Glutamate (excitatory amino acid) transporters

Overview: Plasma membrane located glutamate transporters (nomenclature proposed by Amara and Arriza, 1993) are members of the solute carrier family 1 (SLC1) of sodium-dependent transporters that also includes the neutral amino acid transporters ASCT1 and ASCT2 (Palacín *et al*., 1998; Kanai and Hediger, 2003; 2004; Beart and O'Shea, 2007). Glutamate transporters present the unusual structural motif of 8TM segments and 2 re-entrant loops (Grunwald and Kanner, 2000). The crystal structure of a glutamate transporter homologue (Glt_{Ph}) from *Pyrococcus horikoshii* supports this topology and indicates that the transporter assembles as a trimer, where each monomer is a functional unit capable of substrate permeation (Yernool *et al*., 2004; Boudker *et al*., 2007). These structural data are in agreement with the proposed quaternary structure for EAAT2 (Gendreau *et al*., 2004) and several functional studies that propose the monomer is the functional unit (Ryan *et al*., 2004; Grewer *et al*., 2005; Koch *et al*., 2007; Leary *et al*., 2007). Splice variants of EAAT2 have recently been shown to form homomeric and heteromeric assemblies at the cell surface (Peacey *et al*., 2009). The activity of glutamate transporters located upon both neurones (predominantly EAAT3, 4 and 5) and glia (predominantly EAAT 1 and 2) serves, dependent upon their location, to regulate excitatory neurotransmission, maintain low ambient extracellular concentrations of glutamate (protecting against excitotoxicity) and provide glutamate for metabolism including the glutamate-glutamine cycle. The Na^+/K^+/ATPase that drives transport has been demonstrated to co-assemble with EAAT1 and EAAT2 (Rose *et al*., 2009). Recent evidence supports novel roles in brain for splice variants of EAAT1 and EAAT2 (Macnab and Pow, 2007; Sullivan *et al*., 2007). Enhanced expression of EAAT2 resulting from administration of ß-lactam antibiotics (e.g. ceftriaxone), or the neuroimmunophilin GPI-1046, is neuroprotective (Rothstein *et al*., 2005; Ganel *et al*., 2006). Enhanced expression by ceftriaxone has been proposed to occur through NF-kappaB-mediated EAAT2 promoter activation (Lee *et al*., 2008), although the protective affects of ceftriaxone in a mouse model of multiple sclerosis are thought to mediated by a reduction in T cell activation with no effect on EAAT2 expression, or function (Melzer *et al*., 2008). A thermodynamically uncoupled Cl^- flux, activated by Na^+ and glutamate (Kanner and Borre, 2002; Kanai and Hediger, 2003; Grewer and Rauen, 2005) (or aspartate in the case of Glt_{Ph}, Ryan and Mindell, 2007), is sufficiently large, in the instances of EAAT4 and EAAT5, to influence neuronal excitability (Veruki *et al*., 2006). In the kidney, EAAT3 located in the apical membrane of proximal tubular cells is responsible for the reabsorption of glutamate (Hediger, 1999). Three structurally and functionally distinct vesicular glutamate transporters (VGLUT1, 2 and 3) of the SLC17 family are responsible for concentrating glutamate within synaptic vesicles (Reimer and Edwards, 2004).

Nomenclature	EAAT1	EAAT2	EAAT3
Other names	GLAST, SLC1A3	GLT1, SLC1A2	EAAC1, SLC1A1
Ensembl ID	ENSG00000079215	ENSG00000110436	ENSG00000106688
Endogenous substrates	L-glutamate, L-aspartate	L-glutamate, L-aspartate	L-glutamate, L-aspartate
Inhibitors (K_B or K_i)	UCPH-101 (IC_{50} = 120 nM – membrane potential assay, Jensen *et al.* (2009), DL-TBOA (9 μM)	WAY-213613 (IC_{50} = 130 nM), DL-TBOA (0.12 μM), (2*S*,4*R*)-4-methylglutamate (3.4 μM), dihydrokainate (9 μM), *Threo*-3-methylglutamate (18 μM)	NBI-59159 (IC_{50} = 25 nM), DL-TBOA (IC_{50} = 8 μM), L-β-BA (IC_{50} = 0.8 μM – [^{3}H]-D-aspartate uptake assay)
Probes	[^{3}H]-ETB-TBOA (K_D = 15.5 nM), [^{3}H]-[(2*S*,4*R*)-4-methylglutamate, [^{3}H]-D-aspartate, [^{3}H]-L-aspartate	[^{3}H]-ETB-TBOA (K_D = 16.2 nM), [^{3}H]-[(2*S*,4*R*)-4-methylglutamate, [^{3}H]-D-aspartate, [^{3}H]-L-aspartate	[^{3}H]-ETB-TBOA (K_D = 320 nM), [^{3}H]-D-aspartate, [^{3}H]-L-aspartate
Stoichiometry	–	$3Na^+$: $1H^+$: 1glutamate (in): $1K^+$ (out)	$3Na^+$: $1H^+$: 1glutamate (in): $1K^+$ (out)

Nomenclature	EAAT4	EAAT5
Other names	SLC1A6	SLC1A7
Ensembl ID	ENSG00000105143	ENSG00000162383
Endogenous substrates	L-glutamate, L-aspartate	L-glutamate, L-aspartate
Inhibitors (K_B or K_i)	DL-TBOA (4.4 μM), *Threo*-3-methylglutamate (50 μM)	DL-TBOA (3.2 μM)
Probes	[^{3}H]-ETB-TBOA (K_D = 24.8 nM), [^{3}H]-D-aspartate, [^{3}H]-L-aspartate	[^{3}H]-ETB-TBOA (K_D = 29.5 nM), [^{3}H]-D-aspartate, [^{3}H]-L-aspartate

The K_B (or K_i) values reported, unless indicated otherwise, are derived from transporter currents mediated by EAATs expressed in voltage-clamped *Xenopus laevis* oocytes (Vandenberg *et al*., 1997; Shimamoto *et al*., 1998; Eliasof *et al*., 2001; Shigeri *et al*., 2001). K_B (or K_i) values derived in uptake assays are generally higher (e.g. Shimamoto *et al*., 1998). In addition to acting as a poorly transportable inhibitor of EAAT2, (2*S*,4*R*)-4-methylglutamate, also known as SYM2081, is a competitive substrate for EAAT1 (K_M = 54μM; Vandenberg *et al*., 1997; Huang *et al*., 2009) and additionally is a potent kainate receptor agonist (Zhou *et al*., 1997), which renders the compound unsuitable for autoradiographic localisation of EAATs (Apricò *et al*., 2007). Similarly, at concentrations that inhibit EAAT2, dihydrokainate binds to kainate receptors (Shimamoto *et al*., 1998). WAY-855 and WAY-213613 are both non-substrate inhibitors with a preference for EAAT2 over EAAT3 and EAAT1 (Dunlop *et al*., 2003; Dunlop *et al*., 2005). NBI-59159 is a non-substrate inhibitor with modest selectivity for EAAT3 over EAAT1 (>10-fold) and EAAT2 (fivefold) (Coon *et al*., 2004; Dunlop, 2006). Analogously, L-β-*threo*-benzyl-aspartate (L-β-BA) is a competitive non-substrate inhibitor that preferentially blocks EAAT3 versus EAAT1, or EAAT2 (Esslinger *et al*., 2005). [^{3}H]-[(2*S*,4*R*)-4-methylglutamate demonstrates low affinity binding ($K_D \cong 6.0$ μM) to EAAT1 and EAAT2 in rat brain homogenates (Apricò *et al*., 2001) and EAAT1 in murine astrocyte membranes (Apricò *et al*., 2004), whereas [^{3}H]-ETB-TBOA binds with high affinity to all EAATs other than EAAT3 (Shimamoto *et al*., 2007). The novel isoxazole derivative (-)-HIP-A may interact at the same site as TBOA and preferentially inhibit reverse transport of glutamate (Colleoni *et al*., 2008). *Threo*-3-methylglutamate induces substrate-like currents at EAAT4, but does not elicit heteroexchange of [^{3}H]-aspartate in synaptosome preparations, inconsistent with the behaviour of a substrate inhibitor (Eliasof *et al*., 2001). Parawixin1, a compound isolated from the venom from the spider *Parawixia bistriata* is a selective enhancer of the glutamate uptake through EAAT2 but not through EAAT1 or EAAT3 (Fontana *et al*., 2003; 2007). In addition to the agents listed in the table, DL-*threo*-β-hydroxyaspartate and L-*trans*-2,4-pyrolidine dicarboxylate act as non-selective competitive substrate

inhibitors of all EAATs. Zn^{2+} and arachidonic acid are putative endogenous modulators of EAATs with actions that differ across transporter subtypes (reviewed by Vandenberg *et al.*, 2004).

Abbreviations: **DL-TBOA**, DL-*threo*-β-benzyloxyaspartate; **GPI-1046**, (3-(3-pyridyl)-1-propyl (2*S*)-1-(3,3-dimethyl-1,2-dioxopentyl)-2-pyrrolidinecarboxylate; **ETB-TBOA**, (2*S*, 3*S*)-3-{3-[4-ethylbenzoylamino]benzyloxy}aspartate; **(-)-HIP-A**, (-)-3-hydroxy-4,5,6,6a-tetrahydro-3a*H*-pyrrolo[3,4-d]-isoxazole-4-carboxylic acid; **NBI-59159** (also known as WAY-209429), (*N*-4-(9*H*-fluoren-2-yl)-L-asparagine; **WAY-855**, 3-amino-tricyclo[2.2.1.$0^{2,6}$]heptane-1,3-dicarboxylic acid; **WAY-213613**, *N*(4)-[4-(2-bromo-4,5-difluorophenoxy)phenyl]-L-asparagine; **UCPH-101**, 2-amino-4-(4-methoxyphenyl)-7-(naphthalene-1-yl)-5-oxo-5,6,7,8-tetrahydro-4*H*-chromene-3-carbonitrile

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