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Current Opinion in Pharmacology

Excitatory amino acid transporters: recent insights into molecular mechanisms, novel modes of modulation and new therapeutic possibilities

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The five excitatory amino acid transporters (EAAT1–5) mediating the synaptic uptake of the major excitatory neurotransmitter glutamate are differently expressed throughout the CNS and at the synaptic level. Although EAATs are crucial for normal excitatory neurotransmission, explorations into the physiological functions mediated by the different transporter subtypes and their respective therapeutic potential have so far been sparse, in no small part due to the limited selection of pharmacological tools available. In the present update, we outline important new insights into the molecular compositions of EAATs and their intricate transport process, the novel approaches to pharmacological modulation of the transporters that have emerged, and interesting new perspectives in EAAT as drug targets proposed in recent years.

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Current Opinion in Pharmacology 2015, **20**:116–123

This review comes from a themed issue on **Neurosciences**

Edited by **Pierre Paoletti** and **Jean-Philippe Pin**

For a complete overview see the Issue and the Editorial

Available online 5th November 2014

http://dx.doi.org/10.1016/j.coph.2014.10.008

Introduction

The synaptic reuptake of glutamate (Glu), the major excitatory neurotransmitter in the CNS, following its release from presynaptic terminals is mediated by excitatory amino acid transporters (EAATs). The EAAT family consists of five transporters, the human EAAT1–5 subtypes corresponding to GLAST, GLT-1, EAAC1, EAAT4 and EAAT5, respectively, in rodents [1–4]. EAAT2 is the major physiological subtype responsible for >90% of total Glu uptake in the brain. EAAT1 and EAAT3 also are abundantly distributed in the CNS, whereas EAAT4 and EAAT5 are expressed almost exclusively in cerebellar Purkinje cells and retina, respectively [1]. Whereas EAAT1 and EAAT2 predominantly are expressed in glia cells, EAAT3–5 are neuronal-specific transporters [1–4]. Thus, the EAAT-mediated regulation of glutamatergic signaling is delicately balanced by the regional and cellular distribution of the five transporters. In this update, we outline new insights into EAAT structure–function aspects, novel approaches to drug development in the field, and interesting findings about the physiological functions mediated by the transporters reported in recent years.

Recent insights into EAAT structure/function aspects

The EAATs are secondary-active transporters coupling the movement of one Glu with the symport of three Na^+ and one proton and the counter-transport of one K^+ [3,4]. The transporters are trimeric assemblies of protomers that function independently [5,6], and although most EAATs are assembled as homotrimers, the recent *in vitro* demonstration of heterotrimeric EAAT3/EAAT4 formation indicate that native EAAT populations may comprise more than five trimeric assemblies [7]. Crystal structures of the prokaryotic EAAT homologues Glt_{Ph} or Glt_{Tk}, sodium/aspartate symporters from *Pyrococcus horikoshii* and *Thermococcus kodakarensis*, have revealed that each protomer consists of an immobile trimerization domain and a transport domain that encompasses the substrate, Na^+, H^+ and K^+ binding sites (Figure 1a). The trimerization domain mediates inter-protomeric interactions in the trimer and constitutes a rigid scaffold for the dramatic movements of the transport domain during the transport process (Figure 1b,c).

The transport process. During the last decade high resolution structures of Glt_{Ph} and Glt_{Tk} in multiple conformations have provided highly detailed information about the structural transformations during the transport cycle (outlined in Figure 1c, reviewed in [3,8]). Importantly, recent fluorescence spectroscopy studies have provided insight into the dynamics underlying these conformational changes, thus breathing life into the structures. Perhaps the most interesting observations to come out of these studies are the insights into the driving force of the transport process and the anarchistic nature of operations in the trimer. As outlined in Figure 1c, substrate binding to the transporter is coupled to the binding of all three Na^+ in a highly cooperative manner at both the outward-facing

and inward-facing conformations [9••]. Binding of the initial two Na^+ as well as of the third Na^+ to Glt_{Ph} have been shown to be associated with great energy costs, indicative of the ions inducing major rearrangements in the substrate binding site rather than binding to pre-existing sites [9••]. Thus, the key role of Na^+ in the transport process appears to be routed in its induction of a binding site capable of binding the substrate and of undergoing the transmembrane movement of the transport domain the rather than stabilizing any of these subsequent structural transitions. Analogously, K^+ binding to the inward-facing EAAT conformation has been proposed to enable the re-translocation of the substrate-free transport domain to the outward-facing conformation [10]. Two recent single-molecule FRET studies of Glt_{Ph} have reported transport events mediated by the three protomers in the trimer to take place in an uncoordinated and stochastic manner [11••,12••]. Thus, while trimeric EAAT assembly undoubtedly is essential for cell membrane expression and stability, the transport seems to be orchestrated by completely independent units tightly regulated by cation binding and unbinding events.

EAATs as anion channels. EAATs are not only secondary-active Glu transporters, but also anion-selective channels and thus represent prototypical dual function membrane transport proteins [13,14]. EAAT anion conduction is mediated by a perfectly anion-selective conduction pathway and exhibits unitary current amplitudes similar to those of specialized anion channels [15]. Macroscopic EAAT anion currents are substrate-dependent and voltage-dependent and follow closely transitions within the uptake cycle [16]. Noise analysis has revealed unitary current amplitudes that are identical in the presence as well as in the absence of Glu [17]. These results support the notion that anion permeation occurs through a defined ion conduction pathway that is opened and closed by conformational changes coupled to the transport process rather than by multiple distinct conduction pathways associated with different transporter states.

On the basis of their relative Glu transport rates and anion currents, EAATs group into two functionally distinct classes, EAAT1–3 being efficient Glu transporters with small associated macroscopic anion currents and EAAT4–5 low-capacity transporters with predominant anion conductance [18]. The differences are due to largely differing Glu transport rates, as the conduction properties of separate EAAT anion channels are very similar [15]. So far, neither the localization of this conduction pathway, the underlying conformation of the transporter, nor the mechanisms of anion permeation are sufficiently understood. EAAT anion conduction appears to play a role in synaptic transmission in neurons [19] and may contribute to the regulation of intracellular chloride concentrations in glial cells [20••] (see below).

Novel generations of EAAT modulators

Considering the immense therapeutic potential in pharmacological intervention into glutamatergic neurotransmission and the fact that other neurotransmitter transporters are targeted by clinically administered drugs against epilepsy, depression and attention deficit hyperactivity disorder [21], EAATs have historically received surprisingly little attention as putative drug targets [2]. EAAT modulation as a therapeutic concept faces the same inherent obstacle as drugs acting through other glutamatergic mechanisms: the considerable risk of inducing adverse effects due to the abundance of glutamatergic neurons in the CNS and the importance of the neurotransmitter for most central processes. Furthermore, most EAAT ligands published to date have been derived from the use of Glu, aspartate or other α-amino acids as lead structures. These efforts have admittedly spawned some important ligands for the transporters, and seemingly simple amino acids can exhibit surprisingly complex pharmacology, as recently demonstrated for the bicyclic Glu analog (+)-HIP-B [22]. Nevertheless, this strategy has some major shortcomings when it comes to developing pharmacological tools. The conserved nature of the substrate binding sites in the five EAATs has, with the exception of a few EAAT2-selective inhibitors, so far been an insurmountable obstacle to the development of truly subtype-selective ligands [2]. Moreover, since both substrates and inhibitors acting through this site will inhibit EAAT-mediated Glu uptake, the therapeutic potential in augmentation of EAAT function cannot be addressed with orthosteric ligands [2]. These realisations have fuelled two alternative approaches for ligand development.

Allosteric modulators. Several endogenous nutrients and exogenous compounds have been found to modulate EAATs, however, the majority of these exhibit rather promiscuous pharmacological profiles and/or low potencies at the transporters (Figure 2a) [23–27]. In recent years we have developed a series of potent EAAT1/GLAST-inhibitors, including UCPH-101 and UCPH-102, from a hit identified in a screening of a small compound library (Figure 2a) [28,29]. The compounds are highly selective for EAAT1 over the other four EAAT subtypes, and they act as negative allosteric modulators (NAMs) inhibiting Glu uptake through EAAT1 non-competitively through an intra-protomeric site involving regions from both the transport and trimerization domains (Figure 2a) [30•]. Interestingly, close structural analogs from this series exhibit profoundly different kinetic properties as EAAT1 inhibitors, with UCPH-101 and UCPH-102 exhibiting slow and fast unbinding kinetics from the transporter, respectively [30•].

The distinct mechanisms of action and binding sites proposed for a couple of allosteric EAAT modulators [24,27,30•] combined with the dramatic 'reshuffling' of