Heteromeric amino acid transporters in brain: from physiology to pathology.

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Abstract

In humans, more than 50 transporters are responsible for the traffic and balance of amino acids within and between cells and tissues, and half of them have been associated with disease [1]. Covering all common amino acids, Heteromeric Amino acid Transporters (HATs) are one class of such transporters. This review first highlights structural and functional studies that solved the atomic structure of HATs and revealed molecular clues on substrate interaction. Moreover, this review focuses on HATs that have a role in the central nervous system (CNS) and that are related to neurological diseases, including: i) LAT1/CD98hc and its role in the uptake of branched chain amino acids trough the blood brain barrier and autism. ii) LAT2/CD98hc and its potential role in the transport of glutamine between plasma and cerebrospinal fluid. iii) y^+LAT2/CD98hc that is emerging as a key player in hepatic encephalopathy. xCT/CD98hc as a potential therapeutic target in glioblastoma, and v) Asc-1/CD98hc as a potential therapeutic target in pathologies with alterations in NMDA glutamate receptors.

Keywords

HAT, LAT, amino acid transporter, CNS, SLC7, disease

Introduction

Amino acid availability regulates cell physiology. The transfer of amino acids across the plasma membrane is mediated by specific transporter proteins that recognise, bind and transport these amino acids from the extracellular medium into the cell, or vice versa. Heteromeric Amino acid Transporters (HATs) are one of the 10 types of amino acid transporters present in humans. These molecules comprise a heavy subunit and a light subunit linked by a conserved disulphide bridge (Fig. 1) [2]. The heavy subunits of HATs appeared in metazoans, whereas the light subunits can be traced to prokaryotes. Two homologous heavy subunits and eight light subunits belonging to the SLC3 and SLC7 protein families, respectively, have been identified in humans. Heavy subunits, namely rBAT (SLC3A1) and CD98hc (also named 4F2hc) (SLC3A2), are ancillary proteins required for trafficking the holotransporter to the plasma membrane [2].

The mammalian SLC7 family is part of the APC (amino acid, polyamine and organocation transporters) superfamily. SLC7 members comprise two families, namely Cationic Amino acid Transporters (CATs) [3] and the light subunits (LATs) of HATs [2]. Six of these light subunits heterodimerize with CD98hc (LAT1, LAT2, y^+LAT1, y^+LAT2, Asc-1 and xCT) [4-9] and two with rBAT ($b^{0,+}$AT and AGT1) [10,11], conferring substrate specificity [2]. LATs are polytopic and non-N-glycosilated antiporters that cover a full range of amino acid substrates.

Several human pathologies highlight the physiological roles of HATs. Four transporters of this family are responsible for or contribute to inherited diseases. Thus, mutations in either of the two genes coding for the subunits of system $b^{0,+}$ (rBAT and $b^{0,+}$AT) lead to cystinuria (MIM 220100) [12,10], whereas mutations in y+LAT1 (a CD98hc-associated system y+L) result in lysinuric protein intolerance (LPI) (MIM 222700) [13]. In addition, mutations in LAT1 and LAT2 (CD98hc-associated system L transporters) cause autism-related disorders and contribute to age-related hearing loss and cataracts, respectively [14-16]. xCT (the CD98hc-associated system xc−), which mediates cystine uptake and glutamate efflux, is essential for Kaposi's sarcoma-associated Herpesvirus infection [17]. By regulating the basal level of extra-synaptic glutamate, this transport system is also involved in cocaine relapse [18]. Finally, as is also the case for xCT, LAT1 (a CD98hc-associated system L) is overexpressed in many human tumours, thereby suggesting that these amino acid transporters are essential for tumour cell survival and progression [19,20].

HATs are amino acid exchangers and as such they harmonise amino acid concentrations at each side of the plasma membrane [21,1]. Moreover, by functional coupling with other transporters, HATs mediate the net balance of amino acid transport. Thus, glutamine uptake by ASCT2 coupled with the exchange of branched chain amino acids (BCAAs) (glutamine efflux / BCAA influx) by LAT1/CD98hc occurs in tumour cells [22]. Similarly, there is functional cooperation between TAT1 and LAT2/CD98hc in the renal reabsorption of neutral amino acids in the basolateral membrane of the renal epithelium, where TAT1 mediates the efflux of aromatic amino acids, which is coupled with the exchange of neutral amino acids (aromatic AA influx / other neutral amino acids efflux) by LAT2/CD98hc [23].

HAT structure and transport mechanisms

In the last three years, several atomic structures of HATs [24-27] and LATs [28] have been solved, paving the way for the dissection of the molecular transport mechanisms. LATs present the APC superfamily fold, with 12 transmembrane (TM) domains, where the first 10 TMs have an internal symmetry with a pseudo 2-fold axis in the middle of the plane of the membrane that relate the first five (TM1 to 5) and the second five (TM6 to 10) domains. Interestingly, all LAT structures solved (human LAT1 and $b^{0,+}$AT and bacterial Asc (BasC)) are in an inward-facing conformation (i.e., the substrate vestibule open to the cytosol and closed to the extracellular space) (Fig. 1).

The substrate binding site of LATs solved with substrate bound [28,26,27] shows a basic conserved design: the α-amino and carboxyl moiety of the substrate bind to unwound segments of TM1 and TM6 to interact mainly with atoms of the protein backbone (Fig. 1). Functional studies with amines or alkyl derivatives of the amino acid substrates suggest that the α-amino and carboxyl groups are necessary for proper binding and/or to trigger the transport cycle [28]. A conserved aromatic residue in TM6a (i.e., the external α helical segment of TM6) acts as an external gate that interacts with the substrate (Fig. 1) [28,26,27]. The substrate cavity design locates the side chain of the amino acid substrates mainly towards TM3 and TM8. Functional studies with human LAT1 suggest that the lateral chain of residues in the substrate cavity determines the size of the substrates [24].

The atomic structure of bacterial GkApcT [29] provides clues as to how the transporter closes the substrate vestibule upon substrate binding. GkApcT is a bacterial homologue of CATs, the subfamily that, together with LATs, conforms the SLC7 family. GkApcT has been solved in an inward-facing conformation, with the substrate bound and occluded. A comparison of the structures of BasC and GkApcT reveals the TM movements that close access of the substrate to the cytosol. Mainly, but not only, tilting of TM1a and TM6b is responsible for closing the vestibule. Interestingly, a fully conserved Lys residue in TM5, essential for transporter activity, connects TM1a and TM8 by an H-bond in the occluded conformation [28,29].

Functional studies had revealed that LATs present asymmetrical interaction with substrates, with K_m values (apparent affinity) in the μM range on the extracellular side and in the mM range on the cytosolic side [28,30,31]. Interestingly, the conserved Lys residue in TM5 (Lys194 in human Asc-1 and Lys 154 in BasC) is key for transport function and contributes to the high apparent affinity of these transporters on the extracellular side in human Asc-1 and bacterial BasC [28]. Of note, mutation K191E of this residue in y^+LAT1 causes LPI [32]. The mechanism that links the role of this Lys residue to the occlusion of the substrate vestibule on the cytosolic side and the high apparent affinity on the extracellular side is unknown.

Given that there are no structures of human LATs in outward-facing conformations, the substrate binding site facing the extracellular space has only been modelled. Thus, structural models based on the atomic structure of distant homologues (e.g., AdiC) [33-37] provide clues about the substrate binding site and vestibule from the extracellular side [38]. These models have been used to moderately improve inhibitors that target human LATs of clinical interest [39].

Recent cryo-EM studies of LAT1/CD98hc (Fig. 1) and $b^{0,+}$AT/rBAT revealed the molecular organisation of HAT heterodimers [24-27]. CD98hc is a type II membrane glycoprotein with a large N-glycosylated ectodomain that presents sequence and structural homology with bacterial glucosidases [40]. CD98hc shows a short cytosolic α helix contacting a similar α helix in the C-terminal end of LAT1. Then, CD98hc TM (TM1') domain interacts with residues in TM4 of LAT1. The short sequence ("neck"), which connects TM1' with the ectodomain, interacts with a few residues in the extracellular loop 2 (EL2) of LAT1, and this interaction is stabilised by a disulphide bond between the two subunits in this

location. Finally, the CD98hc ectodomain is located over the extracellular side of LAT1, where polar and ionic interactions between the two subunits are established (Fig. 1) [24,27]. This quaternary structure and the topological organisation are in full agreement with previous biochemical and structural studies of the human LAT2/CD98hc transporter [41,42]. A similar design is present in the human $b^{0,+}$AT/rBAT transporter [25,26]. In this case, a dimer of heterodimers, which interfaces between the two heterodimers is located in the rBAT ectodomains. This oligomeric state is in full agreement with previous biochemical studies indicating that rBAT determines the oligomeric state [41]. Finally, despite sequence and structural homology with bacterial glycosidases, neither CD98c nor rBAT present catalytic activity [40,26].

HATs in CNS and neurological diseases

The CD98hc-associated transporters LAT1, LAT2, y^+LAT2, xCT and Asc-1 are involved, or postulated to be involved, in inherited and acquired diseases of the CNS (Table 1). Mutations in the CD98hc-associated subunit y+LAT1 that cause LPI, characterised by hepatic neurotoxicity caused by hyperammonemia due to a defective urea cycle in the liver [43,44], will not be considered further in this review.

LAT1/CD98hc

LAT1 (L-type amino acid transporter-1)/CD98hc (SLC7A5/SLC3A2) was identified on the basis of its capacity to transport large neutral amino acids (LNAA) when expressed with CD98hc [24,5,27]. Its uptake is sodium-independent and its selectivity range is relatively broad, the apparent affinity for the uptake of branched and aromatic amino acids being quite high (μM range) [45,5,30]. LAT1/CD98hc is an obligatory exchanger that is overexpressed in a wide range of solid tumours, including the most frequently diagnosed types of cancer and brain tumours [46-49]. High LAT1 expression in brain tumours in which LAT1 is upregulated has been associated with significantly shorter survival [48].

Large neutral essential amino acids are key for cancer cell proliferation, suggesting that increased amino acid uptake is required to maintain protein synthesis in highly proliferative cancers and to enhance certain signal transduction pathways [50]. In this regard, the increase in leucine uptake mediated by LAT1 in cancer cells promotes the activity of Mechanistic Target of Rapamycin Kinase Complex 1 (mTORC1). Aberrant mTORC1 activation is common in cancer, where it stimulates pathways that support cancer

cell growth, proliferation and resistance to apoptosis [51]. Nevertheless, LAT1 not only supports mTORC1 activity but also reinforces MYC and EZH2 signalling in cancer cells. In addition, low nutrient levels and hypoxia in the tumour microenvironment might increase LAT1 levels. In this regard, leucine or glutamine starvation was shown to increase LAT1 expression in normal kidney (NRK) and prostate cancer cell lines [52,53], while hypoxia increased *SLC7A5* transcription *in vivo* through transcriptional activation by HIF2α [54].

BCAAs play a key role in brain metabolism as nitrogen donors. In fact, approximately 30% of brain glutamate/glutamine nitrogen derives from leucine [55]. Thus, LAT1 would participate in brain glutamate recycling through the glutamate/GABA-glutamine cycle, whose malfunction would be related to brain diseases [56]. The relevance of BCAAs for brain development has been highlighted by the observation that loss-of-function mutations in the catabolic pathway of BCAAs and in the BCAA transporter LAT1 cause autism spectrum disorders [57,58,16]. Mutations in the BCKDK gene, which codes for the kinase responsible for the negative regulation of the branched-chain α-keto acid dehydrogenase complex (BCKD), are associated with autism spectrum disorders in several families. In this condition, the fully active BCKD depletes BCAA levels in the plasma and cerebrospinal fluid (CSF) of patients. Interestingly, a protein-rich diet plus oral BCAA supplementation normalises plasma BCAA levels and improves growth, developmental and behavioural variables [57].

LAT1/CD98hc exchanges LNAA, including BCAAs (Table 1), and it is located in the blood-brain barrier (BBB) [16,59] (Fig. 2). Deletion of *Slc7a5* from the endothelial cells of the BBB in mice leads to an atypical brain amino acid profile, abnormal mRNA translation, and severe neurological abnormalities. Intracerebroventricular administration of BCAAs ameliorates abnormal behaviour in adult mutant mice. Moreover, two loss-of-function mutations in homozygosis were identified in several members of two families with autistic traits. Mutation Ala246Val presents an almost complete loss-of-function in reconstituted proteoliposomes [16], and its position in the N-terminal part of TM6a [24,27] suggests that it compromises the closing of the thick external gate of LAT1. Mutation Pro375Leu presents partial defective transport activity [16]. Proline 375 is located in TM9 [24,27] and the structure does not reveal clues as to the molecular defect associated with this mutation. In this regard, it is not clear whether this mutation affects protein folding or intrinsic transport function. Therapeutic interventions at the protein level would require a greater understanding of the molecular defects associated with these mutations.

LAT2/CD98hc

LAT2 (L-type amino acid transporter-2)/CD98hc (SLC7A8/SLC3A2) is a sodium-independent transporter that equilibrates the relative concentrations of neutral amino acids across the plasma membrane, including, to a lesser extent, the small ones (e.g., alanine, glycine, cysteine and serine) [30,7] (Table 1). Due to the epithelial localisation of SLC7A8, research into this transporter has been focused mainly on amino acid (re)absorption. In polarised cells such as the renal proximal tubules, and the intestinal, ciliary and lens epithelia, LAT2/CD98hc is restricted to the basolateral membrane domain [60-62,15,63], although in the placental syncytiotrophoblast it is distributed on both the apical (maternal) and basolateral (fetal) surfaces, showing colocalisation with the apically expressed LAT1 [64]. This particular localisation suggests that LAT2 plays an essential role in renal and intestinal neutral amino acid (re)absorption processes [60-63], as well as in the transport of essential neutral amino acids from blood to the aqueous humour and lens [15], and in transplacental amino acid flux [65]. Nevertheless, although functional cooperation between TAT1 and LAT2/CD98hc for the renal reabsorption of neutral amino acids has been recently reported, compensation by y^+LAT1/CD98 in the event of their defect has also been demonstrated [23]. In fact, compensations for the basolateral efflux of neutral amino acids by other transporters most probably explain why no neutral aminoaciduria caused by the defect of basolateral LAT2 transporter has been uncovered.

More recently, SLC7A8 expression has also been reported in the plasma membrane of microglia, astrocytes, and neuronal axons, and in various brain regions, such as the choroid plexus, subfornical organ, cerebral cortex and hypothalamus [66,67,14]. This specific localisation in the brain indicates that the absence of the transporter may potentially lead to neurological disorders. Nevertheless, full ablation of SLC7A8 in mice only resulted in a hearing loss [14] and cataracts [15]. In addition, behavioural screening showed that the absence of SLC7A8 in mice does not affect learning or memory [14]. In contrast, three coding variants (p.Pro16Arg, p.Gly18Trp, p.Ser29Phe), as well as one intronic SNP (c.1016-49T > C), have recently been associated with increased risk of autism spectrum disorder, probably via restricting the availability of essential amino acids in the developing brain [68], as shown for LAT1/CD98 [16]. Nevertheless, LAT2 expression in the BBB is a matter of discussion and its role in regulating the availability of neutral amino acids in the brain is still unclear.

Recent studies have shown that LAT2, in cooperation with SNAT3, in the mouse choroid plexus is key for the luminal release of non-essential amino acids, in particular glutamine, into CSF [67]. Nevertheless, the unidirectional transporters SNAT1 (SLC38A1) (Na+-cotransporter) and SNAT3 (SLC38A3) (Na^+ cotransporter and H^+ exchanger) would dominate the transfer of glutamine to the CSF. In this regard, with its antiporter function, LAT2 therefore appears to reuptake essential neutral amino acids from the CSF and thus to participate in the maintenance of the amino acid concentration gradient between the plasma and CSF [69,70]. This amino acid transport across the choroid plexus, would be crucial for brain amino acid homeostasis and thus for brain function.

However, the impact of LAT2 on brain pathophysiology is yet to be fully understood. In fact, CNS disorders such as schizophrenia, depression and Parkinson's disease appear to be dependent upon the brain uptake of LNAA (e.g., L-tryptophan and L-tyrosine). This uptake is proposed to be performed through LAT1, suggesting that LAT1 and LAT2 functions are complementary. In this regard, LAT2 would mediate the outward transport of LNAA that are not competently transported through LAT1, as is the case of glutamine [46,45,49]. Thus, LAT2 has been shown to be involved in the efflux of glutamine from astrocytes as a part of the glutamate/GABA-glutamine cycle in the brain [71] (Fig. 2). Brain glutamate regulation is primarily mediated by this cycle, where excess glutamate remaining after excitation is taken up by astrocytes [56]. Glutamate conversion to glutamine is mediated by the astrocyte-specific microsomal enzyme glutamine synthetase.

Given that LAT2 is expressed in the plasma membrane of astrocytes, it may participate in the release of extracellular fluid glutamine from the brain for reuptake by pre-synaptic neurons [72]. Additionally, transporters of the SLC38 family mediate glutamine efflux from astrocytes (SLC38A3 and SLC38A5) and glutamine influx in neurons (SLC38A1, 2, 7 and 8) [73,74]. Transporter SLC38A10, located in neurons and astrocytes, might participate in the glutamate/GABA-glutamine cycle [75]. In this context, LAT1/CD98hc and LAT2/CD98hc would harmonize the astrocytic and neuronal intracellular concentrations of BCAAs and LNAA, and glutamine and BCAAs, respectively. The glutamate/GABA-glutamine cycle is of great importance for brain physiology for two reasons, first, because glutamate uptake from the blood is minimal and, second, because the deregulation of glutamate levels can trigger pathological conditions [76].

y^+LAT2/CD98hc

y+LAT2 (system y^+L transporter 2)/CD98hc (SLC7A8/SLC3A2) exchanges cationic amino acids with neutral amino acids plus sodium [9] (Table 1) and is highly efficient for the exchange of L-arginine (efflux)/L-glutamine plus Na^+ (influx) [77]. This transporter is widely expressed, being found in the brain, heart, testis, kidney, small intestine and parotis [77]. In the brain, y+LAT2 mRNA presents low expression in the microvascular endothelial cells of the murine BBB and its expression increases upon in vitro culture [78]. Similarly, y^+LAT2 protein has been detected in cultured rat cortical astrocytes and neurons [73]; mouse single-cell transcriptomics show robust expression in neurons, and lower in astrocytes, being absent in endothelial cells (www.dropviz.org). However, to the best of our knowledge, the cellular distribution of this protein *in vivo* has not been reported in the brain.

Defects in the urea cycle in liver, due to loss-of-function mutations in key enzymes or transporters of the cycle, or by liver failure, cause hepatic encephalopathy [79]. Similarly, in LPI, as a result of loss-of-function mutations in y^+LAT1, hypoargininemia causes hyperammonemia, which results in astrocytosis and brain edema [43]. There is evidence that y^+LAT2/CD98hc plays a role in ammonium toxicity in the brain [73,80]. Acute hyperammonemia activates the glutamate-NO-cGMP pathway by a mechanism that is at least partially dependent on the overactivation of glutamate NMDA receptors. This activation results in ATP depletion and inactivation of glutamine synthase, thereby decreasing ammonium consumption and thus increasing toxicity [81]. Increased production of NO causes oxidative/nitrosative stress (ONS), resulting in astrocyte swelling, mitochondrial disfunction and brain edema [82]. In advanced hepatic encephalopathy, there is decreased NO and cGMP levels in the brain, which would contribute to the motor and cognitive alterations of the condition [83]. The Albrecht lab has proposed that extracellular accumulation of glutamine, derived from glutamine synthethase in astrocytes, depletes intracellular arginine, thereby contributing to a decrease in the production of NO and cGMP [84].

Interestingly, this glutamine/arginine exchange has the pharmacological profile of y^+LAT2/CD98hc [84]. Moreover, in rat hepatic encephalopathy, ammonium upregulates y^+LAT2, thereby specifically increasing the transport of arginine [85,86]. These results support the notion that ammonium-dependent y^+LAT2 upregulation triggers iNOS induction and NO production [87]. The mechanism underlying y^+LAT2 upregulation by ammonium is not fully understood [73]. Nevertheless, the upregulation of this transporter would modulate ONS gliotoxicity, a characteristic of hepatic encephalopathy. Specific ablation of *Slc7a6* in

astrocytes and/or neurons would help to dissect the pathological mechanisms of y^+LAT2 in hyperammonemic toxicity in the brain.

xCT/CD98hc

Under normal conditions, xCT (system x_c^- transporter)/CD98hc (SLC7A11/SLC3A2) is found mainly in the native brain (hypothalamic area, meninges) and in macrophages, as well as in most cell culture lines [88,8]. Specific labelling with antibodies validated using tissue from xCT knockout mice, revealed expression in mouse brain only in astrocytes (Fig. 2) and with higher expression in blood/brain/CSF interface areas [89]. It is a sodium-independent electroneutral transporter and it follows an obligatory exchange mode, exchanging extracellular anionic cystine for glutamate (Table 1). The driving force for this exchange is generated by the cystine concentration gradient (intracellular reduction) and the high intracellular concentration of glutamate. High xCT expression in border regions between brain and periphery is in accordance with a role of xCT/CD98hc in the maintenance of the cysteine/cystine redox balance in the CSF and plasma [90].

As cystine uptake and reduction are rate-limiting for glutathione (GSH) synthesis, xCT/CD98hc activity directly controls intracellular GSH levels, thereby preventing iron-dependent cell death (ferroptosis) [91]. Consequently, xCT expression is elevated in cells requiring high GSH synthesis, for instance activated macrophages, glial cells and a wide range of cancer cells [92,8]. In addition, xCT is necessary for Kaposis' sarcoma herpes virus infection [17]. The inhibition of xCT has been correlated with the regression of Kaposis' sarcoma herpes virus-associated lymphoma [93], as well as with stroke and multiple sclerosis [94,95].

The inactivation of xCT is emerging as a promising therapeutic target also in oncology, as high xCT expression correlates with poor prognosis and metastasis across several tumour types, as well as with chemotherapeutic resistance to cisplatin or temozolomide (TMZ) [96-98]. The inhibition of xCT transport by small molecules in preclinical models of pancreatic, gastrointestinal and colorectal cancers reduces metastases and tumour growth [99,100], and immunotherapy targeting epitope-specific anti-xCT inhibits the progression of metastatic breast cancer in mice [101]. In particular, xCT is a promising therapeutic target for high-grade gliomas, including glioblastoma (GBM) and paediatric diffuse intrinsic pontine glioma (DIPG), the latter characterised by resistance to

chemotherapy. Both tumour types have limited treatment options and a very low average survival expectancy (i.e. between one and two years after diagnosis). Initial studies on neoantigen vaccination have shown that it elicits intratumoral T cell responses in GBM but with very limited therapeutic efficiency [102,103].

It has been reported that 80% of glioma patients suffer associated epilepsy due to glutamate release via xCT [104]. This observation thus points to this transporter as a marker for epilepsy and poor overall survival [105,106]. In addition, the genetic or pharmacological inhibition of xCT in GBM mice xenographs (GBM PDXs) abrogates glutamate release and neurodegeneration, and reduces tumour growth and associated seizures, thereby prolonging survival [107]. It has also been reported that the inhibition of xCT in rodents sensitises GBM PDXs to radiation and chemotherapy (TMZ) [108,107,109,110].

Despite the availability of compounds and derivatives that inhibit xCT activity, their cross-reactivity with other key molecular functions, their limited potency and/or difficulty in crossing the BBB make them of little value for clinical use. Among them are S-4-carboxyphenylglycine (S-4-CPG), sulfasalazine (SSZ), erastin and sorafenib, which are known as multikinase or VDAC inhibitors [111-114,104]. In addition, some series of amino-3-carboxy-5-methylisoxazole propionic acid derivatives show greater specificity but lower potency (~50 microM) [115], and optimised TFMIH, the first non-competitive xCT inhibitor, also shows low potency [116]. Moreover, the mechanisms involved in ferroptosis induction by xCT inhibitors (erastin, SSZ and sorafenib) are not clear, and whether this process involves direct and/or AMPK-BECN1-mediated inhibition of xCT has been recently challenged [117]. It is therefore necessary to design and put into practice guided strategies for the identification of specific xCT inhibitors with real clinical value for the treatment of brain tumours.

Asc-1/CD98hc

Asc-1 (system asc transporter 1)/CD98hc (SLC7A10/SLC3A2) mediates sodium-independent transport of small neutral amino acids such as glycine, L-alanine, L-serine, L-threonine and L-cysteine. It also transports D-isomers, including D-serine, with high apparent affinity (Table 1). It functions preferentially, but not exclusively, in an exchange mode [4,118]. Asc-1 mRNA is expressed in the brain, lung, small intestine and placenta. Although the functional significance of Asc-1 has not yet been fully determined, it is notable

that it transports D-serine, an endogenous modulator of NMDA-type glutamate receptors (NMDARs), and thus might play a role in regulating synaptic transmission.

NMDARs play a central role in long-term potentiation (LTP), synapse formation, plasticity, learning and memory [119]. NMDAR hypofunction may underlie the cognitive impairment of schizophrenia, while NMDAR over-activation leads to excitotoxicity [120,121]. D-serine (D-Ser) and glycine (Gly) are co-agonists of NMDARs and are required for neurotransmission and excitotoxicity triggered by L-glutamate [121]. Neurons contain D-Ser and its biosynthetic enzyme serine racemase (SR) [122]. L-serine (L-Ser) shuttled from astrocytes through amino acid transporter ASCT1 feeds neuronal SR to generate D-Ser (Fig. 2), which sustains extracellular D-Ser, LTP of glutamatergic transmission, memory and learning [123].

Neuronal D-Ser dynamics is regulated by the neutral amino acid transporter Asc-1/CD98hc [124], which mediates the exchange of D-Ser with neutral amino acids, referred to as the exchange mode [4,118]. However, Asc-1/CD98hc also releases substrates by facilitated diffusion [4,118]. Asc-1 ablation, as well as efflux of D-Ser and Gly trans-stimulated by the specific substrate D-isoleucine (D-Ile), showed that this transporter mediates tonic D-Ser and Gly release, which is required for optimal NMDAR activation, LTP, and synaptic plasticity in the hippocampus and cortical brain synapsis [125-127]. Neuronal SR and Asc-1/CD98hc, on the one hand, and astrocytic glycolysis and ASCT1, on the other, define the serine astrocytic/neuronal shuttle (Fig. 2). Asc-1/CD98hc also regulates Gly metabolism. Asc-1 knockout mice show a global reduction of Gly in brain associated with impaired glycinergic inhibitory transmission in the spinal cord and brain stem, and a hyperekplexia-like phenotype caused by deficient Gly synthesis from L-Ser catalysed by serine hydroxymethyltransferase [128].

Asc1/CD98hc has been proposed to contribute to schizophrenia and excitotoxicity. Schizophrenia is a debilitating mental illness that affects 1% of the population (>5 million people in the EU) and has major public health implications. The aetiology of schizophrenia is complex and not fully understood, and current antipsychotics, based on the dopaminergic hypothesis, are characterised by severe limitations [129]. In this regard, they are efficient for only about half the patients, they ameliorate mainly positive symptoms and have serious side-effects. The dopaminergic hypothesis is complemented by the glutamatergic hypothesis (impaired glutamatergic NMDA neurotransmission) [122]. To avoid excitotoxicity, indirect

approaches to increase concentrations of agonists by blocking the glycine transporter GLYT1 have been attempted but with no consistent results [122]. In this regard, the development of specific and efficient substrates that trans-stimulate D-Ser and Gly efflux from neurons via Asc-1 to activate NMDAR signalling emerges as an alternative approach [130]. The identification of determinants of substrate interaction and transport would facilitate the design of specific and efficient substrates of human Asc-1/CD98hc.

Excitotoxicity is the principle mechanism underlying neuronal death following cerebral ischemia and in other neurodegenerative diseases (e.g., brain trauma, Huntington's disease, Alzheimer's disease, and Amyotrophic lateral sclerosis). Over-excitation of the NMDAR subtype containing the GluN2B subunit seems to underlie neuronal death after ischemia [120]. Approaches to inhibit the release of glutamate, NMDAR antagonists and calcium channel blockers have resulted in side-effects or no improvement in stroke outcome, while selective GluN2B receptor antagonists prevent most of the side-effects but have not passed the clinical testing required for broad clinical use [120]. Targeting the interaction of GluN2B with neuronal-death effectors is very promising but not yet available in the clinical setting [131]. Blocking hAsc-1 transporter to decrease the extracellular concentration of the co-agonists of NMDAR D-Ser and Gly is therapeutic option to be explored [126].

Pharmacological interest in human Asc-1/CD98hc in the context of schizophrenia and excitoxicity has led to the identification of small-molecule interactors by means of functional screening and chemical biology improvement. The following are of note: i) Lu AE00527 (Lundbeck pharma) and ACPP (Takeda pharma) block the transporter competitively, with medium (IC_{50}: 5 μM) and high (IC_{50}: 0.7 μM) potency, respectively [24,132,133,127]. These inhibitors decrease D-Ser concentration in CSF and might be considered leads for the treatment of excitotoxicity. Lu AE00527 has very low BBB permeability in mice [127], a feature likely to be shared by ACPP; ii) S-methyl L-cysteine (SMLC), a non-selective moderate-affinity substrate that inhibits D-Ser influx (IC_{50}: 78 μM) and trans-stimulates D-Ser efflux. Accordingly, SMLC increases extracellular D-Ser concentration in the rat medial frontal cortex [130]. Such a compound with satisfactory affinity, selectivity and BBB permeability would have potential therapeutic value for schizophrenia; and iii) the competitive inhibitor BMS-466442 (Bristol-Myers-Squibb and Janssen companies), proposed to bind to the orthosteric site in a model of hAsc-1 based on the outward-facing conformation of remote bacterial homologs [39].

Future directions

HATs have relevant functions in the CNS. Defects in these transporters result in brain disorders (e.g., LAT1/CD98hc, and potentially LAT2/CD98hc, in autism) and their exacerbated function confers an advantage to tumour cells in the CNS (xCT/CD98hc and LAT1/CD98hc). Transporter y+LAT2/CD98hc might modulate hepatic encephalopathy. Moreover, these transporters have the potential to be used as therapeutic targets in diseases of the CNS (Asc-1/CD98hc in schizophrenia, stroke and other excitotoxic-related conditions). The revolution brought about by cryo-EM in structural biology opens the possibility to shed light into the structural features of HATs with relevance in CNS physiopathology. Ahead of these studies are the dissection of the specific molecular mechanisms of these transporters and the development of new drugs with potential therapeutic value.

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Light subunit	SLC member	Transport mode	Substrate selectivity	Distribution in CNS	Role in CNS
LAT1	SLC7A5	Exchange	L-Leu, L-Ile, L-Val, L-Met, L-His, L-Phe, L-Trp, L-Tyr, L-Cys, D-Leu, D-Phe, D-Met > L-Asn, L-Gln [134]	- Luminal and contra-luminal membranes of endothelial cells in BBB [16,59] - Astrocytes [135]	Transfer of essential LNAA from blood to brain [16]
LAT2	SLC7A8	Exchange	L-Leu, L-Ile, L-Val, L-Met, L-Asn, L-His, L-Gln, L-Phe, L-Trp, L-Tyr > L-Ala, L-Ser, L-Thr, L-Cys >> Gly [7]	- Luminal membrane of the choroid plexus epithelium [67] - Astrocytes [71]	Harmonization of L-Gln and LNAA in astrocytes and neurons [73]
y^+LAT2	SLC7A6	Exchange (AA+/AA0 +Na^+)	L-Gln, L-Leu, L-Arg, Na^+ [77,9]	- Astrocytes and neurons in culture [72]	L-Arg/L-Gln exchange in astrocytes [73]
xCT	SLC7A11	Exchange	Cystine, L-Asp, L-Glu [88]	- Astrocytes in brain/CSF/plasma interface [88]	Cystine uptake to sustain glutathione synthesis [90]
Asc-1	SLC7A10	Exchange and Facilitated diffusion	Gly, L-Ala, L-Ser, L-Thr, L-Cys, D-Ser > L-Leu, L-Ile, L-Val > L-Met, L-Asn, L-His [118]	- Neurons [123]	L-Ser/D-Ser shuttle in neurons [136]

Table 1. CD98hc-associated amino acid transporters in brain.

Figure legends

Figure 1. Heteromeric Amino acid Transporters structure and binding site design. Left, inward-facing crystal structure of 2-amino-bicyclo[2.2.1]heptane-2-carboxylic acid (BCH)-bound hLAT1/CD98hc heterodimer structure (PDB ID 6IRT). hLAT1 helices are coloured blue to red from the N-termini and CD98hc is shown in magenta. Right, detailed region of the hLAT1 binding site, showing residues in TM1 and TM6 interacting with BCH (hydrogen bonds are shown as dashed grey lines). Phenylalanine 252 is the external gate that interacts with the substrate. NAG: N-acetyl-β-D-glucosamine.

Figure 2. Heteromeric Amino acid Transporters in brain. LAT1/CD98hc, located in the luminal and contraluminal cell membrane of the cerebral vascular endothelium, is responsible for the transfer of BCAAs across the BBB in exchange with LNAAs [16,59]. LAT2/CD98hc in the luminal cell membrane of the choroid plexus epithelium participates, together with SNAT3 in the release of non-essential amino acids, in particular glutamine, into CSF (luminal space marked in light blue), in exchange with LNAAs. LAT1/CD98hc and LAT2/CD98hc, together with transporters of the SLC38 family (not depicted here for clarity), are suggested to be regulators of the glutamate/GABA-glutamine cycle. Glutamine synthetase (GS) in astrocytes and glutaminase (GlnAse) in neurons would close the glutamate/GABA-glutamine cycle. Transamination reactions between glutamate (L-Glu) and BCAAs mediated by cytosolic branched chain amino acid transaminase (cBCAT) in neurons and mitochondrial branched chain amino acid transaminase (mBCAT) in astrocytes contribute to harmonize L-Glu and L-Gln concentrations. Different SLC1A transporters (Excitatory Amino acid Transporters), in astrocytes (EAAT) and neurons (not depicted for simplicity) withdraw L-Glu from the glutamatergic synapses. The shuttle of L-serine (L-Ser) from astrocytes to neuronal serine racemase (SR) generates D-serine (D-Ser), co-agonist of the NMDA glutamate receptor (NMDAR). The astrocytic transporter ASCT1 (SLC1A4) and the neuronal Asc1/CD98hc mediate this shuttle. xCT/CD98hc in astrocytes mediates the exchange of cysteine (influx)/glutamate (efflux). The sense of this exchange is favoured by the reduction of intracellular cysteine to cysteine and their utilization for glutathione (GSH) synthesis. L-arginine (L-Arg) uptake in exchange with L-Gln by y^+LAT2/CD98hc in astrocytes and in neurons (not depicted) might participate in the toxicity by hyperammonemia in brain by depleting intracellular L-Arg as a result of the increased extracellular concentration of L-Gln [73].

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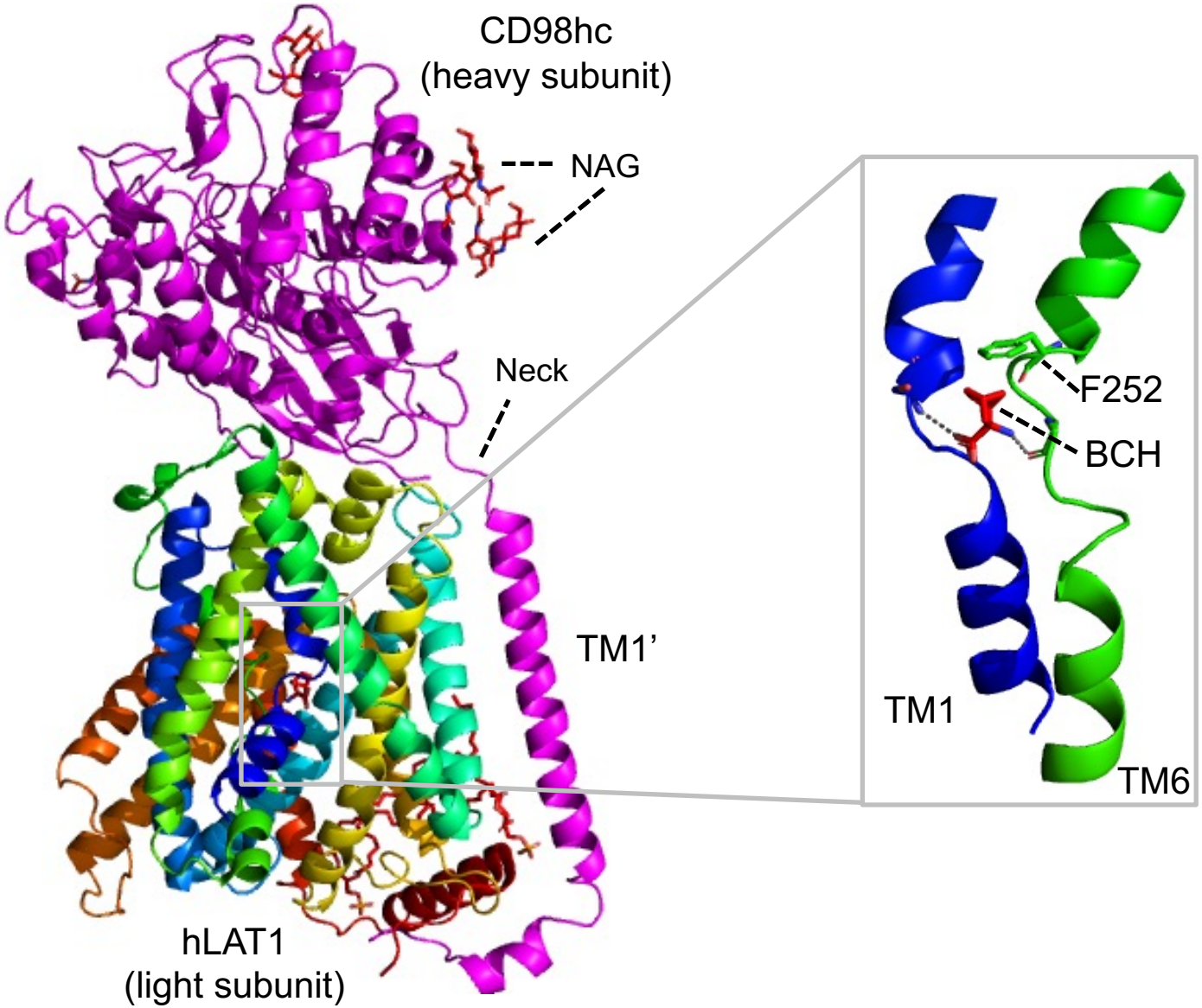
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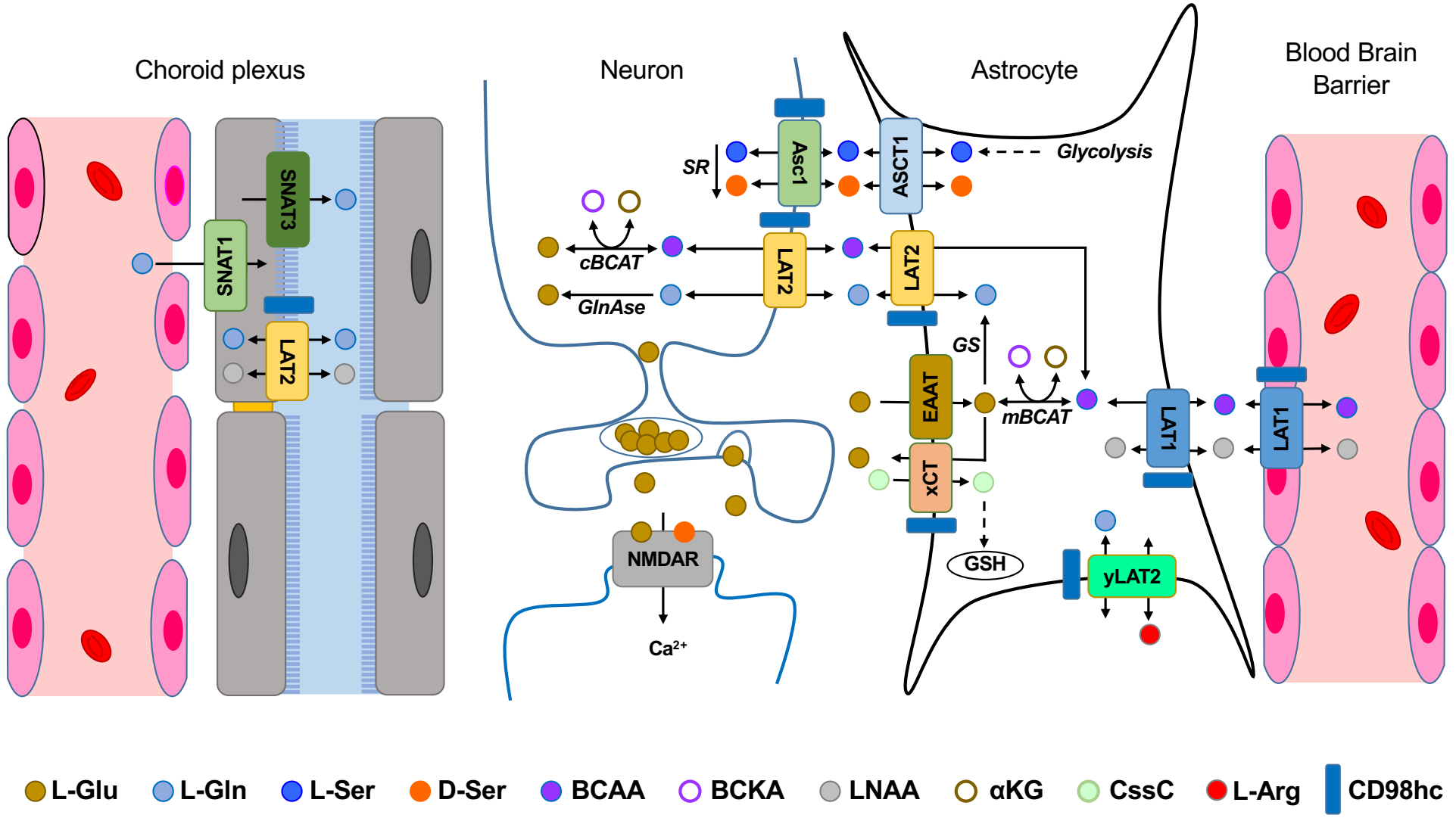
NAG
Neck
TM1'
hLAT1
(light subunit)
F252
BCH
TM1
TM6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LAT2
GS
EAAT
mBCAT
xCT
LAT1
LAT1
NMDAR
GSH
yLAT2
Ca2+
L-Glu
L-Gln
L-Ser
D-Ser
BCAA
BCKA
LNAA
αKG
CssC
L-Arg
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