Modulation of glycine receptor function: a novel approach for therapeutic intervention at inhibitory synapses?

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Transmitter-gated ion channels mediate rapid synaptic transmission in the CNS and constitute important targets for many neuroactive drugs. Inhibitory glycine receptors (GlyRs) are members of the nicotinic acetylcholine receptor superfamily and inhibit neuronal firing by opening Cl^- channels following agonist binding. In this article, we discuss recent developments in GlyR pharmacology, delineate the receptor domains that are involved in binding of agonists and allosteric modulators, and present a molecular model of the extracellular architecture of the receptor. The recent discovery of compounds that act preferentially on specific GlyR isoforms and the differential expression of these isoforms in distinct regions of the developing and adult CNS show considerable promise towards the development of drugs that act in defined glycine-mediated pathways. In particular, compounds that can potentiate GlyR function should provide leads for novel muscle relaxants in addition to sedative and analgesic agents.

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Inhibitory neurotransmission in the mammalian CNS is mainly mediated by the amino acids GABA and glycine, which activate ionotropic $GABA_A$ receptors and glycine receptors (GlyRs), respectively. Both types of receptors are members of the group I ligand-gated ion channel superfamily [1]. $GABA_A$ receptors constitute major targets of widely used drugs such as barbiturates and benzodiazepines, whereas clinically applicable compounds that target GlyRs have yet to be identified. In this article, recent results from studies of the molecular pharmacology of mammalian GlyRs are summarized, and potential leads for clinically useful GlyR modulatory agents are discussed.

Glycinergic synapses are found in many regions of the CNS but are particularly abundant in spinal cord, brain stem, caudal brain and retina, where they are implicated in the control of motor rhythm generation, coordination of reflex responses and processing of sensory signals [2]. The transmitter function of glycine has been studied mostly in spinal cord, where glycine mediates reciprocal inhibition in stretch reflex circuits via interneurons in addition to recurrent inhibition of motoneurons via Renshaw cells [2]. Decreases in glycine-mediated input therefore result in pathologies of muscle tone regulation [3]. The roles of glycine in sensory processing range from modulation of neuronal circuits in the central auditory pathway and of receptive fields in the retina to suppression of nociceptive signals in spinal structures. These multiple functions of glycine transmission correlate with the localization of different types of GlyRs in the respective brain regions [4,5]. Compounds that selectively potentiate GlyR responses are therefore potential therapeutics for spasticity, muscle relaxation and pain relief.

Developmental changes in GlyR function and subunit composition

In adult neurons, activation of GlyRs by presynaptically released glycine or extracellularly applied agonists causes the opening of the anion-selective channel of the receptor, thereby allowing influx of Cl^- ions into the cytoplasm. The resulting hyperpolarization of the postsynaptic membrane stabilizes the resting potential of the cell, and thus inhibits neuronal firing. During early development, glycine acts as an excitatory transmitter because of a more positive Cl^- equilibrium potential in embryonic neurons. Consequently, GlyR activation results in Cl^- efflux, thus causing depolarization of the neuronal plasma membrane and opening of voltage-gated Ca^{2+} channels [6]. The subsequent rise in intracellular Ca^{2+} appears to be important for synapse formation because Ca^{2+} channel antagonists have been found to prevent proper localization of GlyRs at glycinergic nerve terminals [7]. After birth, the Cl^- equilibrium potential shifts to more negative 'hyperpolarizing' values, as a result of active Cl^- extrusion by the K^+/Cl^- cotransporter KCC2 [8]. During the first two postnatal weeks, a change to more rapid channel decay kinetics is also observed [9]. This shift in kinetic properties reflects a change in the subunit composition of GlyRs (Box 1) [10]. In adult spinal cord, the GlyR is a hetero-pentameric membrane protein composed of α1- and β-subunits [11]. By contrast, embryonic GlyRs appear to be predominantly homopentamers of the α2-subunit [12].

GlyR agonists and antagonists

In addition to glycine, the endogenous β-amino acids β-alanine and taurine (Fig. 1) display inhibitory activity when applied to neurons [13]. The agonistic

  PII: S0165-6147(02)02138-7

Box 1. Glycine receptor (GlyR) structure and molecular determinants of GlyR activity

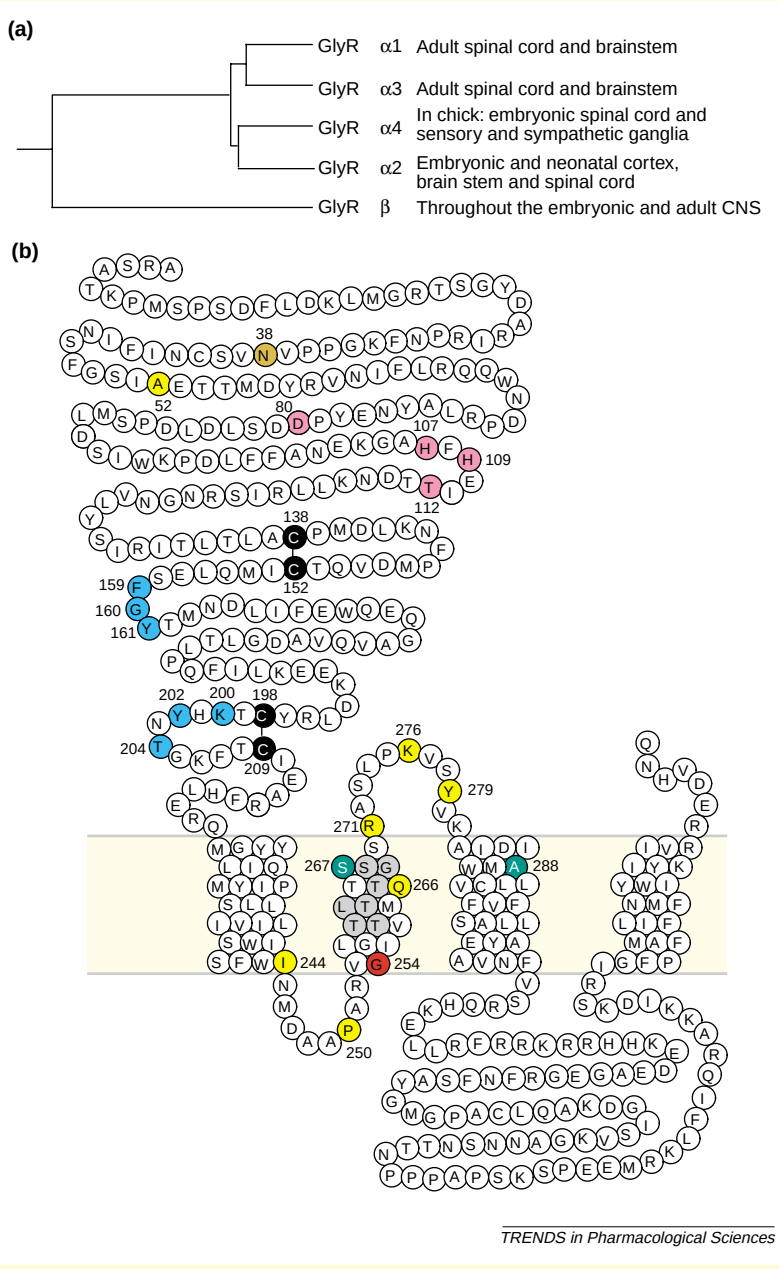

Fig. I. Phylogenetic tree of mammalian glycine receptor (GlyR) subunits and model of their membrane topology. (a) Evolutionary relationships between GlyR subunits. In addition to the individual subunits, their major sites of expression in the mammalian CNS are indicated. (b) Transmembrane topology of the human α1-subunit and location of functionally important amino acids. Conserved cysteine residues that are thought to form disulfide bridges are indicated in black. Residue N38 (brown) constitutes the sole N-glycosylation site. Natural GlyR mutants (yellow residues): mutation A52S is found in the spasmodic mouse; and mutations I244N, P250T, Q266H, R271Q/L, K276E and Y279C are found in different hyperekplexia families. Binding site determinants (blue residues): residues G160, K200 and Y202 contribute to strychnine binding, whereas F159, Y161 and T204 are important determinants of agonist affinity. S267 (green) in the second transmembrane segment (M2) and A288 (green) in the third transmembrane segment (M3) are targets for alcohol and volatile anesthetics. Residue D80 (pink) is an important determinant of Zn^{2+} potentiation, whereas residues H107, H109 and T112 (pink) are important determinants of Zn^{2+} inhibition. Channel function: G254 (red) in M2 of the α1-subunit determines the main-state conductance and sensitivity to the channel blocker cyanotriphenylborate (CTB); E290 and E297 in the homologous region of the β-subunit are crucial for resistance to inhibition by picrotoxinin. Residues of M2 that are thought to line the ion channel, as deduced by molecular modeling [g] and cysteine accessibility studies of the highly homologous $GABA_A$ receptor [h], are shown in gray.

The glycine receptor (GlyR) is a member of the group I ligand-gated ion channel superfamily, which includes nicotinic acetylcholine, 5-HT_3, $GABA_A$ and $GABA_C$ receptors. In adult vertebrates, GlyRs are pentameric membrane proteins composed of two types (α and β) of homologous membrane-spanning subunits [a–c]. The α-subunits contain major determinants of agonist and antagonist binding and exist in four different isoforms (α1–α4) encoded by distinct genes. To date, only a single gene encoding the β-subunit is known. The different GlyR subunit genes show marked regional and temporal differences in their expression patterns (Fig. Ia) [d]. Whereas the gene encoding the β-subunit is widely expressed throughout the embryonic and adult CNS, the gene encoding the α1-subunit and, to a lesser extent, the gene encoding the α3-subunit are mainly active in spinal cord and brain stem at later postnatal stages. By contrast, expression of the gene encoding the α2-subunit is high in the embryonic and perinatal CNS but barely detectable in the adult brain, although some expression persists in higher cortical regions. The gene encoding the α4-subunit has been found to be transcribed in lower vertebrates, but might be a pseudogene in humans [e].

Upon heterologous expression, all α-subunits generate functional homomeric receptors whose properties closely resemble those of native GlyRs [a–c,e]. By contrast, the β-subunit forms channels only upon co-assembly with α-subunits at an α:β stoichiometry of 3:2 [a]. A major function of the β-subunit is synaptic anchoring of the GlyR by binding to the postsynaptic scaffolding protein gephyrin. In addition, incorporation of β-subunits alters the pharmacological and functional properties, such as main channel conductance, gating kinetics, and sensitivity to channel blockers and modulatory compounds. Although adult GlyRs are heteromeric proteins, homo-oligomeric GlyRs appear to be synthesized during embryonic and early postnatal development and might serve as extrasynaptic receptors.

Sequence comparisons and site-directed mutagenesis of different GlyR α-subunits have allowed the identification of major determinants of agonist and antagonist binding (Fig. Ib) [a–c]). These are localized in distinct subdomains of the N-terminal extracellular region. Mutations causing hereditary neuromotor disorders have been identified in mouse and humans [a–c,f]. Residues within the second transmembrane segment have been shown to determine the rate and duration of Cl^- flux through, and channel blocker binding to, the ion channel of the GlyR [a–c].

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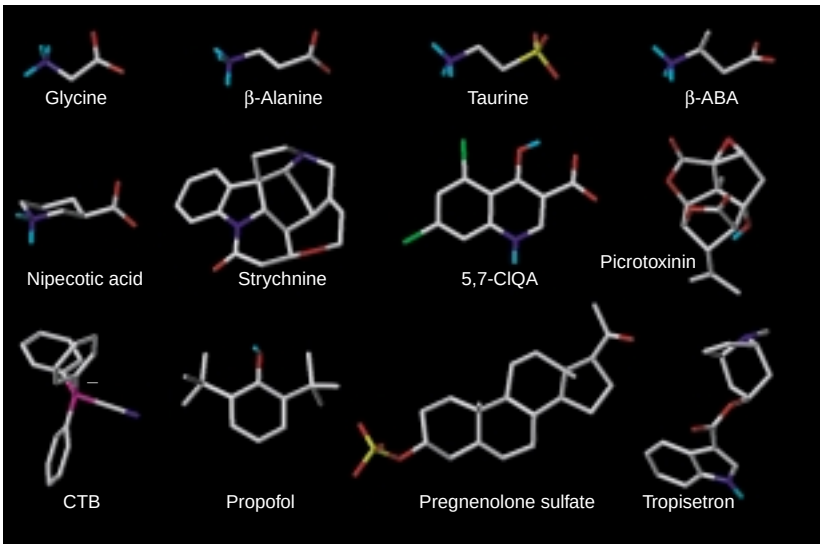

Fig. 1. Chemical structures of selected compounds that act at glycine receptors (GlyRs). The α-amino acid glycine, the β-amino acids β-alanine and taurine, and β-aminobutyric acid (β-ABA) exert agonistic activity. Taurine and β-ABA act as partial agonists at GlyRs. The piperidine derivative nipecotic acid mimicks β-amino acids in *trans*-conformation and behaves as a competitive GlyR antagonist. Strychnine and 5,7-dichloro-4-hydroxyquinoline-3-carboxylic acid (5,7-ClQA) are competitive GlyR antagonists, whereas picrotoxinin and cyanotriphenylborate (CTB) block the Cl^- channel of GlyRs. Propofol, pregnenolone sulfate and tropisetron are modulators of GlyRs. Propofol potentiates neuronal glycine-mediated currents [26] and recombinant α1 GlyR responses, pregnenolone sulfate exhibits full competitive inhibition of GlyR responses, and tropisetron, in spinal neurons, potentiates the effects of low concentrations of glycine but inhibits glycine-mediated currents at higher concentrations.

and antagonistic actions of several α- and β-amino acids have been studied in detail using recombinant GlyRs generated by heterologous expression of the α1-subunit in *Xenopus* oocytes or mammalian cells [14]. This revealed that the agonistic activity of several α-amino acids (e.g. glycine, alanine and serine) exhibits marked stereoselectivity and is sensitive to substitutions at the Cα-atom. By contrast, antagonism as observed with other α-amino acids [13] is neither influenced by C-atom substitutions nor chirality. β-Amino acids such as taurine, β-aminobutyric acid (β-ABA) and β-aminoisobutyric acid (β-AIBA) (Fig. 1) act as partial agonists at GlyRs and competitively inhibit glycine responses at low concentrations, whereas high concentrations elicit significant membrane currents [14,15]. The conformationally constrained nipecotic acid and related piperidine carboxylic acid (Fig. 1) mimic β-amino acids in *trans*-conformation and behave as competitive GlyR antagonists. Ivermectin, an antihelmintic macrocyclic lactone, is an unconventional GlyR agonist [16].

Strychnine (Fig. 1) is a classical GlyR antagonist that causes motor disturbances, increased muscle tone, and hyperactivity of sensory, visual and acoustic perception, with higher doses resulting in convulsions and death [17,18]. Further selective GlyR antagonists have emerged from studies on quinolinic acid derivatives [19]. Both 4-hydroxy-quinoline and 4-hydroxy-quinoline-3-carboxylic acid inhibit the responses of micromolar glycine at recombinantly expressed GlyRs [19]. Substitution of the 5- and 7-positions of the quinoline ring system by chloro (5,7-ClQA; Fig. 1) or trifluoromethyl groups increases their inhibitory potency.

The $GABA_A$ receptor antagonist picrotoxinin (Fig. 1) inhibits GlyR Cl^- channels and has been used to discriminate homo-oligomeric from heteroligomeric GlyRs [20]: α1β GlyRs are relatively resistant to picrotoxinin, whereas α1 GlyRs are blocked at low micromolar concentrations [21,22]. The bulky anion cyanotriphenylborate (CTB) (Fig. 1) is a use-dependent inhibitor of α1-containing GlyRs and is more potent at positive membrane potentials, consistent with open channel blockade [23].

Allosteric modulation of GlyR function by anesthetics, alcohols, steroids and dihydropyridines

At present, only a few agents are known to potentiate GlyR-mediated currents and, unfortunately, most lack receptor specificity. For decades, anesthetics and alcohols have been known to potentiate not only $GABA_A$ receptor responses but also GlyR responses, and the potentiating effect of ethanol has been demonstrated for GlyRs from various sources [24]. The potency of *n*-alcohols on recombinant GlyR responses increase with alkyl chain length up to 12 carbon atoms [25]. Anesthetic concentrations of propofol, an aromatic alcohol (Fig. 1), also potentiate neuronal glycine-mediated currents [26] and recombinant α1 GlyR responses [27], albeit to a lesser extent than $GABA_A$ receptor responses [26]. Furthermore, anesthetic concentrations of trichloroethanol, ethers and volatile halogenated hydrocarbons, such as halothane, enflurane, isoflurane, methoxyflurane and sevoflurane, enhance the effects of low concentrations of glycine [25,28]. The available data are consistent with both alcohols and anesthetics potentiating glycine-mediated inhibition at concentrations reached during intoxication and narcosis, respectively.

Steroids are known to markedly affect inhibitory neurotransmission mediated by both GABA and glycine. Whereas progesterone inhibits GlyRs only partially [29,30], the sulfates of pregnenolone (Fig. 1), androsterone and dehydroepiandrosterone (DHEA) cause a complete block [30]. This inhibition is only seen with negatively charged 3-sulfate and 3-hemisuccinate ester derivatives, whereas pregnenolone, its 3-acetic ester, and minaxolone, a dimethylamino derivative of allopregnanolone, potentiate recombinant GlyR responses [30,31]. Importantly, pregnenolone sulfate and progesterone display distinct modes of GlyR inhibition: full competitive versus partial non-competitive inhibition, respectively [29]. These varying effects on GlyR function suggest heterogeneous binding sites for neurosteroids, which, depending on the ligand bound, might facilitate or inhibit agonist activation.

The bidirectional modulatory effects of neurosteroids on GlyRs depend on subunit composition: pregnenolone potentiates only α1 GlyRs, whereas inhibition by progesterone is seen only at α2 GlyRs [30].

Table 1. Agents reported to inhibit and potentiate GlyR function[a]

Agent	Potency[b]	GlyRs tested[c]	Therapeutic effect	Refs
Inhibition				
Indole alkaloids:				
β-spiropyrrolidinoindoles	++[d]	–	–	[50]
corymine	++	–	Analgetic	[49]
Opioids:				
thebaine	+++[d]	–	Narcotic	[50]
dextromethorphan	++	–	Antitussive	[68]
ω-Phosphono-α-amino acids	++[d]	–	–	[50,69]
Colchicine	++	α2 > α1	Antimitotic	[70]
Thiocolchicoside	++[d]	–	Myorelaxant	[71]
Tropeines:				
atropine	++	α2 > α1	Antispastic	[36]
cocaine	+[d]	–	Anesthetic	[72]
Steroids:				
RU5135	+++[d]	–	–	[50]
deoxycorticosterone	++	–	Mineralo-corticoid	[29]
3α/βandrosterone sulfate	++	–	–	[30]
Isoxazol derivatives:				
isoTHAZ, THIP	++[d]	–	–	[50]
TAG	++	–	–	[50]
PTK inhibitors (genistein and daidzein)	++	–	–	[73]
PKC activators (phorbol ester)	++	–	–	[49]
Benzodiazepines	++[d]	–	Sedative, anxiolytic	[50]
Bicuculline derivatives	++[d]	–	Convulsant	[50]
Pitrazepine	+++[d]	–	–	[50]
Imipramine	++	–	Antidepressant	[50]
Furosemide	+	–	Diuretic	[74]
Riluzole	++	α1β	Anticonvulsant	[75]
Potentiation				
Alkylbenzene sulfonate	++	α2 > α1	Detergent	[76]
Penicillin G	++	–	Antibiotic	[49]
Chlormethiazole	++	–	Anticonvulsant	[77]

[a]Abbreviations: GlyR, glycine receptor; isoTHAZ, 5,6,7,8-tetrahydro-4*H*-isoxazolo-[3,4-*d*]azepin-3-ol; PKC, protein kinase C; PTK, protein tyrosine kinase; TAG, 6–aminomethyl-3-methyl-4*H*-1,2,4-benzo-thiadiazine-1,1-dioxide; THIP, 4,5,6,7-tetrahydroisoxazolo-[5,4-*c*]pyridin-3-ol.
[b]If not otherwise indicated, the potencies were examined in electrophysiological recordings.
[c]In cases where recombinant GlyRs were used for evaluation, the respective subunit composition is indicated.
[d]Potencies were determined by displacement of [^{3}H]strychnine binding. Receptor affinities determined in different vertebrate species are summarized in [49,50]. Potency ranking according to inhibition [M]: +++, <10^{-6}; ++, 10^{-6}–10^{-4}; +, >10^{-4}.

Also, the inhibition patterns of different homo- and hetero-oligomeric GlyRs differ for distinct sulfated steroids. Notably, the inhibitory potencies of DHEA sulfate compared with pregnenolone sulfate are lower at adult α1β GlyRs than at embryonic α2 GlyRs [30]. These findings suggest that neurosteroids might preferentially modulate perinatal GlyR activity. Indeed, neurosteroids such as pregnenolone and progesterone can be present perinatally at concentrations sufficient to alter GlyR responses. Neurosteroids display different structure–activity relationships at GlyRs than at $GABA_A$ receptors. Therefore, the development of high-affinity GlyR-selective derivatives should have considerable promise for therapy.

L-type Ca^{2+} channel blockers verapamil and several dihydropyridines have recently been shown to block glycine responses in rat spinal neurons [32]. Interestingly, the modest structural changes in nitrendipine and nicardipine led to potentiation of GlyR currents elicited by non-saturating glycine concentrations [32]. Table 1 summarizes data on several additional compounds that have been reported to potentiate or inhibit GlyR function.

Tropeines: lead compounds for novel GlyR effectors?

Ionotropic 5-HT_3 receptors are antagonized by several tropeines [33]. Recently, tropeines have been found to act as allosteric modulators of GlyRs. In spinal neurons, responses to low concentrations of glycine are potentiated by submicromolar concentrations of tropisetron (Fig. 1), bemesetron and LY278584, whereas inhibition of glycine-mediated currents is observed at higher micromolar concentrations of these compounds [34].

In the presence of glycine, several tropeines inhibit [^{3}H]strychnine binding to GlyRs with high affinity. These tropeines also increase the displacing potencies of glycine and have therefore been termed 'glycine-positive' agents [35]. By contrast, micromolar concentrations of 'glycine-negative' tropeines such as atropine attenuate the displacement of [^{3}H]strychnine by glycine [35] and act only as inhibitors of GlyR function [34,36]. Glycine-positive tropeines with nanomolar potencies [35] might provide excellent leads for the development of selective GlyR-potentiating agents. Interestingly, the potencies of these tropeines are GlyR-subunit dependent. For example, α1 and α2β GlyRs, but not α2 GlyRs, are potentiated by tropisetron [37]. Moreover, the sites of action of the tropeines are distinct from those of Zn^{2+}, propofol and ethanol, and thus define novel drug target regions on the GlyR.

Zn^{2+}-mediated modulation of the GlyR

In several regions of the mammalian brain and spinal cord the divalent cation Zn^{2+} is highly concentrated in the synaptic vesicles of selected neuronal subpopulations, from which it can be released in an activity-dependent manner [38]. Different lines of evidence support the idea that locally released Zn^{2+} modulates postsynaptic responses by binding to different neurotransmitter receptors [39]. Low concentrations of Zn^{2+} (<10 μM) enhance glycine-mediated currents by increasing the apparent agonist affinity without changing the maximal inducible current, whereas higher concentrations of Zn^{2+} (10 μM – 1 mM) have an opposite inhibitory effect (Fig. 2a) [40,41]. Both potentiation and inhibition of GlyR currents by Zn^{2+} are fully reversible and have also been observed following application of partial agonists such as taurine [41,42]. A remarkable difference in the modulation of glycine- and taurine-gated currents by Zn^{2+} is that maximal inducible currents for taurine but not glycine are increased (Fig. 2b) [43]. Single-channel analysis of glycine-gated currents suggests that Zn^{2+} affects both agonist dissociation and the efficacy of channel opening of the GlyR (Fig. 2c) [43].

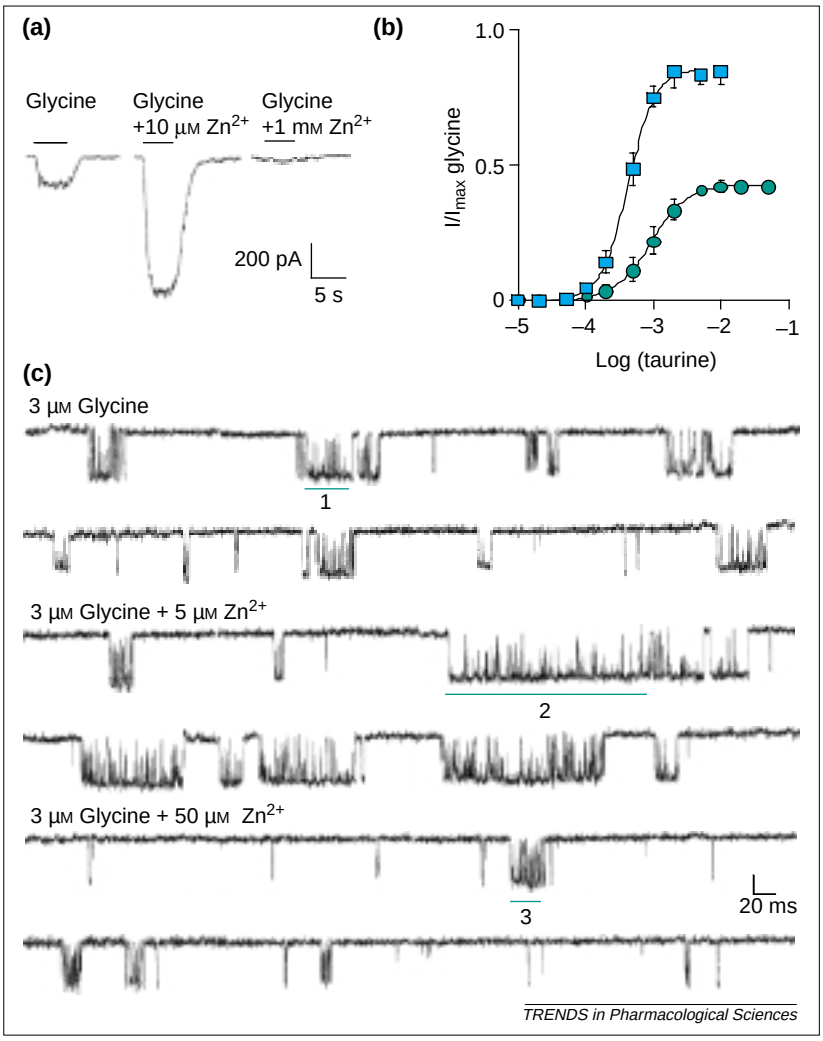

Fig. 2. Allosteric modulation of the inhibitory glycine receptor (GlyR) by Zn^{2+}. (a) Effects of Zn^{2+} on the glycine-mediated response of spinal cord neurons. Glycine-induced whole-cell currents of cultured spinal neurons are potentiated by 10 μM Zn^{2+}, whereas at higher concentrations of Zn^{2+} (1 mM) the current is reduced. Glycine was used at a concentration of 20 μM. (b) Dose–response curves of taurine in the absence (circles) and presence (squares) of 5 μM Zn^{2+}. Zn^{2+} converts taurine from a partial into a full GlyR agonist. (c) Single-channel recordings of glycine-mediated currents in the absence and presence of Zn^{2+}. Outside-out patch recording from HEK293 cells expressing GlyRs in the absence and presence of 5 μM and 50 μM Zn^{2+} are shown. 5 μM Zn^{2+} causes an increase, and 50 μM Zn^{2+} causes a decrease, in the apparent probabilities of channel opening [43]. In addition, the frequency and duration of current bursts are increased by 5 μM Zn^{2+}. Changes in burst durations are indicated (1–3) [43].

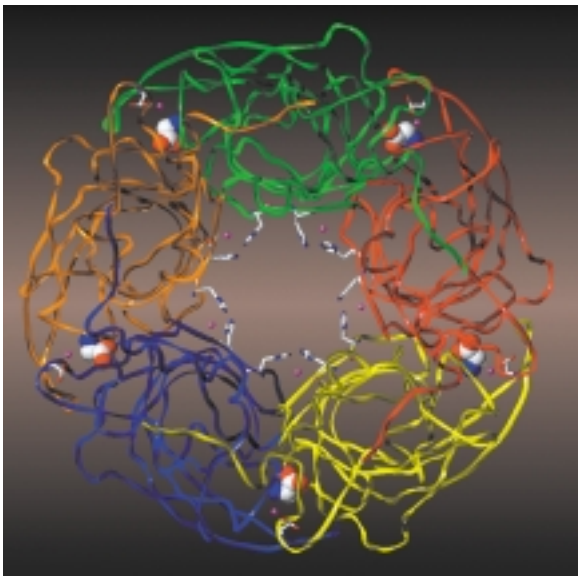

Fig. 3. Model of the N-terminal region of the glycine receptor (GlyR) α1-subunit. Top view of the N-terminal region of the α1 GlyR subunit modeled after the crystal structure of the pentameric acetylcholine binding protein of *Lymnea stagnalis*, as determined at 2.7 Å resolution by X-ray diffraction studies [64]. Molecular modeling and ligand docking was performed as described in [78]. Individual subunit backbones are colored differently. Mutagenesis data predict the glycine and Zn^{2+} binding sites to be located in close association at subunit interfaces. Bound glycine is shown in a space-filling representation, and bound Zn^{2+} ions in magenta. Side-chains of residues implicated in Zn^{2+} binding are indicated by capped sticks.

Molecular determinants of GlyR function

Site-directed mutagenesis of recombinant GlyRs and the analysis of mutations resulting in disease have identified binding regions and specific amino acid residues within GlyR subunits that determine both ligand potency and efficacy (Box 1). Different mutations in the α1-subunit of the GlyR that all have been shown to alter GlyR pharmacology are discussed below.

Mutations affecting agonist binding

The aromatic amino acids F159 and Y161 in the N-terminal extracellular region of the α1-subunit define the core agonist binding region (Box 1) [44]. Altering the position of the phenolic hydroxyl group in the double mutant F159Y–Y161F drastically enhances activation by GABA and serine [44], whereas substitution of glycine at position 160 by glutamate reduces strychnine antagonism by >500-fold [45]. Mutations K104A, F108A and T112A in α1 GlyRs enhance not only the potencies of all agonists but also allow full channel activation by the partial agonists taurine and GABA [46]. In addition, residues K200, Y202 and T204 are crucial for high-affinity binding of agonists and the competitive antagonist strychnine [47,48]. These results indicate that the glycine binding pocket of the α1 GlyR is formed by distinct subdomains of the N-terminal extracellular region.

Mutations causing hyperekplexia

Human startle disease (hyperekplexia) is a hereditary neuromotor disorder caused by different mutations in GlyR structural genes [13,49,50]. To date, several of these natural mutations have been shown to represent single amino acid substitutions in the human α1-subunit of the GlyR. Most of these substitutions are located at the extracellular end of, or in the loops flanking, the channel-lining transmembrane segment M2 (Box 1), and consequently also affect the gating mechanism. Indeed, partial agonists are no longer capable of channel activation in these mutated receptors. Taurine and β-alanine act as antagonists at α1 I244N, Q266H, R271L/Q, K276E and Y279C mutant GlyRs, all of which are identified to cause hyperekplexia [51–55]. Similarly, substitution of alanine at position 254, located at the intracellular end of M2 of the α3-subunit, by glycine converts the substituted butyrolactone α-ethyl-α-methyl-γ-thiobutyrolactone (αEMBTL) from an antagonist into a low-affinity

Box 2. Parameters determining the efficacy of glycine-mediated neurotransmission: considerations for drug targeting

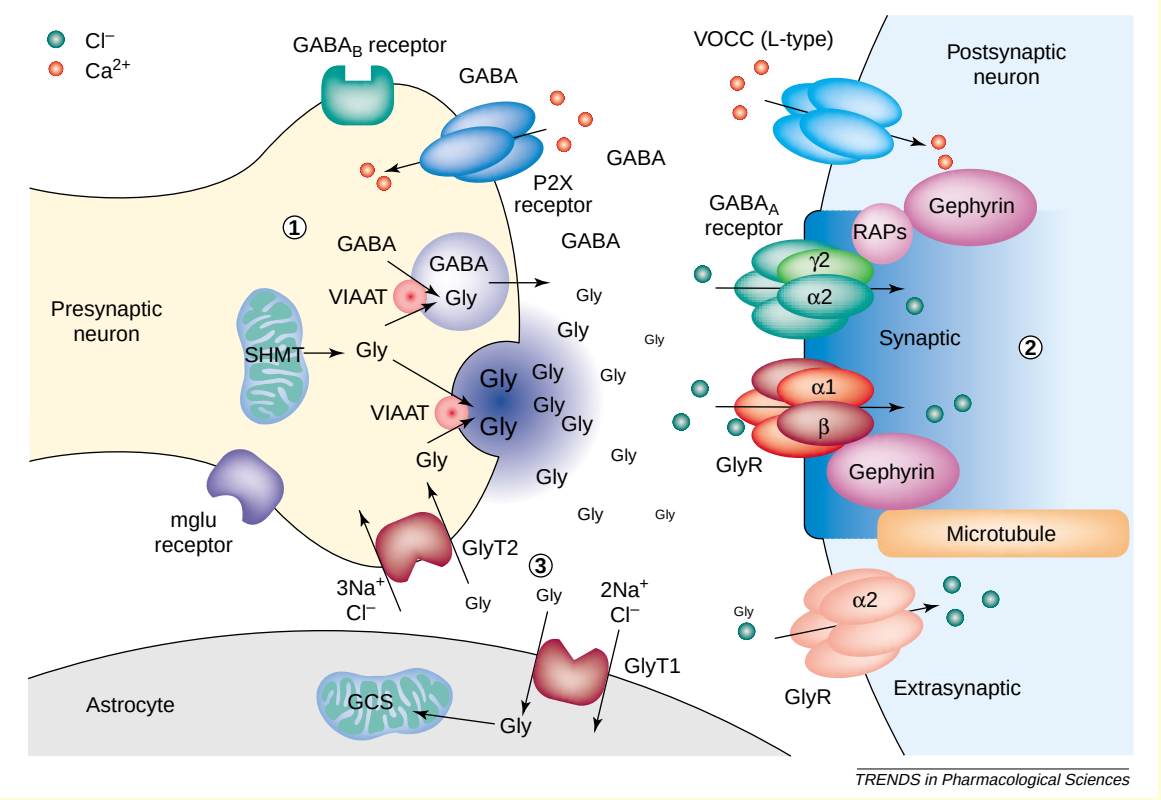

Fig. I. A glycinergic synapse indicating potential non-glycine receptor (GlyR) target sites of drug action. In the terminals of glycine-containing neurons, glycine is synthesized in mitochondria by serine hydroxymethyltransferase (SHMT), released into the cytosol and concentrated in small clear synaptic vesicles by the vesicular inhibitory amino acid transporter (VIAAT), which also mediates GABA uptake. Excitation of the terminal causes Ca^{2+}-triggered glycine (and GABA) release into the synaptic cleft, opens postsynaptic GlyRs and thereby increases Cl^- (green) conductance in response to agonist binding. Transmission is terminated by the reuptake of glycine mediated by Na^+- and Cl^--dependent transporters located in the presynaptic (GlyT2) and glial (GlyT1) plasma membranes. Glycine is then degraded in astroglial mitochondria by the glycine cleavage system (GCS). Synaptic GlyRs are anchored via the β-subunit to the submembraneous scaffolding protein gephyrin, which interacts with cytoskeletal components (tubulin). Gephyrin is also associated with synaptic $GABA_A$ receptors, presumably via receptor-associated proteins (RAPs). Glycine levels in the synaptic cleft could be altered by targeting several mechanisms. (1) Release of glycine: agonists of presynaptic $GABA_B$ receptors attenuate evoked glycine-mediated inhibitory postsynaptic currents (IPSCs) [d]; ATP reversibly increases the amplitude of electrically evoked IPSCs via presynaptic ionotropic P2X receptors [e,f]; activation of presynaptic metabotropic glutamate (mglu) receptors inhibits glycine release by reducing Ca^{2+} influx (red) [g]. (2) Modulation of GlyR function: ligands, such as Zn^{2+} and ethanol, modulate GlyR-mediated responses; there are differential effects on synaptic versus extrasynaptic GlyRs and thus extrasynaptic receptors might play an important role in the tonic inhibition of neurons and could provide a target to selectively affect background inhibition [b]; differential effects could be induced on $GABA_A$ receptors versus GlyRs at mixed GABAergic and glycinergic synapses [h]; cotransmission of GABA and glycine results in a complex time-course of IPSCs [h]; compounds that act differentially on $GABA_A$ receptors and GlyRs might reshape inhibitory postsynaptic currents; Ca^{2+}-permeable channels [e.g. voltage-activated Ca^{2+} channels (VOCC) and P2X receptors], Ca^{2+}-dependent clustering [i] and Ca^{2+}-induced potentiation of GlyRs [j] are crucial for the maturation and plasticity of glycinergic synapses. (3) Uptake and degradation of glycine: inhibition of glycine transporters GlyT1 and GlyT2, which are located in presynaptic terminals and surrounding glial cells, will differentially increase glycine levels in the synaptic cleft [k,l]; primary defects in the GCS within the inner mitochondrial membrane of astrocytes causes metabolic disorders; inhibitors of the GCS should also increase effective extracellular glycine concentrations.

potentiating agent [56]. In conclusion, amino acid exchanges in M2 and adjacent loop regions alter the balance between agonism and antagonism, suggesting that agonist binding induces allosteric transitions of these regions required for channel gating.

Determinants of GlyR modulation

Ser267 within the M2 domain of α1 GlyRs has been found to play a unique role in the effects of ethanol. Replacement of this serine by alanine enhances ethanol potentiation, whereas substitution with bulky amino acids such as phenylalanine results in ethanol inhibition [57]. Introduction of isoleucine, an unbranched amino acid, at this position had no effect on ethanol sensitivity [58], but alkylation of the cysteine in mutant S267C caused constitutive activation of α1 GlyRs [59]. These effects highlight M2 as being crucial for ethanol action. Moreover, residue A288 in the M3 region of α1 GlyRs has been reported to be essential for potentiation by both ethanol and enflurane [60]. Therefore, ethanol might act via a site formed by transmembrane segments M2 and M3 of GlyR α-subunits. This site also seems to be crucial for the action of various halogenated alcohols, such as trichloroethanol [61].

Heterologous expression experiments have shown that the Zn^{2+} binding sites mediating both

The efficacy of glycine-mediated inhibition depends crucially on the topology of individual glycinergic synapses. This puts significant constraints on how transmission at glycinergic synapses might be modulated. First, during synaptic transmission postsynaptic glycine receptors (GlyRs) might be saturated at some but not all synapses [a], indicating that the amplitude of glycine-mediated inhibitory postsynaptic currents (IPSCs) might exhibit variable sensitivity to modulatory compounds in different neurons. Second, extrasynaptic receptors might play an important role in the tonic inhibition of neurons and therefore represent another potential target for drugs that affect glycine-mediated transmission (Fig. I). Notably, tonic inhibition is likely to be mediated by GlyRs differing in molecular composition and response properties from synaptic GlyRs (Box 1). Recently, Flint and colleagues [b] have found that GlyRs in the developing rodent neocortex are activated by non-synaptically released taurine, which is stored in immature cortical neurons. Because fetal taurine deprivation has been linked with cortical dysgenesis [c], taurine has been proposed to influence cortical development by activating extrasynaptic α2 GlyRs. In conclusion, their localization might affect the response properties and functions of GlyRs. Third, the efficacy of GlyR-mediated neurotransmission will also be influenced by the activity of other membrane proteins located either pre- or postsynaptically, or in adjacent glial cells (Fig. I).

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potentiation and inhibition are localized in the N-terminal region of the GlyR α-subunits [41–43,62]. Mutational analysis of the α1-subunit indicates that residues D80 and H107, H109 and T112 are important determinants of Zn^{2+} potentiation and inhibition, respectively (Box 1). Computer-assisted modeling of the extracellular N-terminal region of the GlyR α1-subunit predicts that Zn^{2+} binding to these residues modifies subunit interactions required for agonist binding (see below).

The pharmacological properties of GlyRs are also influenced by incorporation of β-subunits into the GlyR. As mentioned above, coexpression of the β-subunit with α2-subunits revealed potentiating effects of tropisetron in addition to inhibition of α2 GlyRs [37]. Likewise, co-assembly of the β-subunit with an α3-subunit converts αEMBTL from an antagonist into a potentiating agent [56].

Molecular model of the GlyR N-terminal extracellular region

The significant extent of sequence homology between ligand-gated ion channel family group I members [1] (Box 1) indicates a common structural organization of these membrane proteins. Composite ligand binding sites, conserved throughout the receptor family, are thought to be located at extracellular subunit interfaces, and comprise residues assigned by photoaffinity labeling and site-directed mutagenesis to distinct subunit loop regions [63]. Recently, the crystal structure of a soluble acetylcholine binding protein was determined by X-ray diffraction studies [64]. This protein has high homology to the N-terminal extracellular domain of the group I receptors. We have used the structure of this acetylcholine binding protein to model the extracellular domain of the GlyR α1-subunit by homology modeling techniques (Fig. 3). Inspection of this model allows several conclusions. First, mutational analysis has identified several residues whose substitution alters agonist and antagonist binding [44–48,65,66]. Notably, most of these residues (K104, F159, Y161, K200, Y202 and T204) are found to be located at or close to the subunit interfaces. This supports the notion that agonist binding occurs between subunits (Fig. 3). Second, the model explains the importance of distinct residues for potentiation versus inhibition of GlyRs by Zn^{2+} [41–43,62]. Residue D80 is solvent-exposed and again located at the subunit interfaces. This arrangement might explain how Zn^{2+} binding to this site exerts a potentiating effect on GlyR function by affecting subunit interactions. However, Zn^{2+} binding to residues H107 and H109, located at the vestibule of the channel (Fig. 3), might block rotational motion of neighboring subunits thought to occur upon agonist binding [67] by forming a H107–Zn^{2+}–H109 ionic bridge between two subunits. This would impair channel opening and thereby prevent GlyR activation. Similar mechanisms might also underlie the effects of other modulators. Thus, our homology-based model provides a valid template for rational drug design.

Concluding remarks

Considerable progress has been made in understanding the structural basis of ligand binding to postsynaptic GlyRs. Molecular models based on the three-dimensional structure of the soluble acetylcholine-binding protein now shed light on the determinants of agonism, antagonism and allosteric regulation. The potentiating and inhibitory effects of Zn^{2+}, alcohol, neurosteroids and tropeines on GlyR function are well documented. The latter compounds might serve as suitable leads for the development of

Acknowledgements
Work in our laboratories was supported by MPG, DFG, BMBF, Fonds der Chemischen Industrie and OTKA (T29723). We thank S. Malany for critical reading of the manuscript and Maren Baier for secretarial assistance.

new GlyR subtype-selective drugs. In addition, several other parameters determine the efficacy of glycine-mediated transmission. First, the extent of agonist saturation of the GlyR might differ between synapses, thereby altering the response to modulating agents (Box 2). Also synaptic sites located in somatic regions might differ in their architecture and release properties from those found in proximal or distal dendritic regions. Second, a large number of other membrane proteins located either pre- or postsynaptically, or in adjacent glial cells, also affect the amount of and the response to presynaptic released glycine (Box 2). Some of these proteins have recently become additional targets of drug development. Together, these are exciting prospects for a broader pharmacology of glycinergic synapses.

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Chemical names

LY278584: 1-methyl-N-(3α-tropanyl)1*H*-indazole-3-carboxamide
RU5135: 3α-hydroxy-16-imino-5β-17-azaandrostan-11-one

Estrogen and cognitive aging in women

Barbara B. Sherwin

The steady increase in female life expectancy has attracted attention to the importance of preventing cognitive aging and Alzheimer's disease (AD) in women. Evidence from randomized, controlled trials and from cross-sectional and longitudinal studies shows that estrogen-replacement therapy preferentially protects against a decline in verbal memory in healthy postmenopausal women and decreases the risk of AD. Although results are not consistent across studies, they indicate that treatment with estrogen during the postmenopausal years might protect against cognitive aging in women during the latter part of their life.

Published online: 4 October 2002

The fact that female life expectancy has nearly doubled in industrialized countries during the past century means that more and more women are living into old age. In an attempt to preserve the quality of life for elderly women, research efforts have focused on preventing degenerative diseases, such as osteoporosis and coronary heart disease, that might compromise their daily functioning. There is increasing recognition that aspects of cognition also decline with normal aging in women and that this might impact negatively on their quality of life. Moreover, the epidemiological statistic of an ~1.6:1.0 female:male ratio in the incidence of Alzheimer's disease (AD) remains, even when controlling for greater female longevity [1]. The age-related decline in aspects of cognition observed in women has led some researchers to investigate whether changes in the levels of sex hormone in aging women might be influential. Here, I review the most recent evidence on the relationship between estrogen and cognition. This is preceded by a brief

 0165-6147/02/$ – see front matter PII: S0165-6147(02)02093-X