Glycine receptors

Overview: The inhibitory glycine receptor [nomenclature as agreed by the NC-IUPHAR Subcommittee on glycine receptors (Lynch, 2009a)] is a member of the Cys-loop superfamily of ligand-gated ion channels that includes the $GABA_A$, nicotinic acetylcholine and $5\text{-}HT_3$ receptors (Lynch, 2009b). Structurally and functionally, the glycine receptor is most closely related to the $GABA_A$ receptor. The receptor is expressed either as a homo-pentamer of α subunits, or a complex now thought to harbour 2α and 3β subunits (Grudzinska *et al.*, 2005; Betz and Laube, 2006), that contain an intrinsic Cl^- channel. Four differentially expressed isoforms of the α subunit (α1–α4) and one variant of the β subunit (β1, ENSG00000109738) have been identified by genomic and cDNA cloning. Further diversity originates from alternative splicing of the primary gene transcripts for α1 ($\alpha1^{INS}$ and $\alpha1^{del}$), α2 (α2A and α2B), α3 (α3S and α3L) and β (βΔ7) subunits and by mRNA editing of the α3 subunit (Meier *et al.*, 2005; Oertel *et al.*, 2007). Both α2 splicing and α3 mRNA editing can produce subunits (i.e. α2B and α3P185L) with enhanced agonist sensitivity. Predominantly, the mature form of the receptor contains α1 (or α3) and β subunits while the immature form is mostly composed of only α2 subunits. RNA transcripts encoding the α4 subunit have not been detected in adult humans. The N-terminal domain of the α subunit contains both the agonist and strychnine binding sites that consist of several discontinuous regions of amino acids. Inclusion of the β subunit in the pentameric glycine receptor contributes to agonist binding, reduces single channel conductance and alters pharmacology. The β subunit also anchors the receptor, via an amphipathic sequence within the large intracellular loop region, to gephyrin. The latter is a cytoskeletal attachment protein that binds to a number of subsynaptic proteins involved in cytoskeletal structure and thus clusters and anchors hetero-oligomeric receptors to the synapse (see Moss and Smart, 2001; Kirsch, 2006; Kneussel and Loebrich, 2007). G-protein βγ subunits enhance the open state probability of native and recombinant glycine receptors by association with domains within the large intracellular loop (Yevenes *et al.*, 2003; 2006). Intracellular chloride concentration modulates the kinetics of native and recombinant glycine receptors (Pitt *et al.*, 2008). Intracellular Ca^{2+} appears to increase native and recombinant glycine receptor affinity, prolonging channel open events, by a mechanism that does not involve phosphorylation (Fucile *et al.*, 2000).

Nomenclature	α1	α2	α3
Ensembl ID	ENSG00000145888	ENSG00000101958	ENSG00000145451
Selective agonists (potency order)	Glycine > β-alanine > taurine	Glycine > β-alanine > taurine	Glycine > β-alanine > taurine
Selective antagonists and modulators with subunit selectivity	Strychnine, PMBA, bilobalide (IC_{50} = 20 μM; +β = 204 μM), pregnenolone sulphate (K_i = 1.9 μM; +β = 2.7 μM), tropisetron (K_i = 84 μM), colchicine (IC_{50} = 324 μM), nifedepine (IC_{50} = 3.3 μM; +β = 1.2 μM)	Strychnine, PMBA, bilobalide (8 μM +β = 50 μM), pregnenolone sulphate (K_i = 5.5 μM; +β = 10.1 μM), tropisetron (K_i = 13 μM; +β = 5.4 μM), colchicine (IC_{50} = 64 μM), DCKA (IC_{50} = 188 μM)	Strychnine, nifedepine (IC_{50} = 29.2 μM; +β = 11.4 μM)
Selective potentiators	HU210	–	–
Endogenous potentiators (EC_{50})	Zn^{2+} (37 nM) (not affected by β)	Zn^{2+} (540 nM) (not affected by β)	
Endogenous inhibitors (IC_{50})	Zn^{2+} (15 μM; +β = 13 μM), Cu^{2+} (4–15 μM) (not affected by β), H^+	Zn^{2+} (360 μM; +β = 180 μM), Cu^{2+} (17 μM)	Zn^{2+} (150 μM), Cu^{2+} (9 μM),
Channel blockers (IC_{50})	Cyanotriphenylborate (1.3 μM; +β = 2.8 μM), picrotoxin (6.3 μM; +β = 219 μM), picrotoxinin (5.1 μM; +β = 27 μM), picrotin (5.2 μM; +β = 27 μM), ginkgolide B (0.6–8.0 μM; +β = 0.18–2.5 μM)	Cyanotriphenylborate (>>20 μM; +β = 7.5 μM), picrotoxin (2.3 μM; +β = 29.7 μM), picrotoxinin (0.41 μM), picrotin (13.1 μM), ginkgolide B (3.7–11.4 μM; +β = 0.14–0.8 μM)	Picrotoxin (+β weakens block), picrotoxinin (0.43 μM; +β = 8.9 μM), picrotin (6.0 μM; +β = 24 μM), ginkgolide B (1.8 μM; +β = 0.55 μM)
Probes	[^{3}H]strychnine	[^{3}H]strychnine	[^{3}H]strychnine
Functional characteristics	γ = 86 pS (main state) (+β = 44 pS)	γ = 111 pS (main state) (+β = 54 pS)	γ = 105 pS (main state) (+β = 48)

Data in the table refer to homo-oligomeric assemblies of the α subunit; significant changes introduced by co-expression of the β1 subunit are indicated in parenthesis. Not all glycine receptor ligands are listed within the table, but some that may be useful in distinguishing between glycine receptor isoforms are indicated [see Lynch (2009a) for a more comprehensive listing]. Pregnenolone sulphate, tropisetron and colchicine, for example, although not selective antagonists of glycine receptors, are included for this purpose. Strychnine is a potent and selective competitive glycine receptor antagonist with affinities in the range 5–15 nM. RU5135 demonstrates comparable potency, but additionally blocks $GABA_A$ receptors. There are conflicting reports concerning the ability of cannabinoids to inhibit (Lozavaya *et al.*, 2005), or potentiate and at high concentrations activate (Hejazi *et al.*, 2006; Yang *et al.*, 2008; Ahrens *et al.*, 2009; Demir *et al.*, 2009) glycine receptors. Nonetheless, cannabinoid analogues may hold promise in distinguishing between glycine receptor subtypes (Yang *et al.*, 2008). Several analogues of muscimol and piperidine act as agonists and antagonists of both glycine and $GABA_A$ receptors. Picrotoxin acts as an allosteric inhibitor that appears to bind within the pore and shows strong selectivity towards homomeric receptors. While its components, picrotoxinin and picrotin, have equal potencies at α1 receptors, their potencies at α2 and α3 receptors differ modestly and may allow some distinction between different receptor types (Yang *et al.*, 2007). In addition to the compounds listed in the table, numerous agents act as allosteric regulators of glycine receptors [comprehensively reviewed by Laube *et al.* (2002), Lynch (2004) and Webb and Lynch (2007)]. Zn^{2+} acts through distinct binding sites of high- and low-affinity to allosterically enhance channel function at low (<10 μM) concentrations and inhibits responses at higher concentrations in a subunit-selective manner (Miller *et al.*, 2005). The effect of Zn^{2+} is somewhat mimicked by Ni^{2+}. Endogenous Zn^{2+} is essential for normal glycinergic neurotransmission mediated by α1 subunit-containing receptors (Hirzel *et al.*, 2006). Elevation of intracellular Ca^{2+} produces fast potentiation of glycine receptor-mediated responses. Dideoxyforskolin (4 μM) and tamoxifen (0.2–5 μM) both potentiate responses to low glycine concentrations (15 μM), but act as inhibitors at higher glycine concentrations (100 μM). Additional modulatory agents that enhance glycine receptor function include inhalational, and several intravenous general anaesthetics (e.g. minaxolone, propofol and pentobarbitone) and certain neurosteroids. Ethanol and higher order *n*-alcohols also act allosterically to enhance glycine receptor function. Solvents inhaled as drugs

of abuse (e.g. toluene, 1-1-1-trichloroethane) may act at sites that overlap with those recognizing alcohols and volatile anaesthetics to produce potentiation of glycine receptor function. The function of glycine receptors formed as homomeric complexes of α1 or α2 subunits, or hetero-oligomers of α1/β or α2/β subunits, is differentially affected by the 5-HT_3 receptor antagonist tropisetron (ICS 205-930) that may evoke potentiation (which may occur within the femtomolar range at the homomeric glycine α1 receptor), or inhibition, depending upon the subunit composition of the receptor and the concentrations of the modulator and glycine employed. Potentiation and inhibition by tropeines involves different binding modes (Maksay *et al*., 2009). Additional tropeines, including atropine, modulate glycine receptor activity.

Abbreviations: DCKA, dichlorokynurenic acid; **HU210**, 3-(1, 1-dimethylheptyl)-11-hydroxy-Delta8tetrahydrocannabinol; **PMBA**, 3-[2′-phosphonomethyl[1,1′-biphenyl]-3-yl]alanine; **RU5135**, 3α-hydroxy-16-imino-5β-17-azaandrostan-11-one

Further Reading

Betz H, Laube B (2006). Glycine receptors: recent insights into their structural organization and functional diversity. *J Neurochem* **97**: 1600–1610.
Bowery NG, Smart TG (2006). GABA and glycine as neurotransmitters: a brief history. *Br J Pharmacol* **47** Suppl 1: S109–19.
Breitinger, H-G, Becker C-M (2003). The inhibitory glycine receptor – simple views of a complicated channel. *ChemBioChem* **3**: 1042–1052.
Cascio M (2004). Structure and function of the glycine receptor and related nicotinicoid receptors. *J Biol Chem* **279**: 19383–19386.
Colquhoun D, Sivilotti LG (2004). Function and structure in glycine receptors and some of their relatives. *Trends Neurosci* **27**: 337–344.
Harvey RJ, Topf M, Harvey K, Rees MI (2008). The genetics of hyperekplexia: more than startle! *Trends Genet* **24**: 439–447.
Kirsch J (2006). Glycinergic transmission. *Cell Tissue Res* **326**: 535–540.
Kneussel M, Loebrich S (2007). Trafficking and synaptic anchoring of ionotropic inhibitory neurotransmitter receptors. *Biol Cell* **99**: 297–309.
Laube B, Maksay G, Schemm R, Betz H (2002). Modulation of glycine receptor function: a novel approach for therapeutic intervention at inhibitory synapses. *Trends Pharmacol Sci* **23**: 519–527.
Legendre P (2001). The glycinergic inhibitory synapse *Cell Mol Life Sci* **58**: 760–793.
Lewis T M, Schofield PR (1999). Structure-function relationships for the human glycine receptor: insights from hyperekplexia mutations. *Ann NY Acad Sci* **868**: 681–684.
Lobo IA, Harris RA (2005). Sites of alcohol and volatile anesthetic action on glycine receptors. *Int Rev Neurobiol* **65**: 53–87.
Lynch JW (2004). Molecular structure and function of the glycine receptor chloride channel. *Physiol Rev* **84**: 1051–1095.
Lynch JW (2009a). Glycine receptors, introductory chapter. IUPHAR database (IUPHAR-DB), http://www.iuphar-db.org/IC/FamilyIntroductionForward?familyId=9
Lynch JW (2009b) Native glycine receptor subtypes and their physiological roles. *Neuropharmacology* **56**: 303–309.
Lynch JW, Callister RJ (2006). Glycine receptors: a new therapeutic target in pain pathways. *Curr Opin Investig Drugs* **7**: 48–53.
Moss SJ, Smart TG (2001). Constructing inhibitory synapses *Nature Neurosci Rev* **2**: 240–250.
Webb TI, Lynch JW (2007). Molecular pharmacology of the glycine receptor chloride channel. *Curr Pharm Des* **13**: 2350–2367.

References

Ahrens J *et al*. (2009). *Pharmacology* **83**: 217–222.
Demir R *et al*. (2009). *Pharmacology* **83**: 270–274.
Fucile S *et al*. (2000). *Neuron* **28**: 571–583.
Grudzinska J *et al*. (2005). *Neuron* **45**: 727–739.
Hejazi N *et al*. (2006). *Mol Pharmacol* **69**: 991–997.
Hirzel K *et al*. (2006). *Neuron* **52**: 679–690.
Lozavaya N *et al*. (2005). *J Neurosci* **25**: 7499–7506.
Maksay G *et al*. (2009). *J Neurochem* **109**: 1725–1732.
Meier JC *et al*. (2005). *Nat Neurosci* **8**: 736–744.
Miller PS *et al*. (2005). *J Physiol (Lond)* **566**: 657–670.
Oertel J *et al*. (2007). *J Biol Chem* **282**: 2798–2807.
Pitt SJ *et al*. (2008). *J Neurosci* **28**: 11454–11467.
Yang Z *et al*. (2007). *J Neurochem* **103**: 580–589.
Yang Z *et al*. (2008). *Biochem Pharmacol* **76**: 1014–1023.
Yevenes GE *et al*. (2003). *Nat Neurosci* **6**: 819–824.
Yevenes GE *et al*. (2006). *J Biol Chem* **281**: 39300–39307.