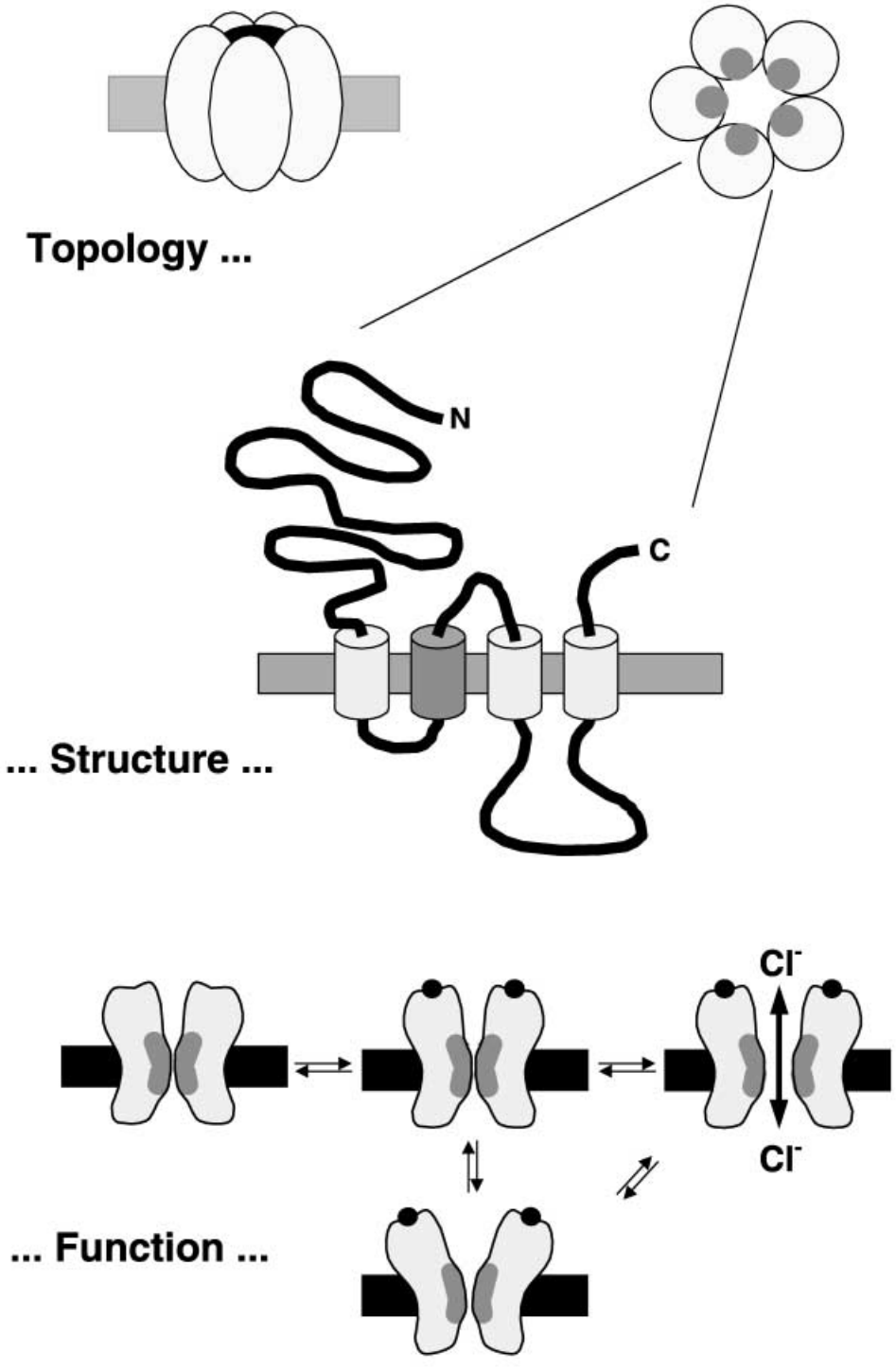
The Inhibitory Glycine Receptor



... of a Ligand-Gated Ion Channel.

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The Inhibitory Glycine Receptor—Simple Views of a Complicated Channel

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The strychnine-sensitive glycine receptor is the principal mediator of fast inhibitory synaptic transmission in the mammalian spinal cord and brain stem. As a member of the ligand-gated ion-channel family, it shares structural homology with the nicotinic acetylcholine, $GABA_{A/C}$ and serotonin 5-HT_3 receptors. Ion-channel activation and desensitisation are controlled by a variety of factors such as subunit composition, posttranslational modification, absence or presence of modulatory ions or other agents and possibly protein–protein interactions. Glycine-receptor mutations, either associated with the human motor disorder hyperekplexia or artificially introduced, have helped to define the regulatory domains of the receptor protein. In addition to their effects on glycine-receptor function, allelic variants of glycine-receptor genes may also affect biogenesis, assembly and degradation of the receptor.

KEYWORDS:

glycine receptor · ion channels · membrane proteins · neurotransmitters · receptors

Introduction

Fast synaptic communication between nerve cells and at the neuromuscular junction comprises the conversion of an electric signal—the action potential arriving at an axon terminal—into a chemical signal, that is, the release of a neurotransmitter into the synaptic cleft. Binding of the neurotransmitter triggers the opening of an intrinsic ion channel at specific postsynaptic receptors. The resulting ion flux alters the transmembrane potential and facilitates or suppresses the generation of a new action potential (Figure 1). Neurotransmitter molecules in the synaptic cleft are subsequently degraded (for example, by acetylcholine esterase) or removed through transporter-mediated uptake into glial or presynaptic nerve cells.[1] The excitatory neurotransmitters acetylcholine, glutamate and serotonin open cation channels (Na^+, Ca^{2+}) which depolarise the postsynaptic cell, thus facilitating the generation of an action potential.[2] Inhibitory neurotransmitters, such as glycine and γ-aminobutyric acid (GABA), activate anion channels (Cl^-, HCO_3^-) that lead to hyperpolarisation, thereby suppressing neuronal firing. When the inhibitory function of glycine receptors is considered, it should be remembered that the expression of chloride transporters, and thus the chloride potential in neurons, is developmentally regulated.[3] Embryonic glycine receptors are in fact excitatory,[4, 5] due to elevated levels of intracellular chloride in early development.

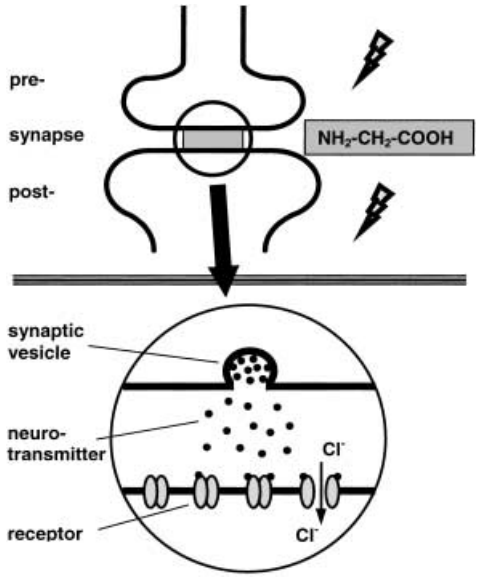

Figure 1. *Signal conversion in fast synaptic transmission.*

Strychnine-sensitive glycine receptors are the predominant carriers of fast inhibitory transmission at synapses in the vertebrate spinal cord and brain stem. Overall protein structure, topology, transmembrane arrangement, pentameric subunit assembly and general mechanism of function are conserved within the family of ligand-gated ion channels (Figure 2 and Table 1).[2] Other members of this receptor family include the nicotinic acetylcholine receptor, $GABA_A$ and $GABA_C$ receptors, and the serotonin 5-HT_3 receptor. These receptor proteins share a high degree of homology at both the protein and nucleic acid level, a fact indicating that ligand-gated ion channels originate from common ancestral genes.[2, 6, 7] Numerous biochemical data,[8, 9] hydropathy assignments and structural data from electron microscopy[10–12] and NMR spectroscopy[13–17] have led to the current four-transmembrane-helix model for ion channels that are nicotinic acetylcholine receptor like (Figure 2 D).[2, 8, 9] While ionotropic glutamate receptors also belong among the group of directly ligand gated ion channels, they are structurally

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more closely related to the voltage-gated receptor family.[2, 18] *N*-methyl-D-aspartate (NMDA) receptors, a subclass of the glutamate-receptor family, carry a glycine binding site and are sometimes referred to as 'non-strychnine-sensitive glycine receptors' in the literature, although in this case glycine modulates the receptor function through an allosteric site and does not actually gate the ion channel.

The Glycine-Receptor Protein Complex

Glycine receptor subunits and genes

To date, four ligand-binding glycine-receptor α subunits (α1 – 4) have been identified, all of which can form functional homomeric channels.[19] One β subunit is known, which is thought to mediate synaptic anchoring and has no ion-channel function on its own.[20–22] Receptor subunits were mostly cloned and isolated from humans, mice or rats,[22] chicks,[23, 24] and zebrafish (*Danio rerio*).[25–28]

The promoter regions of the human glycine-receptor genes GLRA1 and GLRA3 contain a neuron-restrictive silencer element (NRSE, REST), a fact indicating that neuronal expression of these subunits is regulated at the transcription level.[29] In contrast to the predominant expression of α subunits in spinal cord and brain stem, the β subunit is widely distributed throughout the central nervous system.[30, 31] Extraneuronal glycine receptors have been found in Kupffer cells from the liver[32] and in sperm.[33, 34]

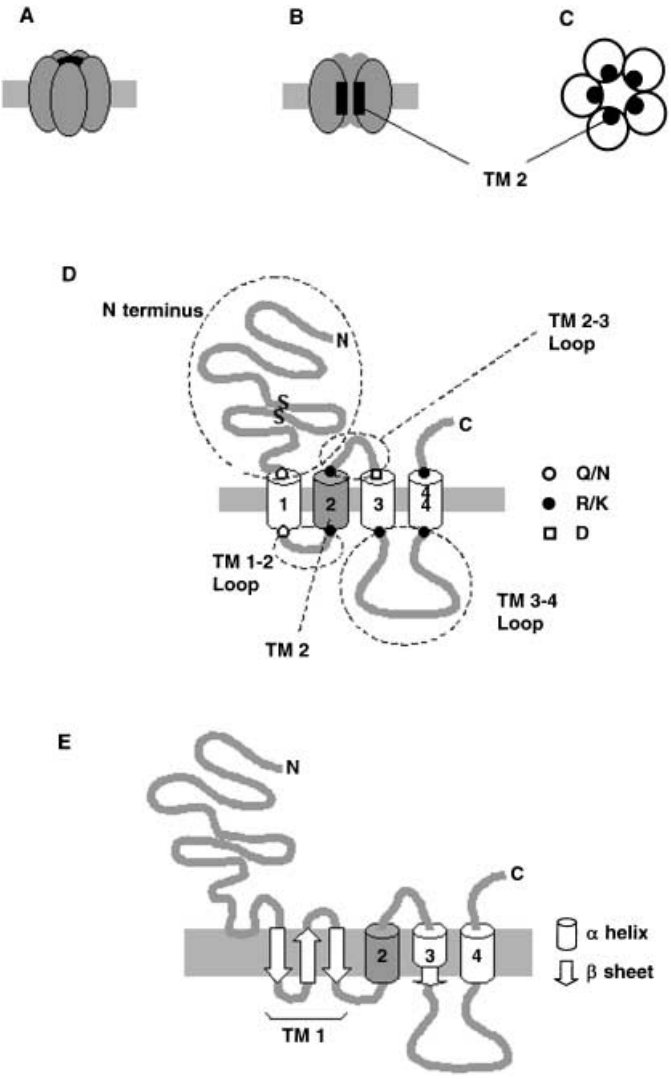

Figure 2. *Structure of a ligand-gated ion channel of the acetylcholine-receptor type. A) Side view of pentameric receptor complex. B) Cut-open view of receptor; the pore-lining transmembrane domain 2 (TM 2) is indicated. C) Top view of receptor; TM 2 is indicated. D) Transmembrane topology and principal structural elements of one receptor subunit; TM 2 is shaded, charged or polar residues (Q/N, R/K, D) that terminate putative transmembrane segments are indicated. E) Alternative transmembrane topology and secondary structure assignments as derived from limited proteolysis of reconstituted receptors.*[156, 157]

Following studies of chemistry in Düsseldorf and London (UK), Hans-Georg Breitinger obtained a PhD in organic chemistry from the Heinrich-Heine-Universität, Düsseldorf, where he investigated modification and immobilisation of polysaccharides in the laboratory of Günter Wulff. He then moved into the field of biophysics as a postdoctoral fellow with George P. Hess at the Department of Biochemistry at Cornell University, Ithaca (NY, USA), where he worked on the synthesis and application of photo-triggered (caged) neurotransmitters and the analysis of ligand-gated ion-channel function by using rapid chemical kinetic techniques in combination with patch-clamp recording methods. In 1997, he took a position as research associate at the Institut für Biochemie of the Friedrich Alexander-Universität Erlangen-Nürnberg, where he is studying the function and regulation of ion-channel receptors.

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Table 1. Functional domains of the glycine receptor.

Function	Structural element	Comments
subunit assembly	N terminus (residues 1 – 100)	8 key interacting residues
ligand binding	N terminus; TM 2 – 3 loop	3-loop model of ligand binding, N-terminal residues 1 – 100 not involved
signal transduction	several protein domains	conformational change after ligand binding leads to movement of TM 2
gating	TM 1 – 2 loop; TM 2 – 3 loop	hinges for TM 2 movement
	(TM 3 – 4 loop, TM residues)	indirect effects on TM 2 motion
channel pore	TM 2	inner wall of ion channel
synaptic anchoring	TM 3 – 4 loop, β subunit	18-residue gephyrin binding motif
functional regulation/ modulation	intracellular TM 3 – 4 loop	intracellular anchoring, phosphorylation, ubiquitination, protein – protein interaction, alternative splicing (additional tissue-specific fine-tuning of glycine-receptor function)

Subunit splicing, receptor composition and assembly

Glycine-receptor α subunits differ subtly with respect to their basic functional and pharmacological properties. Glycine affinity (approximately 10 – 100 μM) and single-channel conductance (multiple conductance states of 50 – 100 pS) are similar for all homomeric glycine receptors.[19, 35–38] Tissue-specific fine-tuning of ion-channel function is accomplished by various mechanisms: 1) alternative splicing, 2) subunit composition and 3) posttranslational modifications.

Alternative splicing within the intracellular loop between transmembrane domains 3 and 4 (TM 3 – 4; see Figure 2) has been observed with α1 and α3 subunits. Glycine receptor α1 splice variants, differing by an 8-residue insert in the intracellular TM 3 – 4 loop (Figure 4 A), had similar functional properties.[39] In contrast, α3 splice variants, characterised by the absence or presence of a 15-residue insert in the similar position (Figure 4 A) show differences in receptor desensitisation.[38] Finally, alternative splicing of the rat α2 subunit results in the exchange of two N-terminal residues (V85I/T86A); this exchange is not associated with functional differences.[40] Aberrant splicing due to insertion of a LINE-1 element leads to $>90\,\%$ loss of functional β subunits. This defect underlies the murine phenotype *spastic*.[41, 42]

Subunit composition: Adult glycine-receptor isoforms are generally assumed to be $\alpha_3\beta_2$ heteromers, where eight key residues close to the N terminus are critical for receptor assembly and form the intersubunit contact surface.[43] While homomeric α_5 receptor complexes, as well as other mixed α/β heteromers, are functional, pure β_5 complexes do not form functional ion channels.[41, 42] Absence or presence of the β subunit in glycine receptors gives rise to subtle changes in single-channel properties, with lower conductance levels being predominantly populated in α1/β heteromeric receptors as compared to $\alpha 1_5$ homomers.[37] However, β subunits exert only a minor influence on macroscopic parameters of glycine-receptor function, such as whole-cell current amplitudes or receptor desensitisation. Presence of the β subunit reduces picrotoxin sensitivity of recombinant glycine receptors.[44] This picrotoxin insensitivity has been found to depend on a single residue within TM 2, namely α1(T258), which corresponds to β(F282). A ring of five threonines (one per subunit) at this position of the ion pore is required for picrotoxin sensitivity. Replacement of one or more of these threonines by phenylalanine through mutagenesis or coexpression of β subunits largely abolishes picrotoxin inhibition.[45]

Synaptic anchoring: Microscopic and electrophysiological studies have demonstrated the interplay of activation and synaptic anchoring of glycine-receptor complexes.[46–50] Glycine-receptor diffusion, as monitored by single-particle tracking, is dramatically reduced but not completely abolished in the presence of the tubulin-binding protein gephyrin. Thus, anchoring through gephyrin locks glycine receptors at the synapse, thereby allowing only a small proportion of receptors to escape from the synaptic complex. This mechanism effectively regulates the receptor turnover rate by extending the amount of time a receptor remains at synaptic locations in the cell membrane.[49–51] Gephyrin mediates synaptic anchoring by specifically binding to glycine-receptor β subunits through a core binding motif of 18 amino acid residues within the intracellular TM 3 – 4 loop of the β subunit (Figure 4 A).[52, 53] Receptor activation is required for initiation of the anchoring process: in the presence of strychnine, that is, under conditions of reduced glycinergic activity, synapse formation and maintenance is impaired.[54, 55]

Posttranslational modification: Glycine-receptor subunits carry consensus motifs for phosphorylation,[56–66] glycosylation[43, 67] and ubiquitination.[68] Receptor function is susceptible to both posttranslational protein modification and the effects of modulatory ions or pharmaceutical compounds.[22, 30]

Receptor degradation: A constant number of neurotransmitter receptors at synaptic sites is the result of a fine balance between the rates of receptor synthesis and degradation. One general pathway of protein degradation, ubiquitin-mediated internalisation and decomposition, has recently been shown to apply to glycine receptors.[68] The intracellular TM 3 – 4 domain of the α1 subunit contains ten lysine residues, one or several of which may constitute a ubiquitination site.[68]

Glycine-receptor biosynthesis and degradation have received close attention only much more recently than the investigation of functional defects. Loss of glycinergic transmission may be a result of both reduced metabolic stability and impairment of glycine-receptor function. In the mouse mutant *spastic*, a $\approx 90\,\%$

reduction of functional glycine receptors due to missing β subunits was found to underlie the observed phenotype.[41, 42, 69] Membrane insertion of subunits was abolished in the case of the human hyperekplexia mutation S231R.[70] Similarly, symptoms of hyperekplexia were observed only in patients showing compound heterozygosity for the two mutant alleles R252H and R392H, which are also supposed to interfere with membrane expression of receptor subunits.[71]

Glycine Receptor Function

A minimum mechanism for ligand-gated ion channels

The activation of neurotransmitter receptors is a complex series of events. For analysis, the simplest possible models—that is, those that use the least number of assumptions and fitted constants—should be used to derive the pertinent kinetic constants. Such a minimum mechanism of receptor activation which represents the elementary steps of ligand-gated ion channel function was originally developed for the nicotinic acetylcholine receptor[72] and was subsequently shown to be valid for other ligand-gated ion channels.[73] One or more molecules of the activating ligand (that is, the neurotransmitter) bind to the receptor and thereby induce a conformational transition which leads to the opening of the intrinsic ion channel. The fully liganded receptor oscillates between the open and closed state, a process referred to as gating. In the continued presence of agonist, the receptor can desensitise, that is, convert into a liganded, closed state (Figure 3). Desensitisation on a timescale of minutes was originally described by Katz and Thesleff[72] for the nicotinic acetylcholine receptor. Faster modes of desensitisation (ms – s timescales) were later identified for ligand-gated ion-channel receptors.[74–77] Note that the kinetic scheme depicted in Figure 3) assumes the binding of two ligand molecules prior to channel opening. This simplified model was chosen for clarity, since it considers the key steps of receptor activation (ligand-binding—channel opening and closing—desensitisation), while the real activation mechanism may be more complicated. Two different glycine-receptor populations were described on mouse cortical neurons, one requiring the binding of two and the other of three ligand molecules prior to channel opening.[78] In fact, several studies suggest a three-ligand model of receptor activation.[79, 80] In homopentameric receptor complexes, the binding of up to five ligand molecules to the receptor complex is actually feasible; this has recently been considered in careful mechanistic investigations.[81]

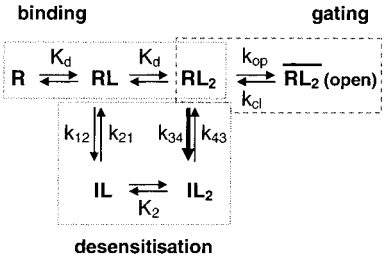

Figure 3. *Minimum mechanism of ligand-gated receptor function. R indicates the active receptor, I is the inactive, desensitised receptor form. L is the activating ligand (here: glycine), with subscripts to indicate the number of ligand molecules bound.* K_d *is the dissociation constant of ligand from the receptor,* k_{op} *and* k_{cl} *are the rates for channel opening and closing, respectively. The transition to and from the desensitised state is described by the rate constants* k_{12}, k_{21}, k_{34} *and* k_{43}. *Desensitisation is usually sufficiently described by* k_{34}, *that is, from the doubly liganded state,* RL_2. *Note that desensitisation from states other than RL, and* RL_2 *is omitted for simplicity, but cannot be excluded; likewise, additional open or closed states may exist but are not considered in this minimum model. Binding of two and three ligands prior to channel activation has been reported; in fact, states up to* RL_5 *could be expected for homopentameric receptors. Here, the simplest mechanism that reflects all the essential steps of glycine receptor activation is shown.*

Electrophysiological techniques[82] generally analyse the function of ion-channel receptors, which by nature is a transient phenomenon. Single-channel measurements give information on channel conductance and the amount of time the receptor spends in the open or closed state, while whole-cell recordings reflect the entire ensemble of receptors on an individual cell and describe macroscopic channel properties.[83] Dose-response analysis, as routinely used in many biological situations, cannot be directly applied to neurotransmitter receptors. The observed half-maximal activation (EC_{50}) comprises the two elementary steps of ligand binding and channel gating which cannot be separated.[84] Thus, concentration-dependence of agonist-evoked currents gives an EC_{50} value that serves as a fingerprint of receptor activity but does not necessarily describe the true ligand affinity of the receptor binding site(s). The minimum model (Figure 3) accounts for the subsequent events of receptor activation—reality may be much more complicated, but the essential steps of channel function are reflected in this simple mechanism.[72, 73, 83]

Generally, ligand-gated ion-channel opening rates are of the order of $10^3 - 10^5\ s^{-1}$, and the determination of these rates requires advanced single-channel analysis and/or sub-ms kinetic techniques.[83] However, the gating equilibrium constant $\Phi = k_{cl}/k_{op}$,[73] or its inverse $E = k_{op}/k_{cl}$,[84] can be determined from whole-cell recordings by using an appropriate kinetic model (Figure 3).[85] Receptor desensitisation usually occurs on a timescale of seconds. If the recording setup provides an appropriate kinetic resolution, the individual steps of channel activation and desensitisation can be spread out on the time axis and analysed independently from each other.[73]

Ligand binding

Kinetics: Similar rates of agonist binding and channel opening have been observed for acetylcholine receptors.[86] For homomeric α1 glycine receptors, ligand association and dissociation rates of $10^7\ M^{-1}\ s^{-1}$ and $1900\ s^{-1}$, respectively, were determined by a laser-pulse photolysis technique for rapid glycine delivery from a caged precursor.[79] The rate constants for the glycine receptor were $2500\ s^{-1}$ for k_{op} and $40\ s^{-1}$ for k_{cl}.[79] Thus, ligand binding to the glycine receptor is in a rapid preequilibrium compared to the subsequent steps of receptor activation.

Ligand affinity: The determination of ligand affinities requires the choice of appropriate methodology, depending on the process under study. Radioligand binding experiments, where thermodynamic equilibrium between all possible receptor states

(that is, R, RL, RL_2, $RL_{2(open)}$, IL and IL_2; see Figure 3) has been reached, are useful for the investigation of clinically relevant compounds that are present in tissues or body fluids for an extended time. In electrophysiological recordings, however, where a concentration jump of ligand is applied,[83] one measures functional data of the active receptor state, R (Figure 3). This situation pertains to synaptic events and is, therefore, suited for the analysis of activating ligands. Data from both methods can be compared if the active and desensitised receptor states are of similar thermodynamic stability and therefore easily interconverted.[84] This situation, although not common for ligand-gated ion-channel receptors, is in fact observed for wildtype glycine receptors.[22, 30]

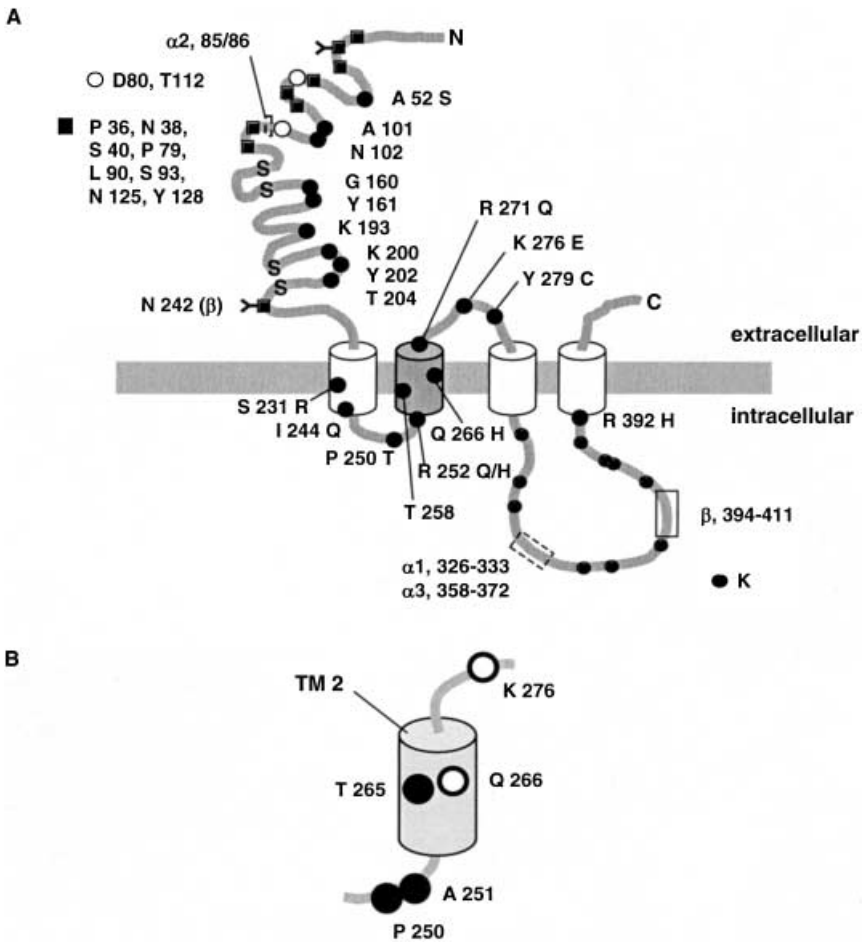

Figure 4. *A) Glycine-receptor subunit; individual residues and domains that affect ion channel function are indicated. Extracellular: ■: N-terminal residues forming the intersubunit contact surface; ○: residues involved in Zn^{2+} modulation; dashed-line box: alternatively spliced region; Y: potential glycosylation sites; ●: functionally relevant single-point mutations. Intracellular: black oval: potential ubiquitination sites (lysine); dashed-line box: alternatively spliced region; □: gephyrin binding motif (β subunit). B) Transmembrane domain 2. ●: residues mutated or deleted for inversion of charge selectivity; ○: residues affecting channel gating.*

Ligand binding domains: The extracellular N terminus of glycine receptors comprises approximately 50 % of the total protein and contains regions that are critical for ligand binding and the subsequent steps of channel gating as well as for subunit assembly. Sequence alignments and molecular modelling suggested SH2- and SH3-like domains within the ligand binding domain of glycine receptors,[67] a fact showing that common motifs of protein structure may be relevant for ligand-gated receptors. Exchange of larger sections of the protein, combined with photocrosslinking and biochemical studies indicate that the N-terminal residues 1 – 100 are not involved in ligand binding.[6] It appears that the region immediately preceding TM 1 is crucial for ligand binding, while domains responsible for subunit association are located close to the N terminus. In fact, six of the eight amino acids forming the intersubunit contact surface reside within the first 100 residues of the receptor protein.[43]

A rigid assignment of functional building blocks may not be sufficient to describe the functional architecture of ligand-gated ion channels, since individual protein regions may overlap or interact. Residues of the N terminus which affect apparent ligand affinity need not only reside within the domains described before (Figure 4 and Table 2), particularly since the consequence of ligand binding is a conformational change within the protein. Thus, residues that directly interact with the ligand and residues involved in the subsequent folding process will both be sensitive to exchange or structural modification. Residues that affect apparent ligand affinity in this way were identified through analysis of spontaneous mutations or amino acid exchange by site-directed mutagenesis. The point mutation α1(A52S) in the mouse mutant *spasmodic* is associated with reduced glycine affinity.[87, 88] Three residues near the first transmembrane domain of the human glycine-receptor α1 subunit, K200, Y202 and T204, plus residues G160 and Y161, were identified as part of the ligand binding pocket.[89, 90] Other residues involved in ligand binding include A101, N102 and K193.[91] The simultaneous interaction of several ligands with the glycine receptor gave evidence for similar, but not identical, ligand binding pockets within the same region of the protein. Equilibrium radioligand binding studies, as well as whole-cell current measurements, suggest that glycine and strychnine bind to partially overlapping sites on the receptor.[92]

Other glycine receptor agonists: An important physiological means of regulating ion-channel function at the ligand binding stage is the choice of activating ligand. Taurine and β-alanine have been proposed as endogenous glycine-receptor agonists in early development.[23, 93, 94] Binding and receptor activation by taurine and β-alanine have been intensively studied on wildtype receptors, where their affinities and efficacies are lower than that of glycine.[22] In some hyperekplexia mutations, however, both

Table 2. *Functionally relevant residues and domains of the inhibitory glycine receptor.*

Residue(s)[a]	Species	Phenotype	Functional effect	Reference(s)
N38	human		glycosylation	[43]
A52S	mouse	*spasmodic*, *spd*	reduced glycine affinity	[87, 88]
P36, N38, S40, P79, L90, S93, N125, Y128	human		contact surface for subunit assembly	[43]
A52, A101, N102, G160, Y161, K193, K200, Y202, T204			involved in glycine and strychnine binding	[87 – 91]
D80, T112	human		involved in Zn^{2+} modulation	[141]
α2(V85/T86)	rat		alternative splicing (→I85/A86)	[40]
βG229D/exon 5 loss	human	hyperekplexia	ligand binding	[163]
β(N242)	human		putative glycosylation site	
I244Q	human	hyperekplexia		[164]
P250T	human	hyperekplexia	low conductance, rapid desensitisation, reduced ligand affinity	[107, 116]
R252Q/H	human	hyperekplexia	membrane insertion	[71]
α1(T258)/β(F282)	human		picrotoxin sensitivity	[45]
V260M	human	hyperekplexia	functional effects not reported	[165]
Q266H	human	hyperekplexia	gating (single-channel open time)	[118]
R271L/Q	human	hyperekplexia	reduced apparent ligand affinity, low conductance levels occupied, altered sensitivity to tropeines, taurine/β-alanine→antagonists	[95, 146, 166, 167]
K276E	human	hyperekplexia	gating (single-channel open time)	[80, 117]
Y279C	human	hyperekplexia	(non-penetrant)	[168, 169]
α1(326 – 333)	rat		alternatively spliced segment; no functional effect described	[39]
α3(358 – 372)	human		alternatively spliced segment; affects desensitisation, gating	[38]
β(394 – 411)	human		gephyrin binding	[52, 53]
K in TM 3 – 4 loop (10×)	human		putative ubiquitination, internalisation, degradation	[68, 170]
R252H/R392H	human	hyperekplexia	membrane insertion, symptoms only in compound heterozygotes	[71]
Δα1[b]	human	hyperekplexia	total loss of α1 subunit	[171]
Δα1[b]	mouse	*oscillator, spd^{ot}*	total loss of adult receptor form, lethal at P14 – 21	[172, 173]
Δα1[b]	bovine	myoclonus	total loss of α1 subunit	[174]
Δβ[b]	mouse	*spastic, spa*	< 10% of normal β-subunit levels, < 10% functional adult receptors	[41, 42, 175]

[a] Numbering refers to glycine-receptor α1 subunit residues, unless otherwise indicated. [b] Total loss of subunit only observed in homozygous individuals.

compounds still bind to the receptor but do not open the ion channel, thus effectively acting as competitive inhibitors.[95]

The receptor ion pore

Structural data from electron microscopy[10, 12, 96] and NMR spectroscopy,[14, 16, 97] as well as biochemical investigations[2, 8, 98–102] and studies of the accessibility of substituted cysteine residues,[103] have indicated that TM 2 of ligand-gated ion channels is an α helix, which forms the inner lining of the receptor ion pore (Figure 2B, C). Ion selectivity is effected through sets of charged residues near the mouth of channel.[2] Cation-conducting acetylcholine receptors can be made anion selective by exchange of only three residues within or close to the ion-channel pore: insertion of P250, which is absent from cation channels, but highly conserved in anion channels, and the exchanges E251A and V265T (all positions numbered as in the glycine receptor α1 subunit, see Figure 4B).[104] A similar conversion of cationic into anionic charge selectivity after the corresponding exchanges (Δ250P, E251A, V265T) has been reported for the 5-HT_3 serotonin receptor.[105] For the glycine receptor, the reverse mutations (P250Δ, A251E, T265V) have been performed, which resulted in cation selectivity.[106] The critical effect of the chemical structure of position α1(250) in the glycine receptor has been further demonstrated in a mutagenesis study, where isotropic forces, charge and hydrophobicity were identified as critical determinants of receptor function.[107] Thus, P250 may serve a dual role by positioning neighbouring residues as well as being a critical part of the protein surface near the inner mouth of the ion channel.

Even if the principal ion selectivity of an ion channel can be inverted by the replacement of only one charge, ion permeation through the ion pore is a complex process. Two anion binding sites within the glycine-receptor channel have been proposed from detailed electrophysiological studies,[35–37] and the complete pathway of ion permeation across the membrane is not yet

understood. In case of the acetylcholine receptor, energy-minimised models of high quality are available for the channel pore and its neighbouring regions.[8, 9, 108, 109] However, the X-ray structures of the $K_{cs}A$[110] and ClC_2[111] channels, as well as electron microscopic[10, 96] and NMR spectroscopic[14, 16, 97] studies demonstrate that the ion-channel pore and its selectivity filter are highly defined, complex structures,[2] which may not be accessible through simulation approaches alone.

Channel gating

The currently accepted model for the architecture of ligand-gated ion channels[2, 11] has several residues within TM 2 forming the narrowest constriction of the receptor ion pore. By using a kinked helix model originally derived for TM 2 of the acetylcholine receptor,[112] Monte-Carlo minimization was used to predict similar surface properties and channel dimensions[113] for the glycine receptor as deduced from the permeation of organic anions.[114] Receptor gating, kinetically defined as the open–close transition of the ion channel (Figure 3) has been interpreted as a movement of TM 2.[10] The short loops flanking TM 2 have been proposed to act as hinges for this conformational transition.[107, 115, 116] The effect of the hyperekplexia mutation P250T, located at the intracellular 'hinge' leads to a lower channel conductance,[116] but does not affect gating, as evident from unaltered channel opening and closing rates (Breitinger et al., *EMBO J.*, submitted). In contrast, the hyperekplexia mutation K276E,[117] situated within the extracellular TM 2–3 loop (Figure 4B), has no effect on channel conductance, but almost exclusively affects gating.[80] The hyperekplexia mutation Q266H, although located next to the 'charge relevant' T265 (Figure 4B), does affect ion-channel gating by altering channel-open times,[118] yet no apparent effect on ionic selectivity has been reported. A 'reluctant' gating mode, characterised by low channel-open probability, has been identified for glycine receptors on neurons from zebrafish larvae.[119]

Receptor desensitisation

The physiological role of receptor desensitisation is not yet fully understood, since neurotransmitter removal by uptake or enzymatic breakdown usually terminates signal transmission. Even rapid current decay may only have a minor effect on the physiological signal of ligand-gated receptors.[120] However, the time course of current desensitisation is a fingerprint of individual receptor species and contains information about refractory states of the receptor.

Glycine-receptor desensitisation in the hyperekplexia mutant α1(P250T) is dramatically increased.[107, 116] Replacing P250 with various other amino acids showed that an increased hydrophobic surface in this position favoured rapid desensitisation and low apparent ligand affinity, while small, or charged residues gave rise to high-affinity, nondesensitising receptors.[107] Surprisingly, if P250 is replaced by glycine, alanine, or serine, wildtype receptor properties will be retained. The determinants in position 250 for glycine-receptor function are volume and charge, rather than a particular conformation of the peptide chain.[107] Desensitisation may be the consequence of an intramolecular transition to a closed state, such as a movement of TM 2,[10] or of an interaction between intracellular domains of the receptor complex. In fact, glycine receptor α3 splice variants that differed by the absence or presence of a 15-residue insert within the large TM 3–4 loop did differ in desensitisation properties.[38] Removal of hydroxylated residues within the alternatively spliced insert lead to recombinant receptors that desensitised faster than their wildtype counterparts, a fact indicating that desensitisation is also sensitive to alterations of the protein surface within the intracellular TM 3–4 region of the protein.[121]

Receptor modulation by external factors

Cellular environment: Glycine-receptor function is sensitive to intracellular factors, such as modulatory proteins, ions or phosphorylation.[22] Functional differences between receptors from neurons and recombinant systems have been observed with acetylcholine[122] and glycine receptors.[123] Expression of glycine-receptor α1(R271Q) subunits in HEK-293 cells results in higher ligand affinity of mutant glycine receptors than expression in rat dorsal horn neurons, a fact attributed to differences in posttranslational modification in the host cells.[123] Two widely used expression systems, *Xenopus* oocytes and HEK-293 cells, both supply a background of functional receptor modulation that is difficult to control and may vary from cell to cell. In the case of homomeric recombinant α1 or α2 glycine receptors from zebrafish (*Danio rerio*), more than tenfold differences were measured in EC_{50} values for the same receptor from one oocyte to the next and also from one HEK-293 cell to the next.[124] These differences could be attributed to alterations in channel gating, but the underlying modification is not known.[124] Modulation of glycine-receptor function by a Ca^{2+} binding factor was proposed from a detailed study on rat neurons and recombinant receptors.[66] Receptor density has been shown to affect receptor properties in oocytes,[125, 126] yet it is not known whether this is due to interactions between neighbouring receptors or to other protein modifications.

Modulatory compounds: Modulation of receptor function by alcohols and anaesthetics has been intensively studied in recent years,[127–132] leading to the identification of individual residues and modifications that are critical for alcohol potentiation of glycine receptors (Figure 4A and Table 2).[64, 128, 133, 134] Glycine-receptor modulation by Zn^{2+} has been studied in great detail, and several residues that are critical for zinc binding have been identified (D80, T112; Figure 4A and Table 2).[28, 135–141]

Glycine receptors as secondary targets: Several pharmaceutically relevant compounds that have other primary targets also interact with glycine receptors. Such cross-reactivity is frequently observed with $GABA_A$ receptor ligands.[22, 142, 143] Calcium-channel antagonists, such as dihydropyridines and verapamil, were found to be direct blockers of the glycine-receptor channel.[144] Several agonists and antagonists of the 5-HT_3 receptor were able to displace [^{3}H]strychnine from glycine receptors with micromolar affinity, that is, an affinity similar to glycine itself.[145]

Tropisetron and atropine were found to be inhibitors,[146] while a number of 5-HT_3 receptor antagonists potentiate glycine receptor currents.[147] Potentiation of α1(5) glycine receptor currents by ICS-205,930 is more pronounced than with α2 homomers.[148] Current potentiation for both subunits is sensitive to the presence or absence of β subunits, a fact indicating a participation of the β subunits in the allosteric potentiation site.[148] Substance P shows both, indirect[149] as well as direct potentiation of glycine-receptor responses.[150] Forskolin, in addition to its activation of protein kinase A (PKA), appears to bind directly to $GABA_A$ and glycine receptors in carp amacrine cells where it accelerates current desensitisation.[151] Direct binding of the neuroprotective drug riluzole induces fast desensitisation of glycine receptors.[152] The anthelmintic ivermectin has recently been identified as a novel glycine-receptor agonist which binds to the receptor and induces channel opening from a site that is different from the glycine and strychnine binding site.[153] The absence of cross-desensitisation suggests an altogether different mechanism of glycine-receptor activation by ivermectine.[153] While such compounds are of great interest for the delineation of the mechanisms of glycine-receptor function, their multiple physiological effects render a therapeutical application rather unlikely.

Glycine-receptor structure—what do we really know?

N terminus: The X-ray structure of an acetylcholine binding protein,[154] which superimposes well with the electron microscopy[10–12] and NMR spectroscopy[15] structures of the N-terminal domain of acetylcholine-receptor subunits, supports the predicted organisation of the receptor. Pentameric coassembly of isolated extracellular domains of acetylcholine-receptor subunits was demonstrated by electron microscopy.[96] However, when assembly of extracellular domains plus TM 1, that is, residues 165 – 291 of the glycine-receptor α1 or $GABA_A$-receptor subunits, was studied by using electron microscopy, pentameric complexes were only found for the $GABA_A$R domain, while the glycine-receptor N termini formed trimers.[155] One explanation would be that too many residues of the assembly cassette[43] of the glycine receptor were missing in these fragments. On a contrasting note, limited proteolysis of glycine receptors previously reconstituted into artificial lipid membranes led to the proposition of a slightly modified transmembrane topology of the glycine-receptor N terminus (Figure 2 E).[156, 157] Note, however, that all functional domains of the receptor protein are retained in this alternative model.

Receptor ion pore: A 'minimum' chloride channel, consisting only of the second transmembrane domain with four attached lysine residues to ensure proper membrane insertion was shown to form functional anion channels,[158–162] probably the clearest independent evidence that TM 2 of ligand-gated ion channels can form an effective channel pore.

At present there is no information regarding the three-dimensional structure of the large intracellular TM 3 – 4 domain and the C-terminal part of the receptor. It is obvious that interactions responsible for cellular anchoring and posttranslational modification must be directed to these domains. Measurements involving intracellular protein regions are often complicated by their increased susceptibility to cellular and environmental factors (see above).

To date, the inhibitory glycine receptor has been established as a model for hereditary channelopathies. The biochemical pathways from gene structure to protein biosynthesis and to the mechanism of function and regulation of the mature receptor complex are understood in their basic form. There is promise for both deeper understanding of the functional complexity of ion channels and improved therapeutic perspectives of channel-associated diseases.

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