

## PERSPECTIVE

# Parawixin1: A Spider Toxin Opening New Avenues for Glutamate Transporter Pharmacology

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### ABSTRACT

Glutamate is the major excitatory neurotransmitter in the mammalian central nervous system. After release from glutamatergic nerve terminals, glial and neuronal glutamate transporters remove glutamate from the synaptic cleft to terminate synaptic transmission and to prevent neuronal damage by excessive glutamate receptor activation. In this issue of *Molecular Pharmacology*, Fontana et al. (p. 1228) report on the action of a venom compound, Parawixin1, on excitatory amino acid transporters (EAATs). They demonstrate that this agent selectively affects a glial glutamate transporter, EAAT2, by specifically

increasing one particular step of the glutamate uptake cycle. Disturbed glutamate homeostasis seems to be a pathogenetic factor in several neurodegenerative disorders. Because EAAT2 is a key player in determining the extracellular glutamate concentration in the mammalian brain, drugs targeting this protein could prevent glutamate excitotoxicity without blocking glutamatergic transmission. Its specificity and selectivity makes Parawixin1 a perfect starting point to design small molecules for the treatment of pathological conditions caused by alterations of glutamate homeostasis.

Secondary-active transport moves substrates against their driving forces, coupling that upward movement to the downward flow of other substrates (Christensen and Riggs, 1952; Crane, 1977; Kaback et al., 1993). The biological significance of these transport processes can hardly be overestimated. Secondary-active transporters are necessary for the absorption of nutrients, transepithelial transport of many substances, substrate accumulation in cellular organelles, and ion homeostasis in the extracellular space. In the central nervous system, secondary-active transporters terminate synaptic transmission by quickly taking up neurotransmitter after their release from presynaptic nerve terminals (Kanner, 1994; Torres and Amara, 2007).

Glutamate is not only the most important excitatory neurotransmitter in the mammalian central nervous system but also a potent neurotoxin. Neurons do not survive at external glutamate concentrations of 100  $\mu$ M if maintained for a few

minutes (Choi et al., 1987). This value imposes an enormous thermodynamic challenge for the glutamate reuptake system. Excitatory amino acid transporters solve this by transporting six ions in one transport cycle, one glutamate coupled to three sodium ions and one proton, in counter transport with one potassium ion (Zerangue and Kavanaugh, 1996; Levy et al., 1998). This complex transport stoichiometry provides a driving force that is sufficient to control the extracellular glutamate concentration to values as low as 2 nM under normal conditions (Levy et al., 1998). However, the variety of cotransported substrates, together with the resulting electrogenicity, makes these transporters susceptible to drastic changes in the transport rate or even inversion of the transport direction upon small alterations of the external milieu. In severe brain ischemia, glutamate is mainly released by reversed glutamate uptake (Rossi et al., 2000). Moreover, there are many neurological diseases that seem to be associated with increased levels of external glutamate, such as amyotrophic lateral sclerosis (Rothstein et al., 1992; Howland et al., 2002), schizophrenia (Laruelle et al., 2003), Alzheimer disease (Scott et al., 2002), and multiple sclerosis (Pitt et al., 2000; Smith et al., 2000). At present, no causal

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**ABBREVIATIONS:** EAAT, excitatory amino acid transporter.

treatment is known for any of these conditions, and drugs that increase glutamate uptake could be helpful in alleviating the disease symptoms of affected persons.

The neuromuscular synaptic junction of insects uses glutamate as excitatory neurotransmitter, and toxins that interfere with glutamatergic synaptic transmissions are used by predators to paralyze their prey (Stromgaard et al., 2001; Estrada et al., 2007). Ionotropic glutamate receptors seem to be the obvious target of such toxins. However, Fontana et al. (2003) showed that a compound from the venom of the spider *Parawixia bistriata* (Parawixin1) enhances glutamate uptake. In a subsequent publication, published in the current issue of *Molecular Pharmacology*, Fontana et al. (2007) further characterize the action of this compound. They demonstrate that Parawixin1 exerts an isoform-specific action, enhancing glutamate transport by excitatory amino acid transporter (EAAT) 2 but not by EAAT1 or EAAT3. Parawixin1 thus specifically affects the glutamate transporter responsible for extracellular glutamate homeostasis in the mammalian brain (Danbolt et al., 1992; Rothstein et al., 1996; Tanaka et al., 1997).

In contrast to various compounds that act as indirect modulators of EAAT activity (Rothstein et al., 2005; Ganel et al., 2006; Li et al., 2006), Parawixin1 affects directly the function of EAAT2. In a series of elegant experiments, Fontana et al. (2007) demonstrate that Parawixin1 does not modify the binding affinities for glutamate and sodium; rather, it facilitates one specific step of the glutamate uptake cycle, the reorientation of the potassium-bound transporter. Thus, by speeding up one partial reaction of the glutamate uptake cycle, Parawixin1 enhances glutamate uptake, without stimulating the potentially harmful reverse glutamate efflux. Parawixin1 thus fulfills many requirements to serve as a paradigm for pharmacological compounds to therapeutically enhance glutamate clearance and to prevent excitotoxicity without affecting the synaptic transmission. An important step toward this ultimate aim will be the determination of the chemical structure of Parawixin1 that is currently not known. It does not seem to be a peptide (Fontana et al., 2003). Spider venoms contain a larger number of polyamine-like compound (Estrada et al., 2007), and Parawixin1 might belong to this substance group.

In addition to these therapeutic implications, Parawixin1 promises to be a great tool to study the function of EAAT glutamate transporters. The future identification of the binding site of Parawixin1 will provide insights into conformational changes of the protein during the reorientation of the empty transporter. The definition of the binding stoichiometry (i.e., whether each subunit of the trimeric transporter binds one molecule of Parawixin1 or one trimer binds only one molecule) will give further information about the ongoing debate on interactions between glutamate transporter subunits (Grewer et al., 2005; Torres-Salazar and Fahlke, 2006; Koch et al., 2007a,b). Knowledge about the binding site might also help to engineer prokaryotic glutamate transporters with  $K^+$  as cosubstrate that are more amenable to crystallography or spectroscopy studies than their mammalian counterparts.

An intriguing aspect of Parawixin1 is its specific action on the insect neuromuscular synapse. It was originally purified from a spider venom that paralyzes insects. Does Parawixin1 block synaptic transmission by enhancing glutamate uptake,

or does it merely support other components of the toxin that target ionotropic glutamate receptors? On the other hand, the toxin might affect other functions of EAAT glutamate transporters. All EAATs exhibit a pore-mediated anion conductance that is gated by the glutamate uptake cycle (Fairman et al., 1995; Wadiche et al., 1995; Watzke et al., 2001). Under certain conditions, this anion channel can change its selectivity and become permeable to cations (Melzer et al., 2005). Such a cation conductance might depolarize the presynaptic nerve terminal and make it unexcitable. One might thus speculate that Parawixin1 could reduce presynaptic excitability and glutamate release by modifying the anion conductance associated with glutamate transporters. In this case, Parawixin1 would promote the EAAT anion conductance from a biophysical peculiarity with unclear physiological significance to an important toxicological and pharmacological target.

We live in exciting times for neurotransmitter transporter research. Biophysical approaches are delineating the structure and function of these molecules, and genetic studies and mouse models are defining their functional role in mammals. The work of Fontana et al. (2007) is a beautiful example of how nature itself can provide tools to enhance our understanding of neurotransmitter transport. Parawixin1 represents a promising tool to further clarify the molecular and structural basis of glutamate transporters function. This agent is a potent candidate for drug design, and we all hope that Parawixin1 will hold this therapeutic promise and will allow the development of better drugs to battle devastating human diseases.

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