Glycine and its synaptic interactions : Functional and clinical implications

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Functional and clinical implications

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Glycine is a major neurotransmitter of inhibitory neurons in the spinal cord and brainstem. In many of these neurons, glycine coexists with γ-aminobutyric acid (GABA). The synaptic availability of glycine is controlled by 2 different transporters, and its postsynaptic effects are mediated by a specific chloride (Cl^-) channel receptor. In the adult nervous system, glycine exerts fast postsynaptic inhibition that is important for control of excitability of motor neurons, auditory processing, pain transmission in the dorsal horn, and other functions. Corelease of GABA may affect the temporal profile of the postsynaptic effects of glycine. Glycine is also a coagonist of glutamate on NMDA receptors (NMDARs). Studies in vitro and in knockout mouse models have provided insight on the complex synaptic interactions among glycine, GABA, and glutamate, and on the consequences of impaired regulation of glycinergic transmission in the nervous system. Excessive synaptic levels of glycine may explain the manifestations of nonketotic hyperglycinemia, whereas loss-of-function mutations impairing glycinergic signaling have been linked to familial hyperekplexia. Autoimmune disorders affecting the glycine receptor (GlyR) complex may also result in excessive motoneuron excitability. Pharmacologic blockade of a glycine transporter may constitute a novel approach to increase NMDAR activity in schizophrenia. Many of these topics have been recently reviewed.[1–9]

GLYCINE AS A NEUROTRANSMITTER: VESICULAR STORAGE AND TRANSPORT MECHANISM

Synthesis and storage. Glycine is the primary neurotransmitter of many inhibitory interneurons in the spinal cord and brainstem, including the ventral horn and motor cranial nerve nuclei, dorsal horn and trigeminal nuclei, and auditory and vestibular systems; it also mediates inhibitory effects of some amacrine cells and Golgi cells of the cerebellum.[1] Glycine also has an essential role in intermediate metabolism; for example, it is the precursor to the one-carbon pool of folic acid intermediates that are fundamental to many synthetic reactions. Glycine is catabolized via the glycine cleavage system, a group of mitochondrial proteins that mediate the interconversion of glycine and serine.[8] Glycine is incorporated into synaptic vesicles via the vesicular inhibitory amino acid transporter (VIAAT), which also mediates vesicular uptake of GABA (hence it is also referred to as vesicular GABA transporter, VGAT)[10–12] (figure). The shared vesicular carrier explains the frequent colocalization and corelease of glycine and GABA in the inhibitory terminals in the spinal cord and brainstem.[12–14]

Glycine transporters. The concentration of glycine at synapses is controlled by 2 high-affinity sodium (Na^+) and chloride (Cl^-) dependent transporters, GlyT1 and GlyT2.[2,3] These transporters belong to the SLC6 family, which also includes the transporters for GABA and monoamines. GlyT1 and GlyT2 are integral membrane proteins with 12 transmembrane domains and exist in multiple variants generated by alternative splicing.[2,3] These transporters have a complementary distribution in the nervous system and differ in their expression patterns, function, and pharmacologic properties (table). GlyT1 is expressed throughout most regions of the CNS, primarily in astrocytic processes ensheathing glycinergic synapses; it is also expressed at lower levels in some glutamatergic terminals and in the postsynaptic membrane at glutamatergic synapses.[2,3] In contrast, GlyT2 expression is restricted to regions rich in glycinergic synapses, such as the spinal cord, brainstem, and cerebellum, where it is present in the presynaptic terminals of glycinergic neurons.[2,3]

GLOSSARY

Cys = cysteine; **GABA** = γ-aminobutyric acid; **GlyR** = glycine receptor; **KCC2** = K^+, Cl^- cotransporter 2; **NKH** = nonketotic hyperglycinemia; **NMDAR** = NMDA receptor; **VGAT** = vesicular GABA transporter; **VIAAT** = vesicular inhibitory amino acid transporter.

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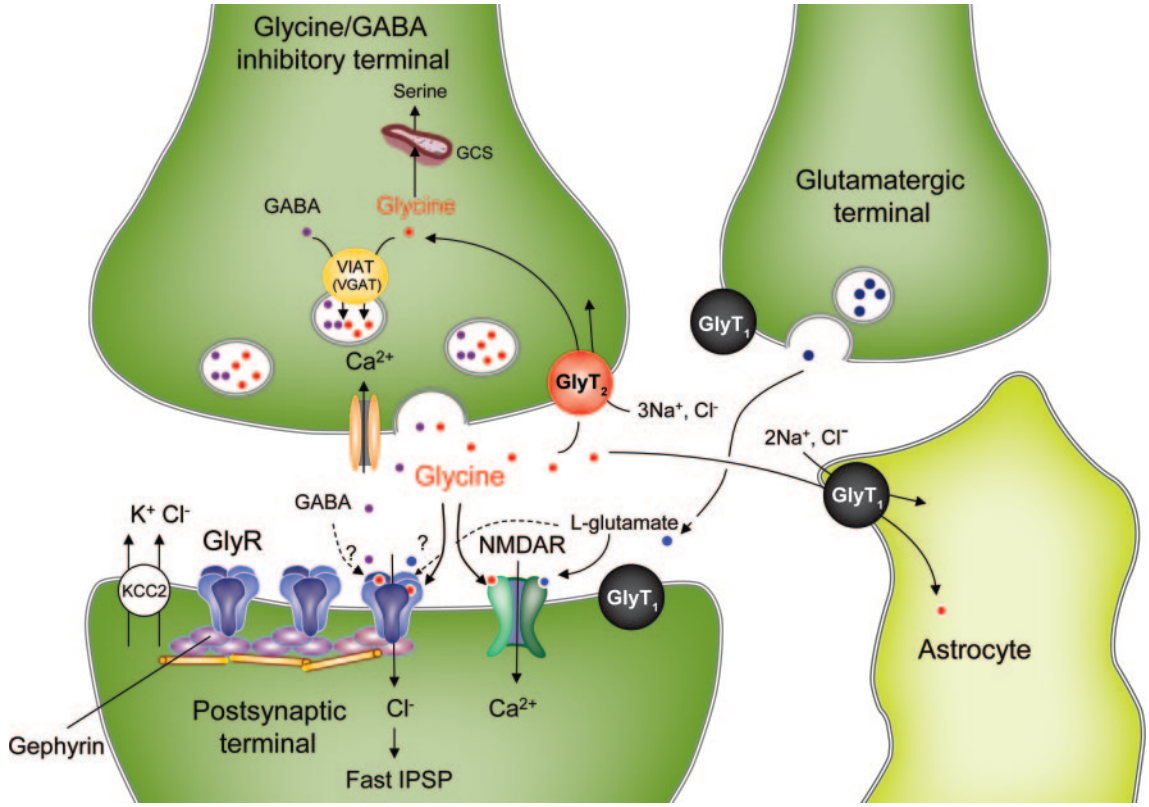
Figure Glycinergic neurotransmission



Glycine is a nonessential amino acid that acts as the primary neurotransmitter of many inhibitory interneurons in the spinal cord and brainstem. Glycine is catabolized via the glycine cleavage system (GCS). Glycine is incorporated into synaptic vesicles via the vesicular inhibitory amino acid transporter (VIAAT), which also mediates vesicular uptake of γ-aminobutyric acid (GABA) (VGAT). The concentration of glycine at synapses is controlled by 2 high-affinity sodium (Na^+) and chloride (Cl^-) dependent transporters, GlyT1, located primarily in astrocytes, and GlyT2, located at presynaptic glycinergic terminals. The postsynaptic effects of glycine are mediated by the glycine receptor (GlyR), which is clustered at the postsynaptic membrane by interactions with gephyrin. Glycine binding to GlyRs opens the Cl^- channel; the resulting change in membrane potential is determined by the activity of the K^+, Cl^- cotransporter 2 (KCC2). In mature neurons, GlyR activation elicits fast postsynaptic inhibition. Many inhibitory neurons in the spinal cord and brainstem release both glycine and GABA, which may act as coagonists of GlyRs. Glycine is an obligatory coagonist of glutamate required for the activation of NMDA receptors (NMDAR). Reciprocally, glutamate may allosterically potentiate GlyR-mediated currents. IPSP = inhibitory postsynaptic potential.

Studies on knockout mice indicate a differential role of GlyT1 and GlyT2 in shaping inhibitory neurotransmission.[2,3,15,16] These studies indicate that the major function of GlyT1 is to decrease the levels of glycine at glycinergic synapses (the classic role of membrane transporters). In contrast, the primary role of GlyT2 is replenishment of the presynaptic pool of glycine necessary to refill synaptic vesicles.[2,3] The phenotype of each of these knockout lines resembles that of human hereditary disorders of glycinergic transmission (table). Homozygous GlyT1 knockout mice exhibit severe lethargy and hypotonia and die within 6–14 hours of birth due to respiratory failure[15]; these manifestations resemble those of human glycine encephalopathy in nonketotic hyperglycinemia.[8] GlyT2-deficient mice die during the second postnatal week after developing a disorder characterized by motor hyperexcitability, resembling the manifestations of hereditary hyperekplexia.[16]

The mechanisms of regulation of expression, trafficking, and degradation of GlyT1 or GlyT2 are incompletely understood.[2,3,17] The state of glycosylation of these transporters during transit through the Golgi apparatus affects their trafficking to the membrane. Calcium-triggered insertion of GlyT2 at the presynaptic membrane depends on its interaction with syntaxin 1A[17]; protein kinase C promotes the downregulation of both GlyT1 and GlyT2.[2,3] Inhibition of GlyT1 by arachidonic acid, zinc, and protons, which are also modulators of NMDARs, may help to coordinate glycine availability and NMDAR responses.[3,17]

GLYCINE RECEPTORS

Structure. The GlyR is a ligand-gated Cl^- channel that is a member of the cysteine (Cys) loop ion channel receptor family, which also includes the nicotinic, $GABA_A$, and serotonin $5\text{-}HT_3$ receptors.[4,5] Like other Cys loop family

Table Glycine transporters

Transporter	GlyT1	GlyT2
Distribution	Throughout the nervous system	Spinal cord, brainstem, cerebellum
Location	Astrocytic processes ensheathing glycinergic synapses, glutamatergic terminals, postsynaptic membrane (together with NMDA receptor)	Presynaptic membrane of glycinergic nerve terminals
Stoichiometry	2 Na^+/Cl^-/glycine	3 Na^+/Cl^-/glycine
Transmembrane glycine gradient maintained	Relatively low	High
Possibility to act as reverse transporters (non-Ca^{2+} dependent vesicular release)	Likely	Unlikely
Inhibition by sarcosine	Yes	No
Primary function	Removal of glycine from the synapse	Replenishment of presynaptic pool of glycine
Other functions	Controls availability of glycine as coagonist of NMDA receptor	May contribute to glycine removal from some synapses
Phenotype of homozygous knockout mice	Reflects hyperglycinergic state: lethargy, hypotonia, and respiratory failure (heterozygous have evidence of increased NMDA receptor activation)	Reflects hypoglycinergic state: motor hyperexcitability, spasticity, ataxia, tremor
Human disorder mimicked by transporter knockout	Nonketotic hyperglycinemia (glycine encephalopathy)	Familial hyperekplexia, strychnine poisoning, tetanus

receptors, GlyRs are pentameric oligomers with each of their 5 subunits arranged symmetrically around a central ion-conducting pore. Functional GlyRs are formed by the association of one of 4 α-subunit subtypes (α1–α4, encoded by the *GLRA*1–4 genes, respectively) and a β-subunit (encoded by a single *GLRB* gene), with a putative 2α:3β stoichiometry.[4,5] Heteromeric α1β GlyRs mediate most glycinergic inhibition in the adult CNS. The GlyRα subunit contains the major determinants of agonist and antagonist binding; the β-subunit is required for the postsynaptic clustering of the GlyRs. GlyR subunits contain a large extracellular amino (N)-terminal domain that harbor the glycine binding site in the α-subunit, 4 α-helical transmembrane domains (M1–M4), and a short extracellular carboxy (C)-terminal tail. The M2 domain of each subunit contributes to the lining of the ion channel; a sequence in the M3-M4 loop region of the β subunit binds the postsynaptic scaffolding protein gephyrin (figure).

Distribution of receptor subtypes. High levels of GlyRs are found in the ventral and dorsal horn of the spinal cord; motor, auditory, vestibular, and sensory nuclei of the brainstem; superior colliculus; granular cell layer of the cerebellum; retina; olfactory bulb; and hippocampus.[4,5] Heteromeric α1β GlyRs are highly expressed in the adult spinal cord and brainstem; GlyRs composed by α2β subunits mediate inhibition on amacrine cells in the adult rat retina; and α3β GlyRs mediate glycinergic inhibitory neurotransmission in laminae I and II of the dorsal horn.[4] Homomeric α2 GlyRs are abundantly expressed in embryonic neurons.

Gephyrin and postsynaptic clustering of GlyRs. Postsynaptic clustering of GlyRs at mature synapses critically depends on their interactions with gephyrin.[6,7] Gephyrin is a scaffold protein that consists of 3 functional domains and binds both to the M3-M4 loop of the GlyR β subunit and to the microtubules.[7] Gephyrin also has a major role in dynamic activity-dependent regulation of GlyR trafficking and expression. This scaffold protein is translocated to the cell membrane by the GDP-GTP exchange factor collybistin,[18] and its dynamic interactions with both microtubules and microfilaments contribute to homeostatic regulation of surface expression and distribution of GlyRs.[6,7] During maturation of inhibitory synapses, glycine-triggered depolarization and postsynaptic calcium (Ca^{2+}) influx (see below) may regulate the interactions between gephyrin and GlyRs.[6,7] Gephyrin is also involved of postsynaptic clustering of $GABA_A$ receptors.[6,7]

Effects mediated via glycine receptors. The GlyRs are ligand gated Cl^- permeable anion channels; they require binding of glycine to 2 or 3 sites at their α/β interfaces for full activation. The GlyRs can also be activated by D-alanine and the sulfonic acid taurine, and are selectively blocked by the competitive antagonist strychnine. Binding of glycine to the GlyR elicits opening of the Cl^- channel; this effect is similar to that of GABA acting on $GABA_A$ receptors ($GABA_A$ Rs).[4,5] The resulting change in membrane potential elicited by GyR or $GABA_A$R activation de-

pends on the transmembrane Cl^- concentration, which is determined by the activity of the K^+, Cl^- cotransporter 2 (KCC2). This transporter extrudes Cl^- from the cell and its expression increases in parallel to neuronal maturation.[19] Developmental changes in KCC2 receptor expression result in a shift in the direction of transmembrane Cl^- flux and thus the change in membrane potential elicited by GlyR (or $GABA_AR$) activation.[19] In mature neurons, where there is low intracellular Cl^- concentration maintained by KCC2, activation of GlyRs elicits influx of Cl^- leading to fast hyperpolarization and postsynaptic inhibition.[4,5] In contrast, in immature neurons activation of GlyRs results in efflux of Cl^-, leading to neuronal depolarization; this opens voltage-dependent Ca^{2+} channels, elicits action potentials, and establishes early network activity in the developing nervous system.[19] The high Cl^- permeability of homomeric $\alpha 2$ GlyRs expressed in embryonic neurons may promote this excitatory action.[5] Modulators of GlyR activity in vitro include zinc, neurosteroids, cannabinoids, alcohol, general anesthetics (such as propofol), picrotoxin, cocaine, and some anticonvulsants.[4]

GLYCINE AND GABA AS COTRANSMITTERS IN INHIBITORY NEURONS

Glycine and GABA as cotransmitters. Many interneurons in the spinal cord[20,21] and brainstem[22,23] as well as cerebellar Golgi cells[24] express both glycine and GABA and are thus mixed inhibitory interneurons. There is a differential distribution of GlyR and $GABA_A$ receptor subunits in spinal and brainstem nuclei.[25] Glycine/GABA cotransmission in the spinal cord and brainstem provides for a wide range of time courses of postsynaptic inhibition at single synapses; GlyR-mediated responses are faster than those mediated by $GABA_A$Rs.[26,27] Whereas there is a postnatal shift from glycine/GABA cotransmission to glycine-only transmission in several brain regions,[28,29] corelease of glycine and GABA may also occur in adult animals.[30] Mixed inhibitory neurons may perform cotransmission at some of their synapses, pure glycinergic or GABAergic inhibition at others, or both.[13] For example, Golgi cells of the cerebellum elicit pure $GABA_A$ receptor-mediated inhibition of their granule cells and GlyR-mediated inhibition of unipolar bushy cells in the vestibulocerebellum.[13]

Lu et al.[30] described a unique form of glycine/GABA cotransmission at the inhibitory synapse in the medial nucleus of the trapezoid body, an auditory brainstem nucleus that is part of the neural circuitry underlying sound localization. At this level, glycine and GABA act as coagonists at GlyRs; the joint action of GABA (a weak agonist) and glycine (a strong agonist) accelerates the rate of inactivation of GlyRs, thereby accelerating the time course of glycinergic inhibition.[30] Thus, GABA/glycine cotransmission may provide a flexible mechanism for adjustments of the time course of glycinergic inhibition to match the requirements of different neural circuits.[30,31]

Effects of glycine on motor nuclei. There is abundant expression of GlyRs in cranial and spinal motoneurons.[32] In the spinal cord and brainstem, glycinergic interneurons are involved in the coordination of reflex responses and motor rhythm generation.[1] For example, spinal IA interneurons mediate reciprocal inhibition in muscle stretch reflex circuits thus allowing coordinated contraction of agonists and relaxation of antagonist muscles.[33] Renshaw interneurons produce recurrent negative feedback inhibition that regulates the excitability of motoneurons.[1,34] Corelease of GABA and glycine determines the strength and timing of inhibition through interactions between a fast, GlyR-mediated and a slow, $GABA_A$ receptor–mediated component, thus optimizing inhibition of motoneuron function.[27] Glycinergic postsynaptic inhibition of brainstem and spinal motoneurons may be responsible for muscle atonia during REM sleep[35,36] although there is some evidence suggesting the contrary.[37]

Glycinergic inhibition in the dorsal horn. There is colocalization of glycine and GABA in synaptic terminals in the superficial dorsal horn (lamina I–II)[21]; some evidence indicates that GlyRs are located postsynaptically whereas $GABA_A$ receptors appear to be located extrasynaptically.[38] Postsynaptic GlyRs with $\alpha 3\beta$ subunits mediate glycinergic inhibitory neurotransmission in nociceptive sensory neuronal circuits in laminae I and II of the dorsal horn.[5,39] Some of these neurons receive input from low threshold cutaneous afferents and may provide an inhibitory gate that prevents the disynaptic excitation of lamina I nociceptive neurons by these low threshold afferents.[40]

SYNAPTIC CROSSTALK BETWEEN GLYCINE AND GLUTAMATE

Glycine as a coagonist of NMDA receptors. Glycine is an obligatory coagonist of glutamate required for the activation of NMDARs.[41] Although it remains unclear whether glycine can be coreleased with glutamate from presynaptic glutamatergic terminals, glycine released from glycinergic terminals might reach nearby glutamatergic synapses by spillover. GLYT1, which is expressed in astrocytes, glutamatergic terminals, and in their postsynaptic targets, may have a major role in controlling the availability of glycine for binding to the NMDARs.[2,3] Glycine may have an important role in promoting synaptic plasticity and network interactions mediated by NMDARs. Inhibition of GlyT1 enhances

NMDAR-mediated long-term potentiation in the hippocampus in vitro[42]; heterozygous GlyT1 knockout mice with reduced GlyT1 expression have enhanced hippocampal NMDAR function and memory retention.[43] D-serine, an endogenous amino acid released from astrocytes, may substitute for glycine at the costimulatory site in the NMDA receptor.[44] A particular subtype of NMDA receptor, consisting of NR1/NR3 subunits, is highly expressed in myelin and can be activated by pure glycine-site agonists such as D-serine, in the absence of glutamate.[45] These NR1/NR3 receptors may participate in mechanisms of excitotoxic injury to myelin.[45]

Glutamate-induced allosteric facilitation of GlyR responses. Liu et al.[46] found that glutamate allosterically potentiates GlyR-mediated currents; this effect is mediated by a binding site that is probably on the α-subunit of the GlyRs and is pharmacologically different for that of the known inotropic glutamate receptors. These results suggest a new model of functional crosstalk between glycine and glutamate via the reciprocal allosteric enhancement of receptor function for each neurotransmitter, providing a rapid homeostatic control mechanism for neuronal excitability.[46]

CLINICAL CORRELATIONS

Nonketotic hyperglycinemia. Nonketotic hyperglycinemia (NKH), also known as glycine encephalopathy, is an autosomal recessive disorder caused by deficiency in the glycine cleavage system.[8,47] This disorder affects term neonates within the first or second day of life and manifests with progressive lethargy, severe seizures associated with hypsarrhythmia or burst-suppression pattern on EEG, myoclonic jerks, hiccupping, and severe hypotonia. Whereas hypotonia and respiratory depression may reflect excessive inhibitory glycinergic effects, seizures may reflect excessive activation of NMDA receptors.[47] NKH may also manifest in older children with intermittent choreoathetosis.[48] NKH has been linked to mutations of the *GLDC* (glycine decarboxylase) gene.[49]

Hereditary hyperekplexia. Hereditary hyperekplexia, or human startle disease, is a paroxysmal motor disorder caused by a defect of glycinergic inhibition of motor neurons.[9,50] Hyperekplexia is characterized by an exaggerated startle reflex in response to tactile or acoustic stimuli. This disorder first manifests as neonatal hypertonia, followed in some cases by episodes of life-threatening apnea. Hereditary hyperekplexia is genetically heterogeneous.[9] Most commonly, it is due to missense or nonsense mutations in the *GLRA1* gene encoding the postsynaptic GlyR α1 subunit. More recently, missense, nonsense, and frameshift mutations in the *SLC6A5* gene encoding the glycine transporter GlyT2 gene have been identified as the second most common cause. Rare causes include mutations in the *GLRB* gene encoding GlyR β subunit, the *GPHN* gene encoding gephyrin, and the *ARHGEF9* gene encoding collybistin. The disease is not lethal in humans and the condition improves after 1 year of age in most infants. This might be due to compensatory effects of GABAergic transmission.[50]

Autoimmune disorders. Autoimmune disorders may also impair glycinergic transmission resulting in motoneuron hyperexcitability. Butler et al.[51] reported a patient with clinical features resembling stiff-man syndrome associated with paraneoplastic autoantibodies directed against gephyrin, in the setting of a mediastinal undifferentiated carcinoma. More recently, Hutchinson et al.[52] detected autoantibodies against GlyR in a patient with hyperekplexia, rigidity, and brainstem signs consistent with progressive encephalomyelitis, rigidity, and myoclonus syndrome.

Neuropathic pain. Impaired glycinergic inhibition in the dorsal horn may underlie mechanisms of inflammatory and neuropathic pain.[39,40] Prostaglandin E2, a major product of inflammation, inhibits $\alpha3\beta$ GlyRs in the dorsal horn via protein kinase A–mediated phosphorylation[39]; this mechanism may contribute to sensitization of dorsal horn neurons in the setting of inflammation. Impaired glycinergic inhibition in the setting of nerve injury may in part reflect a downregulation of KCC2 expression in the dorsal horn, thereby shifting the equilibrium potential of Cl^- to depolarized values, rendering the effects of glycine excitatory rather than inhibitory.[53] Disinhibition in the dorsal horn may result in tactile allodynia by allowing low threshold afferent stimuli to polysynaptically activate lamina I neurons.[40] Impaired glycinergic inhibition may also allow tactile afferents to activate astrocytes, which may provide D-serine to enable NMDA receptor activation and thus contributing to development of allodynia.[54]

Schizophrenia. GLYT1 is a potential target for the treatment of schizophrenia.[3] Evidence from experimental models and effects of drugs such as ketamine and phencyclidine indicate that symptoms of schizophrenia may, at least in part, reflect impaired NMDAR-mediated glutamatergic transmission. Experimental studies indicate that increased availability of glycine as a coagonist of the NMDAR may be useful to treat schizophrenia; selective GLYT1 inhibitors are being assessed in preclinical studies.[55,56]

PERSPECTIVE

Glycine, the simplest amino acid, has multiple synaptic interactions with GABA and glutamate; these interactions provide for flexible reg-

ulation of neuronal excitability both during development and in the adult nervous system. In addition to the dramatic and life-threatening motor hyperexcitability resulting from intoxications with tetanus toxin (which impairs glycine release) or strychnine (which selectively blocks GlyRs), mutations affecting proteins involved in glycinergic transmission either presynaptically (GlyT2) or postsynaptically (GlyR, gephyrin, collybistin) emphasize the importance of this amino acid in controlling motoneuron excitability in the spinal cord and brainstem. Autoantibodies against components of the GlyR complex, including not only GlyRs but also gephyrin (and perhaps collybistin and other yet unidentified molecules), are increasingly recognized as an important cause of subacute onset motor hyperactivity syndromes. Pharmacologic approaches to promote glycinergic transmission, for example by GlyT1 inhibitors, may be potentially helpful in these disorders. Similar approaches may be used as adjuvant treatment of neuropathic pain.

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