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Glycine as a neurotransmitter in the forebrain: a short review

Marina Sorrentino Hernandes · Lanfranco R. P. Troncone

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Abstract Since the late 1970s glycine has been considered an important inhibitory neurotransmitter in brain stem and medulla. The description of its involvement in the mechanism of action of the potent neurotoxin strychnine pushed further the concept of inhibitory transmitter. The significant concentrations of glycine in forebrain motivated investigators to evaluate different aspects of glycinergic transmission under the ontogenetic, physiologic and pathologic standpoints. This review encompasses a few of these aspects as the role of the different glycine receptors (GlyRs) in intracellular chloride balance, glycine transporters, GABA/Glycine co-release, glycine/NMDA receptor interaction, glycine receptors in acute alcohol effects and advocates a more relevant role for glycine as a stimulatory transmitter in forebrain areas. Finally, the possible co-release of glycine and GABA is considered as an important process to understand the role of glycine in forebrain neural transmission.

Keywords Glycine · Forebrain · GABA · NMDA · Alcohol · Neurotransmitters

M. S. Hernandes
Department of Physiology and Biophysics,
Institute of Biomedical Sciences,
University of São Paulo, Av. Prof. Lineu Prestes,
1524, São Paulo, SP 05508-900, Brazil

L. R. P. Troncone (✉)
Laboratory of Pharmacology, Instituto Butantan,
Av. Vital Brasil, 1500, São Paulo, SP 05503-900, Brazil
e-mail: ltroncone@butantan.gov.br

Glycine metabolism

Glycine is a simple amino acid found in all mammalian body fluids and tissue proteins in substantial amounts. Aprison and Werman (1965) first proposed that glycine acts as a neurotransmitter in mammalian central nervous system (CNS). They noted that the concentration of glycine in the spinal cord is higher than elsewhere in the brain. Later, Hopkin and Neal (1969) revealed that this amino acid could be released from spinal cord slices after stimulation. After many neurochemical and electrophysiological investigations, glycine fulfilled many of the criteria for a substance to be accepted as a neurotransmitter: release after appropriate stimulation, the presence of a mechanism to stop or limit the transmission after release, the existence of specific receptors sensitive to glycine and the ability of other ligands to antagonize the action of glycine (Hopkin and Neal 1970; Shank and Aprison 1970).

It is now more than 40 years since glycine was believed to play a role as a neurotransmitter and at the present time only scanty information is available on the factors regulating the concentration of glycine in the CNS. The knowledge about glycine metabolism in the nervous tissue is still poorly understood, but its biosynthetic pathway in other tissues is well known.

Glycine has a simple molecular structure with a side-chain consisting of one single hydrogen atom. It can be formed from serine by a reversible folate-dependent reaction catalyzed by the enzymes glycine decarboxylase (GDC, also named glycine-cleavage-system or glycine dehydrogenase) and serine hydroxymethyltransferase (SHMT). GDC and SHMT are both responsible for the inter-conversion of glycine and serine, an essential step of the primary metabolism by providing one-carbon units for many biosynthetic reactions. GDC is a multienzyme

complex which occurs in prokaryotes and eukaryotes (Bauwe and Kolukisaoglu 2003). It is composed of four different subunits, designated P-protein (a homodimer containing pyridoxal phosphate, 200 kDa), H-protein (a monomeric lipoamide-containing protein, 14 kDa), T-protein (a tetrahydrofolate-requiring cofactor, 41 kDa), and L-protein, a dihydrolipoamide dehydrogenase (a homodimer containing FAD and a redox active cysteine residue, 100 kDa) (Douce and Neuburger 1999). In eukaryotes, GDC is found in the mitochondria and catalyzes the oxidative decarboxylation and deamination of glycine. Isoforms of SHMT occur in the cytosol and catalyzes the reversible transfer of a methylene group from serine to tetrahydrofolate (THF) with the formation of glycine and 5,10-methylenetetrahydrofolate. Mutations in the genes coding some of these enzymes can result in non-ketotic hyperglycinemia, a metabolic disorder with autosomal recessive inheritance causing severe, frequently lethal, neurological symptoms in the neonatal period. Most infants affected by the disease have defects either in the P or in the T subunits of the GDC complex (Kure et al. 1997).

The term "glycine–serine inter-conversion" might suggest that the central importance of this pathway is just the synthesis of serine from glycine and vice versa. However, in both directions of the concerted reaction of GDC and SHMT, THF becomes N^5,N^{10}-methylated making these reactions the most important source of active one-carbon-units for a number of biosynthetic processes such as the biosynthesis of methionine, pyrimidines and purines. Glycine and serine are precursors for chlorophyll, tryptophan and ethanolamine (Bauwe and Kolukisaoglu 2003). Inasmuch as these amino acids are also precursors of phosphatidylcholine and phosphatidylserine, both are important components of the cell membrane and proteins (Xu et al. 1991).

There are several alternative sources of glycine: (1) it can be formed from glyoxylate via a transaminase reaction with glutamate, (2) a limited amount of glycine can also be synthesized from catabolism of threonine by the threonine cleavage complex and (3) betaine metabolism (or degradation of its precursor, choline) can generate glycine by successive removal of the methyl groups from the amino group of betaine. This leads to the formation of dimethylglycine and monomethylglycine (sarcosine) and, finally, glycine (Datta and Maclean 2007). Although controversial, there is significant evidence supporting serine as the major precursor of glycine in the CNS.

Glycine transporters

Classically, the glycinergic neurotransmission involves the storage of the transmitter in synaptic vesicles, neuron depolarization, transmitter release in the synaptic cleft and glycine binding to GlyRs in the post-synaptic neuron. Once released from the pre-synaptic neuron, glycine is rapidly removed from the synaptic cleft by specific high-affinity transporters located in the neuronal and glial plasma membranes (Neal and Pickles 1969). Two genes encoding glycine transporters (GLYT) have been cloned, GLYT1 and GLYT2, with approximately 50% amino acid sequence identity but differing in pharmacology and tissue distribution (Eulenburg et al. 2005). Both transporters belong to the family of Na^+- and Cl^--dependent neurotransmitter transporter proteins as the GABA, proline, monoamines and several other orphan transporters (Aragon and Lopez-Corcuera 2003). Extracellular binding of glycine together with Na^+ and Cl^- can induce a conformational change in the transporter that switches from an outward to an inward facing state (Eulenburg et al. 2005). It is also known that when the Na^+ gradient is dissipated, the direction of transport changes from inward (uptake) to outward (release). Five GLYT1 variants were identified (a, b, c, e, f). In contrast, only two variants of GLYT2 (a and b) have been described in the rat brain. It is widely accepted that GLYT1 is found on astrocytes, whereas GLYT2 is located in glycinergic neurons. Opposing this view, Raiteri et al. (2008) showed that functional GLYT1s are present on neuronal axon terminals and functional GLYT2s are predominantly expressed in astrocytes, questioning the glial versus neuronal localization of these transporters in the spinal cord. The presence of these transporters may change the concentration of glycine in restricted cellular territories by several orders of magnitude (Supplisson and Bergman 1997).

Interestingly, glycine release in the brain stem can be stimulated by glycine itself, but not by GABA or taurine, suggesting the involvement of transporters functioning in a reverse direction. Furthermore, in Na^+-free superfusion media, the transporters were not functional, and accordingly, addition of extracellular glycine caused no effect (Saransaari and Oja 2009).

When considering the storage and release of a chemical transmitter, it is of great importance to take into account the vesicular transporters. The vesicular storage of glycine is carried out by proteins named vesicular inhibitory amino acid transporters (VIAAT) or vesicular GABA transporters (VGAT), which stores GABA, glycine and, sometimes, both in the same vesicles (Aubrey et al. 2007). The co-release of GABA and glycine by the same pre-synaptic terminal will be specifically addressed below.

Glycine receptors

Glycine receptors could be purified from rat spinal cord using affinity chromatography on aminostrychnine–agarose

(Pfeiffer et al. 1982). Subsequent analyses showed that the GlyRs are composed of five distinct glycosylated integral membrane proteins that are derived from two separate gene families, named α and β subunits. Four genes encoding α subunits exist (α1–α4), whereas only one gene encoding the β subunit was identified in mammals. Initial studies suggested that in adult tissue the heteromeric GlyRs have a 3α:2β stoichiometry but more recent studies suggest that the stoichiometry is 2α:3β (Cheng et al. 2007). The β subunit, which is unable to form homomeric receptors, carries a binding motif to gephyrin, a peripheral membrane protein that mediates synaptic clustering and heteromeric GlyRs anchoring by interacting with the subsynaptic cytoskeleton. In the absence of the β subunit, α subunits are able to form homomeric GlyRs. The characteristics of the various subtypes of GlyRs can be explained by their content of α subunits (Malosio et al. 1991; Harvey et al. 2004). For example, α1 homomeric GlyRs expressed in *Xenopus* oocytes can be activated by glycine, taurine and β-alanine, but homomeric GlyRs formed only by α2 or α3 subunit are not activated by taurine or β-alanine (Kuhse et al. 1990; Schmieden et al. 1992).

Apart from its role in anchoring GlyR to the cytoskeleton, β subunit decreases ionic currents and stabilizes lower conductance states of the receptor (Bormann et al. 1993) and may also influence receptor sensitivity to glycine (Rees et al. 2002) and drugs such as tropisetron, a 5-HT3 receptor antagonist (Supplisson and Chesnoy-Marchais 2000), the neurosteroid pregnenolone sulfate (Maksay et al. 2001) and picrotoxin analogs (Shan et al. 2001).

Investigations conducted by Shan and collaborators in HEK293 cells expressing recombinant GlyRs demonstrated that α and β subunits contribute asymmetrically to the activation of GlyRs, suggesting important structural differences between the subunits (Shan et al. 2003). According to the authors, β subunit seems to be involved in structural transitions between open and close states of the receptor, and this effect probably involves allosteric interactions with the α subunits.

It is well known that many ion channels functionally interact with other membrane proteins. By the same way, in vitro studies suggested that leptin receptors may modulate GlyRs structure and function via specific interactions with its α1 subunit. This interaction was confirmed in binding studies that used the cytoplasmic loop of the GlyR as an affinity ligand for homogenized tissue from rat spinal cords and lower brainstem. This interaction can influence receptor targeting, localization, regulation and/or modulation of its function (Leite et al. 2002).

Beta subunit presents four transmembrane domains (TM1–TM4), a large N-terminal and a small extracellular C-terminal domain (Grenningloh et al. 1987).There is a large intracellular loop between TM3 and TM4 responsible for interactions with cytoplasmic proteins, cytoskeleton and neuroreceptors involved in turnover, clustering, modulation and traffic of these receptors (Karlin and Akabas 1995).

Several inherited disorders have been associated with mutations in GlyRs subunits. As reviewed by Jentsch et al. (2002), startle syndromes caused by impaired glycinergic neurotransmission include familial startle disease in humans, the murine mutations spastic, spasmodic and oscillator, and the bovine, equine and canine forms of myoclonus. In humans, mutations in the α1 gene of the GlyR cause hyperekplexia, an autosomal dominant neurological disorder characterized by an intense muscle tone and strong startle reflex (Shiang et al. 1993). However, dysfunctions of other proteins related to the GlyR, as the β subunit and gephryrin, can also give rise to a hyperekplexia phenotype (Rees et al. 2002, 2003). In mouse, both the spasmodic and oscillator phenotype are caused by mutations in α1 subunit that reduces the receptor sensitivity to glycine (Saul et al. 1994).

The distribution pattern of GlyRs has been studied using many in vitro methods: immunohistochemistry, autoradiography of [^{3}H]-strychnine and [^{3}H]-glycine binding sites, for example. These methods revealed high densities of GlyRs in the gray matter but not in the white columns of the spinal cord. In contrast, GlyRs in the brain stem are localized in some specific nuclei as cuneate nucleus, gracile nucleus, hypoglossal motor nucleus, reticular nuclei, trigeminal nuclei and cochlear nuclei. In the midbrain the GlyRs are present in the thalamus and hypothalamus (Aprison and Werman 1965). These receptors were also described in retina (Vitanova et al. 2004), striatum and cerebellar cortex (Zarbin et al. 1981; Araki et al. 1988; Friauf et al. 1997).The presence of receptors in these areas argues in favor of a more widespread role for glycine.

Glycine as an inhibitory neurotransmitter

Glycine is recognized as the major inhibitory neurotransmitter in the spinal cord. The GlyR is an oligomeric glycoprotein that forms a transmembrane channel selective to chloride ions. When glycine binds to the receptor, the pore opens allowing Cl^- to passively diffuse across the membrane. The Cl^- conductance thus leads to neuron hyperpolarization, besides preventing depolarization and neuronal firing induced by excitatory neurotransmitters (Stein and Nicoll 2003).

In the mammalian brain stem and medulla the glycine-mediated inhibitory transmission is essential for the voluntary motor control as well as for processing of sensorial inputs and generation of reflex responses. In the brain stem, glycine is involved in auditory, cardiovascular and respiratory functions, for example. The release of [^{3}H]-glycine

from mouse brain stem slices in a superfusion system revealed that it is a Ca^{2+}-dependent exocytosis and it could be mediated by Na^+ and Cl^- channels. Furthermore, according to the authors, the release was affected by multiple regulatory systems, being reduced by increasing cGMP levels, phospholipase-A2 inhibition by quinacrine and genistein inhibition of tyrosine kinase, while the activation of protein kinase C by PMA increased basal release (Saransaari and Oja 2009).

Spinal inhibitory reflexes involve recurrent inhibitory circuits in which glycine takes a fundamental role. Medullary glycinergic Ia interneurons reciprocally inhibit the stretching reflex allowing the relaxation of antagonistic muscles and coordinating the agonistic muscles. On its turn, Renshaw interneurons using glycine as a transmitter control the excitability of motoneurons producing a recurrent inhibition by negative feed-back (Lundberg et al. 1975; Betz and Laube 2006).

The involvement of GlyRs in the sensory system of spinal cord was also described. Harvey et al. (2004) have been able to show that the inhibition of α3-GlyR by prostaglandin-E2-dependent phosphorylation underlies central inflammatory pain sensitization.

Glycine as an excitatory neurotransmitter

In several parts of the developing CNS, the intracellular Cl^- concentration is increased in comparison with the extracellular medium. Thus, the activation of GlyRs induces outflow of Cl^-, leading to a strong depolarization and neurotransmitter release, instead of hyperpolarization (Ben-Ari 2002). Although still unclear, this early excitatory action has been proposed as a physiologically relevant event. It is believed important in neuronal differentiation, proliferation, neuronal network stability and synaptogenesis (Wester et al. 2008). During synaptogenesis, for example, GlyR activation increases the amount of intracellular Ca^{2+}, which has a crucial role in the correct formation of postsynaptic glycinergic membrane specializations (Kirsch and Betz 1998).

The switch to the mature neuron phenotype involves the expression of K^+/Cl^- co-transporters (KCCs), which lowers the internal Cl^- concentration, thereby shifting the Cl^- equilibrium potential to more negative values and converting the action of the GlyR from excitatory to inhibitory (Plotkin et al. 1997). It is accepted that Na^+K^+/Cl^- (NKCCs) co-transporters expression dominates in immature neurons of cortex and hippocampus, whereas KCC expression is only induced after birth. This developmental change in chloride co-transporters expression coined the term "chloride switch" (Zhu et al. 2008).

Several lines of evidence indicate that during CNS development and the postnatal period, the GlyRs are composed mainly of α2 subunit (Hoch et al. 1989). These subunits are progressively replaced by the α1 subunit during the second postnatal week in most of the brain structures (Akagi and Miledi 1988; Malosio et al. 1991). The α1 subunit is expressed at low levels in embryonic and newborn rat spinal cord (Aguayo et al. 2004). Curiously, the developmental switch from depolarizing to hyperpolarizing GlyRs activity coincides with the developmental switch from the early postnatal form of GlyR composed of α subunit only, to heteromeric receptors essentially composed of three β and two α subunits (Hoch et al. 1989). A role for the α2 subunit during neuronal development has been suggested by McDearmid et al. (2006). These authors demonstrated that α2-GlyRs regulate interneuron differentiation during spinal cord early development. It is not surprising that some studies suggested that homomeric α2-GlyRs could not participate in inhibitory transmission in the adult brain. It seems that the activation of these homomeric α2-GlyRs is rather slow and is unrelated to rapid transmission at the synapses. Probably these receptors are located extra-synaptically, being activated by basal levels of glycine, whereas heteromeric α2β-GlyRs, which are able to bind to the cytoskeleton, have a preferential synaptic location (Mangin et al. 2003).

A few studies suggest that glycine can act as an excitatory neurotransmitter in mature neurons (Hernandes et al. 2007; Sanchez et al. 2008). Similar to immature neurons, a possible explanation is based on conditions under which intracellular Cl^- concentrations can be sufficiently high, compared to the extracellular medium, to favor depolarization. According to this hypothesis, when glycine binds to the GlyR, the resulting Cl^- flux moves the membrane potential rapidly toward the Cl^- equilibrium potential. Depending on the value of the equilibrium potential relative to the cell resting potential, the Cl^- flux can cause a depolarization or a hyperpolarization. As a result of the Cl^- efflux, calcium voltage-gated channels can be activated and the neuron can be depolarized (Lynch 2004). The most interesting is that in mature neurons a small change in Cl^- concentration is necessary to cause a large impact in the membrane gradient, altering the current through the GlyR (De Koninck 2007).

It is well known that acetylcholine plays a central role in the extrapyramidal control of motor function. Tonically active cholinergic interneurons, also known as giant aspiny neurons (GANs), in the striatum give rise to dense axonal arborizations and significantly shape striatal output. These neurons receive abundant synaptic glutamatergic inputs from the ipsilateral thalamus at their somata, proximal and distal dendrites, besides relatively sparse glutamatergic inputs from the ipsilateral and contralateral cortex. Moreover, dopaminergic inputs from the substantia nigra pars compacta and also projections from local GABAergic

interneurons (Lapper and Bolam 1992; Reynolds and Wickens 2004). Glycine may also play a modulatory role in the striatum but this possibility has been poorly explored. Semba et al. (1995) investigated the characteristics of extracellular amino acids released from the striatum by means of in vivo microdialysis in freely moving rats. Glycine release was stimulated by high K^+ and veratrine. The effect was not abolished with Ca^{2+} omission or tetrodotoxin inclusion in the perfusion medium. In addition, functional studies showed the presence of strychnine-sensitive GlyRs in GABAergic interneurons and GANs (Sergeeva 1998). However, glycine sources and their roles in the striatal microcircuitry are not clear because no glycinergic pathways/projections have been described in these structures. To understand the role of glycine in the striatum and to elucidate its involvement in neurotransmitter release, our group have evaluated whether glycine alters acetylcholine, glutamate or dopamine release using an in vitro superfusion approach. We found that glycine stimulated ^{3}H-acetylcholine release (^{3}H-ACh) in a dose- and calcium-dependent manner, but failed at releasing ^{3}H-glutamate or ^{3}H-dopamine. Tetrodotoxin inhibited about 75% of the release demonstrating a predominant dendritic and cell body location of glycine receptors. The prototypical glycine receptor antagonist strychnine at 10 μM completely abolished ^{3}H-ACh release. These effects could be attributed to changes in chloride transporters expressed in the GANs as well as glycine receptor subunit composition and finally to GABA/glycine co-release in this tissue (Hernandes et al. 2007).

Chloride ions and GlyR function

Chloride transporters

The cation-chloride co-transporters (CCCs) constitute a family of seven known Na–Cl (NCCs), NKCCs and KCCs co-transporters that perform a wide variety of physiological roles and differ in tissue and cellular distribution (Delpire and Mount 2002). Under physiological conditions, NCCs and NKCCs provide the main route for Cl^- uptake, whereas KCCs are responsible for Cl^- extrusion from the neuron (Gamba et al. 1994). NKCC and KCC are of particular interest because they are critically involved in Cl^- homeostasis in the adult brain. NKCCs were found in neurons and also in glial cells. All four known KCC isoforms are expressed in neuronal tissue (KCC1–KCC4). KCC1, KCC3 and KCC4 have been found with a more limited expression in neurons than KCC2, which is exclusively expressed in mature neurons (Le Rouzic et al. 2006). NKCC1 and KCC2 are co-localized in retinal neurons, illustrating that the two proteins are functionally coupled in the control of intracellular Cl^- concentration (Billups and Attwell 2002).

Several studies have suggested that the balance between inhibition and excitation could be determined by Cl^- uptake and Cl^- extrusion processes active in each neuron. Thus, CCCs play a key role in shaping GABA- and glycine-mediated signaling, influencing not only fast cell-to-cell communication but also many aspects of neuronal development, plasticity and trauma (Payne et al. 2003).

Recently, it has been discussed that traumatic injury, neurological disorders and synaptic plasticity can interfere with chloride homeostasis. An important loss of KCC2 protein expression followed by an increased intracellular Cl^- concentration was observed in an in vivo model of epilepsy. These changes induced a depolarizing action of GABAA receptors instead of the classical hyperpolarizing action (van den Pol et al. 1996). In an experimental model of neuropatic pain, neurons of the superficial dorsal horn of the spinal cord showed a decrease in KCC2 expression and an associated depolarizing shift in the anion gradient (Coull et al. 2003). Down regulation of KCC2 mRNA was also found in the hippocampus of drug-resistant epileptic patients (Palma et al. 2006). According to Payne et al. (2003), trauma might cause neurons to revert to a state where they have greater developmental flexibility, which is perhaps needed in sprouting and retargeting. In cortical neurons, differences in the expression of Cl^- transporters create subcellular differences in Cl^- concentration that determines the effect of GABA as an inhibitory or excitatory neurotransmitter (Szabadics et al. 2006).

Besides being controlled by chloride transporters, the Cl^- concentrations are constantly regulated by endogenous modulators as the brain-derived neurotrophic factor (BDNF) (De Koninck 2007). The exposition of hippocampal brain slices to BDNF caused down regulation of KCC2 mRNA and protein on a rapid time-scale, with a corresponding deficit in Cl^- extrusion capacity (Rivera et al. 2002, 2004). On the other hand, during the early postnatal period, BDNF is involved in ontogeny of Cl^- homeostasis by promoting the up regulation of KCC2 (Aguado et al. 2003).

Glycine and NMDA receptors

NMDA glutamate receptors are ligand-gated ion channels conducting Na^+, K^- and Ca^{2+}. They are tetrameric structures composed of combinations of NR1, NR2A-D and NR3A-B subunits. NMDARs have a specific recognition site for glutamate or aspartate or yet NMDA and a second site that recognizes glycine or serine. Both these sites have to be occupied to activate the ion channel (Johnson and Ascher 1987; Kleckner and Dingledine 1988).

The glycine recognition site is located on NR1 while the recognition site for glutamate is on subunit NR2 (Hirai et al. 1996; Cull-Candy et al. 2001). Johnson and Ascher (1987) employed primary cortical neuron cultures to first demonstrate that glycine may facilitate excitatory transmission in the brain through the activation of the NMDA receptor. These cells showed a stronger response to glutamate or NMDA when glycine was present, an effect that was insensitive to strychnine. The exact role of glycine in glutamatergic synapses is yet poorly understood because the real synaptic concentration of this amino acid is unknown and the different glutamate receptor isoforms have different affinity for glycine (Kuryatov et al. 1994).

In addition, it has been shown that glutamate receptors could be activated by glycine even in the absence of glutamate or NMDA (Meguro et al. 1992; Pace et al. 1992). Recent studies using heterologous expression of NR1-NR3A or B receptors demonstrated that these combinations of subunits produced a receptor that responds to glycine but not to glutamate or NMDA and can be inhibited by D-serine. These receptors are relatively impermeable to Ca^{2+} and resistant to Mg^{+} blockade. The prototypical NMDA antagonists including D-2-amino-5-phosphonovaleric acid, ifenprodil, memantine, (5*R*,10*S*)-(_)-5-methyl-10,11-dihydro-5*H*-dibenzo[*a*,*d*]cyclohepten-5,10-imine hydrogen maleate (MK-801) or acamprosate did not inhibit glycine-activated NR1/NR3A/NR3B receptors (Chatterton et al. 2002; Smothers and Woodward 2007). According to some authors these data suggest the existence of excitatory glycine receptors in the brain with novel functional and pharmacological properties (Barth et al. 2005; Smothers and Woodward 2007).

The brain areas that express glycine transporters and NMDA receptors are greatly overlapping. Interestingly, Chen et al. (2003) demonstrated by electrophysiological experiments that the inhibition of GLYT1s increases the activation of NMDA receptors. This implies that the concentration of glycine in the glutamate synaptic cleft is tightly controlled by glycine transporters (Aragon and Lopez-Corcuera 2005).

Glycine and GABA co-release

The co-release of GABA and glycine by the same presynaptic terminal was described in cerebellar Golgi cells (Dumoulin et al. 2001), in the spinal cord (Sagne et al. 1997; Supplisson and Bergman 1997) and in the superior olivary complex (Smith et al. 2000). However, very little is known about possible interactions between the two neurotransmitters at the post-synaptic level, a field that should receive more attention. In the same fashion as glutamate/glycine interactions and co-agonistic interplay, glycine may well interact with other neurotransmitters and offer a better explanation to still obscure phenomena like those involving GABA agonists and antagonists and their role in anxiety and depression.

Alcohol and anesthetics

The mechanism of action of alcohol and general anesthetics involve glycine neurotransmission, as reviewed by many authors (Vengeliene et al. 2008; Ye 2008; Zeller et al. 2008). Although a subject for many investigations, there is no consensus about the ultimate molecular mechanism of anesthesia or any volatiles including alcohol. Since the objective of this review is to raise the attention of researchers to the potential stimulatory actions of glycine and considering the numerous reviews on anesthetics molecular actions, we will not explore this specific subject in this review and only a few considerations will be made on the actions of alcohol as a prototype drug.

Celentano et al. (1988) first demonstrated that exposure to pharmacologically relevant (50–100 mM) concentrations of alcohol potentiated glycine-activated chloride currents in chick spinal cord neurons. Ethanol is able to increase the amplitude of glycine-induced current (I_{Gly}) by apparently changing the receptor affinity for glycine (Aguayo and Pancetti 1994). Despite the fact that ethanol increases the amplitude of the synaptic current in mature neurons, experiments in immature cultured spinal neurons showed that GlyRs have not been affected by 40 mM of ethanol (Tapia and Aguayo 1998). Analogously, neonate hypoglossal motoneurons (P1-3) exposed to 30 mM ethanol did not significantly change the spontaneous miniature inhibitory post-synaptic currents (Eggers et al. 2000). These findings support the idea that the receptor affinity for alcohol depends on neural development. Apparently, the adult form of the native GlyR is more sensitive to alcohol than the fetal form.

According to Brown (1994), G proteins are able to open ion channels directly without employing second messengers. Neurochemical methods and electrophysiological recordings showed the involvement of G proteins in alcohol–GlyRs interaction. In isolated ventral tegmental area neurons, for example, G proteins are involved in the function of GlyRs and in the alcohol potentiation of these receptors. In this study, when G proteins were maximally activated by GTP-g-S [guanosine-5V-0-(2-thiotriphosphate)] 2 mM, the action of alcohol was partially occluded, indicating that some other factors, in addition to G proteins, may also contribute to the action of alcohol on GlyRs (Zhu and Ye 2005).

Alcohol seems to be a rather non-specific agent and only a few targets have been positively related to its action

(Davies et al. 2004; Yevenes et al. 2006; Crawford et al. 2007). These are the NMDA, GABAA, glycine, 5-hydroxytryptamine 3 (serotonin) and nicotinic acetylcholine receptors as well as L-type Ca^{2+} channels and G-protein-activated inwardly rectifying K^+ channels (Vengeliene et al. 2008). To date, it is also known that GlyRs containing the α1 subunit seem to be more sensitive to low concentrations of alcohol than those containing the α2 (Mascia et al. 1996). Some studies showed that serine-267 (S267) and alanine-288 (A288) in TM segments 2 and 3 of the α1-GlyR subunit are important for allosteric modulation by alcohol (Mihic et al. 1997). In the same way, as increased atmospheric pressure antagonizes alcohol effects, recent studies have used pressure to identify alcohol targets in GlyRs (Davies et al. 2003, 2004). It was reported that A52S mutation eliminated the sensitivity of α1-GlyRs to a direct alcohol antagonist, suggesting that the extracellular domain holds the target for alcohol action (Davies et al. 2004). Basically the same research group showed recently that pressure counteracted alcohol effects in α1-GlyRs that contains a non-polar residue at position 52, but did not antagonize alcohol effects in receptors with a polar residue at this position, indicating that polarity at this position plays a key role in determining sensitivity to alcohol and pressure antagonism of alcohol (Perkins et al. 2008). According to Crawford et al. (2007) these studies suggest that there are multiple sites for alcohol action in α1-GlyRs, with at least one site located in the TM domain (e.g., position 267) and another one in the extracellular domain (e.g., position 52).

Conclusions

Considering the above, the potential involvement of glycine in forebrain functioning and modulation of numerous neuronal circuits may have been overlooked. Neurotransmission employing GABA may employ glycine as a co-transmitter as already described for glutamate. Also, glycine may well be a principal transmitter in the forebrain with excitatory function besides the well-known spinal inhibitory role. Also, the possible involvement of glycine receptors in alcohol and anesthetic's acute effects suggest a major involvement in neural transmission, consciousness and higher level integrative functions. It could be particularly interesting to investigate the involvement of glycine in learning and memory and motor functions, as well as its involvement in neurodegenerative diseases such as Parkinson's and Alzheimer's as potential sites of pharmacological intervention may arise.

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