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The glutamate/neutral amino acid transporter family SLC1: molecular, physiological and pharmacological aspects

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Abstract The solute carrier family 1 (SLC1) includes five high-affinity glutamate transporters, EAAC1, GLT-1, GLAST, EAAT4 and EAAT5 (SLC1A1, SLC1A2, SLC1A3, SLC1A6, and SLC1A7, respectively) as well as the two neutral amino acid transporters, ASCT1 and ASCT2 (SLC1A4 and ALC1A5, respectively). Although each of these transporters have similar predicted structures, they exhibit distinct functional properties which are variations of a common transport mechanism. The high-affinity glutamate transporters mediate transport of L-Glu, L-Asp and D-Asp, accompanied by the cotransport of 3 Na^+ and 1 H^+, and the countertransport of 1 K^+, whereas ASC transporters mediate Na^+-dependent exchange of small neutral amino acids such as Ala, Ser, Cys and Thr. The unique coupling of the glutamate transporters allows uphill transport of glutamate into cells against a concentration gradient. This feature plays a crucial role in protecting neurons against glutamate excitotoxicity in the central nervous system. During pathological conditions, such as brain ischemia (e.g. after a stroke), however, glutamate exit can occur due to "reversed glutamate transport", which is caused by a reversal of the electrochemical gradients of the coupling ions. Selective inhibition of the neuronal glutamate transporter EAAC1 (SLC1A1) may be of therapeutic interest to block glutamate release from neurons during ischemia. On the other hand, upregulation of the glial glutamate transporter GLT1 (SLC1A2) may help protect motor neurons in patients with amyotrophic lateral sclerosis (ALS), since loss of function of GLT1 has been associated with the pathogenesis of certain forms of ALS.

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Introduction

The SLC1 family consists of five high-affinity glutamate transporters and two neutral amino acid transporters. The different properties of each of these gene products is summarized in Table 1 and their phylogenetic relationships is shown in Fig. 1.

The identification of the high-affinity glutamate transporters started in 1992 when three isoforms (EAAC1, GLT1 and GLAST) were independently identified using different approaches. Expression cloning with *Xenopus* oocytes was used by Kanai and Hediger to isolate a cDNA encoding the neuronal and epithelial high-affinity glutamate transporter EAAC1 [37]. Kanner and coworkers [12] purified a 73-kDa glycoprotein (GLT1) from crude

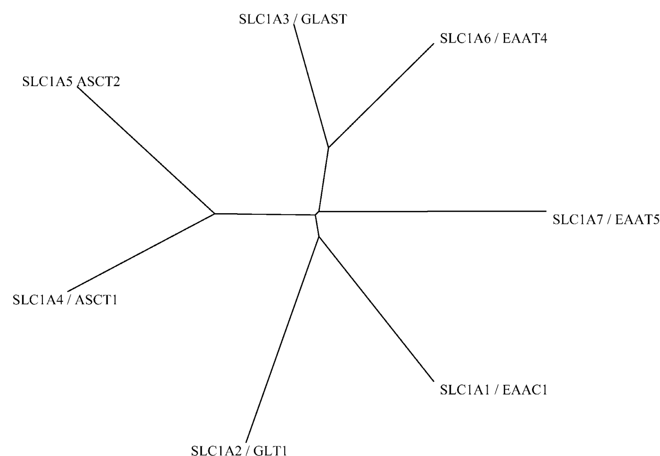

Fig. 1 Phylogenetic tree of the transporters enhanced by the SLC family. The SLC1 family is composed of two subfamilies that encodes the high-affinity glutamate transporters (*SLC1A1*, *2*, *3*, *6* and k) and the system ASC neutral amino acid transporters (*SLC1A4* and *5*)

Table 1 SLC1: the high-affinity glutamate and neutral amino acid transporter family

Human gene name	Protein name	Aliases	Predominant substrates	Transport type/coupling ions[a]	Tissue distribution and cellular/subcellular expression	Link to disease[b]	Human gene locus	Sequence Accession ID	Splice variants and their specific features
SLC1A1	EAAC1 EAAT3	System X^-_{AG}	L-Glu, D/L-Asp	C/Na^+, H^+ and K^+	Brain (neurons), intestine, kidney, liver, heart	G/dicarboxylic amino aciduria?	9q24	NM_004170	
SLC1A2	GLT-1 EAAT2	System X^-_{AG}	L-Glu, D/L-Asp	C/Na^+, H^+ and K^+	Brain (astrocytes), liver	A/amyotrophic lateral sclerosis	11p13-p12	NM_004171	C-terminal spliced variants expressed in neurons
SLC1A3	GLAST EAAT1	System X^-_{AG}	L-Glu, D/L-Asp	C/Na^+, H^+ and K^+	Brain (astrocytes), heart, skeletal muscle, placenta		5p13	NM_004172	
SLC1A4	ASCT1 SATT	System ASC	L-Ala, L-Ser, L-Cys	C/Na^+, E/amino acids	Widespread		2p15-p13	NM_003038	
SLC1A5	ASCT2 AAAT	System ASC	L-Ala, L-Ser, L-Thr, L-Cys, L-Gln	C/Na^+, E/amino acids	Lung, skeletal muscle, large intestine, kidney, testis, adipose tissue		19q13.3	U53347 NM_005628	
SLC1A6	EAAT4	System X^-_{AG}	L-Glu, D/L-Asp	C/Na^+, H^+ and K^+	Cerebellum (Purkinje cells)		19	NM_005071	
SLC1A7	EAAT5	System X^-_{AG}	L-Glu, D/L-Asp	C/Na^+, H^+ and K^+	Retina		1p	U76362 NM_006671	

[a] *C* cotransporter, *E* exchanger, *F* facilitated transporter, *O* orphan transporter
[b] *A* acquired defect, *G* genetic defect

synaptosome fraction P_2, which when reconstituted into liposome was demonstrated to exhibit high-affinity glutamate transport. An antibody was then raised against the purified protein and used to isolate a clone from a rat brain cDNA library that encodes the glial glutamate transporter GLT1 [56]. Stoffel and colleagues [74] copurified a 66-kDa hydrophobic glycoprotein, the glial glutamate transporter GLAST, during the isolation of UDPgalactose:ceramide galactosyltransferase, and obtained its cDNA based on partial amino acid sequencing. Two additional glutamate transporters, EAAT4 and EAAT5, and two Na^+-dependent neutral amino acid transporters, ASCT1 and ASCT2, were subsequently identified based on sequence homology to those glutamate transporters [2, 4, 18, 43, 71, 74, 81]. Glutamate transporters exhibit 44–55% amino acid sequence identity with each other. The neutral amino acid transporters ASCT1 and ASCT2 possess 57% identity. The identity between glutamate transporters and ASC transporters is 40–44%. High-affinity glutamate transporters are coupled to the inwardly directed electrochemical potential gradients of Na^+ and H^+, and to the outwardly directed gradient of K^+, whereas system ASC transporters are coupled only to the Na^+ electrochemical potential gradient.

Glutamate transporters

Brief description of the individual glutamate transporter isoforms

SLC1A1 (protein names: EAAC1, EAAT3)

SLC1A1[1] encodes the glutamate transporter EAAC1 (also known as EAAT3). EAAC1 is a neuronal and epithelial type high-affinity glutamate transporter [37]. It is predominantly expressed in neurons of various brain areas, particularly in hippocampus, cerebral cortex, olfactory bulb, striatum, superior colliculus and thalamus [7, 40, 64]. In the kidney, EAAC1 is present in the apical membrane of proximal tubules [72]. EAAC1-knockout mice did not develop remarkable neurological symptoms and neurodegeneration during a period of more than 12 months, except that homozygous mutants display a significantly reduced spontaneous locomotor activity [55]. EAAC1 knockout mice developed dicarboxylic aminoaciduria, confirming the role of EAAC1 in the reabsorption of glutamate from the renal proximal tubules [55].

SLC1A2 (protein names: GLT1, EAAT2)

SLC1A2 encodes the glial type high-affinity glutamate transporter GLT1 [56], also known as EAAT2. It is

[1] Note: capital letters are used for the human "SLC" gene names, whereas lower case "slc" names refer to non-human versions (rodents, etc.)

expressed in astrocytes of various brain regions particularly in cerebral cortex and hippocampus. The GLT1-knockout mice showed dramatic lethal spontaneous epileptic seizures with behavioral patterns similar to those of NMDA-induced seizures [77]. The mice also exhibited increased susceptibility to acute cortical injury. Histological examination of GLT1-knockout mice revealed selective neuronal degeneration in the hippocampal CA1 region, consistent with the roles GLT1 plays in neuroprotection [77].

Electrophysiological analysis of the CA1 pyramidal neurons of these knockout mice revealed that the inhibition of the NMDA-receptor-mediated EPSC in the presence of the rapidly dissociating NMDA-receptor antagonist was significantly less in the knockout mice than in the wild type mice, indicating that GLT1 contributes to the removal of glutamate from the synaptic cleft [86]. In the retina, GLT1 is found only in cones and various types of bipolar cells. GLT1-deficient mice show almost normal electroretinograms and mild increased damage after ischemia, which is in contrast to the prominent changes of electroretinograms and sever retinal damage after ischemia [29].

Pharmacologically, GLT1 is distinct from the other high-affinity glutamate transporter isoforms. GLT1 is selectively inhibited by dihydrokainate and kainate, which are non-transportable inhibitors of GLT1 [3]. Several reports have indicated the presence of spliced variants for GLT1 [26, 33, 50, 82].

SLC1A3 (protein names: GLAST, EAAT1)

SLC1A3 encodes the glutamate transporter GLAST, also known as EAAT1. GLAST is a glial type high-affinity glutamate transporter which is particularly abundant in the cerebellum [74]. It is expressed in astrocytes and cerebellar Bergmann glia [64]. Although GLAST-knockout mice developed normally and could manage simple coordinated tasks, they exhibited motor discoordination for more difficult tasks, consistent with the abnormality in cerebellum [86]. Electrophysiologically, it was demonstrated that cerebellar Purkinje cells in the knockout mice remained to be innervated by climbing fibers even at the adult stage. The knockout mice, furthermore, exhibited increased susceptibility to cerebellar injury [86].

In the retina, GLAST is expressed in Muller cells. In GLAST-deficient mice, the electroretinogram beta-wave and oscillatory potentials are reduced, indicating that GLAST is required for normal signal transmission between photoreceptors and bipolar cells. In addition, retinal damage after ischemia is exacerbated in the GLAST knockout mice [29]. In the peripheral auditory system, it is suggested that GLAST plays an important role in keeping the concentration of glutamate in the perilymph at a nontoxic level during acoustic overstimulation, because GLAST-deficient mice showed increased accumulation of glutamate in perilymphs after acoustic overstimulation, resulting in exacerbation of hearing loss [28]. The effect of the disruption of the GLAST gene on epileptogenesis was also investigated. In GLAST knockout mice, the generalized seizure duration of amygdala-kindled seizures was significantly prolonged compared with that of wild type mice. Furthermore, GLAST knockout mice showed more severe stages of pentylenetetrazol-induced seizures than wild type mice, and the latency to the onset of seizures was significantly shorter for the mutant mice, indicating that GLAST is one of the determinant of seizure susceptibility [85].

SLC1A6 (protein name: EAAT4)

SLC1A6 encodes the neuronal high-affinity glutamate transporter EAAT4 which is predominantly expressed in cerebellar Purkinje cells on postsynaptic dendritic spines [18, 51]. This transporter shows a remarkable thermodynamically uncoupled chloride conductance associated with substrate transport [18].

SLC1A7 (protein name: EAAT5)

SLC1A7 encodes the a high-affinity glutamate transporter EAAT5 which is primarily expressed in the retina [4]. Immunocytochemical studies of the rat retina showed that EAAT5 is associated with rod photoreceptors and some bipolar cells [57, 58]. Transporter currents elicited by glutamate are largely carried by chloride ions, indicating that EAAT5 exhibits a prominent chloride conductance [4]. These properties of EAAT5 are similar to the glutamate-elicited chloride conductances previously described in retinal neurons, suggesting that the EAAT5-associated chloride conductance may participate in visual processing.

Functional properties of glutamate transporters

Ion-coupling stoichiometry

Cellular uptake of glutamate must occur against a steep electrochemical potential gradient. Glutamate transporters couple glutamate uptake to the transport of inorganic ions, thereby utilizing the free energy stored as electrochemical potential gradients of these ions to power uphill transport. This coupling mechanism is essential for the efficient removal of glutamate from extracellular fluids such as the cerebrospinal fluid, the intestinal lumen and the lumen of renal proximal tubules. The coupling stoichiometry of the cloned glutamate transporter (EAAC1) was analyzed in *Xenopus* oocyte expression system. It is now generally accepted that 3 Na^+ ions and 1 H^+ are co-transported and 1 K^+ is counter-transported with each glutamate molecule [89] (see Fig. 2, left). Based on this stoichiometry, it was calculated that glutamate transporters can concentrate glutamate 5×10^6-fold inside cells under physiological conditions [89]. Assuming a concentration of 10 mM

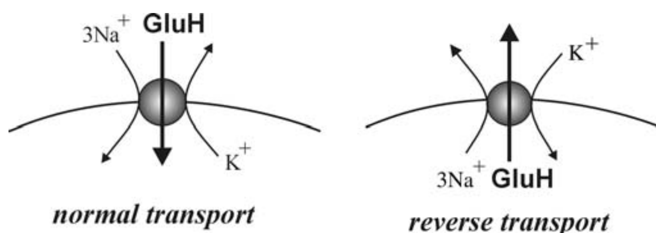

Fig. 2 Stoichiometry as well as normal and reversed transport of glutamate transporters. *GluH* protonated form of glutamate

inside glutamatergic neurons, the extracellular concentration which can be achieved with this stoichiometry is ~2 nM.

The H^+ co-transport was demonstrated by voltage clamp studies with a pH-sensitive fluorescent dye, showing that the transport of an equivalent amount of L-cysteine, a neutral amino acid substrate of EAAC1, did not result in the intracellular acidification, in spite of the marked acidification induced by L-glutamate or L-cysteate [89]. After intracellular release, L-cysteine (pK=8.3) remains predominantly protonated, whereas glutamic and cysteic acids (pK<5) release the proton. An alternative model was recently proposed, indicating that glutamate removal does not require binding of an extracellular H^+ [5]. According to this model, the translocation of an H^+ occurs within the K^+-transporting portion of the transport cycle, when glutamate is not bound to the transporter. Further studies are required to determine the exact role of protons during the glutamate transport cycle.

Steady-state and pre-steady-state kinetics

The detailed analysis of the steady-state and pre-steady-state currents displayed by glutamate transporters in response to step changes of the membrane potential has provided important clues to their dynamics and structure. With respect to the glutamate transporter GLT1, kainate, a non-transported inhibitor, was used to “freeze” the transporter-inhibitor complex in a non-transporting form, in order to isolate pre-steady-state currents [84]. Based on the Na^+-dependence of the pre-steady-state currents, it was concluded that the currents reflect the voltage-dependent binding and unbinding of Na^+ near the extracellular surface of the transporter (Fig. 3, step 1) and that they do not reflect conformational changes of charged residues of the transporter molecule in the membrane electrical field (Fig. 3, step 6) [84]. This is in agreement with the previous speculation that the empty carrier of glutamate transporters behaves “electroneutral” within the membrane electric field [32]. Analysis of the steady-state currents of the glutamate transporters revealed a strong voltage-dependence of the glutamate-evoked currents [18, 39, 41, 44, 84]. A specific voltage-dependent step, the so-called “charge translocation step” (i.e., step 3), was proposed to be rate-limiting during the

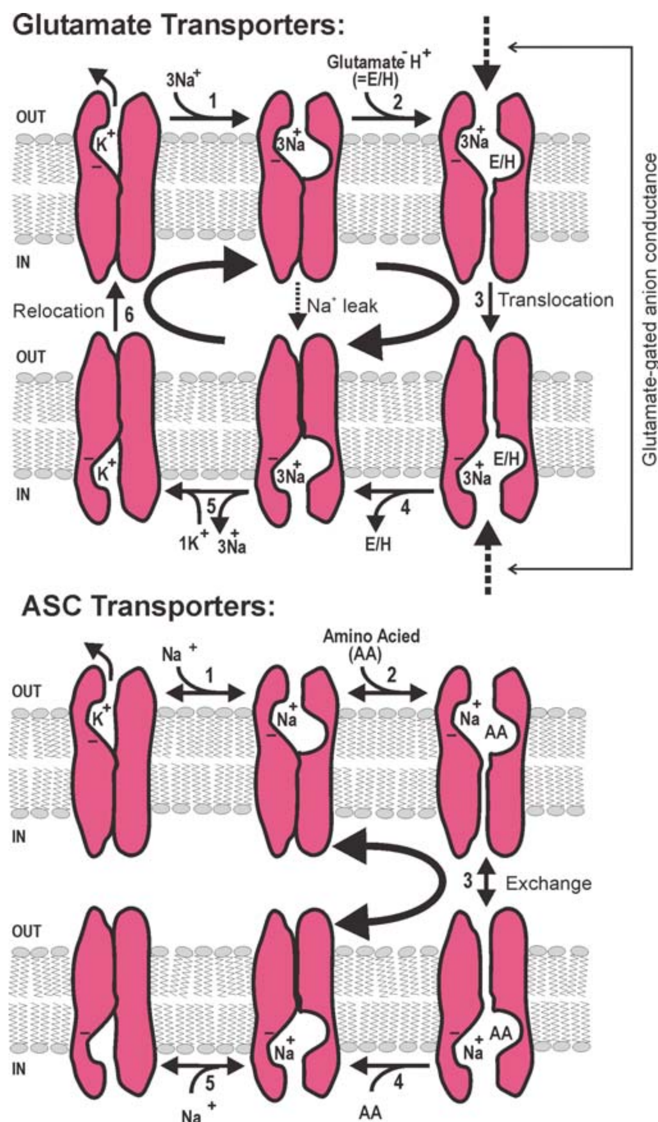

Fig. 3 Kinetic models of glutamate (*top*) and ASC transporters (*bottom*) (hypothetical). *Top part*: under normal conditions, glutamate transport involves loading the empty glutamate carrier with glutamate$^-$/H^+ and 3 Na^+ (steps 1 and 2), followed by translocation of the fully loaded carrier across the plasma membrane (charge translocation step; step 3) and release of the substrates at the intracellular face (steps 4 and 5). Thereafter, K^+ binds to the carrier inside and promotes the relocation of the empty carrier (step 6). For net uptake of glutamate, glutamate transporters have to complete this cycle. If it is not completed because the empty carrier cannot enter the relocation step, the empty carrier binds Na^+ and glutamate again at the inside of the cells and translocates back in the reverse direction (steps 5 to 1). In this case, the transporter behaves like an exchanger. The substrate-gated anion conductance is indicated on the right (hypothetical). *Bottom part*: Because the GLT1 mutant, Glu404Asp, and the ASC transporters lack the K^+-coupling step, they cannot enter the relocation step and will only work in the exchange mode. Similar to glutamate transporters, ASCT transporters also exhibit substrate-gated anion conductances (not shown)

transport cycle [39, 41]. The countertransport of K^+ (step 6) appears to speed up the relocation of the empty carrier so that the relocation step is faster than the charge translocation step.

Anion conductance

The functional characterization of glutamate transporter EAAT4 has led to the identification of an additional feature of high-affinity glutamate transporters, a substrate gated anion conductance [18] (see Fig. 3, top right). This is also displayed by other SLC1 family members [4, 8, 18, 83, 88]. The anion permeability of glutamate transporters decreases in the order EAAT4/5>GLAST >EAAC1>>GLT1. The anion conductance has the characteristics of a substrate-gated anion channel with a selectivity order $NO_3^- > I^- > Br^- > Cl^- > F^-$ [18, 83]. Cl^- is mainly translocated in the presence of glutamate or related substrates, and Cl^- movement is not thermodynamically coupled to the substrate transport. Cl^- is therefore not necessary for substrate translocation [18, 83]. By using rapid applications of glutamate to outside-out patches excised from transfected human embryonic kidney 293 cells, it was demonstrated that both anion and stoichiometric currents display similar kinetics, suggesting that anion channel gating and stoichiometric charge movement are linked to early transitions in the transport cycle [52]. It was shown that EAAT5 exhibits a prominent chloride conductance compared with its amino acid fluxes [4]. Thus, glutamate transporters appear to possess structures that can function as chloride channels.

Exchange mode

The glutamate transporter EAAC1 can also facilitate substrate exchange, in addition to electrogenic glutamate uptake [90] (Fig. 3, steps 1–5). However, under normal conditions, the exchange component is small compared to the uptake component [89, 90]. In contrast, ASC transporters ASCT1 and ASCT2 mediate exclusively Na^+-dependent exchange of substrate amino acids [8, 88] (see Fig. 3, bottom). Site-directed mutagenesis provided the answer to the question of why glutamate transporters mediate two transport modes, whereas ASC transporters mediate only the exchange mode: Mutation of Glu 404 or Tyr 403 resulted in loss of K^+-coupling in rat GLT1 [42, 91] (see Fig. 4). Those GLT1 mutants displayed only the exchange mode without regular uptake, similar to ASC transporters. Interestingly, the Glu and Tyr residues are conserved in all glutamate transporters but not in ASCT1 and ASCT2. ASC transporters were reported not to be coupled to the K^+ [88]. Thus, it was concluded that these Glu and Tyr residues are crucial for the K^+-coupling which drives the relocation step of glutamate transporters. Because K^+-coupling was disrupted in those Glu and Tyr mutants, the mutant GLT1 was not able to facilitate the relocation step, that is the conversion of the empty carrier from the inwardly facing to the outwardly facing state after releasing glutamate at the intracellular surface. The only relocation for this mutant is the reversal of the Na^+-coupled uptake step, resulting in glutamate efflux (Fig. 3, top, steps 5–1).

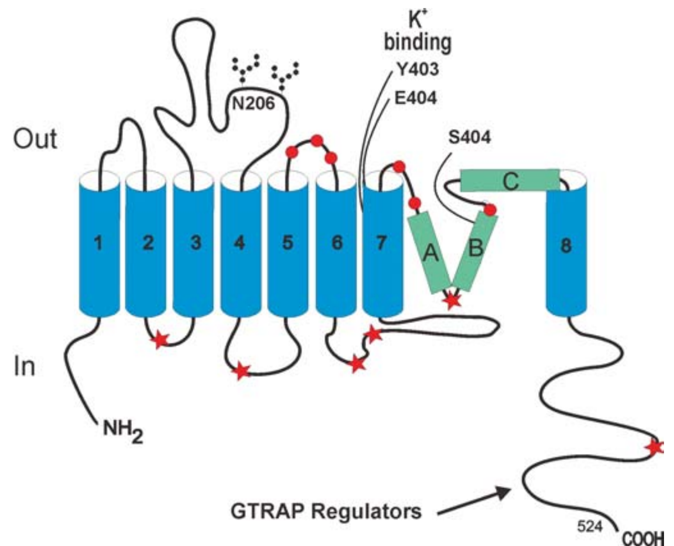

Fig. 4 Membrane topology model of glutamate transporters. The topology shown is based on experiments involving biotinylation of single cysteine mutants of the glutamate transporter GLT1 from rat brain [24, 25]. The results of labeling with a biotinylation reagent are shown. *Full circles* refer to labeling with the biotinylation agent in the absence of permeabilization. *Stars* refer to labeling after permeabilization. The transmembrane domains which are long enough to span the membrane as α-helices are indicated by *arabic* numbers. The legs of the reentrant loop are indicated by *A* and *B*. *C* corresponds to a "loop" which is predicted to extend partially into the "translocation pore" between trans-membrane domains 7 and 8

Structure-function relationship of glutamate transporters

The SLC1 family members have a unique, highly conserved long hydrophobic stretch near the *C*-terminus (Fig. 4). Cysteine-scanning mutagenesis was performed to examine the accessibility of amino acid residues in the hydrophobic stretch using sulfidyl-reactive reagents [24, 70]. The model in Fig. 4 features 8 predicted α-helical trans-membrane domains (#1–8), a large extracellular glycosylated loop between transmembrane domains 3 and 4, a "reentrant loop" (A/B) between trans-membrane domains 7 and 8 similar to the ion-permeating pore of ion channels, and a "loop" which is predicted to extend partially into the "translocation pore" between trans-membrane domains 7 and 8.

In the 7th transmembrane domain, there are two amino acid residues, Tyr 403 and Glu 404 that are important for the coupling with K^+ and the relocation of the carrier [42, 91]. With respect to the GLT1 mutant at position Tyr 403, it was further reported that it functions not only in the presence of Na^+ but also in presence of Li^+ or Cs^+, in contrast to wild type GLT1 which cannot use Li^+ or Cs^+ as a coupling ion [91], suggesting that Tyr 403 is associated with the Na^+-binding site or the structure responsible for the selectivity of inorganic cations. It was also reported that mutation at Ser 440 which resides at the extracellular face of the re-entrant loop (B in Fig. 4) alters the selectivity to inorganic cations and the sensitivity to the glutamate transport inhibitors [92]. Therefore, Ser 440

is important for the Na^+-coupling mechanism as well and it is tightly associated with the substrate binding site of glutamate transporters.

Although the three-dimensional structure of glutamate transporters is not available at present, freeze-fracture electron microscopy of *Xenopus* oocytes overexpressing human EAAC1 (EAAT3) revealed distinct 10-nm freeze-fracture particles which appeared in the protoplasmic face only after EAAC1 expression [17]. The cross-sectional area of the human EAAC1 particles in the plasma membrane (48 ± 5 nm^2) predicted 35 ± 3 transmembrane α-helices in the transporter complex. This information along with secondary structure models (6–10 transmembrane α-helices) suggests that human EAAC1 particles were pentagonal in which five domains could be identified. It is speculated that, although the EAAC1 monomer can perform secondary active transport, the chloride channel mode seen in glutamate transporters is related to the oligomeric assembly [17].

Physiological significance of glutamate transporters

Role of glutamate transporters in glutamatergic transmission

It is now generally accepted that glutamate transporters play significant roles in terminating glutamatergic transmission (Fig. 5). The contribution of glutamate transporters to shape the excitatory postsynaptic potential at glutamatergic synapses has been controversial because of the rapid diffusion of glutamate from the synaptic cleft and the fast decay of the postsynaptic potential due to the rapid desensitization of non-NMDA type glutamate-receptors [34, 49, 68, 78]. Glutamate transporters, however, play important roles in removing released glutamate from the synaptic cleft. Although the turnover rate of glutamate transporters estimated based on the analysis of steady-state and pre-steady-state currents is low [84], binding of glutamate to glutamate transporters exhibits fast kinetics, which significantly contributes to the glutamate clearance in the synaptic cleft [78]. Recently the laser-pulse photolysis technique of caged glutamate [23] demonstrated that EAAC1-mediated pre-steady-state currents are composed of two components: a transport current generated by substrate-coupled charge translocation across the membrane and an anion current that is not thermodynamically coupled to glutamate transport. It was concluded that glutamate translocation occurs within a few milliseconds after binding. The transition to an anion-conducting state is, however, delayed with respect to the onset of glutamate transport [23].

Role of glutamate transporters in maintaining extracellular glutamate concentration

Based on the stoichiometry of glutamate transporters and the prevailing ionic environment, glutamate transporters

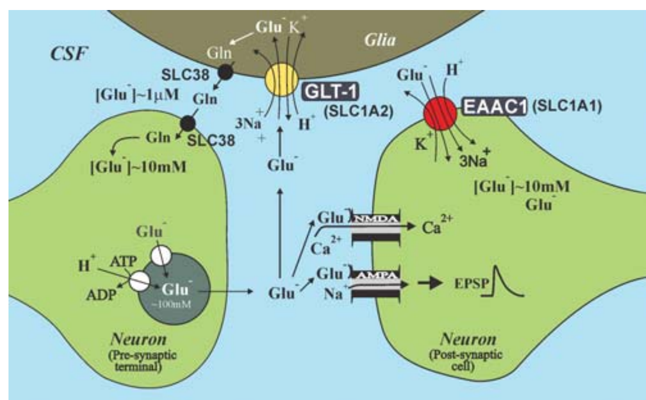

Fig. 5 Glutamate transporters and glutamatergic synapses. The excitatory neurotransmitter L-glutamate is stored in synaptic vesicles at presynaptic terminals. Glutamate is transported into these vesicles by the vesicular glutamate transporters VGluT1, VGluT2 and VGluT3 (SLC17A6, SLC17A7 and SLC17A8, respectively). Glutamate is released into the synaptic cleft to act on glutamate receptors. The *AMPA* receptors mediate fast excitatory postsynaptic potentials, whereas the *NMDA* receptors possesses a cation channel which is permeable to Ca^{2+}. High-affinity glutamate transporters play essential roles in removing released glutamate from the synaptic cleft. These transporters are also crucial for maintaining the extracellular glutamate concentration of the cerebrospinal fluid (CF) below neurotoxic levels. The high accumulative power of glutamate transporters is provided by the coupling transport to the co- or countertransport of the ions Na^+, H^+ and K^+ (see Fig. 1). The figure shows the neuronal glutamate transporter EAAC1 (*SLC1A1*) and the glial glutamate transporter GLT1 (*SLC1A2*). Much of the glutamate taken up into glial cells via GLT1 (SLC1A2) or GLAST (SLC1A3) is metabolized to glutamine by glutamine synthase (with hydrolysis of ATP). Glial cells in turn supply the nerve terminal with glutamine which serve as precursors of glutamate synthesis. Glutamine exits astrocytes via an SLC38 (system N) transporter and then enters neurons via another SLC38 (system A) transporter

can concentrate glutamate more than 10^6-fold across cell membranes (see above and [45]). Because of this high concentrating capacity, high-affinity glutamate transporters are thought to play a major role in maintaining the extracellular glutamate concentration at low levels and to protect neurons from the excitotoxic action of glutamate [63]. Using antisense oligonucleotides corresponding to each glutamate transporter isoform, it was demonstrated that glial but not neuronal isoforms are critical to maintain extracellular glutamate concentration and to protect neurons from glutamate excitotoxicity, which is understood when considering the difference in the intracellular glutamate concentration between neurons and glial cells [66]. The importance of glial glutamate transporters in protecting neurons from glutamate was also demonstrated in the glutamate transporter knockout mice (see below).

Regulation of glutamate transporters

Several studies have indicated that neuronal and non-neuronal factors are required for the expression and

maintenance of functionally active glutamate transporters [15, 20, 69, 75]. For example, GLT1 can be induced in astroglial cultures by the pituitary adenylate cyclase-activating peptide (PACAP), a neuron-derived peptide [19], by brain-derived neurotrophic factor (BDNF), a neurotrophin that is predominantly expressed in neurons [21], or by epidermal growth factor (EGF) receptor activation [87]. In C6 glioma, the Wnt-1 gene product, an autocrine- and paracrine-soluble factor, induces GLT1 expression [53]. Growth hormone stimulates GLT1 expression in mouse placenta, whereas insulin-like growth factor II (IGF-II) downregulates EAAT4. Physiological concentrations of IGF-II ensure maintenance of GLT1, GLAST and EAAC1 at normal levels [48]. Several growth factors that are neuroprotective also increase transport activity. For example, platelet-derived growth factor (PDGF) increases cell surface expression of EAAC1 in C6 glioma cells, but has no effect on transporter expression [73]. Activity-dependent neurotrophic factor (ADNF) enhances basal glutamate transport in neocortical synaptosomes and attenuates oxidative stress-induced impairment of glutamate uptake, as does basic fibroblast growth factor (bFGF) and nerve growth factor (NGF) [27].

The functional activity of membrane proteins is often regulated by rapidly changing their expression at the plasma membrane. Recently, it was shown that EAAC1 expressed in *Xenopus* oocytes is downregulated by activation of protein kinase C (PKC) [80]. This downregulation was found to be associated with a decrease in the maximal transport rate (V_{max}) and a movement of the transporter from the plasma membrane to intracellular compartments, with no change in the affinity for glutamate (K_m^{Glu}). The findings suggest that protein kinase C does not directly phosphorylate EAAC1 but rather alters indirectly the distribution of EAAC1 in the plasma membrane and intracellular vesicles. Another study also showed that activation of PKC induces a consistent decrease in the activity of EAAC1 expressed in the human U373 astrocytoma cell line [16]. In contrast, experiments with C6 glioma cells, which endogenously express EAAC1 only, showed that activation of PKC increases glutamate transport activity within minutes by mobilizing EAAC1 in the plasma membrane [13, 14, 22]. Studies of other glutamate transporter isoforms showed that activation of PKC down- or up-regulates the activity of the glutamate transporters: Glutamate transporter GLAST expressed in *Xenopus* oocytes or HEK293 cells was downregulated by PKC, possibly via direct phosphorylation at a non-specified non-consensus site of GLAST [11]; glutamate transporter GLT1 expressed in HeLa cells was upregulated by PKC via direct phosphorylation at serine residues 113 of GLT1 [10]. In both cases, PKC activation was not altered by transporter expression in the plasma membrane.

Among EAAC1, GLT1 and GLAST, only EAAC1 appears to be regulated by PKC via intracellular trafficking events, and probably this occurs in cell-type specific fashion, since activation of PKC leads to opposite effects on EAAC1 expressed in different culture models. Since EAAC1 is expressed in many different tissues (brain, intestine, kidney, liver, heart, etc) where it serves different purposes [30, 31], regulation by phosphorylation will likely depend on the local phosphorylation-sensitive trafficking systems. In post-synaptic elements of neurons, the metabotropic glutamate receptor mGluR1a is coupled to phospholipase C and often co-localizes with EAAC1. Activation of mGluR1 may downregulate EAAC1 to elevate extracellular glutamate concentration and increase synaptic efficacy. Alternatively, prolonged activation will sustain neurotoxic events.

In addition to growth factors or protein phosphorylation, glutamate transporters are regulated by their associated proteins. By means of yeast two-hybrid screening, it was demonstrated that distinct proteins interact with EAAT4 and EAAC1. Two proteins, called GTRAP41 and GTRAP48 (for glutamate transporter EAAT4 associated proteins) were found to specifically interact with the intracellular carboxyl-terminal domain of EAAT4 and to modulate its glutamate transport activity [35]. The expression of either GTRAP41 or GTRAP48 resulted an increase in V_{max} of glutamate transport without altering the K_m value. EAAC1 interacts with the completely different protein called GTRAP3–18 which interacts with the intracellular carboxyl-terminal domain of EAAC1 [47]. GTRAP3–18 reduces the EAAC1-mediated glutamate transport by decreasing the affinity of the transporter for glutamate.

Pathological and pharmacological relevance of glutamate transporters

Glutamate transporters and ischemia

Glutamate neurotoxicity in brain areas of severe ischemia is mainly caused by reversal of glutamate transporters, most likely neuronal glutamate transport [62]. As already noted, glutamate transport is driven by the free energy stored in the form of electrochemical potential transmembrane gradients of the coupling ions. The disruption of these gradients due to insufficient energy supply during ischemia will result either in decreased glutamate uptake or reversed glutamate transport, i.e., cellular release of glutamate (see Fig. 1, right). Since neurons have a much higher content of glutamate than glial cells, neuronal glutamate transporters are more likely to run in reverse in ischemia and to contribute to the extracellular rise in glutamate to excitotoxic levels [38]. Selective inhibitors of neuronal glutamate transporters may therefore be of therapeutic interest to prevent reversed glutamate transport without affecting glial glutamate transporters, thereby minimizing the raise in extracellular glutamate concentration.

Role of glutamate transporters in amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive neurological disorder characterized by degeneration of upper and lower motor neurons. Although about 20% of cases arise because of mutations in the gene for Cu/Zn superoxide dismutase (SOD1) [9, 60, 61], the primary pathogenic trigger is still unknown. A decrease in the glutamate transporter activity due to the reduction of the GLT1 (EAAT2) isoform in motor and sensory cortex of sporadic ALS patients has been reported [64, 65]. More recently, aberrant splicing of the GLT1 transcript was suggested to be the cause of a reduced expression of GLT1 in ALS [46]. Another factor which may contribute to loss of GLT1 in ALS brains is the fact that the GLT1 glutamate transporter is highly vulnerable to oxidative stress [54]. It was reported that the activity of ALS-associated SOD1 mutants directly damage GLT1 function through the action of hydrogen peroxide produced by these mutants (gain of function of SOD1) [79]. The oxidant-vulnerable site of GLT1 resides within its intracellular carboxyl-terminal domain. Proteins exposed to oxidative damage have altered structure, and are likely to undergo spontaneous internalization and increased proteolytic fragmentation. This process may account for the loss of GLT1 immunoreactivity detected in a transgenic model of ALS as well as in human patients.

Using single-strand conformation polymorphism analysis of genomic DNA, a mutation in the GLT1 gene was reported to be associated with sporadic ALS. This mutation substitutes an asparagine for a serine at position 206 (N206S) [1]. The GLT1-N206S mutant has reduced glutamate transport activity and a dominant negative impact on wild-type GLT1 activity. Moreover, the GLT1-N206S exhibited an increased reverse transport capacity. The evidence that a missense mutation of GLT1 is present in a patient with sporadic ALS, and that such a mutation affects the capacity of a cell to regulate the glutamate concentration at synapses, supports the concept that this mutation contributes to excitotoxicity that occurs in ALS.

Neutral Amino Acid Transporters

Description of the ASCT amino acid transporter isoforms

SLC1A4 (protein name ASCT1; also known as SATT)

SLC1A4 encodes the Na^+-dependent neutral amino acid transporter ASCT1 which has the properties of system ASC [2, 71]. It accepts L-alanine, L-serine, L-cysteine and L-threonine in a stereospecific manner. The electrical currents associated with ASCT1-mediated transport result from activation of a thermodynamically uncoupled chloride conductance with permeation properties similar to those described for the glutamate transporter subfamily [88]. Unlike glutamate transporters which mediate net flux and complete a transport cycle by countertransport of K^+, ASCT1 mediates only obligatory exchange of amino acids and is insensitive to K^+ [88]. In brain, ASCT1 is preferentially expressed in glial cells, with L-serine biosynthetic enzyme 3-phosphoglycerate dehydrogenase [67]. It is therefore suggested that a large amount of L-serine is synthesized and stored in these glial cells and is released through ASCT1 in exchange for other extracellular substrates. This exchange is presumed to be regulated to meet the metabolic demands of neurons through the transport of glial-borne small neutral amino acids [67].

SLC1A5 (ASCT2; also known as AAAT or hATB0)

SLC1A5 encodes a second isoform of the ASC transport system, called ASCT2. ASCT2 exhibits distinctive properties in substrate selectivity [43, 81]. In addition to the typical system ASC substrates L-alanine, L-serine, L-cysteine and L-threonine, ASCT2 also transports L-glutamine and L-asparagine at high affinity, as well as some other neutral amino acids with lower affinity [8, 81]. ASCT2 transports glutamate even though with low affinity. Glutamate transport via ASCT2 is enhanced at low pH [81]. Similar to ASCT1, ASCT2 mediates Na^+-dependent obligatory exchange of substrate amino acids [8]. In the kidney and intestine, ASCT2 was shown to be present in the brush-border membranes of proximal tubule cells and enterocytes, respectively [6]. Recently, ASCT2 was shown to be a retrovirus receptor [59]. This finding is reminiscent of the cationic amino acid transporter 1 (CAT1; SLC7A1) which was originally identified as a viral receptor.

Functional properties of ASC transporters

The ASC neutral amino acid transporters exhibit the properties of the classical Na^+-dependent amino acid transport system ASC [2, 43, 71, 81]. ASC transporters have a high-affinity for alanine, serine, threonine and cysteine. The two ASC transporters ASCT1 and ASCT2 exhibit distinct substrate selectivity. In addition to the common substrates of ASC transporters, ASCT2 also accepts glutamine and asparagine as high-affinity, and methionine, leucine and glycine as low-affinity substrates, whereas ASCT1 does not accept these substrates [2, 43, 71, 81].

Despite the distinctive substrate selectivity displayed among glutamate and ASC transporters, they still exhibit common properties in substrate recognition reflecting their structural similarity. For example, glutamate transporters, particularly EAAC1, transport the neutral amino acid cysteine [90] and, vice versa, neutral amino acid transporter ASCT2 transports glutamate, even though with low affinity [81]. Glutamate transport via ASCT2 is enhanced at low pH [81], and ASCT1 becomes inhibited by acidic amino acids such as glutamate, aspartate, cysteate and cysteinesulfinate by lowering pH [76]. These

findings, together with the high conservation of amino acid sequences between glutamate and ASC transporters, suggest that both transporter types have structurally similar substrate binding sites [36].

In analogy to glutamate transporters, ASC transporters also exhibit an anion conductance. Thus, these transporters can behave like ligand-gated anion channels [8, 88]. In contrast to glutamate transporters which require K^+ to fulfill their relocation step, ASC transporters are not coupled to the countertransport of K^+ [8, 88] (Fig. 3, bottom). Consistent with this, Glu404 and Tyr403 (rat GLT1 residue numbers) responsible for the K^+-coupling and the relocation step in glutamate transporters are not conserved in ASC transporters. ASC transporters, in contrast to glutamate transporters, do also not appear to be coupled with H^+-transport [8, 88]. ASC transporters are proposed to function exclusively as Na^+-dependent amino acid exchangers, while glutamate transporters can mediate both uptake and exchange (Fig. 3).

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