Molecular Pharmacology Fast Forward. Published on August 28, 2007 as doi:10.1124/mol.107.041020

Parawixin1: a spider toxin opening new avenues for glutamate transporter pharmacology

Delany Torres-Salazar[*] and Christoph Fahlke[*,‡]

*Institut für Neurophysiologie, Medizinische Hochschule Hannover, Germany, ‡Zentrum für Systemische Neurowissenschaften Hannover (ZSN), Germany

Molecular Pharmacology Fast Forward. Published on August 28, 2007 as DOI: 10.1124/mol.107.041020
This article has not been copyedited and formatted. The final version may differ from this version.

Running title: A natural agonist of EAAT2 glutamate transporters

Address correspondence to: Christoph Fahlke, Institut für Neurophysiologie, Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1 , D – 30625 Hannover, Tel +49 511 532 2777, Fax +49 511 532 2776, Email: fahlke.christoph@mh-hannover.de

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. study 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 appears to be a pathogenetic factor in several neurodegenerative disorders. Since 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.

Molecular Pharmacology Fast Forward. Published on August 28, 2007 as DOI: 10.1124/mol.107.041020
This article has not been copyedited and formatted. The final version may differ from this version.

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 μM (Choi et al., 1987), and 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 co-transported substrates, together with the resulting electrogenecity, 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 appear 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 treatment is known for any

Molecular Pharmacology Fast Forward. Published on August 28, 2007 as DOI: 10.1124/mol.107.041020
This article has not been copyedited and formatted. The final version may differ from this version.

of these conditions, and drugs that increase glutamate uptake could be helpful in alleviating the disease symptoms of affected individuals.

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 appear to be the obvious target of such toxins. However, Fontana and coworkers recently showed that a compound from the venom of the spider *Parawixia bistriata* (Parawixin1) enhances glutamate uptake (Fontana et al., 2003). In a subsequent publication, published in the current issue of *Molecular Pharmacology*, the authors further characterize the action of this compound (Fontana et al., 2007). They demonstrate that Parawixin1 exerts an isoform-specific action enhancing glutamate transport by EAAT2, 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. demonstrate that Parawixin1 does not modify the binding affinities for glutamate and sodium, but rather facilitate 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 towards this ultimate aim will be the determination of the chemical structure of Parawixin1 that is currently not known. It does not appear to be a peptide (Fontana et al., 2003). Spider venoms

Molecular Pharmacology Fast Forward. Published on August 28, 2007 as DOI: 10.1124/mol.107.041020
This article has not been copyedited and formatted. The final version may differ from this version.

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 whether 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;Koch et al., 2007b). Knowledge about the binding site might also help to engineer prokaryotic glutamate transporters with K^+ as co-substrate 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 paralyses 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? Alternatively, the toxin might impact 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.

Molecular Pharmacology Fast Forward. Published on August 28, 2007 as DOI: 10.1124/mol.107.041020
This article has not been copyedited and formatted. The final version may differ from this version.

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 and colleagues 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.

Acknowledgement
We would like to thank Dr. Patricia Hidalgo for helpful discussions.

References

Choi DW, Maulucci-Gedde M and Kriegstein AR (1987) Glutamate Neurotoxicity in Cortical Cell Culture. *J Neurosci* **7**:357-368.

Christensen HN and Riggs TR (1952) Concentrative Uptake of Amino Acids by the Ehrlich Mouse Ascites Carcinoma Cell. *J Biol Chem* **194**:57-68.

Crane RK (1977) The Gradient Hypothesis and Other Models of Carrier-Mediated Active Transport. *Rev Physiol Biochem Pharmacol* **78**:99-159.

Danbolt NC, Storm-Mathisen J and Kanner B I (1992) An [Na^+ + K^+] Coupled L-Glutamate Transporter Purified From Rat Brain Is Located in Glial Cell Processes. *Neuroscience* **51**:295-310.

Estrada G, Villegas E and Corzo G (2007) Spider Venoms: a Rich Source of Acylpolyamines and Peptides As New Leads for CNS Drugs. *Nat Prod Rep* **24**:145-161.

Fairman WA, Vandenberg RJ, Arriza JL, Kavanaugh MP and Amara SG (1995) An Excitatory Amino-Acid Transporter With Properties of a Ligand-Gated Chloride Channel. *Nature* **375**:599-603.

Fontana AC, Beleboni RO, Wojewodzic MW, dos Santos WF, Coutinho-Netto J, Grutle NJ, Watts SD, Danbolt NC and Amara SG (2007) Enhancing Glutamate Transport: Mechanism of Action of Parawixin1, a Neuroprotective Compound From Parawixia Bistriata Spider Venom. *Mol Pharmacol*.

Fontana AC, Guizzo R, de Oliveira BR, Meirelles E Silva AR, Coimbra NC, Amara SG, dos Santos WF and Coutinho-Netto J (2003) Purification of a Neuroprotective Component of Parawixia Bistriata Spider Venom That Enhances Glutamate Uptake. *Br J Pharmacol* **139**:1297-1309.

Ganel R, Ho T, Maragakis N J, Jackson M, Steiner J P and Rothstein J D (2006) Selective Up-Regulation of the Glial Na^+-Dependent Glutamate Transporter GLT1 by a Neuroimmunophilin Ligand Results in Neuroprotection. *Neurobiol Dis* **21**:556-567.

Grewer C, Balani P, Weidenfeller C, Bartusel T, Tao Z and Rauen T (2005) Individual Subunits of the Glutamate Transporter EAAC1 Homotrimer Function Independently of Each Other. *Biochemistry* **44**:11913-11923.

Howland DS, Liu J, She YJ, Goad B, Maragakis NJ, Kim B, Erickson J, Kulik J, DeVito L, Psaltis G, DeGennaro LJ, Cleveland DW and Rothstein JD (2002) Focal Loss of the Glutamate Transporter EAAT2 in a Transgenic Rat Model of SOD1 Mutant-Mediated Amyotrophic Lateral Sclerosis (ALS). *Proceedings of the National Academy of Sciences of the United States of America* **99**:1604-1609.

Kaback HR, Jung K, Jung H, Wu J, Prive GG and Zen K (1993) What's New With Lactose Permease. *J Bioenerg Biomembr* **25**:627-636.

Kanner BI (1994) Sodium-Coupled Neurotransmitter Transport: Structure, Function and Regulation. *J Exp Biol* **196**:237-249.

Koch HP, Brown RL and Larsson HP (2007a) The Glutamate-Activated Anion Conductance in Excitatory Amino Acid Transporters Is Gated Independently by the Individual Subunits. *J Neurosci* **27**:2943-2947.

Koch HP, Hubbard JM and Larsson HP (2007b) Voltage-Independent Sodium-Binding Events Reported by the 4B-4C Loop in the Human Glutamate Transporter EAAT3. *J Biol Chem*.

Laruelle M, Kegeles LS and bi-Dargham A (2003) Glutamate, Dopamine, and Schizophrenia: From Pathophysiology to Treatment. *Ann N Y Acad Sci* **1003**:138-158.

Levy LM, Warr O and Attwell D (1998) Stoichiometry of the Glial Glutamate Transporter GLT-1 Expressed Inducibly in a Chinese Hamster Ovary Cell Line Selected for Low Endogenous Na^+-Dependent Glutamate Uptake. *Journal of Neuroscience* **18**:9620-9628.

Li LB, Toan SV, Zelenaia O, Watson DJ, Wolfe JH, Rothstein JD and Robinson MB (2006) Regulation of Astrocytic Glutamate Transporter Expression by Akt: Evidence for a Selective Transcriptional Effect on the GLT-1/EAAT2 Subtype. *J Neurochem* **97**:759-771.

Melzer N, Torres-Salazar D and Fahlke C (2005) A Dynamic Switch Between Inhibitory and Excitatory Currents in a Neuronal Glutamate Transporter. *Proc Natl Acad Sci U S A* **102**:19214-19218.

Pitt D, Werner P and Raine CS (2000) Glutamate Excitotoxicity in a Model of Multiple Sclerosis. *Nat Med* **6**:67-70.

Rossi DJ, Oshima T and Attwell D (2000) Glutamate Release in Severe Brain Ischaemia Is Mainly by Reversed Uptake. *Nature* **403**:316-321.

Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, Kanai Y, Hediger MA, Wang Y, Schielke JP and Welty DF (1996) Knockout of Glutamate Transporters Reveals a Major Role for Astroglial Transport in Excitotoxicity and Clearance of Glutamate. *Neuron* **16**:675-686.

Rothstein JD, Martin LJ and Kuncl RW (1992) Decreased Glutamate Transport by the Brain and Spinal Cord in Amyotrophic Lateral Sclerosis. *N Engl J Med* **326**:1464-1468.

Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, Jin L, Hoberg MD, Vidensky S, Chung DS, Toan SV, Bruijn LI, Su ZZ, Gupta P and Fisher PB (2005) Beta-Lactam Antibiotics Offer Neuroprotection by Increasing Glutamate Transporter Expression. *Nature* **433**:73-77.

Scott HL, Pow D V, Tannenberg A E G and Dodd P R (2002) Aberrant Expression of the Glutamate Transporter Excitatory Amino Acid Transporter 1 (EAAT1) in Alzheimer's Disease. *Journal of Neuroscience* **22**: RC206:1-5

Smith T, Groom A, Zhu B and Turski L (2000) Autoimmune Encephalomyelitis Ameliorated by AMPA Antagonists. *Nat Med* **6**:62-66.

Stromgaard K, Andersen K, Krogsgaard-Larsen P and Jaroszewski JW (2001) Recent Advances in the Medicinal Chemistry of Polyamine Toxins. *Mini Rev Med Chem* **1**:317-338.

Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Hori S, Takimoto M and Wada K (1997) Epilepsy and Exacerbation of Brain Injury in Mice Lacking the Glutamate Transporter GLT-1. *Science* **276**:1699-1702.

Torres GE and Amara SG (2007) Glutamate and Monoamine Transporters: New Visions of Form and Function. *Curr Opin Neurobiol* **17**:304-312.

Torres-Salazar D and Fahlke C (2006) Intersubunit Interactions in EAAT4 Glutamate Transporters. *J Neurosci* **26**:7513-7522.

Wadiche JI, Amara SG and Kavanaugh MP (1995) Ion Fluxes Associated With Excitatory Amino Acid Transport. *Neuron* **15**:721-728.

Watzke N, Bamberg E and Grewer C (2001) Early Intermediates in the Transport Cycle of the Neuronal Excitatory Amino Acid Carrier EAAC1. *J Gen Physiol* **117**:547-562.

Zerangue N and Kavanaugh MP (1996) Flux Coupling in a Neuronal Glutamate Transporter. *Nature* **383**:634-637.

Footnotes

This work was supported by the Deutsche Forschungsgemeinschaft (FOR450) to Ch.F.