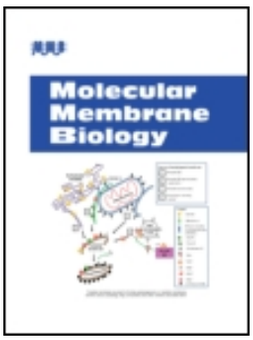

Molecular Membrane Biology

ISSN: 0968-7688 (Print) 1464-5203 (Online) Journal homepage: https://www.tandfonline.com/loi/imbc20

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To cite this article: Beatriz López-Corcuera, Arjan Geerlings & Carmen Aragón (2001) Glycine neurotransmitter transporters: an update, Molecular Membrane Biology, 18:1, 13-20, DOI: 10.1080/09687680010028762

To link to this article: https://doi.org/10.1080/09687680010028762

Molecular Membrane Biology, 2001, 18, 13–20

Glycine neurotransmitter transporters: an update

Beatriz López-Corcuera, Arjan Geerlings and Carmen Aragón*

Departamento de Biología Molecular, Centro de Biología Molecular 'Severo Ochoa', Facultad de Ciencias, Universidad Autónoma de Madrid, 28049-Madrid, Spain

Summary

Glycine accomplishes several functions as a transmitter in the central nervous system (CNS). As an inhibitory neurotransmitter, it participates in the processing of motor and sensory information that permits movement, vision, and audition. This action of glycine is mediated by the strychnine-sensitive glycine receptor, whose activation produces inhibitory post-synaptic potentials. In some areas of the CNS, glycine seems to be co-released with GABA, the main inhibitory amino acid neurotransmitter. In addition, glycine modulates excitatory neurotransmission by potentiating the action of glutamate at N-methyl-D-aspartate (NMDA) receptors. It is believed that the termination of the different synaptic actions of glycine is produced by rapid re-uptake through two sodium-and-chloride-coupled transporters, GLYT1 and GLYT2, located in the plasma membrane of glial cells or pre-synaptic terminals, respectively. Glycine transporters may become major targets for therapeutic of pathological alterations in synaptic function. This article reviews recent progress on the study of the molecular heterogeneity, localization, function, structure, regulation and pharmacology of the glycine transporter proteins.

Keywords: Glycinergic neurotransmission, glycine transport, NMDA receptor, central nervous system, transporter function.

Introduction

Glycine, the smallest amino acid, plays an important role as a transmitter in the vertebrate central nervous system (CNS) where several functions are accomplished by this molecule. At glycinergic pathways of caudal brain, spinal cord and retina, glycine acts as a classic inhibitory neurotransmitter and participates in the processing of motor and sensory information that permits movement, vision, and audition (Werman *et al.* 1967, Aprison and Daly 1978, Aprison 1990, Cortes and Palacios 1990, Daly 1990, Pourcho and Goebel 1990, Wenthold and Hunter 1990). This glycine-mediated function involves storage of the transmitter in synaptic vesicles (Sagné *et al.* 1997, Chaudhry *et al.* 1998, Domoulin *et al.* 1999), release following neuron depolarization (Jonas *et al.* 1998), and glycine binding to a specific ligand-gated Cl^- channel receptor on the post-synaptic cell. Glycine-induced channel opening generates an inhibitory post-synaptic potential that is antagonized by strychnine (Werman *et al.* 1967). In some areas of the CNS, glycine seems to be co-released with GABA, the main inhibitory transmitter amino acid, with which glycine shares a common vesicular transporter that accumulates the amino acids into synaptic vesicles (McIntire *et al.* 1997, Sagné *et al.* 1997, Jonas *et al.* 1998, O'Brien and Berger 1999). The synaptic action of glycine ends by reducing extracellular transmitter concentrations, a task that is achieved by specific high-affinity transporters located in neuronal and glial plasma membranes (Neal and Pickles 1969). The reuptake of glycine is an active process energized by the electrochemical gradient of sodium through the plasma membrane that is maintained by the Na^+-K^+ ATPase. The process is also chloride-dependent. Glycine transport into pre-synaptic terminals or surrounding glial processes permits glycine accumulation against its concentration gradient and controls the availability of neurotransmitter in the synaptic cleft (Kuhar and Zarbin 1978).

Besides its inhibitory action, glycine exerts a positive modulation on excitatory glutamatergic neurotransmission. Although a vesicular glycine release seems not to take place at glutamatergic synapses, glycine behaves as a necessary coagonist of glutamate on N-methyl-D-aspartate (NMDA) receptors (Johnson and Ascher 1987), and externally applied glycine has been found to potentiate NMDA-evoked neuronal activity both *in vitro* and *in vivo* (Salt 1989, Budai *et al.* 1992, Thiels *et al.* 1992). Glycine binding to its strychnine-insensitive site on the NMDA receptor, allosterically enhances glutamate binding and reduces antagonists binding to the glutamate recognition site. The binding site of glycine on the NMDA receptor exhibits its own pharmacological profile, being sensitive to several agonists such as (D)-serine and antagonists as HA-966. This site is also subjected to allosteric regulation by polyamines and glutamate itself (Leeson and Iverson 1994). Plasma membrane glycine transporters play a critical role in the control of extracellular glycine concentrations below saturating levels for the NMDA receptor glycine site (Berger *et al.* 1998, Bergeron *et al.* 1998). Additionally, Na^+-and-Cl^- coupled glycine transporters may, in certain conditions, pump transmitter out of the cells and serve as a calcium-independent non-vesicular mechanism for neurotransmitter release (Adam-Vizi 1992, Attwell *et al.* 1993). This mechanism might be especially relevant at glutamatergic synapses where neither glycine vesicular release nor glycine vesicular transporter have been found (Chaudhry *et al.* 1998).

Since glycine exerts multiple functions in central neurotransmission, compounds able to potentiate glycine-mediated actions may find applications in the treatment of psychiatric diseases and spasticity (Krystal and D'Souza 1998). Hypofunction of glutamatergic (NMDA receptor-mediated) transmission has been proven to be involved in symptoms of schizophrenia (Tsai *et al.* 1998). It is also likely that a decrease in the glycinergic inputs is involved in pathologies of the muscle tone regulation (Floeter and Hallet 1993, Simpson *et al.* 1995). Therefore, a new role for the glycine transporters as targets of future therapeutic drugs is now emerging.

*To whom correspondence should be addressed.
e-mail: caragon@cbm.uam.es

Molecular Membrane Biology
ISSN 0968-7688 print/ISSN 1464-5203 online © 2001 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/09687680010028762

Within this context, the study of the molecular properties of the plasma membrane glycine transporters may reveal interesting structural, functional, pharmacological or regulatory characteristics that may help to understand the physiology and pathologies of glycine synaptic action.

Biochemical studies on plasma membrane glycine transporters

A number of research groups contributed to the functional identification of high-affinity active transport systems for glycine in nerve terminals and glial cells. Several aspects of the transporters' activity were known prior to their molecular cloning by using brain-derived preparations (Johnston and Iversen 1971, Logan and Snyder 1972, Kuhar and Zarbin 1978, Mayor *et al.* 1981, Zafra and Giménez 1986, Fedele and Foster 1992). The study of their Na^+- and Cl^--dependence, stoichiometry, electrogenicity, and pharmacology (Aragón *et al.* 1987, Zafra and Giménez 1988, 1989), ran parallel to the discovery of glycine transporter heterogeneity (Johnston and Iversen 1971, Logan and Snyder 1972). In the early 1990s, a glycine transporter from pig brain stem was solubilized, reconstituted and purified to apparent homogeneity by this group (López-Corcuera and Aragón 1989, López-Corcuera *et al.* 1989, 1991, 1993, Alcántara *et al.* 1991). This was the first direct evidence indicating the existence of several glycine transporter subtypes. The purified transporter was obtained by sequential chromatography on phenyl-Sepharose, wheat germ agglutinin-Sepharose, and hydroxylapatite columns, followed by a 5–20% sucrose gradient fractionation. After reconstitution into liposomes, the purified transporter retained all the functional properties of the native high-affinity glycine transport. However, it was resistant to the inhibition by sarcosine (*N-methyl glycine*), the only high-affinity glycine uptake inhibitor which was then known. This indicated that a single subtype was isolated, which subsequently proved to be GLYT2. The structural analysis of the carbohydrate moiety of the purified glycine transporter revealed that sugar chains represent ~ 30% of their 100 kDa apparent molecular mass and are predominantly tri- or tetra-antennary complex *N*-linked oligosaccharides containing sialic acid residues (Núñez and Aragón 1994, Zafra *et al.* 1997, Aragón and López-Corcuera 1998).

Two genes encoding glycine transporters (GLYT1 and GLYT2) were cloned (Guastella *et al.* 1992, Liu *et al.* 1992a, 1993a, Smith *et al.* 1992), taking advantage of their sequence similarity with the GABA transporter GAT1, the first neurotransmitter transporter whose primary structure was known (Guastella *et al.* 1990). Hence, GLYT1 and GLYT2 belong to the Na^+- and Cl^--dependent neurotransmitter transporter superfamily (Pacholczyk *et al.* 1991, Liu *et al.* 1992b, 1993b, c, López-Corcuera *et al.* 1992, uhl and Hartig, 1992, Amara and Kuhar 1993, Kanai *et al.* 1994, Malandro and Kilberg 1996), a group of glycoproteins that includes transporters for GABA, monoamines, proline and glycine, among others (for a review see Nelson 1998). These proteins present a common structure with 12 transmembrane domains and amino and carboxyl-terminal ends intracellularly oriented (Olivares *et al.* 1994, Zafra *et al.* 1995a). GLYT1 and GLYT2 share 48% identity at the amino acid sequence level and 50% identity with the proline transporter (PROT). Together with the latter, glycine transporters have been grouped in the subfamily of amino acid transporters within the superfamily. The molecular cloning of the two glycine transporter genes opened a new research area and allowed one to study their localization, pharmacology, regulation and structure-function relationships.

Molecular heterogeneity of glycine transporters

GLYT1 has been cloned from different sources including human, rat and mouse (Guastella *et al.* 1992, Liu *et al.* 1992a, 1993a, Smith *et al.* 1992, Kim *et al.* 1994). This transporter presents several isoforms that, in the retina, have been termed GLYT1a, GLYT1b, GLYT1e and GLYT1f. These variants result from the combination of different amino terminal ends (1a and 1b) (Borowsky *et al.* 1993, Liu *et al.* 1993a, Kim *et al.* 1994, Adams *et al.* 1995), originated from transcription initiated at alternate promoters (Borowsky and Hoffman 1998), and different carboxylterminal ends (1D and 1E; Hanley *et al.* 2000), produced by alternative splicing, with the same protein core. Alternative splicing also produces another *N*-terminal end (1c) not found in retina cells (Adams *et al.* 1995). Therefore, GLYT1a and GLYT1b contain *C*-terminal D in conjunction with *N*-terminal a or b, respectively, and GLYT1e and GLYT1f contain *C*-terminal E in conjunction with *N*-terminal a or b, respectively (Hanley *et al.* 2000). Variation in the *N*-terminal domain of these GLYT1 proteins had no apparent effect in their uptake properties. This has been confirmed by experiments in which the differential amino-termini were deleted by site-directed mutagenesis and the resultant truncated proteins retained the kinetics and energetic parameters of the intact transporter (Olivares *et al.* 1994). However, the *C*-terminal domain of GLYT1 transporter might be involved in protein–protein interactions, since the recently identified *C*-terminal end 1E has been reported to interact with GABA receptor ρ1 subunit (Hanley *et al.* 2000).

The GLYT2 transporter is generated from a separate gene and has been cloned from rat and human sources (Liu *et al.* 1993a, Morrow *et al.* 1998, Gallagher *et al.* 1999). Rat and human proteins exhibit 93% amino acid sequence identity, the greatest divergence being found at the amino terminus, where only 74% of the amino acids are conserved among residues 1–190 (Gallagher *et al.* 1999). The human GLYT2 gene has been localized on the short arm of chromosome 11 (11p15.1–15.2) (Morrow *et al.* 1998).

The rat GLYT2 also presents some degree of heterogeneity. Two alternative spliced isoforms termed GLYT2a and GLYT2b have been described by using rapid amplification of GLYT2 cDNA ends (RACE) on rat brain DNA. The GLYT2b isoform presents five amino acids more at the *N*-terminal region than the previously identified GLYT2a (Liu *et al.* 1993a, Ponce *et al.* 1998). As in the case of the GLYT1 variants, no significant kinetic or energetic differences were detected between GLYT2 isoforms. More experiments have to follow to understand the role of the different amino termini of the GLYT2 transporter.

Distribution pattern of glycine transporters

The detailed anatomical localization of GLYT1 and GLYT2 in the CNS has been performed by using a list of immunological and molecular techniques such as immunocytochemistry, Northern and Western blotting and *in situ* hybridization (Guastella *et al*. 1992, Liu *et al*. 1992a, Smith *et al*. 1992, Borowsky *et al*. 1993, Adams *et al*. 1995, Zafra *et al*. 1995a, b). GLYT1 is more abundant in glycinergic areas. However, areas devoid of inhibitory glycinergic neurotransmission show considerable levels of the transporter. Recent electrophysiological and pharmacological observations have reinforced the possibility that GLYT1 plays a critical role in the control of extracellular glycine concentrations at the NMDA-mediated glutamatergic terminals (Smith *et al*. 1992, Supplisson and Bergman 1997, Berger *et al*. 1998, Bergeron *et al*. 1998). Light and electron microscopy have been used to study the cellular distribution of GLYT1. This transporter is mainly a glial isoform localized in astrocyte-like pericarya and glial processes of the spinal cord, pons/medulla, and midbrain areas. It is also present in glial cells of the thalamus, hypothalamus and olfactory bulb (Zafra *et al*. 1995a). In addition, the regional and developmental pattern of expression of the GLYT1 variants GLYT1a and GLYT1b have been shown to be distinct, with GLYT1a being expressed in gray matter and GLYT1b in white matter (Borowsky *et al*. 1993, Adams *et al*. 1995). Although the distribution of the recently identified isoforms GLYT1e and GLYT1f have not been studied yet, it is possible that they represent the atypical neuronal form of GLYT1 that is present in amacrine neurons of the retina and other CNS areas (Smith *et al*. 1992, Borowsky *et al*. 1993, Zafra *et al*. 1995a).

By contrast to GLYT1, GLYT2 is exclusively expressed in glycinergic CNS regions containing the strychnine-sensitive glycine receptor, the highest levels being found in the dorsal and ventral horn of the spinal cord, in the auditory system, and in the nuclei of the cranial nerves. Therefore, GLYT2 is abundant in caudal areas of the brain (Luque *et al*. 1995, Zafra *et al*. 1995a, b), and its distribution is parallel to that of the glycine receptor, as proved by different techniques like immunocytochemistry (Araki *et al*. 1988, van den Pol and Gorcs 1988, Wenthold *et al*. 1988) and strychnine binding (Zarbin *et al*. 1981, Jursky and Nelson 1995). At the level of cellular distribution, GLYT2 is a neuronal transporter found almost exclusively in axons (Zafra *et al*. 1995a). GLYT2 has been visualized by electron microscopy in the plasma membrane of the axonal boutons in spinal dorsal horn (Spike *et al*. 1997). The transporter seems to be present in discrete patches outside the active zone, suggesting an interaction of GLYT2 with cytoskeletal elements. From the experimental point of view, a new role for GLYT2 as a biochemical tool has been reported, since double-immunofluorescence experiments showed a perfect co-localization of GLYT2 and glycine immunoreactivities, indicating that this transporter can be used as a marker for glycine-immunoreactive neurons (Poyatos *et al*. 1997).

An asymmetric subcellular distribution of GLYT1 isoforms has been described upon heterologous expression of GLYT1a or GLYT1b in polarized MDCK cells and hippocampal neurons (Poyatos *et al*. 2000). GLYT1a shows an apical localization in epithelial cells, whereas GLYT1b exhibits a basolateral distribution in the same cellular system. However, both variants behave in neurons as somatodendritic proteins. The reason for such discrepancy is presently unknown, but seems to be related to cell-type-specific requirements for protein distribution. The topological determinants of GLYT1a and GLYT1b sorting have been mapped by the use of site-directed mutagenesis. The differential amino termini of GLYT1 and two dileucine motifs located in the 1D carboxyl tail of this protein seem to accommodate the sorting signals. This is in agreement with a previous observation showing that the *C*-terminal region of GLYT1 was necessary to avoid retention of the protein in the endoplasmic reticulum (Olivares *et al*. 1994).

Conversely to GLYT1b, GLYT2 was strongly apical in MDCK cells and not polarized in transfected neurons. No differences in the sorting of the GLYT2 variants a and b were detected. The use of GLYT2 *N*- or *C*-terminal deletion mutants allowed one to discard these two regions of the GLYT2 transporter as areas containing the apical localization signals (Poyatos *et al*. 2000). Recent experimental work has allowed the identification of the determinants of GLYT2 sorting. For this transporter, apical distribution seems to be directed by the carbohydrate moiety (Martínez-Maza *et al*. 2001).

Functional differences of glycine transporter isoforms

By using a stable expression system on HEK-293 cells, the functional properties of the two brain-specific glycine transporters, GLYT1b and GLYT2a, were compared utilizing biochemical and electrophysiological techniques (López-Corcuera *et al*. 1998). Replacement of external Na^+ and Cl^- by isotonic choline and acetate, respectively, allowed the examination of the ionic dependence of glycine transport on the stable cells. The two glycine transporters require one Cl^- ion in their transport cycles, but their apparent Cl^- affinities are different. GLYT1b has at least a three times higher affinity for this ion than GLYT2a. The Na^+ dependence of glycine uptake followed a sigmoidal curve in the two cases, indicating that more than one Na^+ ion is involved in the transport cycles. From these results, a higher Na^+ coupling of GLYT2 was deduced, since non-linear regression analysis of the experimental data yielded the best fit, assuming a 2:1 stoichiometry for GLYT1 but a 3:1 for GLYT2 (López-Corcuera *et al*. 1998, Núñez *et al*. 2000a). In this stable expression system, the number of expressed transporters was sufficient to allow electrophysiological recordings of the uptake current. The voltage dependence of GLYT2 was higher in both its glycine-evoked current and its capacitive current recorded in the absence of substrate. These differences are indeed compatible with a higher Na^+ coupling of the neuronal GLYT2 than by the glial GLYT1. The different stoichiometry of the glycine transporters has recently been proved in a report where electrophysiological and radiotracing techniques were combined (Roux and Supplisson 2000). Therefore, GLYT2a has a stoichiometry of 3 Na^+/1 Cl^-/1 glycine, which predicts effective glycine accumulation in all physiological conditions. Yet, GLYT1b presents a 2 Na^+/1 Cl^-/1 glycine stoichiometry, which predicts that glycine

can be exported or imported, depending on the ionic or electrical environment.

Glycine release mediated by GLYT1 was also studied in HEK-293 cells that stably expressed the transporter. Although efflux and exchange of glycine through the transporters were previously demonstrated in brain-derived preparations (Aragón and Giménez 1986, López-Corcuera *et al.* 1989), in this work the ionic requirements of GLYT1-mediated release were investigated. An increase in transporter-mediated release after the removal of external Na^+, Cl^-, Ca^{2+} or K^+ was observed. The importance of this mechanism in the modulation of the NMDA receptor activity was discussed by the authors (Sakata *et al.* 1997).

All these observations support the role of GLYT1b in the modulation of glycine concentrations around receptors in glutamatergic NMDA-mediated synapses. The observed functional differences between GLYT1 and GLYT2 indicate that glycine transporters are involved in complementary duties: phasic synaptic uptake for the neuronal form (GLYT2a) and tonic homeostatic uptake for the glial isoform (GLYT1b).

Structure-function studies on glycine transporters

The structure and the topology of the glycine transporters have also been studied. By using GLYT1 as a paradigm of the Na^+- and Cl^--coupled neurotransmitter transporters, the 12 transmembrane domain structural model that was predicted for the family has been tested by two complementary methods. As the first approach, the introduction of glycosylation consensus sequences along the entire length of the protein was used as a reporter for an extracellular location. The second approach was an *in vitro* transcription/translation assay where the different GLYT1 segments were placed in a vector before a glycosylation reporter gene whose glycosylation state allowed the analysis of the topogenic properties of the tested fragment. The obtained data confirmed the theoretical model, but in the amino-terminal third of the protein, an important rearrangement was proposed (Olivares *et al.* 1997). The modelling of the amino terminal region of the neurotransmitter transporters is a question that for some time remained controversial (Bennett and Kanner 1997, Olivares *et al.* 1997). However, the originally proposed topology has recently been confirmed by chemical labelling of extracellular residues on the serotonin transporter (Chen *et al.* 1998).

Glycine transporters are proteins containing *N*-linked carbohydrates. A study was performed to investigate the role of GLYT1 *N*-glycosylation by analysing the effects of *N*-glycosylation site removal on protein size and glycine transport activity. The four potential glycosylation sites present on the second hydrophilic loop of GLYT1 seem to indeed be used. Moreover, since the totally unglycosylated GLYT1 was not able to reach the plasma membrane, sugar chains seem to be necessary for trafficking of the protein to the plasma membrane. However, no involvement of the oligosaccharides in transport activity was concluded (Olivares *et al.* 1995).

Recently, the first study about the structure-function relationship of the GLYT2 transporter was published (Ponce *et al.* 2000). The importance of transmembrane domain III in the mechanism of transport that was demonstrated for other transporters of the family (Bismuth *et al.* 1997, Chen *et al.* 1997, Chen and Rudnick 1999) was stressed by these results. It was demonstrated that tyrosine at position 289 of GLYT2a (in this transmembrane domain) is crucial for ion coupling, glycine affinity and sodium selectivity. This residue was changed by site-directed mutagenesis and even conservative substitutions rendered GLYT2 unable to catalyze glycine uptake. However, glycine evoked steady-state currents could be measured in HEK-293 cells expressing the point mutants by using the whole cell patch clamp technique. This allowed the study of the functional effects of tyrosine 289 substitutions. Tyrosine 289 mutants showed decreased apparent affinity for glycine and severely altered sodium dependence, both at the level of apparent affinity and cooperativity. Accordingly, sodium selectivity was lost and chloride dependence was decreased upon tyrosine 289 modification. These results support the hypothesis of transmembrane domain III being part of a common permeation pathway for substrate and cotransported ions.

The critical role for a transport function that transmembrane domain III seems to play, has centred the investigations on the NH2-terminal third of the glycine transporters. In a subsequent work, the functional involvement of the first hydrophilic loop (EL1), a short protein stretch contained in this region, has been analysed by using the scanning cysteine accessibility method. The sensitivity to small, charged sulphydryl-specific methanethiosulphonate (MTS) derivatives of a GLYT2a-EL1 chimera, constructed by replacing the EL1 region of GLYT2a with the corresponding sequence of GLYT1b, and of three cysteine-substituted mutants of single positions within the EL1 region of GLYT2a, was investigated. The conclusion of this work was that Cys62 of GLYT1b and the Cys introduced in the homologous position of GLYT2a (position 223) are responsible for the inhibition of transport activity produced by extracellular MTS derivatives. Furthermore, cotransported ions and substrates decreased cysteine sensitivity to the reagents. Thus, these residues, or the loop in which they are located, may participate in the conformational rearrangements associated with substrate translocation (Lòpez-Corcuera *et al.* submitted).

The study of the *N*-glycosylation of GLYT2 and the role of this protein modification in glycine transport activity, intracellular trafficking and neuronal sorting has recently been investigated. The necessity of the carbohydrate moiety for intracellular trafficking of the protein, as previously proved for GLYT1, was also demonstrated for GLYT2. However, a new role of the sugar moiety, not previously reported for a transporter, as a determinant of the GLYT2 apical sorting, has been discovered (Martínez-Maza *et al.* 2001).

Future investigations in the field of structure-function relationships of the glycine transporters will be addressed to the mapping of inhibitor binding sites through the study of the functional properties exhibited by point mutants and GLYT1-GLYT2 chimeric transporters.

Regulation of glycine transporters

The regulation of the glycine transporters activity is a central aspect of their physiology, although much less experimental work has been done in this field. A dose and time-dependent inhibition of GLYT1 activity by the PKC activator phorbol 1-myristate 13-acetate (PMA) in several experimental systems was reported (Gomeza *et al.* 1995). Although these data suggest that glycine transport can be regulated by second messenger systems, the mode of regulation remains to be elucidated.

By using mixed cultures of glial and neuronal cells, a neuronal dependency of the glycine transporter GLYT1 expression in glial cells has been observed. The possible factors involved in the cross-talk between neurons and glial cells have not been investigated (Zafra *et al.* 1997).

Following the pioneering studies by Quick and coworkers, who described a regulatory interaction between the SNARE protein syntaxin1A and the GABA transporter GAT1 (Beckman *et al.* 1998, Deken *et al.* 2000), a possible regulation of the glycine transporters GLYT1 and GLYT2 by syntaxin 1A has been described (Geerlings *et al.* 2000). The two transporters show a functional and physical interaction with syntaxin 1A. This was demonstrated upon co-expression of syntaxin 1A and GLYT2 (or GLYT1), since an inhibition of glycine uptake was observed in co-transfected cells. Additionally, glycine transporters and syntaxin were co-immunoprecipitated both from transfected cells and brain tissue. Syntaxin 1A-mediated glycine uptake modulation was prevented by the syntaxin 1A-binding protein Munc 18, thus confirming the specificity of this effect. In addition, syntaxin 1A seems to control the number of glycine transporters on the plasma membrane, as revealed by biotinylation experiments. This effect is also reversed by Munc 18 (Geerlings *et al.* 2000).

Recently, it has been demonstrated for the first time that syntaxin is involved in the trafficking of a transporter. It is, however, only involved in the arrival of GLYT2 to the plasma membrane and not in its retrieval. GLYT2 is subjected to a fast regulation mechanism initiated by calcium and, actually, it seems that GLYT2 behaves in a similar way as do some other synaptic proteins that are trafficked and regulated by calcium. This points to the idea that GLYT2 is being transported by synaptic vesicles, or some other similar vesicles. To solve this question, synaptic vesicles were isolated from the rat brain and, by histochemical studies, a GLYT2 localization in small synaptic vesicles was demonstrated (Geerlings *et al.* submitted).

All these new results shed light on the regulation and trafficking of neurotransmitter transporters and, perhaps, open new ways for pharmacological intervention in the duration of the neurotransmission signal.

Pharmacology of glycine transporters

As mentioned before, compounds able to modulate glycine-mediated neurotransmission may find applications in the treatment of psychiatric diseases. Consequently, the potential of the glycine transporters as targets of pharmacological manipulations is clear. However, the pharmacology of the glycine transporters is in its infancy. GLYT1 and GLYT2 activities can be pharmacologically distinguished by the sensitivity of the former to sarcosine (López-Corcuera *et al.* 1989). More recently, a novel GLYT1-specific inhibitor, N [3-(4′-fluorophenyl)-3-(4′phenylphenoxy) propyl] sarcosine (NFPS), has been developed and used to study the contribution of GLYT1 to the modulation of the NMDA receptor function (Bergeron *et al.* 1998). Hence, one area of interest is the study of the pharmacological profile of the glycine transporter isoforms.

Recently, the effects were tested of several tricyclic antidepressant drugs on the glycine uptake mediated by GLYT1b and GLYT2a in a stable expression system (Núñez *et al.* 2000a). A selective inhibition of GLYT2a by amoxapine was observed. This compound behaved as a 10-fold more efficient inhibitor of this isoform than of GLYT1b, and represents the first GLYT2-specific inhibitor described so far. Amoxapine behaved as a competitive inhibitor of both glycine and chloride and a mixed type inhibitor with respect to sodium. A kinetic model was developed to explain the experimental data obtained after a detailed kinetic analysis of the mechanism of amoxapine inhibition on GLYT2a. As predicted by the adopted kinetic model, the simultaneous binding of amoxapine and either glycine or chloride to the transporter is not possible. A complete or partial overlap between amoxapine, glycine and chloride binding sites may explain this observation. However, the results do not rule out the possibility of a conformational change of transporter upon inhibitor binding that prevents binding of chloride or glycine. Differences in chloride affinity between GLYT1 and GLYT2 were previously described (López-Corcuera *et al.* 1998). The different sensitivity of the transporters to amoxapine may suggest a Cl^- binding-site-related structural difference between the two transporter isoforms. The identification of the structural determinants of this and other isoform-specific functional properties may arise from the construction and analysis of chimeric proteins between these two transporters. As also predicted by the kinetic model adopted to explain the results of this work, ternary complexes between Na^+, inhibitor and transporter, may exist, therefore suggesting that Na^+ may bind to the glycine transporter independently of Cl^- and glycine.

The effects of several alkanols, including ethanol, on the function of stably expressed GLYT1b and GLYT2a were investigated in a recent study (Núñez *et al.* 2000b). A differential behaviour in the presence of ethanol at clinically relevant concentrations was exhibited by the two glycine transporters. GLYT2 was allosterically modulated by ethanol when acutely administered to the stable cell lines, although no effect was observed on GLYT1. A direct interaction of alcohols with a discrete site on GLYT2a was suggested. Alternatively, a protein specifically interacting with this glycine transporter may be the target to which alcohols may access from within the membrane. The alcohol-binding pocket in the protein could accommodate alkyl chains of at least four methylene groups, as deduced from 'cut-off' experiments. Another aspect of this work dealt with the effects of chronic administration. Chronic treatment with ethanol caused differential adaptive responses on these transporters. A decrease in activity and surface expression

was observed for the neuronal GLYT2a isoform, whereas the mainly glial GLYT1b slightly increased in function and surface density. These results demonstrated for the first time that acute and chronic effects of ethanol might modulate expression and activity of the glycine transporters. Together with the changes described for other neurotransmitter systems, these changes may support some of the effects observed in glycine-mediated neurotransmission under ethanol intoxication. The mechanisms underlying these changes may be revealed on further studies using brain-derived preparations.

Conclusions and perspectives

The molecular cloning of the two plasma membrane glycine transporter genes GLYT1 and GLYT2 opened an interesting research area for studying the molecular aspects of these proteins. The glycine transporters mediate the rapid reuptake of glycine at the inhibitory glycinergic synapses and may contribute to the modulation of NMDA receptor function in glutamatergic neurotransmission. The involvement of these proteins in the multiple actions of glycine supports their potential use as major targets for therapeutic actions of glycine synaptic function. The initial picture of the structure and functionality of these carriers emerged during the last few years. However, still several areas are uncovered. For instance, the pharmacology of glycine transporters is still lacking. Its regulation will provide promising directions for future research regarding the organization of the transporters in the pre-synaptic membrane, its possible interaction with modulatory or structural elements, and its contribution to the mechanism of glycine release. Other challenges are the knowledge of primary and secondary structures of these proteins at the atomic level, as well as the dissection of the binding sites for substrates and modulators. The molecular study of the plasma membrane glycine transporters will help to understand the physiology of glycine synaptic action and will sustain the development of new therapeutic approaches for glycine-mediated neurotransmission pathologies.

Acknowledgements

We are grateful for financial support received from the Spanish Dirección General de Investigación Científica y Técnica (PM95-0026 and PM98-0013), the BIOMED program of the European Union (BMH1-CT93-1110 and BMH4-CT95-0571), the European Union TMR Program (FMRX-CT98-0228), and an institutional grant from the Fundación Ramón Areces.

References

Adams, R. H., Sato, K., Shimada, S., Tohyama, M., Püschel, A. W. and Betz, H., 1995, Gene structure and glial expression of the glycine transporter GLYT1 in embryonic and adult rodents. *Journal of Neuroscience*, 15, 2524–2532.

Adam-Vizi, V., 1992, External Ca2+-independent release of neurotransmitters. *Journal of Neurochemistry*, 58, 395–405.

Alcantara, R., López-Corcuera, B. and Aragón, C., 1991, Group-selective reagent modification of the sodium-and chloride-coupled glycine transporter under native and reconstituted conditions. *Biochimica et Biophysica Acta*, **1067**, 64–70.

Amara, S. G. and Kuhar, M. J., 1993, Neurotransmitter transporters: recent progress. *Annual Review in Neuroscience*, **16**, 73–93.

Aprison, M. H., 1990, The discovery of the neurotransmitter role of glycine; In *Glycine Neurotransmission*, O. P. Ottersen and J. Storm-Mathisen, eds (New York: John Wiley and Sons), pp. 1—24.

Aprison, M. H. and Daly, E. C., 1978, Biochemical aspects of transmission at inhibitory synapses; the role of glycine. *Advances in Neurochemistry*, **3**, 203–295.

Aragón, C. and Giménez, C., 1986, Efflux and exchange of glycine by synaptic plasma membrane vesicles derived from rat brain. *Biochimica et Biophysica Acta*, **855**, 257–264.

Aragón, C. and López-Corcuera, B., 1998, Purification, hydrodynamic properties and glycosylation analysis of glycine transporters. In *Methods in Enzymology*, S. G. Amara, ed. (London: Academic Press), **296**, pp. 1–17.

Aragón, C., Giménez, C. and Mayor, F., 1987, Stoichiometry of sodium- and chloride-coupled glycine transport in synaptic plasma membrane vesicles derived from rat brain. *FEBS Letters*, **212**, 87–90.

Araki, T., Yamano, M., Murakami, T., Wanaka, A., Betz, H. and Tohyama, M., 1988, Localization of glycine receptor in the rat central nervous system: an immunocytochemical analysis using monoclonal antibody. *Neuroscience*, **25**, 613–624.

Attwell, D., Barbour, B. and Szatkowski, M., 1993, Nonvesicular release of neurotransmitter. *Neuron*, **11**, 401–407.

Beckman, M. L., Bernstein, E. M. and Quick, M. W., 1998, Protein kinase C regulates the interaction between a GABA transporter and syntaxin 1A. *Journal of Neuroscience*, **18**, 6103–6112.

Bennett, E. and Kanner, B. I., 1997, The membrane topology of GAT-1, a (Na+/Cl(-)-coupled gamma-aminobutyric acid transporter from rat brain. *Journal of Biological Chemistry*, **272**, 1203–1210.

Berger, A. J., Dieudonne, S. and Ascher, P., 1998, Glycine uptake governs glycine site occupancy at NMDA receptors of excitatory synapses. *Journal of Neurophysiology*, **80**, 3336–3340.

Bergeron, R., Meyer, T. M., Coyle, J. T., and Greene, R. W., 1998, Modulation of N-methyl-D-aspartate receptor function by glycine transport. *Proceedings of the National Academy of Sciences (USA)*, **95**, 15730–15734.

Bismuth, Y., Kavanaugh, M. P. and Kanner, B. I., 1997, Tyrosine 140 of the γ-aminobutyric acid transporter GAT1 plays a critical role in neurotransmitter recognition. *Journal of Biological Chemistry*, **272**, 16096–16102.

Borowsky, B. and Hoffman, B. J., 1998, Analysis of a gene encoding two glycine transporter variants reveals alternative promoter usage and a novel gene structure. *Journal of Biological Chemistry*, **273**, 29077–29085.

Borowsky, B., Mezey, E. and Hoffman, B., 1993, Two glycine transporters variants with distinct localization in the CNS and peripheral tissues are encoded by a common gene. *Neuron*, **10**, 851–863.

Budai, D., Wilcox, G. L. and Larson, A. A., 1992, Enhancement of NMDA-evoked neuronal activity by glycine in the rat spinal cord in vivo. *Neuroscience Letters*, **135**, 265–268.

Chaudhry, F. A., Reimer, R. J., Bellocchio, E. E., Danbolt, N. C., Osen, K. K., Edwards, R. H. and Storm-Mathisen, J., 1998, The vesicular GABA transporter, VGAT, localizes to synaptic vesicles in sets of glycinergic as well as GABAergic neurons. *Journal of Neuroscience*, **18**, 9733–9750.

Chen, J. G. and Rudnick, G., 1999, Permeation and gating residues in serotonin transporter. *Proceedings of the National Acadamy of Sciences (USA)*, **97**, 1044–1049.

Chen, J. G., Liu-Chen, S. and Rudnick, G., 1998, Determination of external loop topology in the serotonin transporter by site-directed chemical labeling. *Journal of Biological Chemistry*, **273**, 12675–12681.

Chen, J. G., Sachpatzidis, A. and Rudnick, G., 1997, The third transmembrane domain of the serotonin transporter contains residues associated with substrate and cocaine binding. *Journal of Biological Chemistry*, **272**, 28321–28327.

Cortes, R. and Palacios, J. M., 1990, Autoradiographic mapping of glycine receptors by [^{3}H] strychnine binding, In *Glycine Neurotransmission*, O. P. Ottersen and J. Storm-Mathisen, eds (New York: John Wiley and Sons), pp. 240–263.

Daly, E. C., 1990, The biochemistry of glycinergic neurons, In *Glycine Neurotransmission*, O. P. Ottersen and J. Storm-Mathisen, eds (New York: John Wiley and Sons), pp. 25–66.

Deken, S. L., Beckman, M. L., Boos, L. and Quick, M. W., 2000, Transport rates of GABA transporters: regulation by the N-terminal domain and syntaxin 1A. *Nature Neuroscience,* **3**, 998–1003.

Dumoulin, A., Rostaing, P., Bedet, C., Levi, S., Isambert, M. F., Henry, J. P., Triller, A. and Gasnier, B., 1999, Presence of the vesicular inhibitory amino acid transporter in GABAergic and glycinergic synaptic terminal boutons. *Journal of Cell Science,* **112**, 811–823.

Fedele, E. and Foster, A. C., 1992, [3H]Glycine uptake in rat hippocampus: kinetic analysis and autoradiographic localization. *Brain Research,* **572**, 154–163.

Floeter, M. K. and Hallet, M., 1993, Glycine receptors: a startling connection. *Nature Genetics,* **5**, 319–320.

Gallagher, M. J., Burgess, L. H. and Brunden, K. R., 1999, Characterization of multiple forms of the human glycine transporter type-2. *Molecular Brain Research,* **70**, 101–115.

Geerlings, A., López-Corcuera, B. and Aragón, C., 2000, Characterization of the interactions between the glycine transporters GLYT1 and GLYT2 and the SNARE protein syntaxin 1A. *FEBS Letters,* **470**, 51–54.

Gomeza, J., Zafra, F., Olivares, L., Giménez, C. and Aragón, C., 1995, Regulation by phorbol esters of the glycine transporter (GLYT1) in glioblastoma cells. *Biochimica et Biophysica Acta,* **1233**, 41–46.

Guastella, J., Brecha, N., Weigmann, C., Lester, H. A. and Davidson, N., 1992, Cloning, expression, and localization of a rat brain high-affinity glycine transporter. *Proceedings of the National Acadamy of Sciences (USA),* **89**, 7189–7193.

Guastella, J., Nelson, N., Nelson, H., Czyzyk, L., Keynan, S., Miedel, M. C., Davidson, N., Lester, H. and Kanner, B. I., 1990, Cloning and expression of a rat brain GABA transporter. *Science,* **249**, 1303–1306.

Hanley, J. G., Jones, E. M. C. and Moss, S. J., 2000, GABA receptor rho1 subunit interacts with a novel splice variant of the glycine transporter, GLYT1. *Journal of Biological Chemistry*, **275**, 840–846.

Johnson, J. W. and Ascher, P., 1987, Glycine potentiates the NMDA response in cultured mouse brain neurons. *Nature,* **325**, 529–531.

Johnston, G. A. R. and Iversen, L. L., 1971, Glycine uptake in rat central nervous system slices and homogenates: evidence for different uptake systems in spinal cord and cerebral cortex. *Journal of Neurochemistry*, **18**, 1951–1961.

Jonas, P., Bischofberger, J. and Sandküler, J., 1998, Corelease of two fast neurotransmitters at a central synapse. *Science,* **281**, 419–424.

Jursky, F. and Nelson, N., 1995, Localization of glycine transporter (GLYT2) reveals correlation with the distribution of glycine receptor. *Journal of Neurochemistry,* **64**, 1026–1033.

Kanai, Y., Smith, P. and Hediger, M. A., 1994, A new family of neurotransmitter transporters: the high-affinity glutamate transporters. *FASEB Journal,* **8**, 1450–1459.

Kim, K. M., Kingsmore, S. F., Han, H., Yang-Feng, T. L., Godinot, N., Seldin, M. F., Caron, M. G. and Giros, B., 1994, Cloning of the human glycine transporter type 1: molecular and pharmacological characterization of novel isoform variants and chromosomal localization of the gene in the human and mouse genomes. *Molecular Pharmacology,* **45**, 608–617.

Krystal, J. H. and D'Souza, D. C., 1998, D-serine and the therapeutic challenge posed by the N-methyl-D-aspartate antagonist model of schizophrenia. *Biological Psychology,* **44**, 1075–1076.

Kuhar, M. J. and Zarbin, M. A., 1978, Synaptosomal transport: a chloride dependence for choline, GABA, glycine and several other compounds. *Journal of Neurochemistry*, **31**, 251–256.

Leeson, P. D. and Iversen, L. L., 1994, The glycine site on the NMDA receptor: structure-activity relationships and therapeutic potential. *Journal of Medical Chemistry,* **37**, 4053–4067.

Liu, Q. R., López-Corcuera, B., Mandiyan, S., Nelson, H. and Nelson, N., 1993a, Cloning and expression of a spinal cord- and brain-specific glycine transporter with novel structure features. *Journal of Biological Chemistry*, **268**, 22802–22808.

Liu, Q. R., López-Corcuera, B., Mandiyan, S., Nelson, H. and Nelson, N., 1993b, Molecular characterization of four pharmacologically distinct gamma-aminobutiric acid transporters in mouse brain. *Journal of Biological Chemistry*, **268**, 2106–2112.

Liu, Q. R., López-Corcuera, B., Nelson, H., Mandiyan, S. and Nelson, N., 1992b, Cloning and expression of a cDNA encoding the transporter of taurine and (-alanine in mouse brain. *Proceedings of the National Acadamy of Sciences (USA),* **89**, 12145–12149.

Liu, Q. R., Mandiyan, S., López-Corcuera, B., Nelson, H. and Nelson, N., 1993c, A rat brain cDNA encoding a neurotransmitter transporter with an unusual structure. *FEBS Letters,* **315**, 114–118.

Liu, Q. R., Nelson, H., Mandiyan, S., López-Corcuera, B. and Nelson, N., 1992a, Cloning and expression of a glycine transporter from mouse brain. *FEBS Letters,* **305**, 110–114.

Logan, W. J. and Snyder, S. H., 1972, High-affinity uptake systems for glycine, glutamic and aspartic acids in synaptosomes of rat central nervous system. *Brain Research,* **42**, 413–431.

López-Corcuera, B. and Aragón, C., 1989, Solubilization and reconstitution of the sodium- and-chloride coupled glycine transporter from rat spinal cord. *European Journal of Biochemistry,* **181**, 519–524.

López-Corcuera, B., Alcántara, R., Vázquez J. and Aragón, C., 1993, Hydrodynamic properties and immunological identification of the sodium- and-chloride-coupled glycine transporter. *Journal of Biological Chemistry*, **268**, 2239–2243.

López-Corcuera, B., Kanner, B. I. and Aragón, C., 1989, Reconstitution and partial purification of the sodium-and chloride-coupled glycine transporter from rat spinal cord. *Biochimica et Biophysica Acta,* **983**, 247–252.

López-Corcuera, B., Liu, Q. R., Mandiyan, S., Nelson, H. and Nelson, N., 1992, Expression of a mouse brain cDNA encoding novel γ-aminobutyric acid transporter. *Journal of Biological Chemistry*, **267**, 17491–17493.

López-Corcuera, B., Martínez-Maza, R., Núñez, E., Roux, M., Supplisson, S. and Aragón, C., 1998, Differential properties of two stably expressed brain-specific glycine transporters. *Journal of Neurochemistry*, **71**, 2211–2219.

López-Corcuera, B., Vázquez, J. and Aragón, C., 1991, Purification of the sodium- and chloride-coupled glycine transporter from central nervous system. *Journal of Biological Chemistry*, **266**, 24809–24814.

Luque, J. M., Nelson, N. and Richards, J. G., 1995, Cellular expression of glycine transporter messenger RNA exclusively in rat hindbrain and spinal cord. *Neuroscience,* **64**, 525–535.

Malandro, M. S. and Kilberg, M. S., 1996, Molecular biology of mammalian amino acid transporters. *Annual Review in Biochemistry,* **65**, 305–336.

Mayor, F. Jr., Marvizón, J. G., Aragón, C., Giménez, C. and Valdivieso, F., 1981, Glycine transport into plasma membrane vesicles derived from rat brain synaptosomes. *Biochemical Journal,* **198**, 535–541.

Martinez-Maza, R., Poyatos, I., Lòpez-Corcuera, B., Núñez, E., Giménez, C., Zafra, F., and Aragón, C., 2001, The role of N-glycosylation in transport to the plasmine membrane and sorting of the neuronal glycine transporter GLYTZ. *Journal of Biological chemistry,* **276**, 2168–2173.

McIntire, S. L., Reimer, R. J., Schuske, K., Edwards, R. H. and Jorgensen, E. M., 1997, Identification and characterization of the vesicular GABA transporter. *Nature,* **389**, 870–876.

Morrow, J. A., Collie, I. T., Dunbar, D. R., Walker, G. B., Shahid, M. and Hill, D. R., 1998, Molecular cloning and functional expression of the human glycine transporter GlyT2 and chromosomal localisation of the gene in the human genome. *FEBS Letters,* **439**, 334–340.

Neal, M. J. and Pickles, H. G., 1969, Uptake of (14C) glycine by spinal cord. *Nature,* **222**, 679–680.

Nelson, N., 1998, The family of Na+/Cl- neurotransmitter transporters. *Journal of Neurochemistry*, **71**, 1785–1803.

Núñez, E. and Aragón, C., 1994, Structural analysis and functional role of the carbohydrate component of glycine transporter. *Journal of Biological Chemistry*, **269**, 16920–16924.

Núñez, E., López-Corcuera, B., Martínez-Maza, R. and Aragón, C., 2000b, Differential effects of ethanol on glycine uptake mediated by the recombinant GLYT1 and GLYT2 glycine transporters. *British Journal of Pharmacology,* **129**, 802–810.

Núñez, E., López-Corcuera, B., Vázquez, J., Giménez, C. and Aragón, C., 2000a, Differential effects of the tricyclic antidepressant amoxapine on glycine uptake mediated by the recombinant GLYT1 and GLYT2 glycine transporters. *British Journal of Pharmacology,* **129**, 200–206.

O'Brien, J. A. and Berger, A. J., 19??, Cotransmission of GABA and glycine to brain motoneurons. *Journal of Neurophysiology,* **82**, 1638–1641.

Olivares, L., Aragón, C., Giménez, C. and Zafra, F., 1994, Carboxyl terminus of the glycine transporter GLYT1 is necessary for correct processing of the protein. *Journal of Biological Chemistry*, **269**, 28400–28404.

Olivares, L., Aragón, C., Giménez, C. and Zafra, F., 1995, The role of Nglycosylation in the targeting and activity of the GLYT1 glycine transporter. *Journal of Biological Chemistry*, **270**, 9437–9442.

Olivares, L., Aragón, C., Giménez, C. and Zafra, F., 1997, Analysis of the transmembrane topology of the glycine transporter GLYT1. *Journal of Biological Chemistry*, **272**, 1211–1217.

Pacholczyk, T., Blakely, R. D. and Amara, S. G., 1991, Expression cloning of a cocaine and antidepressant-sensitive human noradrenaline transporter. *Nature,* **350**, 9389–9393.

Ponce, J., Biton, B., Benavides, J., Avenet, P. and Aragón, C., 2000, Transmembrane domain III plays an important role in ion binding and permeation in the glycine transporter GLYT2. *Journal of Biological Chemistry*, **275**, 13856–13862.

Ponce, J., Poyatos, I., Aragón, C., Giménez, C. and Zafra, F., 1998, Characterization of the 5'region of the rat brain glycine transporter GLYT2 gene: identification of a novel isoform. *Neuroscience Letters,* **242**, 25–28.

Pourcho, R. G. and Goebel, D. J., 1990, Autoradiographic and immunocytochemical studies of glycine containing neurons in the retina, In *Glycine Neurotransmission,* O. P. Ottersen and J. Storm-Mathisen, eds (New York: John Wiley and Sons), pp. 355–389.

Poyatos, I., Ponce, J., Aragón, C., Giménez, C. and Zafra, F., 1997, The glycine transporter GLYT2 is a reliable marker for glycine-immunoreactive neurons. *Molecular Brain Research,* **49**, 63–70.

Poyatos, I., Ruberti, F., Martínez-Maza, R., Giménez, C., Dotti, C. G. and Zafra, F., 2000, Polarized distribution of glycine transporter isoforms in epithelial and neuronal cells. *Molecular and Cellular Neuroscience,* **15**, 99–111.

Roux, M. and Supplisson, S., 2000, Neuronal and glial glycine transporters have different stoichiometries *Neuron,* **25**, 373–383.

Sagné, C., Mestikawy, S. E., Isambert, M. F., Hamon, M., Henry, J. P., Giros, B. and Gasnier, B., 1997, Cloning of a functional vesicular GABA and glycine transporter by screening of genome databases. *FEBS Letters,* **417**, 177–183.

Sakata, K., Sato, K., Schloss, P., Betz, H., Shimada, S. and Tohyama, M., 1997, Characterization of glycine release mediated by glycine transporter 1 stably expressed in HEK-293 cells. *Molecular Brain Research,* **49**, 89–94.

Salt, T. E., 1989, Modulation of NMDA receptor-mediated responses by glycine and D-serine in the rat thalamus in vivo. *Brain Research,* **481**, 403–406.

Simpson, R. K., Jr., Gondo, M., Robertson, C. S. and Goodman, J. C., 1995, The influence of glycine and related compounds on spinal cord injury-induced spasticity. *Neurochemistry Research,* **20**, 1203–1210.

Smith, K. E., Borden, L. A., Harting, P. R., Branchek, T. and Weinshank, R. L., 1992, Cloning and expression of a glycine transporter reveal colocalization with NMDA receptors. *Neuron,* **8**, 927–935.

Spike, R. C., Watt, C., Zafra, F. and Todd, A. J., 1997, An ultrastructural study of the glycine transporter GLYT2 and its association with glycine in the superficial laminae of the rat spinal dorsal horn. *Neuroscience,* **77**, 543–551.

Supplisson, S. and Bergman, C., 1997, Control of NMDA receptor activation by a glycine transporter co-expressed in Xenopus oocytes. *Journal of Neuroscience*, **17**, 4580–4590.

Thiels, E., Weisz, D. J. and Berger, T. W., 1992, In vivo modulation of N-methyl-D-aspartate receptor-dependent long-term potentiation by the glycine modulatory site. *Neuroscience*, **46**, 501–509.

Tsai, G., Yang, P., Chung, L. C., Lange, N. and Coyle, J. T., 1998, D-serine added to antipsychotics for the treatment of schizophrenia. *Biological Psychology,* **44**, 1081–1089.

Uhl, G. R. and Hartig, P. R., 1992, Transporter explosion: update on uptake. *Trends in Pharmaceutical Science,* **13**, 421–425.

van den Pol, A. and Gorcs, T., 1988, Glycine and glycine receptor immunoreactivity in brain and spinal cord. *Journal of Neuroscience*, **8**, 472–492.

Wenthold, R. J. and Hunter, C., 1990, Immunocytochemistry of glycine and glycine receptors in the central auditory system, In *Glycine Neurotransmission*, O. P. Ottersen and J. Storm-Mathisen, eds (New York: John Wiley and Sons), pp. 391–415.

Wenthold, R. J., Parakkal, M. H., Oberdorfer, M. D. and Altschuler, R. A., 1988, Glycine receptor immunoreactivity in the ventral cochlear nucleus of the guinea pig. *Journal of Computational Neurology,* **76**, 423–435.

Werman, R., Davidoff, R. A. and Aprison, M. H., 1967, Inhibition of motoneurons by iontophoresis of glycine. *Nature,* **214**, 681–683.

Zafra, F. and Giménez, C., 1986, Characterization of glycine uptake in plasma membrane vesicles isolated from cultured glioblastoma cells. *Brain Research,* **397**, 108–116.

Zafra, F. and Giménez, C., 1988, Efflux and exchange of glycine by plasma membrane vesicles isolated from glioblastoma cells. *Biochimica et Biophysica Acta,* **946**, 202–208.

Zafra, F. and Giménez, C., 1989, Characteristics and adaptive regulation of glycine transport in cultured glial cells. *Biochemical Journal,* **258**, 403–408.

Zafra, F., Aragón, C. and Giménez, C., 1997, Molecular biology of glycinergic neurotransmission. *Molecular Neurobiology,* **14**, 117–142.

Zafra, F., Aragón, C., Olivares, L., Danbolt, N. C., Giménez, C. and Storm-Mathisen, J., 1995a, Glycine transporters are differentially expressed among CNS cells *Journal of Neuroscience*, **15**, 3952–3969.

Zafra, F., Gomeza, J., Olivares, L., Aragón, C. and Giménez, C., 1995b, Regional distribution and developmental variation of the glycine transporters GLYT1 and GLYT2 in the rat CNS. European *Journal of Neuroscience*, **7**, 1342–1352.

Zafra, F., Poyatos, I. and Gimenez, C., 1997, Neuronal dependency of the glycine transporter GLYT1 expression in glial cells. *Glia,* **20**, 155–162.

Zarbin, M. A., Wamsley, J. K. and Kuhar, M. J., 1981, Glycine receptor: light microscopic autoradiographic localization with [3H]strychnine. *Neuroscience*, **1**, 532–547.

Received in final form 11 December 2000.