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The glutamate and neutral amino acid transporter family: physiological and pharmacological implications

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Abstract

The *s*olute *c*arrier *f*amily 1 (SLC1) is composed of five high affinity glutamate transporters, which exhibit the properties of the previously described system X^-_{AG}, as well as two Na^+-dependent neutral amino acid transporters with characteristics of the so-called "ASC" (alanine, serine and cysteine). The SLC1 family members are structurally similar, with almost identical hydropathy profiles and predicted membrane topologies. The transporters have eight transmembrane domains and a structure reminiscent of a pore loop between the seventh and eighth domains [Neuron 21 (1998) 623]. However, each of these transporters exhibits distinct functional properties. 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. Given the high concentrating capacity provided by the unique ion coupling pattern of glutamate transporters, they play crucial roles in protecting neurons against glutamate excitotoxicity in the central nervous system (CNS). The regulation and manipulation of their function is a critical issue in the pathogenesis and treatment of CNS disorders involving glutamate excitotoxicity. Loss of function of the glial glutamate transporter GLT1 (SLC1A2) has been implicated in the pathogenesis of amyotrophic lateral sclerosis (ALS), resulting in damage of adjacent motor neurons. The importance of glial glutamate transporters in protecting neurons from extracellular glutamate was further demonstrated in studies of the slc1A2 glutamate transporter knockout mouse. The findings suggest that therapeutic upregulation of GLT1 may be beneficial in a variety of pathological conditions. Selective inhibition of the neuronal glutamate transporter EAAC1 (SLC1A1) but not the glial glutamate transporters may be of therapeutic interest, allowing blockage of glutamate exit from neurons due to "reversed glutamate transport" of EAAC1, which will occur during pathological conditions, such as during ischemia after a stroke.

Keywords: Glutamate; Neutral amino acid transporter; ASC

1. Introduction

The glutamate/neutral amino acid transporter family, also known as *s*olute *c*arrier *f*amily 1 (SLC1), is composed of five high affinity glutamate transporters and two Na^+-dependent neutral amino acid transporters. The identification of the genes encoding 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 (1992) to isolate a cDNA encoding the neuronal and epithelial high affinity glutamate transporter EAAC1. Danbolt et al. (1992) purified a 73-kDa glycoprotein (GLT1) from crude 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 (Pines et al., 1992). Storck et al. (1992) copurified a 66-kDa hydrophobic glycoprotein, the glial glutamate transporter GLAST, during the isolation of UDPgalactose:ceramide galactosyltransferase and obtained its cDNA based on its 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 the

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sequence homology to those glutamate transporters (Storck et al., 1992; Arriza et al., 1997, 1993; Fairman et al., 1995; Kekuda et al., 1996; Shafqat et al., 1993; Utsunomiya-Tate et al., 1996). Glutamate transporters exhibit 44–55% amino acid sequence identity with each other. 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 gradients.

2. Functional properties of glutamate transporters

2.1. 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 the 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 three Na^+ ions and one H^+ are co-transported and one K^+ is counter-transported with each glutamate molecule (Zerangue and Kavanaugh, 1996b). Based on the stoichiometry, it was calculated that glutamate transporters can concentrate glutamate 5×10^6 fold inside cells under physiological conditions (Zerangue and Kavanaugh, 1996b). Thus, assuming a concentration of 10 mM inside glutamatergic neurons, the extracellular concentration that can be achieved at equilibrium is ~ 2 nM.

The H^+ co-transport was proved 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 (Zerangue and Kavanaugh, 1996b). 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^+ (Auger and Attwell, 2000). 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 establish the exact role protons during the glutamate transport cycle.

2.2. 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 (Wadiche et al., 1995a,b). 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 and that they do not reflect conformational changes of charged residues of the transporter molecule in the membrane electrical field (Wadiche et al., 1995a,b). This is in agreement with the previous speculation that the empty carrier of glutamate transporters is "electroneutral" (Heinz et al., 1988). Analysis of the steady-state currents of the glutamate transporters revealed a strong voltage-dependence of the glutamate-evoked currents (Fairman et al., 1995; Kanai et al., 1995a,b, 1994; Klockner et al., 1993; Wadiche et al., 1995a,b). A specific voltage-dependent step, the so-called "charge translocation step", was proposed to be rate-limiting during the transport cycle (Kanai et al., 1995a,b, 1994). The countertransport of K^+ appears to speed up the relocation of the empty carrier so that the relocation step is faster than the charge translocation step. A hypothetical kinetic model of glutamate transporters which was derived based on these observations is presented in Fig. 1 (top part).

2.3. 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 (Fairman et al., 1995). This is also displayed by other SLC1 family members (Arriza et al., 1997; Broer et al., 2000; Fairman et al., 1995; Wadiche et al., 1995a,b; Zerangue and Kavanaugh, 1996a). 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^- (Fairman et al., 1995; Wadiche et al., 1995a,b). Cl^- is mainly translocated in the presence of glutamate or related substrates. Cl^- movement is not thermodynamically coupled to the substrate transport. Cl^- is therefore not necessary for substrate translocation (Fairman et al., 1995; Wadiche et al., 1995a,b). 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

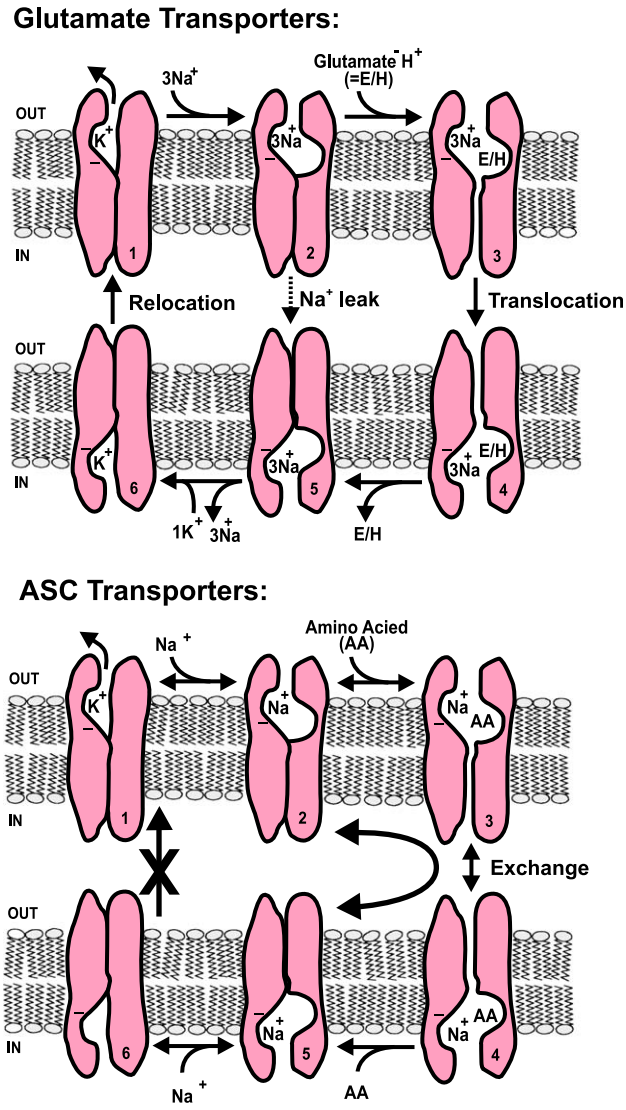

Fig. 1. Kinetic models for glutamate (top) and ASC transporters (bottom) (hypothetical). Under normal conditions, glutamate transport involves loading the empty glutamate carrier with glutamate$^-$/H^+ and 3 Na^+, followed by translocation of the fully loaded carrier across the plasma membrane (charge translocation step) and release of the substrates at the intracellular face. Thereafter, K^+ binds to the carrier inside and promotes the relocation of the empty carrier. 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. In this case, the transporter behaves like an exchanger. Because the GLT1 mutant (Glu404Asp) and the ASC transporters (see below) lack the K^+-coupling step, they cannot enter the relocation step and they will only work in the exchange mode.

gating and stoichiometric charge movement are linked to early transitions in the transport cycle (Otis and Kavanaugh, 2000). It was shown that EAAT5 exhibits prominent chloride conductance compared with its amino acid fluxes (Arriza et al., 1997). Thus glutamate transporters appear to possess structures that can function as chloride channels.

2.4. Exchange mode

The glutamate transporter EAAC1 can also facilitate substrate exchange, in addition to electrogenic glutamate uptake (Zerangue and Kavanaugh, 1996c) (Fig. 1). However, under normal conditions, the exchange component is small compared to the uptake component (Zerangue and Kavanaugh, 1996b,c). In contrast, ASC transporters ASCT1 and ASCT2 mediate exclusively Na^+-dependent exchange of substrate amino acids (Broer et al., 2000; Zerangue and Kavanaugh, 1996a) (Fig. 1, bottom). Site-directed mutagenesis studies 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 (Kavanaugh et al., 1997; Zhang et al., 1998). 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 couple to the K^+ (Zerangue and Kavanaugh, 1996a). 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, i.e. 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 the glutamate efflux (Fig. 1).

3. Functional properties of ASC transporters

The ASC neutral amino acid transporters exhibit the properties of the classical Na^+-dependent amino acid transport system ASC (Arriza et al., 1993; Kekuda et al., 1996; Shafqat et al., 1993; Utsunomiya-Tate et al., 1996). 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 (Arriza et al., 1993; Kekuda et al., 1996; Shafqat et al., 1993; Utsunomiya-Tate et al., 1996).

Despite the distinctive substrate selectivity displayed among glutamate and ASC transporters, they exhibit common properties in substrate recognition reflecting their structural similarity. For example, glutamate transporters, particularly EAAC1, transport the neutral amino acid cysteine (Zerangue and Kavanaugh, 1996c) and, vice versa, neutral amino acid transporter ASCT2 transports glutamate,

even though with low affinity (Utsunomiya-Tate et al., 1996). Glutamate transport via ASCT2 is enhanced at low pH (Utsunomiya-Tate et al., 1996), and ASCT1 becomes inhibited by acidic amino acids such as glutamate, aspartate, cysteate and cysteinesulfinate by lowering pH (Tamarappoo et al., 1996). 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 (Kanai, 1997).

In analogy to glutamate transporters, ASC transporters also exhibit an anion conductance. Thus, these transporters can behave like ligand-gated anion channels (Broer et al., 2000; Zerangue and Kavanaugh, 1996a). In contrast to glutamate transporters that require K^+ to fulfill their relocation step, ASC transporters are not coupled to the countertransport of K^+ (Broer et al., 2000; Zerangue and Kavanaugh, 1996a). Consistent with this, Glu 404 and Tyr 403 (rat GLT1 residue number) 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 couple with H^+-transport (Broer et al., 2000; Zerangue and Kavanaugh, 1996a). ASC transporters are proposed to function exclusively as Na^+-dependent amino acid exchangers, while glutamate transporters can mediate both uptake and exchange modes (Fig. 1).

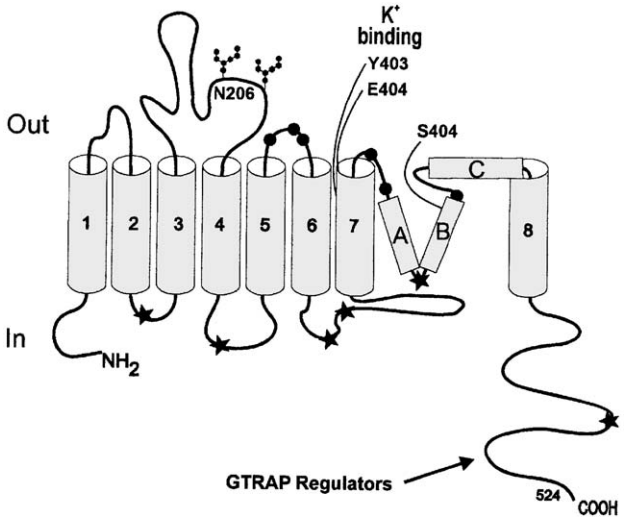

Fig. 2. Membrane topology model of the glutamate transporter GLT1. The topology shown is based on experiments involving biotinylation of single cysteine mutants of the glutamate transporter GLT1 from rat brain (Grunewald et al., 1998; Grunewald and Kanner, 2000). Rat GLT1 numbering is shown in. Residues that were mutated to cysteine residues in GLT1 are indicated by their position number. 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 that 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.

4. Structure function relationship of glutamate transporters

The SLC1 family members have a unique, highly conserved long hydrophobic stretch near the C-terminus (see Fig. 2). Cysteine-scanning mutagenesis was performed to examine the accessibility of amino acid residues in the hydrophobic stretch using sulfidyl-reactive reagents (Grunewald and Kanner, 2000; Seal et al., 2000). The model in Fig. 2 features 8 predicted α-helical transmembrane domains (#1–8), a large extracellular glycosylated loop between transmembrane domains 3 and 4, a "reentrant loop" (A/B) between transmembrane 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 transmembrane domains 7 and 8.

In the seventh transmembrane domain, there are two amino acid residues, Tyr 403 and Glu 404 (rat GLT1 numbering) that are important for the coupling with K^+ and the relocation of the carrier (Kavanaugh et al., 1997; Zhang et al., 1998). In the site-directed mutant of Tyr 403, it was furthermore reported that the mutant GLT1 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 (Zhang et al., 1998), suggesting that Tyr 403 is also associated with the Na^+-binding site or the structure responsible for the selectivity of inorganic cations. It was also reported that the mutation of Ser 440, which resides at the extracellular face of the re-entrant loop (part B in Fig. 2), alters the selectivity to inorganic cations and the sensitivity to the glutamate transport inhibitors (Zhang and Kanner, 1999). Therefore, Ser 440 is also important for the Na^+-coupling mechanism and 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 studies of *Xenopus* oocytes overexpressing human EAAC1 (EAAT3) revealed distinct 10 nm freeze-fracture particles which appeared in the protoplasmic face only after the EAAC1 expression (Eskandari et al., 2000). 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 (Eskandari et al., 2000).

5. Physiological significance of glutamate transporters

5.1. Role of glutamate transporters in glutamatergic transmission

It is now generally accepted that glutamate transporters play important roles in terminating glutamatergic transmission (Fig. 3). 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 fast decay of the postsynaptic potential due to the rapid desensitization of non-NMDA type glutamate-receptors (Isaacson and Nicoll, 1993; Mennerick and Zorumski, 1994; Sarantis et al., 1993; Tong and Jahr, 1994). Glutamate transporters, however, play crucial 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 (Wadiche et al., 1995a,b), binding of glutamate to glutamate transporters exhibits fast kinetics, which significantly contributes to the glutamate clearance in the synaptic cleft (Tong and Jahr, 1994). Recently, the laser-pulse photolysis technique of caged glutamate (Grewer et al., 2000) 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 (Grewer et al., 2000).

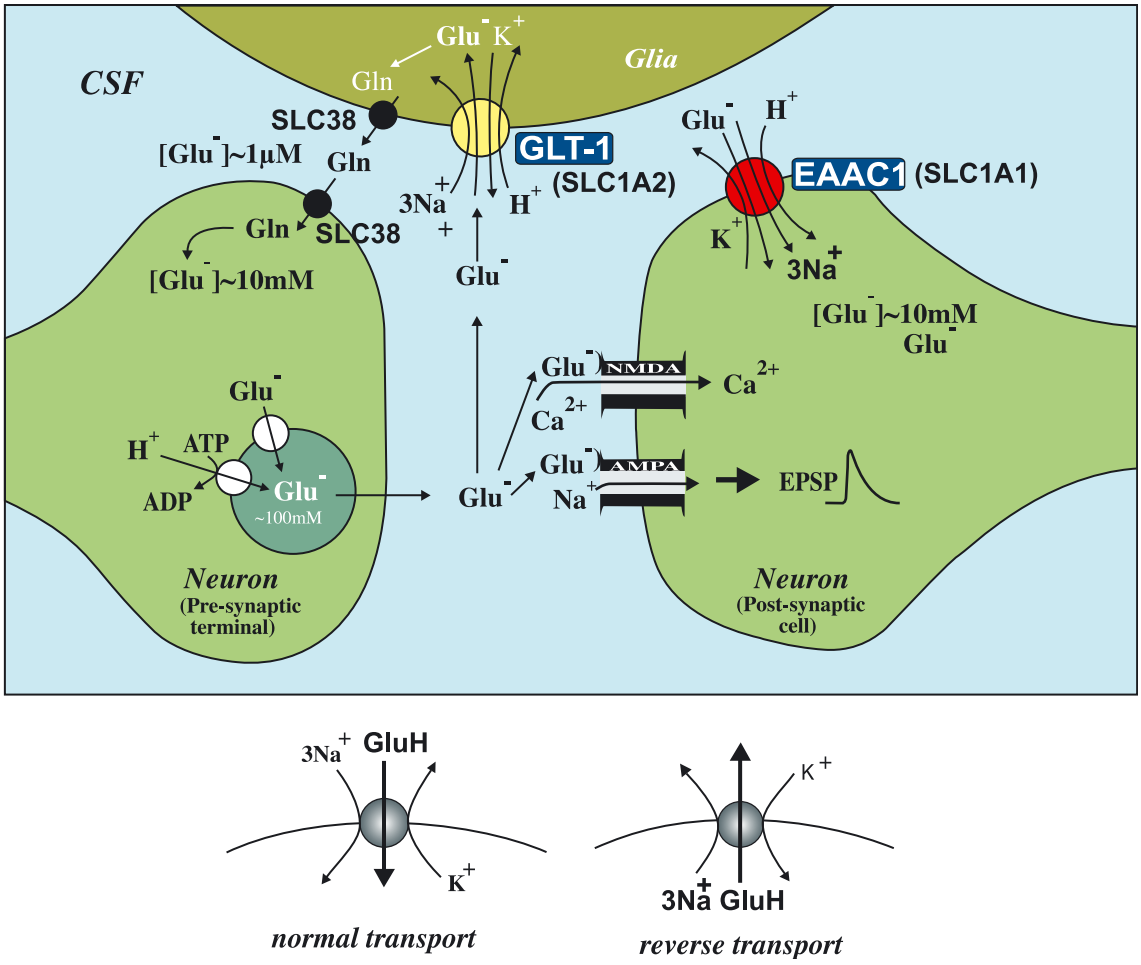

Fig. 3. Top: Role of glutamate transporters at glutamatergic synapses. The excitatory neurotransmitter L-glutamate is stored in synaptic vesicles at presynaptic terminals. Glutamate is transported into these vesicles by the vesicular glutamate transporters VGluT1, VGluT2 and VGluT3 (SLC17A6, SLC17A7 and SLC17A8, respectively). Glutamate is released into the synaptic cleft to act on glutamate receptors. The AMPA receptors mediate fast excitatory postsynaptic potentials (EPSP), whereas the NMDA receptors possess a cation channel that 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 bottom). The figure shows the neuronal glutamate transporter EAAC1 (SLC1A1) and the glial glutamate transporter GLT1 (SLC1A2). Bottom: Forward and reversed mode of glutamate transport.

5.2. Role of glutamate transporters in maintaining extracellular glutamate concentration

Based on the stoichiometry of glutamate transporters and the prevailing ionic environment, it can be calculated that glutamate transporters concentrate glutamate more than 10,000-fold across cell membranes (Levy et al., 1998). 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 (Rothstein et al., 1993). 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 easily understandable when considering the difference in the intracellular glutamate concentration between neurons and glial cells (Rothstein et al., 1996). The importance of glial glutamate transporters to protect neurons from the glutamate was also demonstrated in the glutamate transporter knockout transgenic mice (see below).

6. Regulation of glutamate transporters

Several studies indicate that neuronal and non-neuronal factors are required for the expression and maintenance of functionally active glutamate transporters (Drejer et al., 1983; Gegelashvili and Schousboe, 1997; Schlag et al., 1998; Swanson et al., 1997). For example, GLT1 can be induced in astroglial cultures by the pituitary adenylate cyclase-activating peptide (PACAP), a neuron-derived peptide (Figiel and Engele, 2000), by brain-derived neurotrophic factor (BDNF), a neurotrophin that is predominantly expressed in neurons (Gegelashvili et al., 2000), or by epidermal growth factor (EGF) receptor activation (Zelenaia et al., 2000). In C6 glioma, the Wnt-1 gene product, an autocrine and paracrine soluble factor, induces GLT1 expression (Palos et al., 1999). 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 (Matthews et al., 1999). 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 (Sims et al., 2000). 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) (Guo and Mattson, 2000).

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) (Trotti et al., 2001). 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 (Dunlop et al., 1999). 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 (Davis et al., 1998; Dowd and Robinson, 1996; Gonzalez et al., 2003). Studies of other glutamate transporter isoforms showed that activation of PKC down- or upregulates 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 (Conradt and Stoffel, 1997); glutamate transporter GLT1 expressed in HeLa cells was upregulated by PKC via direct phopshorylation at serine residues 113 of GLT1 (Casado et al., 1993). In both cases, PKC activation was not altered by transporter expression in the plasma membrane. In summary, among the EAAC1, GLT1 and GLAST transporters, 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 (Hediger and Welbourne, 1999; Hediger, 1999), 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 (Jackson et al., 2001). The expression of either GTRAP41 or GTRAP48 resulted in 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 (Lin et al., 2001). GTRAP3-18 reduces the EAAC1-mediated glutamate transport by decreasing the affinity of the transporter for glutamate.

7. Pathological and pharmacological relevance of glutamate transporters

7.1. 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 (Rossi et al., 2000). 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. 3, bottom, right part). 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 (Kanai and Hediger, 2001). Selective inhibitors of neuronal glutamate transporters may therefore be of therapeutic interest to prevent reversed glutamate transport without affecting the capability of glial glutamate transporters, and to keep the extracellular glutamate concentration at low levels.

7.2. Role of glutamate transporters in amyotrophic lateral sclerosis (ALS)

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) (Brown, 1995; Robberecht et al., 1994; Rosen et al., 1994), the primary pathogenic trigger is still unknown. A decrease in the glutamate transporter activity due to the reduction of GLT1 (EAAT2) isoform in motor and sensory cortex of sporadic ALS patients has been reported (Rothstein et al., 1994, 1995). More recently, aberrant splicing of the GLT1 transcript was suggested to be the cause for a reduced expression of GLT1 in ALS (Lin et al., 1998). Another factor which may contribute to loss of GLT1 in ALS brains is the fact that the GLT1 glutamate transporter isoform is highly vulnerable to oxidative stress (Pedersen et al., 1998). 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) (Trotti et al., 1999). 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) (Aoki et al., 1998). 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.

8. Description of the different SLC1 isoforms

8.1. EAAC1 (also known as EAAT3; gene name SLC1A1)

EAAC1 is a neuronal and epithelial type high-affinity glutamate transporter (Kanai and Hediger, 1992). It is predominantly expressed in neurons of various brain areas particularly in hippocampus, cerebral cortex, olfactory bulb, striatum, superior colliculus and thalamus (Berger and Hediger, 1998; Kanai et al., 1995a,b; Rothstein et al., 1994). In the kidney, EAAC1 is present in the apical membrane of proximal tubules (Shayakul et al., 1997). 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 (Peghini et al., 1997). EAAC1 knockout mice developed dicarboxylic aminoaciduria, confirming the role of EAAC1 in the reabsorption of glutamate from the renal proximal tubules (Peghini et al., 1997).

8.2. GLT1 (also known as EAAT2; gene name SLC1A2)

GLT-1 is a glial type high-affinity glutamate transporter (Pines et al., 1992). It is 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 pattern similar to those of NMDA-induced seizures (Tanaka et al., 1997). 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 of GLT1 plays in neuroprotection (Tanaka et al., 1997).

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 that in the wild type mice, indicating that GLT1 significantly contributes to the removal of glutamate from the synaptic cleft (Watase et al., 1998). 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 (Harada et al., 1998).

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 (Arriza et al., 1994). Several reports have indicated the presence of spliced variants for GLT1 (Guo et al., 2002; Honig et al., 2000; Meyer et al., 1999; Utsunomiya-Tate et al., 1997).

8.3. GLAST (also known as EAAT1; gene name SLC1A3)

GLAST is a glial type high-affinity glutamate transporter which is particularly abundant in the cerebellum (Storck et al., 1992). It is expressed in astrocytes and cerebellar Bergmann glia (Rothstein et al., 1994). 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 (Watase et al., 1998). 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 (Watase et al., 1998).

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 (Harada et al., 1998). 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 (Hakuba et al., 2000). 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 (Watanabe et al., 1999).

8.4. ASCT1 (also known as SATT; gene name SLC1A4)

ASCT1 is a Na^+-dependent neutral amino acid transporter with the properties of system ASC (Arriza et al., 1993; Shafqat et al., 1993). 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 (Zerangue and Kavanaugh, 1996a). Unlike glutamate transporters which mediate net flux and complete a transport cycle by countertransport of K^+, ASCT-1 mediates only obligatory exchange of amino acids and is insensitive to K^+ (Zerangue and Kavanaugh, 1996a). In brain, ASCT1 is preferentially expressed in glial cells with L-serine biosynthetic enzyme 3-phosphoglycerate dehydrogenase (Sakai et al., 2003). 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, which is presumed to be regulated to meet metabolic demands by neurons through the transport of glial-borne small neutral amino acids (Sakai et al., 2003).

8.5. ASCT2 (also known as AAAT/hATB0; gene name SLC1A5)

ASCT2 is a second isoform of the ASC transport system. ASCT2 exhibits distinctive properties in substrate selectivity (Kekuda et al., 1996; Utsunomiya-Tate et al., 1996). 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 (Broer et al., 2000; Utsunomiya-Tate et al., 1996). ASCT2 transports glutamate even though with low affinity. Glutamate transport via ASCT2 is enhanced at low pH (Utsunomiya-Tate et al., 1996). Similar to ASCT1, ASCT2 mediates Na^+-dependent obligatory exchange of substrate amino acids (Broer et al., 2000). In the kidney and intestine, ASCT2 was shown to be present in the brush-border membranes of proximal tubule cells and enterocytes, respectively (Avissar et al., 2001). Recently, ASCT2 was shown to be a retrovirus receptor (Rasko et al., 1999). This finding is reminiscent of the cationic amino acid transporter 1 (CAT1; SLC7A1), which was originally identified as a viral receptor.

8.6. EAAT4 (gene name SLC1A6)

EAAT4 is a neuronal high-affinity glutamate transporter which is predominantly expressed in cerebellar Purkinje cells on postsynaptic dendritic spines (Nagao et al., 1997).

This transporter shows a remarkable thermodynamically uncoupled chloride conductance associated with substrate transport (Fairman et al., 1995).

8.7. EAAT5 (gene name SLC1A7)

EAAT5 is a high-affinity glutamate transporter which is primarily expressed in the retina (Arriza et al., 1997). Immunocytochemical studies of the rat retina showed that EAAT5 is associated with rod photoreceptors and some bipolar cells (Pow and Barnett, 2000; Pow et al., 2000). Transporter currents elicited by glutamate are largely carried by chloride ions, indicating that EAAT5 exhibits a prominent chloride conductance (Arriza et al., 1997). 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.

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