



**HAL**  
open science

## Inhibitory Glycine Receptors: An Update

Sébastien Dutertre, Cord-Michael Becker, Heinrich Betz

► **To cite this version:**

Sébastien Dutertre, Cord-Michael Becker, Heinrich Betz. Inhibitory Glycine Receptors: An Update. Journal of Biological Chemistry, 2012, 287 (48), pp.40216-40223. 10.1074/jbc.R112.408229 . hal-02306933

**HAL Id: hal-02306933**

**<https://hal.science/hal-02306933>**

Submitted on 7 Oct 2019

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

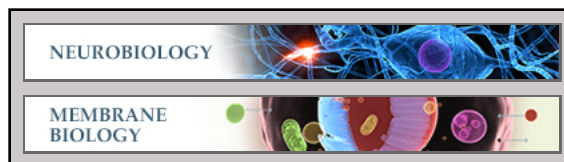
**Minireviews:**

**Inhibitory Glycine Receptors: An Update**

Sébastien Dutertre, Cord-Michael Becker and  
Heinrich Betz

*J. Biol. Chem.* 2012, 287:40216-40223.

doi: 10.1074/jbc.R112.408229 originally published online October 4, 2012



---

Access the most updated version of this article at doi: [10.1074/jbc.R112.408229](https://doi.org/10.1074/jbc.R112.408229)

Find articles, minireviews, Reflections and Classics on similar topics on the [JBC Affinity Sites](#).

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

Supplemental material:

<http://www.jbc.org/content/suppl/2012/10/04/R112.408229.DC1.html>

Read an Author Profile for this article at

[http://www.jbc.org/content/suppl/2012/11/22/R112.408229.DCAuthor\\_profile.html](http://www.jbc.org/content/suppl/2012/11/22/R112.408229.DCAuthor_profile.html)

This article cites 100 references, 41 of which can be accessed free at

<http://www.jbc.org/content/287/48/40216.full.html#ref-list-1>

# Inhibitory Glycine Receptors: An Update<sup>\*[5]</sup>

Published, JBC Papers in Press, October 4, 2012, DOI 10.1074/jbc.R112.408229

Sébastien Dutertre<sup>‡</sup>, Cord-Michael Becker<sup>§</sup>, and Heinrich Betz<sup>¶||1</sup>

From the <sup>‡</sup>Institute for Molecular Bioscience, University of Queensland, Brisbane, Queensland 4072, Australia, the <sup>§</sup>Institute of Biochemistry, University of Erlangen-Nürnberg, 91054 Erlangen, Germany, the <sup>¶</sup>Max-Planck-Institute for Medical Research, 69120 Heidelberg, Germany, and the <sup>||</sup>Department of Molecular Neurobiology, Max-Planck-Institute for Experimental Medicine, 37075 Göttingen, Germany

**Strychnine-sensitive glycine receptors (GlyRs) mediate synaptic inhibition in the spinal cord, brainstem, and other regions of the mammalian central nervous system. In this minireview, we summarize our current view of the structure, ligand-binding sites, and chloride channel of these receptors and discuss recently emerging functions of distinct GlyR isoforms. GlyRs not only regulate the excitability of motor and afferent sensory neurons, including pain fibers, but also are involved in the processing of visual and auditory signals. Hence, GlyRs constitute promising targets for the development of therapeutically useful compounds.**

The normal functioning of the CNS depends on the balanced interplay of both excitatory and inhibitory neurons. Glutamate is the principal excitatory and GABA and glycine are the major inhibitory neurotransmitters in the adult mammalian CNS. Glycine serves, in addition, as a co-agonist of glutamate at the NMDA subtype of excitatory glutamate receptors.

Glycinergic synapses mediate fast inhibitory neurotransmission mainly in the spinal cord, brainstem, and caudal brain and control a variety of motor and sensory functions, including vision and audition (1). Glycine exerts its inhibitory effects via specific glycine receptors (GlyRs)<sup>2</sup> that are highly enriched in the postsynaptic membrane. Binding of glycine leads to the opening of the GlyR integral anion channel, and the resulting influx of Cl<sup>-</sup> ions hyperpolarizes the postsynaptic cell, thereby inhibiting neuronal firing. The alkaloid strychnine antagonizes glycine binding with high affinity and has proven to be a unique tool in radioligand binding studies (2) and affinity purification (3) of GlyRs. Since these original studies, three decades of GlyR research have generated a wealth of genetic, functional, and structural data, which are summarized here.

\* This is the second article in the Thematic Minireview Series on Celebrating the Discovery of the Cysteine Loop Ligand-gated Ion Channel Superfamily.

[5] This article contains supplemental Figs. S1 and S2.

<sup>1</sup> To whom correspondence should be addressed. E-mail: heinrich.betz@mpimf-heidelberg.mpg.de.

<sup>2</sup> The abbreviations used are: GlyR, glycine receptor; LGIC, ligand-gated ion channel; nAChR, nicotinic acetylcholine receptor; GABA<sub>A</sub>R, GABA<sub>A</sub> receptor; ECD, extracellular domain; TM, transmembrane segment; GluCl, glutamate-gated channel; sIPSC, spontaneous inhibitory postsynaptic current; PGE<sub>2</sub>, prostaglandin E<sub>2</sub>; HKPX, hyperekplexia.

## Composition of GlyR Proteins

GlyRs are group I ligand-gated ion channels (LGICs) that belong to the Cys loop receptor family, which, in addition, includes nicotinic acetylcholine (nAChR), serotonin type 3 (5-HT<sub>3</sub>), and the closely related GABA<sub>A</sub> (GABA<sub>A</sub>R) receptors (4, 5). Affinity-purified GlyR preparations contain three polypeptide species (3): the GlyR $\alpha$  (48 kDa) and GlyR $\beta$  (58 kDa) subunits and a tightly bound cytosolic scaffolding protein (93 kDa) later named gephyrin (6). Cross-linking experiments showed that purified GlyRs are heteropentameric proteins, similar to the nAChRs in *Torpedo* electric organ and skeletal muscle (7). Their subunit stoichiometry was originally thought to be 3 $\alpha$ :2 $\beta$  (7, 8), but more recently, this was revised to 2 $\alpha$ :3 $\beta$  (9, 10).

Molecular cloning identified four vertebrate genes (called *Glr1–4*) encoding GlyR $\alpha$  subunits ( $\alpha 1–\alpha 4$ ) and a single gene (*Glrb*) encoding the GlyR $\beta$  subunit (11–15). All GlyR $\alpha$  subunits display high sequence identity (>80%) (supplemental Fig. S1) and, upon heterologous expression, form functional homomeric glycine-gated channels with properties closely resembling those of GlyRs *in vivo* (4, 5). By photoaffinity labeling with [<sup>3</sup>H]strychnine, the GlyR $\alpha$  subunits were shown to possess critical determinants of ligand binding (16). GlyR $\beta$  displays significant sequence differences compared with the  $\alpha$  subunits (<50% identity) (supplemental Fig. S1) and does not generate functional receptors when expressed alone (11) but is retained in the endoplasmic reticulum (17). However, the GlyR $\beta$  subunit is more than simply a structural subunit because it contributes to agonist binding (9) and has an essential role in the intracellular trafficking and synaptic clustering of postsynaptic GlyRs (18, 19). Its extended cytoplasmic loop region binds to the postsynaptic scaffolding protein gephyrin with high affinity (18, 20) and interacts with Vps35 and neurobeachin, proteins implicated in intracellular membrane protein transport (21).

The overall topology of GlyR subunits is shared with that of other group I LGIC proteins (22). All of these polypeptides contain a large N-terminal extracellular domain (ECD), four transmembrane segments (TM1–TM4), a long intracellular loop connecting TM3 and TM4, and a short extracellular C terminus. Sequence homologies are particularly high within the transmembrane segments and a conserved disulfide-bonded motif of 15 amino acids (“Cys loop”) located in the ECD. Although the number of GlyR subunit genes ( $n = 5$ ) appears to be modest compared with those found in the mammalian nAChR ( $n = 17$ ) and GABA<sub>A</sub>R ( $n = 20$ ) families, alternative splicing of exons encoding segments of the extracellular N-terminal or intracellular loop regions further extends GlyR subunit heterogeneity (4, 5, 23). In the case of GlyR $\alpha 2$ , splice variants in the ECD have been shown to differ in agonist efficacies (24), and intracellular loop variants of GlyR $\alpha 3$  have been found to differ in receptor desensitization (25) and subcellular targeting (26). Furthermore, post-transcriptional editing of a minor fraction of the GlyR $\alpha 3$  mRNA at a single nucleotide position has been reported to result in a gain-of-function receptor (27). This editing reaction leads to the substitution of proline 185

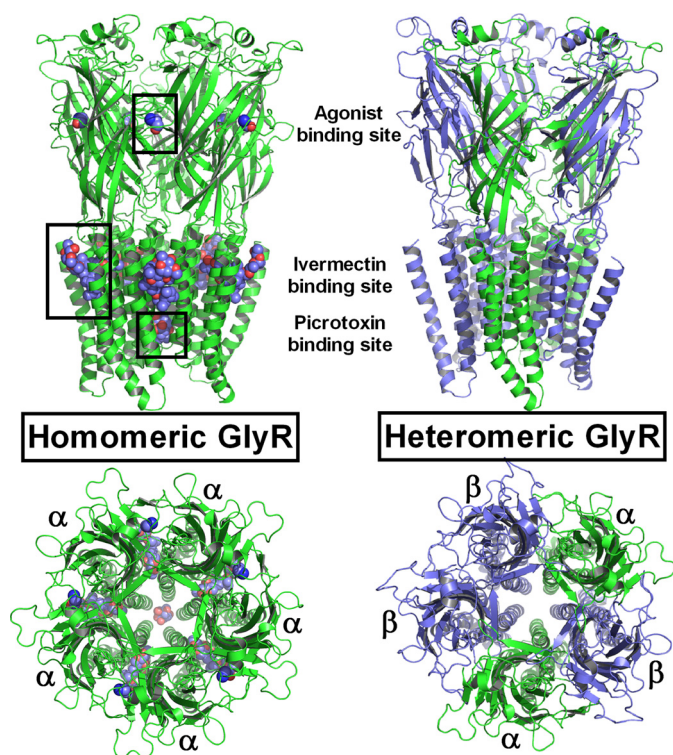


FIGURE 1. Structures of homomeric  $\alpha 1$ - and heteromeric  $\alpha 1\beta$ -GlyRs modeled using GluCl as a template. Left,  $\alpha 1$ -GlyR shown with five glycine and ivermectin molecules each and a single picrotoxin molecule bound (ligands depicted as van der Waals spheres). Right, unliganded heteromeric  $\alpha 1\beta$ -GlyR with the different interfaces indicated.

with leucine in the ECD and increases the apparent agonist affinity of  $\alpha 3$ -GlyRs.

### Structure of the GlyR and Its Extracellular Binding Sites

An atomic resolution structure of a GlyR is not available yet despite efforts to obtain suitable amounts of purified protein for structural studies (28). Therefore, structural information has been inferred from other Cys loop receptors or bacterial homologs of the group I LGIC family. Electron microscopy data collected from the muscle-type nAChR from *Torpedo* (29) and the coordinates of the acetylcholine-binding protein AChBP, a soluble homopentameric protein that is structurally homologous to the ECD of group I LGICs (30), have been widely used to model the ECD of many different receptors, including the GlyR (9, 31, 32). Recently, the first crystal structure of an anion-conducting Cys loop receptor, the glutamate-gated channel (GluCl) from *Caenorhabditis elegans*, was solved at atomic resolution (33). GluCl is a close homolog (up to 43% sequence identity) (supplemental Fig. S1) of GlyR subunits and also contains their hallmark feature, the conserved disulfide bridge in loop C. Therefore, GluCl-based models of GlyRs, as shown in Fig. 1, are considered highly reliable templates for ligand binding studies and the rational design of novel selective drugs.

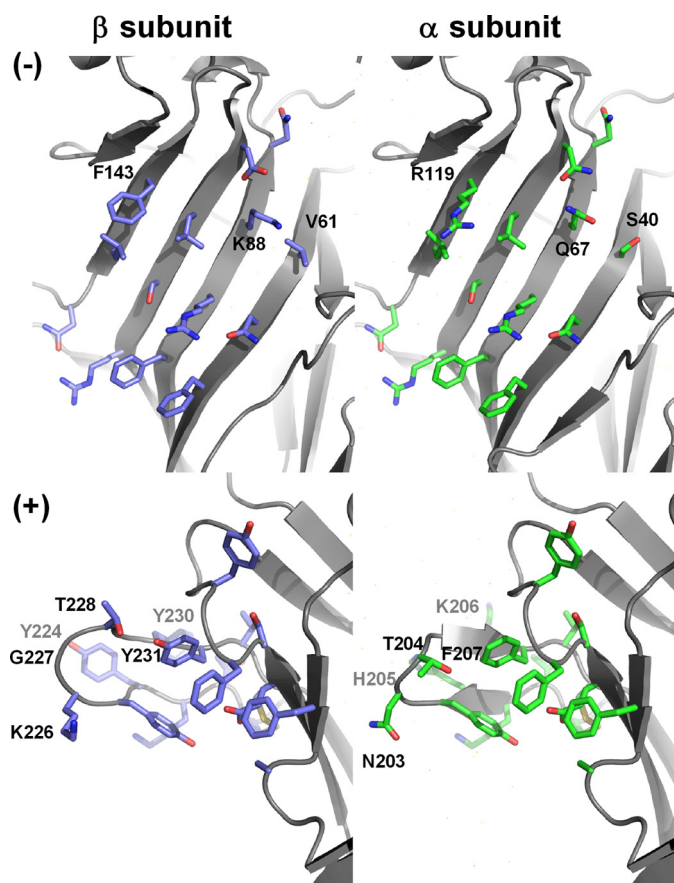
Agonists and competitive antagonists are known to bind to the ECD at the interfaces of two adjacent subunits (4, 5, 22). The GlyR binding pocket is formed by distinct “loop regions” of the principal (+) and complementary (–) subunit surfaces (Fig. 2). Residues whose substitution affects agonist and antagonist binding have been identified in loops D, B, E, and C (4, 5, 31). In

addition, the disulfide bond between  $\alpha 1$ Cys-198 and  $\alpha 1$ Cys-209 constraining loop C appears to be critical for both cell surface expression and ligand binding (34). The binding determinants of the  $\beta$  subunit are less well studied; two GlyR $\beta$  residues (Arg-86 and Glu-180, equivalent to  $\alpha 1$ Arg-65 and  $\alpha 1$ Glu-157, respectively) (Fig. 2) have been found to influence ligand binding to heteromeric  $\alpha 1\beta$ -GlyRs (9). Docking of the agonist glycine and the antagonist strychnine into homology models of the  $\alpha 1$ - $\alpha 1$  and  $\alpha 1$ - $\beta$  interfaces resulted in complex structures that agreed well with previous mutational data (9). However, the properties of the  $\beta$ - $\beta$  interface had remained enigmatic, as GlyR $\beta$  alone does not form functional channels. A novel mutagenesis strategy guided by homology modeling has recently allowed reproduction of all potential agonist-binding sites ( $\alpha$ - $\beta$ ,  $\beta$ - $\alpha$ , and  $\beta$ - $\beta$ ) present in heteromeric GlyRs by single-subunit expression (35). Although fully functional, the resulting  $\beta$ - $\beta$  binding site displayed the most distinct pharmacological profile toward a range of agonists and modulators tested, indicating that it might be selectively targeted to modulate the activity of synaptic GlyRs.

The ECD of GlyRs binds not only agonists and competitive antagonists but also compounds that allosterically modulate the agonist responses of different LGICs. Neurosteroids, general anesthetics, and ethanol potentiate GlyR currents, whereas the divalent cation  $Zn^{2+}$ , tropeines, and endogenous cannabinoids, such as anandamide and 2-arachidonylglycerol, have both potentiating (at low concentrations) and inhibitory (at high concentrations) effects. Because of their potential therapeutic importance, the binding sites and isoform selectivity of these modulators have been intensely investigated (4, 5, 31, 36), and their *in vivo* roles have been studied in transgenic and knock-in mouse models (37, 38).  $Zn^{2+}$  and tropeines bind within or in the vicinity of the extracellular agonist-binding sites (39, 40). In contrast, hydrophobic modulators, such as neurosteroids and endocannabinoids (41) and anesthetics and ethanol (42), all appear to act by binding to the transmembrane domain (see below), although residues located in the ECD and/or the large intracellular loop may also contribute to current modulation (43, 44). Additionally, the principal excitatory transmitter glutamate has been reported to allosterically potentiate native and recombinant GlyRs; this may be important for balancing excitation and inhibition in the CNS (45).

### GlyR Chloride Channel

The transmembrane domain of GlyR subunits and other group I LGICs consists of a four- $\alpha$ -helix bundle, in which the transmembrane segments are arranged in a clockwise order due to multiple interactions between specific hydrophobic residues that are essential for proper receptor assembly (46). TM1-TM2 and TM2-TM3 are connected by short loops, whereas TM3 is linked to TM4 by long intracellular loops that contain phosphorylation and ubiquitination sites as well as binding motifs for interacting proteins. The amphiphilic TM2 forms the ion channel; to facilitate comparisons between different LGICs, its residues are often numbered from 1' to 19', with position 1' corresponding to the cytoplasmic N-terminal end and residue 19' to the extracellular C-terminal end. Substituted cysteine accessibility experiments disclosed that Gly-2', Thr-6',



**FIGURE 2. Models of the principal (+) and complementary (–) surfaces of the GlyR $\alpha$ 1 and GlyR $\beta$  agonist-binding regions.** In heteromeric GlyRs, the + and – surfaces of the  $\alpha$ 1 and  $\beta$  subunit ECDs generate non-equivalent agonist-binding sites at the  $\alpha$ - $\beta$ ,  $\beta$ - $\alpha$ , or  $\beta$ - $\beta$  interface, which are all functional. Note the significant sequence divergence (up to eight substitutions) between  $\alpha$ 1 and  $\beta$ , in particular within loop C.

and Arg-19' line the pore of the  $\alpha$ 1-GlyR (47). Additional pore-lining residues were inferred by sequence comparison with other LGICs and include Thr-7', Leu-9', Thr-10', Thr-13', Ser-16', and Gly-17' (48). A model of the pore domain of GlyRs based on the recently determined structure of GluCl confirms that most of these residues are indeed facing the channel lumen (supplemental Fig. S2).

Agonist binding to the ECD of GlyRs triggers the opening of the anion-selective channel spanning the plasma membrane. Extensive work on different LGICs has demonstrated that the conformational changes within the five subunits induced upon agonist binding are consistent with an allosteric transition model that predicts rotational movements of the TM2 segments around about position 9' (29). This model was recently validated by comparing the structures of two bacterial LGIC homologs crystallized in the open and closed states, respectively (49) and is likely to similarly apply to the GlyR. Residues located at the ends or outside of TM2 have been shown to participate in the gating process, such as the GlyR $\alpha$ 1 residues Arg-271, located in the M2-M3 linker (34), and Lys-276, a pore-facing residue at the extracellular end of TM2 (50). Presumably, these and other residues that reside at the interface between the ECD and transmembrane domain are essential for coupling the

conformational changes occurring in the ECD upon agonist binding to channel opening (4, 5, 51).

The GlyR channel is largely anion-selective but has been reported to also permeate Na<sup>+</sup> and K<sup>+</sup> ions, albeit at low efficiency (52). GlyRs display a permeability sequence of SCN<sup>-</sup> > NO<sub>3</sub><sup>-</sup> > I<sup>-</sup> > Br<sup>-</sup> > Cl<sup>-</sup>, which is inversely correlated to the hydration energies of these ions, implying that removal of hydration water molecules facilitates ion permeation (53). A “ring of charges,” consisting of arginine residues (Arg-0') in TM2, is thought to concentrate permeating chloride ions and to repel oppositely charged cations. Consistent with this view, the subunits of cation-conducting nAChRs have a negatively charged residue (–Glu-1') at the equivalent position, and a respective substitution (A-1'E) was found to convert the GlyR into a cation-selective receptor (52). Residues located at the extracellular ends of the TM2 segments and in the adjacent loops (Arg-19' and Lys-24') provide additional rings of charge, whose substitution also reduced the unitary Cl<sup>-</sup> conductance (54). Besides these positively charged residues, a conserved proline at position 2' appears to play an important role in determining the selectivity and functional properties of homomeric GlyR channels (55); its deletion increased the pore size of the  $\alpha$ 1-GlyR and reduced its anion/cation permeability ratio (56). The new GluCl structure shows that this proline residue is located at the narrowest point of the channel and thus directly restricts ion flow (supplemental Fig. S2).

The GlyR channel is blocked by a number of non-competitive antagonists, such as picrotoxin, ginkgolide B, and cyanotriphenyl borate (4, 5). Previous mutagenesis data had indicated that picrotoxin occludes the channel by binding to pore-exposed residues of TM2 (57), although other sites of interaction have also been proposed (58, 59). More recently, Pro-2' and Thr-6' of TM2 were found to constitute important determinants of picrotoxin block (60). The crystal structure obtained from the GluCl-picrotoxin complex confirmed this assignment by showing that these residues constitute the binding site for this open channel blocker (33). Furthermore, it supports the idea that the low inhibitory potency of picrotoxin observed at heteromeric compared with homomeric GlyRs (57) is due to steric hindrance by the bulky Phe-282 side chain extending from TM2 of GlyR $\beta$  (Fig. 3).

Several hydrophobic modulators of the GlyR are thought to interact with external sites of the transmembrane domain (42, 61). These include neurosteroids, general anesthetics, and alcohols, which all potentiate GlyR function (31). Early mutagenesis experiments had identified a common intrasubunit binding site for these compounds in both GlyRs and GABA<sub>A</sub>Rs (42), which, because of its predicted size and shape, was termed “big cavity” (62). Within this cavity, the polar residues Ser-267 and Thr-264 located in TM2 and TM3 were shown to influence the enhancing effect of volatile anesthetics and high dose ethanol in GlyRs (42). Recently, the crystal structures of complexes between a bacterial LGIC ortholog from *Gloeobacter violaceus* (GLIC) and two anesthetics (propofol and desflurane) have been solved (63). These structures nicely confirmed the concept of a common intrasubunit binding site in group I LGICs, which pre-exists in the GLIC apo structure in the upper part of the transmembrane domain of each monomer and recruits anesthetics

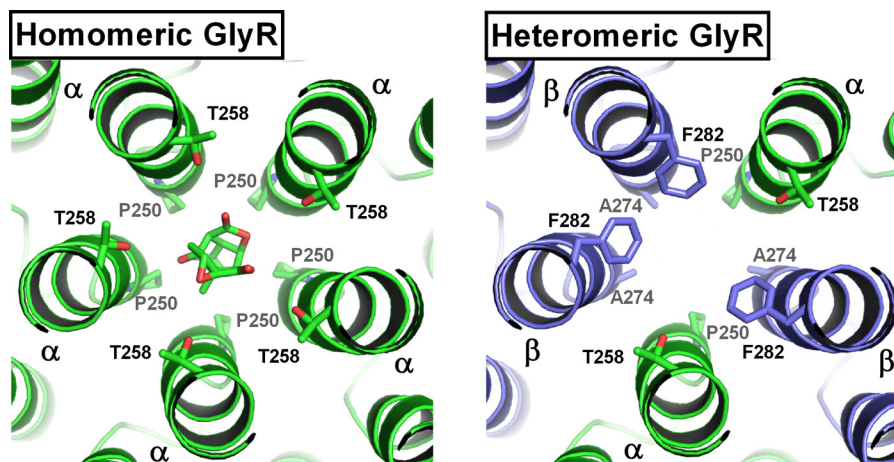


FIGURE 3. **Transmembrane domains of homo- and heteromeric GlyRs.** *Left*, picrotoxin bound in the chloride channel of the homomeric  $\alpha 1$ -GlyR. Binding determinants include five Pro-250 and Thr-258 residues each. *Right*, in the  $\alpha 1\beta$ -GlyR, three of the Thr-258 residues are replaced by Phe-282, and three of the Pro-250 residues by Ala-274 of the  $\beta$  subunit. These substitutions explain the  $\sim 50$ -fold lower affinity of picrotoxin for heteromeric GlyRs.

via van der Waal interactions. This binding may displace crucial lipids and thereby positively or negatively influence channel gating (63). In addition to the big cavity, a second intramembrane site located at the interfaces between adjacent subunits appears to be crucial for potentiation by hydrophobic modulators. The existence of an intersubunit binding site for general anesthetics was first shown in GABA<sub>A</sub>Rs by photoaffinity labeling with etomidate (64). In GlyRs, this site is targeted by the antihelminthic ivermectin, which allosterically activates both GlyR and GluCl currents. The recent crystal structure of the GluCl-ivermectin complex (33) delineates ivermectin bound at the interface between TM3 from the principal (+) and TM1 from the complementary (−) subunits (Fig. 1). Furthermore, it shows that ivermectin stabilizes the open state of GluCl by inducing conformational changes in both the transmembrane domain and ECD. Although most of the ivermectin-binding residues of GluCl appear to be conserved in GlyRs, the GlyR binding site for ivermectin has been suggested to differ (65) and hence should be further challenged experimentally.

#### Roles of GlyR Isoforms in the Mammalian CNS

The physiological functions of the different GlyR isoforms have been incompletely analyzed due to the lack of subtype-specific antagonists. Our present picture of GlyR subtypes is based primarily on immunocytochemical studies and the analysis of knock-out mice.

**$\alpha 1$ -GlyRs**—GlyRs containing the  $\alpha 1$  subunit represent the predominant adult GlyR isoform, and heteromeric  $\alpha 1\beta$  receptors account for the majority of synaptically localized GlyRs in the mammalian CNS. The importance of the GlyR $\alpha 1$  subunit is underlined by the existence of various disease mutations in the *Gla1* genes of different mammalian species, including mouse, cattle, and human (5). The *spasmodic* mouse carries a substitution at position 52 (A52S) of *Gla1* that reduces the agonist binding affinity (66, 67). The *oscillator* strain constitutes a natural *Gla1* null mutation due to a microdeletion in exon 8 that truncates the  $\alpha 1$  subunit after TM3 (68). Notably, GlyR function of the *oscillator* polypeptide can be rescued upon coexpression of a C-terminal fragment containing TM4 (69). This result and complementation experiments with different GlyR and

5-HT<sub>3</sub> receptor fragments (46) have revealed an essential role of TM interactions in group I LGIC assembly.

Upon recombinant expression, the GlyR $\alpha 1$  subunit forms channels characterized by short mean open times and fast decay kinetics, as found for glycinergic spontaneous inhibitory postsynaptic currents (sIPSCs) in adult spinal cord neurons (70). In retinas from *oscillator* mice, glycinergic sIPSCs in A-type ganglion cells are strikingly reduced and their kinetics slowed due to *Gla1* inactivation (71). Together, the available data indicate that the GlyR $\alpha 1$  subunit defines receptors that are crucial for fast regulation of both motor and sensory functions.

**$\alpha 2$ -GlyRs**—This GlyR isoform is highly expressed at the embryonic and neonatal stages but postnatally is largely replaced by  $\alpha 1$  subunit-containing receptors. Synaptically localized  $\alpha 2$  staining has been detected in different adult CNS regions, including the spinal cord, brainstem, midbrain, olfactory bulb, and retina, and corresponds to heteromeric  $\alpha 2\beta$  receptors (72). At early developmental stages, GlyR $\alpha 2$  forms homo-oligomeric receptors (73), which are extrasynaptically localized and thought to mediate non-synaptic tonic transmission caused by non-vesicular glycine release and/or spillover from adjacent nerve terminals.

Despite the availability of *Gla2*<sup>−/−</sup> mice (74, 75), the precise physiological roles of GlyR $\alpha 2$  are still largely enigmatic because the knock-out animals are phenotypically normal. Electrophysiological recordings of neonatal GlyRs in spinal neurons (70) and glycinergic sIPSCs in narrow-field amacrine cells of *Gla2*<sup>−/−</sup> mice (74) indicate that  $\alpha 2$ -GlyRs mediate IPSCs characterized by slow decay kinetics. In *Gla2*<sup>−/−</sup> mice, receptive field center responses in the retinal on and off pathways are impaired (76), and hyperalgesia induced by the injection of zymosan is prolonged compared with wild-type littermates (77). Both its known sites of expression and the available physiological data suggest that, in adult mammals, synaptic GlyR $\alpha 2$  is involved mainly in regulating sensory pathways.

**$\alpha 3$ -GlyRs**—Originally, GlyR $\alpha 3$  was considered a minor adult GlyR isoform with an expression pattern resembling that of GlyR $\alpha 1$  (14, 78). In the spinal cord, GlyR $\alpha 3$  staining is found at synaptic sites in laminae I and II of the dorsal horn, where it

inhibits the propagation of nociceptive signals to higher brain regions and serves as molecular substrate of pain sensitization by the inflammatory mediator prostaglandin  $E_2$  ( $PGE_2$ ) (79).  $PGE_2$  binds to prostaglandin EP2 receptors and thereby activates protein kinase A, which phosphorylates GlyR $\alpha$ 3 and thus down-regulates glycine currents in dorsal horn neurons. In  $Gla3^{-/-}$  mice, this down-regulation of glycine inhibition by  $PGE_2$  is abolished, and  $PGE_2$  fails to sensitize pain responses (79). Also, the analgesic effect of cannabinoids in mouse models of chronic inflammatory and neuropathic pain is absent in  $Gla3^{-/-}$  animals (80). Hence, GlyR $\alpha$ 3 constitutes a promising target for the development of novel drugs for the treatment of chronic pain syndromes.

GlyR $\alpha$ 3 immunoreactivity has also been detected in other pathways involved in sensory processing. In retina, GlyR $\alpha$ 3 is localized at synapses that are distinct from GlyR $\alpha$ 1-containing ones (71). By analyzing  $Gla3^{-/-}$  mice, amacrine cells of the AII subtype have been shown to contain synaptic  $\alpha$ 3-GlyRs, which confer medium-fast kinetics on sIPSCs (74). These receptors regulate the receptive fields of ON-ganglion cells by enhancing the excitatory center response (76). Furthermore, GlyR $\alpha$ 3 has been found at inhibitory synapses in the inner ear (81). Together, the available data indicate that like GlyR $\alpha$ 2,  $\alpha$ 3-GlyRs have an important role in sensory information processing.

**$\alpha$ 4-GlyRs**—GlyR $\alpha$ 4 is the least understood subtype of the GlyR family; this mainly reflects the low abundance of  $\alpha$ 4 mRNA and protein in the mammalian CNS. In chicken embryos, GlyR $\alpha$ 4 transcripts have been detected in the spinal cord, peripheral ganglia, and male genital ridge (82). In the adult rodent retina, GlyR $\alpha$ 4-immunoreactive synapses have been found on displaced ON-cholinergic amacrine cells (71). Upon heterologous expression,  $\alpha$ 4-GlyRs form channels with pharmacological properties largely resembling those of  $\alpha$ 1 receptors (82). Notably, in humans, the *GLRA4* gene is a pseudogene. Based on the analysis of sIPSCs in narrow-field amacrine cells of  $Gla2^{-/-}$  mice,  $\alpha$ 4 receptors have been suggested to display ultra-slow decay kinetics (74).

**GlyR $\beta$  Subunit**—The *Glr $\beta$*  gene encodes the rodent  $\beta$  subunit, which is present three times in adult pentameric  $\alpha$ 1 $\beta$ -GlyRs (9, 10). By *in situ* hybridization, high levels of GlyR $\beta$  mRNA have been detected throughout the embryonic and postnatal CNS (78). However, this widespread transcription of *Glr $\beta$*  is not reflected in a corresponding abundance of the  $\beta$  protein. A recently generated monoclonal antibody revealed GlyR $\beta$  immunoreactivity exclusively at GlyR-positive synapses but not in CNS regions lacking glycinergic neurons, such as the cortex (72). This discrepancy between GlyR $\beta$  transcript and protein levels likely reflects a retention and rapid degradation in the endoplasmic reticulum of GlyR $\beta$  in the absence of  $\alpha$  subunits. Immunostainings of adult mouse retina have confirmed a high extent (>90%) of co-localization of GlyR $\beta$  with GlyR $\alpha$ 1–3 (72). In the case of GlyR $\alpha$ 4, however, immunoreactive synapses that lack GlyR $\beta$  staining were found in the inner plexiform layer. Thus, a minor subpopulation of synaptic GlyRs might not be heteromeric.

The functional importance of GlyR $\beta$  is underlined by the *spastic* mutation in mice, which produces a phenotype identical

to that seen in *spasmodic* animals (83). *Spastic* animals express reduced levels of the major adult GlyR isoform  $\alpha$ 1 $\beta$  due to an intronic insertion of a LINE-1 element in the *Glr $\beta$*  gene (84, 85). Causative for the reduced expression is exon skipping resulting from a polymorphism of a splice signal amplified by the LINE-1 insertion (86).

### Developmental Changes in GlyR Function and Isoform Expression

In contrast to its hyperpolarizing action on adult neurons, glycine depolarizes motor neurons during embryonic development and around birth (87). Due to high intracellular chloride concentrations at these stages, GlyR channel opening leads to chloride efflux and thereby may induce neuronal firing, although inhibition also may occur as a result of the shunting conductance produced by GlyR activation. A depolarizing excitatory function of GlyRs at early developmental stages may be important for synaptogenesis because GlyR-triggered activation of voltage-gated  $Ca^{2+}$  channels appears to be crucial for GlyR clustering at postsynaptic sites (88). Also, it may be essential for the regulation of glutamate release by presynaptic GlyRs that have been detected electrophysiologically in large adult nerve terminals, such as the calyx of Held (89) and hippocampal mossy fiber terminals (90). Postnatally, the neuronal chloride equilibrium potential shifts to negative values due to chloride extrusion upon expression of the  $K^+/Cl^-$  cotransporter KCC2 (91). Thus, GlyR currents become hyperpolarizing, *i.e.* inhibitory.

In the spinal cord and brainstem, the developmental change in GlyR function described above is paralleled by changes in subunit composition (92). Embryonic and neonatal GlyRs are thought to be extrasynaptically localized homopentamers of  $\alpha$ 2 subunits (73), whereas adult synaptic GlyRs are heteromers containing  $\alpha$ 1 (or other  $\alpha$ ) and  $\beta$  subunits (7, 20). This change in subunit composition alters the biophysical properties of GlyR currents, resulting in faster decay kinetics and a smaller channel conductance (70, 93). Recombinant  $\alpha$ 2-GlyRs show slower response kinetics and larger subconductance state distributions than  $\alpha$ 1 $\beta$  receptors, consistent with the *in vivo* properties of neonatal *versus* adult GlyRs (94). Apparently, the postnatal change in GlyR subunit composition fine-tunes inhibitory transmission by shortening the mean channel open time and accelerating the decay of glycinergic IPSCs.

### Mutations in Human GlyR Genes Cause Hyperekplexia

Mutations affecting glycinergic neurotransmission cause the hereditary neuromotor disorder hyperekplexia (HKPX; startle disease). The hallmark symptoms of this genetically heterogeneous disorder are an exaggerated startle response to auditory or tactile stimuli and, particularly in neonates, transient muscle rigidity (“stiff baby syndrome”). In 1993, positional cloning disclosed mutations in the *GLRA1* gene localized on chromosome 5q33.1 (termed the *HKPX1* locus) as a major cause of hyperekplexia (95). In this pioneering study, substitutions of the highly conserved residue Arg-271 within the extracellular loop connecting TM2 and TM3 were found in four families with autosomal dominant hyperekplexia. These substitutions decrease the agonist sensitivity and single-channel conductance of

recombinant GlyR $\alpha$ 1 (54, 96). Subsequently, additional dominant and recessive inheritance patterns and compound heterozygosity have been described for other patient families in which various *GLRA1* missense or null mutations have been identified (4, 5, 97). Some of these mutations have been found to affect GlyR intracellular trafficking rather than agonist binding or channel gating (69, 98). Consistent with an important role of the  $\beta$  subunit in postsynaptic GlyR function, mutations in the *GLRB* gene have been associated with HKPX2 (97, 99). A third major form of hyperekplexia of presynaptic origin (HKPX3) is due to mutations in the gene encoding the neuronal glycine transporter GlyT2 (*SLC6A5*) (97).

Recent studies have implicated other GlyR genes, in particular *GLRA3*, in the pathology of other neurological disorders, such as autism, human immunodeficiency virus-associated dementia, generalized epilepsy, and amyotrophic lateral sclerosis (4, 5). Furthermore, autoantibodies against GlyR $\alpha$ 1 have been found in patients suffering from progressive encephalomyelitis with rigidity and myoclonus (100). Together, all of these studies underline the importance of proper GlyR function for human health.

### Conclusion and Perspectives

Since its purification in 1982, considerable progress has been made in elucidating the structure and pharmacology of the GlyR and in identifying physiological functions of its distinct isoforms. Furthermore, mouse models for different GlyR subunit deficiencies have become available, and the importance of GlyR mutations for the pathogenesis of human neuromotor disease is now well understood. However, many unanswered questions await further investigation, including the precise mechanism of GlyR channel gating, the individual roles of the different GlyR isoforms in various normal and diseased brain regions, and the development of isoform-specific ligands.

*Acknowledgments*—We thank all colleagues on whose work this mini-review is based.

### REFERENCES

- Legendre, P. (2001) The glycinergic inhibitory synapse. *Cell. Mol. Life Sci.* **58**, 760–793
- Young, A. B., and Snyder, S. H. (1973) Strychnine binding associated with glycine receptors of the central nervous system. *Proc. Natl. Acad. Sci. U.S.A.* **70**, 2832–2836
- Pfeiffer, F., Graham, D., and Betz, H. (1982) Purification by affinity chromatography of the glycine receptor of rat spinal cord. *J. Biol. Chem.* **257**, 9389–9393
- Betz, H., and Laube, B. (2006) Glycine receptors: recent insights into their structural organization and functional diversity. *J. Neurochem.* **97**, 1600–1610
- Lynch, J. W. (2004) Molecular structure and function of the glycine receptor chloride channel. *Physiol. Rev.* **84**, 1051–1095
- Prior, P., Schmitt, B., Grenningloh, G., Pribilla, I., Multhaup, G., Beyreuther, K., Maulet, Y., Werner, P., Langosch, D., and Kirsch, J. (1992) Primary structure and alternative splice variants of gephyrin, a putative glycine receptor-tubulin linker protein. *Neuron* **8**, 1161–1170
- Langosch, D., Thomas, L., and Betz, H. (1988) Conserved quaternary structure of ligand-gated ion channels: the postsynaptic glycine receptor is a pentamer. *Proc. Natl. Acad. Sci. U.S.A.* **85**, 7394–7398
- Burzomato, V., Groot-Kormelink, P. J., Sivilotti, L. G., and Beato, M. (2003) Stoichiometry of recombinant heteromeric glycine receptors revealed by a pore-lining region point mutation. *Receptors Channels* **9**, 353–361
- Grudzinska, J., Schemm, R., Haeger, S., Nicke, A., Schmalzing, G., Betz, H., and Laube, B. (2005) The  $\beta$  subunit determines the ligand binding properties of synaptic glycine receptors. *Neuron* **45**, 727–739
- Yang, Z., Taran, E., Webb, T. I., and Lynch, J. W. (2012) Stoichiometry and subunit arrangement of  $\alpha$ 1 $\beta$  glycine receptors as determined by atomic force microscopy. *Biochemistry* **51**, 5229–5231
- Grenningloh, G., Pribilla, I., Prior, P., Multhaup, G., Beyreuther, K., Taleb, O., and Betz, H. (1990) Cloning and expression of the 58-kDa  $\beta$  subunit of the inhibitory glycine receptor. *Neuron* **4**, 963–970
- 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
- Grenningloh, G., Schmieden, V., Schofield, P. R., Seeburg, P. H., Sidique, T., Mohandas, T. K., Becker, C. M., and Betz, H. (1990)  $\alpha$  subunit variants of the human glycine receptor: primary structures, functional expression and chromosomal localization of the corresponding genes. *EMBO J.* **9**, 771–776
- Kuhse, J., Schmieden, V., and Betz, H. (1990) Identification and functional expression of a novel ligand binding subunit of the inhibitory glycine receptor. *J. Biol. Chem.* **265**, 22317–22320
- Matzenbach, B., Maulet, Y., Sefton, L., Courtier, B., Avner, P., Guénet, J. L., and Betz, H. (1994) Structural analysis of mouse glycine receptor  $\alpha$  subunit genes. Identification and chromosomal localization of a novel variant. *J. Biol. Chem.* **269**, 2607–2612
- Graham, D., Pfeiffer, F., and Betz, H. (1983) Photoaffinity labelling of the glycine receptor of rat spinal cord. *Eur. J. Biochem.* **131**, 519–525
- Griffon, N., Büttner, C., Nicke, A., Kuhse, J., Schmalzing, G., and Betz, H. (1999) Molecular determinants of glycine receptor subunit assembly. *EMBO J.* **18**, 4711–4721
- Kneussel, M., and Betz, H. (2000) Clustering of inhibitory neurotransmitter receptors at developing postsynaptic sites: the membrane activation model. *Trends Neurosci.* **23**, 429–435
- Maas, C., Tagnaouti, N., Loebrich, S., Behrend, B., Lappe-Siefke, C., and Kneussel, M. (2006) Neuronal cotransport of glycine receptor and the scaffold protein gephyrin. *J. Cell Biol.* **172**, 441–451
- Meyer, G., Kirsch, J., Betz, H., and Langosch, D. (1995) Identification of a gephyrin binding motif on the glycine receptor  $\beta$  subunit. *Neuron* **15**, 563–572
- del Pino, I., Paarmann, I., Karas, M., Kilimann, M. W., and Betz, H. (2011) The trafficking proteins vacuolar protein sorting 35 and neurobeachin interact with the glycine receptor  $\beta$  subunit. *Biochem. Biophys. Res. Commun.* **412**, 435–440
- Corringer, P. J., Le Novère, N., and Changeux, J. P. (2000) Nicotinic receptors at the amino acid level. *Annu. Rev. Pharmacol. Toxicol.* **40**, 431–458
- Oertel, J., Villmann, C., Kettenmann, H., Kirchhoff, F., and Becker, C. M. (2007) A novel glycine receptor  $\beta$  subunit splice variant predicts an unorthodox transmembrane topology. Assembly into heteromeric receptor complexes. *J. Biol. Chem.* **282**, 2798–2807
- Miller, P. S., Harvey, R. J., and Smart, T. G. (2004) Differential agonist sensitivity of glycine receptor  $\alpha$ 2 subunit splice variants. *Br. J. Pharmacol.* **143**, 19–26
- Nikolic, Z., Laube, B., Weber, R. G., Lichter, P., Kioschis, P., Poustka, A., Mühlhardt, C., and Becker, C. M. (1998) The human glycine receptor subunit  $\alpha$ 3. *GLRA3* gene structure, chromosomal localization, and functional characterization of alternative transcripts. *J. Biol. Chem.* **273**, 19708–19714
- Melzer, N., Villmann, C., Becker, K., Harvey, K., Harvey, R. J., Vogel, N., Kluck, C. J., Kneussel, M., and Becker, C. M. (2010) Multifunctional basic motif in the glycine receptor intracellular domain induces subunit-specific sorting. *J. Biol. Chem.* **285**, 3730–3739
- Meier, J. C., Henneberger, C., Melnick, I., Racca, C., Harvey, R. J., Heinemann, U., Schmieden, V., and Grantyn, R. (2005) RNA editing produces glycine receptor  $\alpha$ 3(P185L), resulting in high agonist potency. *Nat. Neu-*



- roschi*. **8**, 736–744
28. Breitinger, U., Breitinger, H. G., Bauer, F., Fahmy, K., Glockenhammer, D., and Becker, C. M. (2004) Conserved high affinity ligand binding and membrane association in the native and refolded extracellular domain of the human glycine receptor  $\alpha 1$  subunit. *J. Biol. Chem.* **279**, 1627–1636
  29. Miyazawa, A., Fujiyoshi, Y., and Unwin, N. (2003) Structure and gating mechanism of the acetylcholine receptor pore. *Nature* **423**, 949–955
  30. 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
  31. Laube, B., Maksay, G., Schemm, R., and Betz, H. (2002) Modulation of glycine receptor function: a novel approach for therapeutic intervention at inhibitory synapses? *Trends Pharmacol. Sci.* **23**, 519–527
  32. Nevin, S. T., Cromer, B. A., Haddrill, J. L., Morton, C. J., Parker, M. W., and Lynch, J. W. (2003) Insights into the structural basis for zinc inhibition of the glycine receptor. *J. Biol. Chem.* **278**, 28985–28992
  33. Hibbs, R. E., and Gouaux, E. (2011) Principles of activation and permeation in an anion-selective Cys loop receptor. *Nature* **474**, 54–60
  34. Rajendra, S., Lynch, J. W., Pierce, K. D., French, C. R., Barry, P. H., and Schofield, P. R. (1995) Mutation of an arginine residue in the human glycine receptor transforms  $\beta$ -alanine and taurine from agonists into competitive antagonists. *Neuron* **14**, 169–175
  35. Dutertre, S., Drwal, M., Laube, B., and Betz, H. (2012) Probing the pharmacological properties of distinct subunit interfaces within heteromeric glycine receptors reveals a functional  $\beta\beta$  agonist-binding site. *J. Neurochem.* **122**, 38–47
  36. Miller, P. S., Topf, M., and Smart, T. G. (2008) Mapping a molecular link between allosteric inhibition and activation of the glycine receptor. *Nat. Struct. Mol. Biol.* **15**, 1084–1093
  37. Blednov, Y. A., Benavidez, J. M., Homanics, G. E., and Harris, R. A. (2012) Behavioral characterization of knock-in mice with mutations M287L and Q266I in the glycine receptor  $\alpha 1$  subunit. *J. Pharmacol. Exp. Ther.* **340**, 317–329
  38. Hirzel, K., Müller, U., Latal, A. T., Hülsmann, S., Grudzinska, J., Seeliger, M. W., Betz, H., and Laube, B. (2006) Hyperekplexia phenotype of glycine receptor  $\alpha 1$  subunit mutant mice identifies  $Zn^{2+}$  as an essential endogenous modulator of glycinergic neurotransmission. *Neuron* **52**, 679–690
  39. Maksay, G., Laube, B., Schemm, R., Grudzinska, J., Drwal, M., and Betz, H. (2009) Different binding modes of tropeines mediating inhibition and potentiation of  $\alpha 1$  glycine receptors. *J. Neurochem.* **109**, 1725–1732
  40. Miller, P. S., Da Silva, H. M., and Smart, T. G. (2005) Molecular basis for zinc potentiation at strychnine-sensitive glycine receptors. *J. Biol. Chem.* **280**, 37877–37884
  41. Xiong, W., Wu, X., Lovinger, D. M., and Zhang, L. (2012) A common molecular basis for exogenous and endogenous cannabinoid potentiation of glycine receptors. *J. Neurosci.* **32**, 5200–5208
  42. Mihic, S. J., Ye, Q., Wick, M. J., Koltchine, V. V., Krasowski, M. D., Finn, S. E., Mascia, M. P., Valenzuela, C. F., Hanson, K. K., Greenblatt, E. P., Harris, R. A., and Harrison, N. L. (1997) Sites of alcohol and volatile anaesthetic action on GABA<sub>A</sub> and glycine receptors. *Nature* **389**, 385–389
  43. Perkins, D. I., Trudell, J. R., Crawford, D. K., Alkana, R. L., and Davies, D. L. (2010) Molecular targets and mechanisms for ethanol action in glycine receptors. *Pharmacol. Ther.* **127**, 53–65
  44. Yévenes, G. E., and Zeilhofer, H. U. (2011) Molecular sites for the positive allosteric modulation of glycine receptors by endocannabinoids. *PLoS ONE* **6**, e23886
  45. Liu, J., Wu, D. C., and Wang, Y. T. (2010) Allosteric potentiation of glycine receptor chloride currents by glutamate. *Nat. Neurosci.* **13**, 1225–1232
  46. Haeger, S., Kuzmin, D., Detro-Dassen, S., Lang, N., Kilb, M., Tsetlin, V., Betz, H., Laube, B., and Schmalzing, G. (2010) An intramembrane aromatic network determines pentameric assembly of Cys loop receptors. *Nat. Struct. Mol. Biol.* **17**, 90–98
  47. Lynch, J. W., Han, N. L., Haddrill, J., Pierce, K. D., and Schofield, P. R. (2001) The surface accessibility of the glycine receptor M2-M3 loop is increased in the channel open state. *J. Neurosci.* **21**, 2589–2599
  48. Xu, M., and Akabas, M. H. (1996) Identification of channel-lining residues in the M2 membrane-spanning segment of the GABA<sub>A</sub> receptor  $\alpha 1$  subunit. *J. Gen. Physiol.* **107**, 195–205
  49. Bocquet, N., Nury, H., Baaden, M., Le Poupon, C., Changeux, J. P., Delarue, M., and Corringer, P. J. (2009) X-ray structure of a pentameric ligand-gated ion channel in an apparently open conformation. *Nature* **457**, 111–114
  50. Lewis, T. M., Sivilotti, L. G., Colquhoun, D., Gardiner, R. M., Schoepfer, R., and Rees, M. (1998) Properties of human glycine receptors containing the hyperekplexia mutation  $\alpha 1(K276E)$ , expressed in *Xenopus* oocytes. *J. Physiol.* **507**, 25–40
  51. 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
  52. Keramidas, A., Moorhouse, A. J., Pierce, K. D., Schofield, P. R., and Barry, P. H. (2002) Cation-selective mutations in the M2 domain of the inhibitory glycine receptor channel reveal determinants of ion-charge selectivity. *J. Gen. Physiol.* **119**, 393–410
  53. Bormann, J., Hamill, O. P., and Sakmann, B. (1987) Mechanism of anion permeation through channels gated by glycine and  $\gamma$ -aminobutyric acid in mouse cultured spinal neurons. *J. Physiol.* **385**, 243–286
  54. Langosch, D., Laube, B., Rundström, N., Schmieden, V., Bormann, J., and Betz, H. (1994) Decreased agonist affinity and chloride conductance of mutant glycine receptors associated with human hereditary hyperekplexia. *EMBO J.* **13**, 4223–4228
  55. Gunthorpe, M. J., and Lummis, S. C. (2001) Conversion of the ion selectivity of the 5-HT<sub>3A</sub> receptor from cationic to anionic reveals a conserved feature of the ligand-gated ion channel superfamily. *J. Biol. Chem.* **276**, 10977–10983
  56. Lee, D. J., Keramidas, A., Moorhouse, A. J., Schofield, P. R., and Barry, P. H. (2003) The contribution of proline 250 (P-2') to pore diameter and ion selectivity in the human glycine receptor channel. *Neurosci. Lett.* **351**, 196–200
  57. Pribilla, I., Takagi, T., Langosch, D., Bormann, J., and Betz, H. (1992) The atypical M2 segment of the  $\beta$  subunit confers picrotoxinin resistance to inhibitory glycine receptor channels. *EMBO J.* **11**, 4305–4311
  58. Dibas, M. I., Gonzales, E. B., Das, P., Bell-Horner, C. L., and Dillon, G. H. (2002) Identification of a novel residue within the second transmembrane domain that confers use-facilitated block by picrotoxin in glycine  $\alpha 1$  receptors. *J. Biol. Chem.* **277**, 9112–9117
  59. Lynch, J. W., Rajendra, S., Barry, P. H., and Schofield, P. R. (1995) Mutations affecting the glycine receptor agonist transduction mechanism convert the competitive antagonist picrotoxin into an allosteric potentiator. *J. Biol. Chem.* **270**, 13799–13806
  60. Yang, Z., Cromer, B. A., Harvey, R. J., Parker, M. W., and Lynch, J. W. (2007) A proposed structural basis for picrotoxinin and picrotin binding in the glycine receptor pore. *J. Neurochem.* **103**, 580–589
  61. Lu, H., and Xu, T. L. (2002) The general anesthetic pentobarbital slows desensitization and deactivation of the glycine receptor in the rat spinal dorsal horn neurons. *J. Biol. Chem.* **277**, 41369–41378
  62. Jenkins, A., Greenblatt, E. P., Faulkner, H. J., Bertaccini, E., Light, A., Lin, A., Andreasen, A., Viner, A., Trudell, J. R., and Harrison, N. L. (2001) Evidence for a common binding cavity for three general anesthetics within the GABA<sub>A</sub> receptor. *J. Neurosci.* **21**, RC136
  63. Nury, H., Van Renterghem, C., Weng, Y., Tran, A., Baaden, M., Dufresne, V., Changeux, J. P., Sonner, J. M., Delarue, M., and Corringer, P. J. (2011) X-ray structures of general anaesthetics bound to a pentameric ligand-gated ion channel. *Nature* **469**, 428–431
  64. Li, G. D., Chiara, D. C., Sawyer, G. W., Husain, S. S., Olsen, R. W., and Cohen, J. B. (2006) Identification of a GABA<sub>A</sub> receptor anesthetic binding site at subunit interfaces by photolabeling with an etomidate analog. *J. Neurosci.* **26**, 11599–11605
  65. Lynagh, T., Webb, T. I., Dixon, C. L., Cromer, B. A., and Lynch, J. W. (2011) Molecular determinants of ivermectin sensitivity at the glycine receptor chloride channel. *J. Biol. Chem.* **286**, 43913–43924
  66. Ryan, S. G., Buckwalter, M. S., Lynch, J. W., Handford, C. A., Segura, L., Shiang, R., Wasmuth, J. J., Camper, S. A., Schofield, P., and O'Connell, P. (1994) A missense mutation in the gene encoding the  $\alpha 1$  subunit of the

- inhibitory glycine receptor in the *spasmodic* mouse. *Nat. Genet.* **7**, 131–135
67. Saul, B., Schmieden, V., Kling, C., Müllhardt, C., Gass, P., Kuhse, J., and Becker, C. M. (1994) Point mutation of glycine receptor  $\alpha 1$  subunit in the *spasmodic* mouse affects agonist responses. *FEBS Lett.* **350**, 71–76
  68. Buckwalter, M. S., Cook, S. A., Davisson, M. T., White, W. F., and Camper, S. A. (1994) A frameshift mutation in the mouse  $\alpha 1$  glycine receptor gene (*Glr1*) results in progressive neurological symptoms and juvenile death. *Hum. Mol. Genet.* **3**, 2025–2030
  69. Villmann, C., Oertel, J., Ma-Högemeier, Z. L., Hollmann, M., Sprengel, R., Becker, K., Breitinger, H. G., and Becker, C. M. (2009) Functional complementation of *Glr1<sup>spad-ot</sup>*, a glycine receptor subunit mutant, by independently expressed C-terminal domains. *J. Neurosci.* **29**, 2440–2452
  70. Singer, J. H., Talley, E. M., Bayliss, D. A., and Berger, A. J. (1998) Development of glycinergic synaptic transmission to rat brainstem motoneurons. *J. Neurophysiol.* **80**, 2608–2620
  71. Wässle, H., Heinze, L., Ivanova, E., Majumdar, S., Weiss, J., Harvey, R. J., and Haverkamp, S. (2009) Glycinergic transmission in the mammalian retina. *Front. Mol. Neurosci.* **2**, 6
  72. Weltzien, F., Puller, C., O'Sullivan, G. A., Paarmann, I., and Betz, H. (2012) Distribution of the glycine receptor  $\beta$  subunit in the mouse CNS as revealed by a novel monoclonal antibody. *J. Comp. Neurol.* **520**, 3962–3981
  73. Hoch, W., Betz, H., and Becker, C. M. (1989) Primary cultures of mouse spinal cord express the neonatal isoform of the inhibitory glycine receptor. *Neuron* **3**, 339–348
  74. Weiss, J., O'Sullivan, G. A., Heinze, L., Chen, H. X., Betz, H., and Wässle, H. (2008) Glycinergic input of small-field amacrine cells in the retinas of wild-type and glycine receptor-deficient mice. *Mol. Cell. Neurosci.* **37**, 40–55
  75. Young-Pearse, T. L., Ivic, L., Kriegstein, A. R., and Cepko, C. L. (2006) Characterization of mice with targeted deletion of glycine receptor  $\alpha 2$ . *Mol. Cell. Biol.* **26**, 5728–5734
  76. Nobles, R. D., Zhang, C., Müller, U., Betz, H., and McCall, M. A. (2012) Selective glycine receptor  $\alpha 2$  subunit control of crossover inhibition between the on and off retinal pathways. *J. Neurosci.* **32**, 3321–3332
  77. Kallenborn-Gerhardt, W., Lu, R., Lorenz, J., Gao, W., Weiland, J., Del Turco, D., Deller, T., Laube, B., Betz, H., Geisslinger, G., and Schmidtke, A. (2012) Prolonged zymosan-induced inflammatory pain hypersensitivity in mice lacking glycine receptor  $\alpha 2$ . *Behav. Brain Res.* **226**, 106–111
  78. Malosio, M. L., Marquèze-Pouey, B., Kuhse, J., and Betz, H. (1991) Widespread expression of glycine receptor subunit mRNAs in the adult and developing rat brain. *EMBO J.* **10**, 2401–2409
  79. Harvey, R. J., Depner, U. B., Wässle, H., Ahmadi, S., Heindl, C., Reinold, H., Smart, T. G., Harvey, K., Schütz, B., Abo-Salem, O. M., Zimmer, A., Poisbeau, P., Welzl, H., Wolfer, D. P., Betz, H., Zeilhofer, H. U., and Müller, U. (2004) GlyR  $\alpha 3$ : an essential target for spinal PGE<sub>2</sub>-mediated inflammatory pain sensitization. *Science* **304**, 884–887
  80. Xiong, W., Cui, T., Cheng, K., Yang, F., Chen, S. R., Willenbring, D., Guan, Y., Pan, H. L., Ren, K., Xu, Y., and Zhang, L. (2012) Cannabinoids suppress inflammatory and neuropathic pain by targeting  $\alpha 3$  glycine receptors. *J. Exp. Med.* **209**, 1121–1134
  81. Długaiczek, J., Singer, W., Schick, B., Iro, H., Becker, K., Becker, C. M., Zimmermann, U., Rohbock, K., and Knipper, M. (2008) Expression of glycine receptors and gephyrin in the rat cochlea. *Histochem. Cell Biol.* **129**, 513–523
  82. Harvey, R. J., Schmieden, V., Von Holst, A., Laube, B., Rohrer, H., and Betz, H. (2000) Glycine receptors containing the  $\alpha 4$  subunit in the embryonic sympathetic nervous system, spinal cord and male genital ridge. *Eur. J. Neurosci.* **12**, 994–1001
  83. White, W. F., and Heller, A. H. (1982) Glycine receptor alteration in the mutant mouse *spastic*. *Nature* **298**, 655–657
  84. Kingsmore, S. F., Giros, B., Suh, D., Bieniarz, M., Caron, M. G., and Seldin, M. F. (1994) Glycine receptor  $\beta$  subunit gene mutation in *spastic* mouse associated with LINE-1 element insertion. *Nat. Genet.* **7**, 136–141
  85. Müllhardt, C., Fischer, M., Gass, P., Simon-Chazottes, D., Guénet, J. L., Kuhse, J., Betz, H., and Becker, C. M. (1994) The *spastic* mouse: aberrant splicing of glycine receptor  $\beta$  subunit mRNA caused by intronic insertion of L1 element. *Neuron* **13**, 1003–1015
  86. Becker, K., Braune, M., Benderska, N., Buratti, E., Baralle, F., Villmann, C., Stamm, S., Eulenburg, V., and Becker, C. M. (2012) A retroelement modifies pre-mRNA splicing. The murine *Glr<sup>spad</sup>* allele is a splicing signal polymorphism amplified by long interspersed nuclear element insertion. *J. Biol. Chem.* **287**, 31185–31194
  87. Reichling, D. B., Kyrozi, A., Wang, J., and MacDermott, A. B. (1994) Mechanisms of GABA and glycine depolarization-induced calcium transients in rat dorsal horn neurons. *J. Physiol.* **476**, 411–421
  88. Kirsch, J., and Betz, H. (1998) Glycine receptor activation is required for receptor clustering in spinal neurons. *Nature* **392**, 717–720
  89. Turecek, R., and Trussell, L. O. (2002) Reciprocal developmental regulation of presynaptic ionotropic receptors. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 13884–13889
  90. Kubota, H., Alle, H., Betz, H., and Geiger, J. R. (2010) Presynaptic glycine receptors on hippocampal mossy fibers. *Biochem. Biophys. Res. Commun.* **393**, 587–591
  91. Rivera, C., Voipio, J., Payne, J. A., Ruusuvoori, E., Lahtinen, H., Lamsa, K., Pirvola, U., Saarma, M., and Kaila, K. (1999) The K<sup>+</sup>/Cl<sup>-</sup> cotransporter KCC2 renders GABA hyperpolarizing during neuronal maturation. *Nature* **397**, 251–255
  92. Becker, C. M., Hoch, W., and Betz, H. (1988) Glycine receptor heterogeneity in rat spinal cord during postnatal development. *EMBO J.* **7**, 3717–3726
  93. Takahashi, T., Momiyama, A., Hirai, K., Hishinuma, F., and Akagi, H. (1992) Functional correlation of fetal and adult forms of glycine receptors with developmental changes in inhibitory synaptic receptor channels. *Neuron* **9**, 1155–1161
  94. Bormann, J., Rundström, N., Betz, H., and Langosch, D. (1993) Residues within transmembrane segment M2 determine chloride conductance of glycine receptor homo- and hetero-oligomers. *EMBO J.* **12**, 3729–3737
  95. Shiang, R., Ryan, S. G., Zhu, Y. Z., Hahn, A. F., O'Connell, P., and Wasmuth, J. J. (1993) Mutations in the  $\alpha 1$  subunit of the inhibitory glycine receptor cause the dominant neurologic disorder hyperekplexia. *Nat. Genet.* **5**, 351–358
  96. Rajendra, S., Lynch, J. W., Pierce, K. D., French, C. R., Barry, P. H., and Schofield, P. R. (1994) Startle disease mutations reduce the agonist sensitivity of the human inhibitory glycine receptor. *J. Biol. Chem.* **269**, 18739–18742
  97. Davies, J. S., Chung, S. K., Thomas, R. H., Robinson, A., Hammond, C. L., Mullins, J. G., Carta, E., Pearce, B. R., Harvey, K., Harvey, R. J., and Rees, M. I. (2010) The glycinergic system in human startle disease: a genetic screening approach. *Front. Mol. Neurosci.* **3**, 8
  98. Chung, S. K., Vanbellinghen, J. F., Mullins, J. G., Robinson, A., Hantke, J., Hammond, C. L., Gilbert, D. F., Freilinger, M., Ryan, M., Kruer, M. C., Masri, A., Gurses, C., Ferrie, C., Harvey, K., Shiang, R., Christodoulou, J., Andermann, F., Andermann, E., Thomas, R. H., Harvey, R. J., Lynch, J. W., and Rees, M. I. (2010) Pathophysiological mechanisms of dominant and recessive *GLRA1* mutations in hyperekplexia. *J. Neurosci.* **30**, 9612–9620
  99. Al-Owain, M., Colak, D., Al-Bakheet, A., Al-Hashmi, N., Shuaib, T., Al-Hemidan, A., Aldhalaan, H., Rahbeeni, Z., Al-Sayed, M., Al-Younes, B., Ozand, P. T., and Kaya, N. (2012) Novel mutation in *GLRB* in a large family with hereditary hyperekplexia. *Clin. Genet.* **81**, 479–484
  100. Hutchinson, M., Waters, P., McHugh, J., Gorman, G., O'Riordan, S., Connolly, S., Hager, H., Yu, P., Becker, C. M., and Vincent, A. (2008) Progressive encephalomyelitis, rigidity, and myoclonus: a novel glycine receptor antibody. *Neurology* **71**, 1291–1292