma membranes, with each nerve ending using particular neurotransmitters to activate various receptor subsets selectively localized at distinct postsynaptic inputs. The diffusion dynamics of GlyR in live cultured spinal neurons was very recently visualized using quantum dots, and the receptors were observed in mobile clusters over large surfaces of the membrane (with synaptic, perisynaptic, and extrasynaptic localization) (75). The complex dynamics of GlyR organization, or more generally, any neurotransmitter-gated ion channel, are affected by interactions with cellular components that may also allosterically modulate the function of the receptor. A significant fraction of these interactions occur in interactions between subtype-specific cytoplasmic loops in the nicotinicoid receptors with scaffold, anchoring, and adapter proteins. In the GlyR and other members of the nicotinicoid superfamily the amino acids between the third and fourth putative transmembrane domains are postulated to form a large intracellular loop (see Fig. 1). This region (residues 311–392 in the a1 subunit of the GlyR) contains the greatest sequence divergence among the members of the superfamily, with loop sizes ranging from 80 to 265 amino acids (8). This region contains elements involved in recognition and binding of various cytoplasmic proteins, cytoskeletal-linking elements, and other neuroreceptors. Protein-protein contacts via the large variable loop of these receptors are hypothesized to effect receptor assembly, trafficking, clustering, targeting, modulation, turn-
over, and related processes. Throughout this region are Ser, Thr, and Tyr residues that have been shown to be targets for activity-modulating kinases and phosphatases in the various members and subtypes of the nicotinicoid superfamily (76). Phosphorylation of α7 GlyR homomers appears to accelerate receptor desensitization and slow deactivation and recovery from desensitization (77). To date, the best characterized cytoplasmic interacting element with the GlyR is the protein gephyrin, which was co-purified with the receptor in original purifications from rat spinal cord (1). The large intracellular M3-M4 loop of both the GlyR and GABAAR bind the gephyrin-binding element glycophorin, which has been shown to be essential in receptor targeting and clustering (78). In developing neurons GlyR activation, with subsequent Ca2+ influx, is required for the clustering of gephyrin and GlyR at developing postsynaptic membranes (79). In addition to its interaction with gephyrin, the activity of GlyR is modulated by the G protein βγ dimer (Gβγ), with this interaction acting to regulate motor and sensory functions in the central nervous system (80). With the recent development of sensitive proteomic methods capable of finely mapping interactions between cellular components it is likely that the role of many other proteins in GlyR activity and function will soon be elucidated. Other recent examples of the interaction of cellular proteins with nicotinicoid receptors include GABAAR cross-talk with dopamine D1 receptors (81) and role of the tumor-supressor protein adenomatous polyposis coli in nACHR clustering (82).

**Summary**

Although many of the details of GlyR dynamics still remain elusive, the extensive studies conducted on GlyR and other members of the nicotinicoid superfamily in recent years provide us with an emerging picture of the structure and function of these receptors. As novel studies continue to examine these receptors, we are confident that these ion channels will reveal their molecular mechanisms. These details are significant in that they will allow us to develop novel therapeutics and pharmacological tools to modulate channel activity in the central nervous system.

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