

Glycine transporters: structural insights from biophysics, physiology and human disease

Stephane Supplisson, F. Rousseau, M.J. Roux, F.M. Rossi, K.R. Aubrey

Laboratoire de Neurobiologie, Ecole Normale Supérieure, 46 rue d'Ulm, 75005 Paris

Abstract. Sequence alignment shows that the three SLC6 glycine transporters, GlyT1, GlyT2 and ATB0,+ are clustered in a subfamily, despite their important differences in Na⁺ coupling, reversibility property, substrate specificity and uncoupled conductance. We will examine how the kinetic and energetic properties of these three transporters shape their physiological functions. (www.actabiomedica.it)

Key words: GlyT2, SLC6, hyperekplexia

Introduction

The recapture of neurotransmitters by neuronal and glial plasma membrane transporters is the principal mechanism of termination of synaptic transmission in the CNS. The rapid depletion of neurotransmitters in the extracellular space is facilitated by the low extra/intracellular volume ratio of the Vertebrate brain. In contrast, this low volume ratio constitutes a handicap when uptake is used to increase the cytoplasmic concentration of neurotransmitters. To overcome this limitation, the neuronal glycine transporter GlyT2 (1) features many unusual properties among the Na⁺,Cl⁻ coupled SLC6 transporters family (2), that altogether reinforce its critical capacity to accumulate high cytoplasmic glycine concentrations. GlyT2 has a remarkable specificity for glycine, as it does not tolerate any substitution of the C α , thus eliminating the risk of efflux by heteroexchange with false substrate. GlyT2 has a tight flux-coupling with 3 Na⁺ and 1 Cl⁻ which provides a large driving force for uphill transport and limits the risk of reverse transport. Finally, GlyT2 shows a kinetic limitation for efflux that allows this transporter to sustain near "infinite" outwardly directed glycine gradient (3). Therefore,

GlyT2 which is a marker of glycinergic terminal is now considered as a likely presynaptic determinant of glycinergic transmission. Indeed its genetic inactivation is sufficient to disrupts glycine transmission in GlyT2^{-/-} mice and lead to severe and rapidly lethal neuromuscular disorders (4). Recently, several mutations in the human SLC6A5 gene that encodes for the presynaptic GlyT2 have been identified by M. Rees and RJ Harvey (5), in an international cohort of patients suffering from hyperekplexia (6), a rare neuromuscular genetic disorder. This startle disease is associated with dysfunction of glycinergic transmission but all these patients were lacking mutations of the postsynaptic determinants of glycinergic transmission such as the α and β glycine receptor subunits and the scaffolding proteins gephyrin and collybistin (5). While most of these patients were compound heterozygote with mutations altering GlyT2 expression or plasma membrane expression, two of the GlyT2 mutations were interesting in term of structure function. These mutations (W482R and N509S) were predicted to be located at critical positions of the substrate and Na⁺ binding sites, as indicated by the sequence alignment of GlyT2 with the bacterial Na⁺-coupled leucine transporter LeuTaa (7).

Materials and Methods

Transporters wild type and mutants were expressed by mRNA injection in *Xenopus* oocytes. Steady state and presteady-state currents were recorded using two-electrode voltage clamp as described previously (8).

Outcomes

GlyT2 electrophysiological characterisation has shown a tight coupling between the steady-state current evoked by glycine and its uptake in individual oocytes (3). While the energetic role of Na⁺ for uphill uptake of glycine is undisputed, the kinetic and energetic contribution of Cl⁻ to the transporter cycle is less well understood, in particular because the Cl⁻ electrochemical gradient undergoes change in direction during the postnatal maturation of neurons. We will examine how Cl⁻ contributes to the steady-state glycine coupled and uncoupled currents of GlyT2.

In response to voltage steps, Na⁺- and Cl⁻- dependent transient currents are recorded from *Xenopus* oocytes expressing glycine transporters. The kinetic and voltage dependence of these transient currents constitute for a given transporter a specific kinetic signature of the Na⁺-binding steps. With wild type transporters, these transient currents are suppressed by the addition of substrate which evoked steady-state current. Surprisingly, we noticed that the transient currents recorded from oocytes expressing GlyT2:W482R, one of the GlyT2 alteration identified in a hyperekplexia patient (5), were totally insensitive to glycine (up to 10 mM) whereas they were blocked by a non-competitive GlyT2 inhibitor. Because glycine failed to evoke inward current, this result suggests that W482R abolishes glycine binding to the transporter as predicted from LeuTaa structure. Expression of the GlyT2:N509S shows a large reduction in glycine sensitivity due to a voltage dependent increase in EC₅₀ which suggests that this asparagine, which is conserved in SLC6 transporters and LeuTaa, participates to the allosteric interaction between Na⁺ and glycine, in agreement with the idea that LeuTaa structure can be used as a working template for neurotransmitter transporters.

Finally, we will present evidence that GlyT2 properties are not unique to this transporter and thus contribute to shape an “accumulation” phenotype for transport (Rousseau et Supplisson, manuscript in preparation).

Conclusion

Combining studies from structural, genetic and functional analysis help to understand how transporters specialize, and appear fitted for their physiological functions.

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Correspondence: Stéphane Supplisson
Laboratoire de Neurobiologie,
Ecole Normale Supérieure, Paris, France
Tel.: 331 44 32 37 51;
Fax: 331 44 32 38 87;
E-mail: supplis@ens.fr