Acidic Amino Acid Transport Characteristics of a Newly Developed Conditionally Immortalized Rat Type 2 Astrocyte Cell Line (TR-AST)

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ABSTRACT. To characterize acidic amino acid transport in type 2 astrocytes, we established conditionally immortalized rat astrocyte cell lines (TR-AST) from newly developed transgenic rats harboring temperature-sensitive SV40 large T-antigen gene. TR-AST exhibited positive immunostaining for anti-GFAP antibody and A2B5 antibody, characteristics associated with type 2 astrocytes, and expressed glutamine synthetase. Acidic amino acid transporters, GLT-1 and system xC⁻, which consists of xCT and 4F2hc, were expressed in all TR-ASTs by RT-PCR. On the other hand, GLAST expression was found in TR-AST3 and 5. The characteristics of [³H]L-glutamic acid (L-Glu) uptake by TR-AST3 include an Na⁺-dependent and Na⁺-independent manner, concentration-dependence, and inhibition by L-aspartic acid (L-Asp) and D-aspartic acid (D-Asp). The corresponding Michaelis-Menten constants for the Na⁺-dependent and Na⁺-independent process were 36.3 μM and 155 μM, respectively. [³H]L-Asp and [³H]D-Asp uptake by TR-AST5 had an Na⁺-dependent and Na⁺-independent manner. This study demonstrated that GLT-1, system xC⁻, and GLAST were expressed in TR-AST, which has the characteristics of type 2 astrocytes and is able to transport acidic amino acids.

Key words: acidic amino acid/GLT-1/GLAST/system xC⁻/transport/type 2 astrocyte

Acidic amino acids have conflicting roles at the central nervous system (CNS) because they contribute both to excitatory neurotransmission and excitatory damage (Zorumski and Olney, 1993). Therefore, acidic amino acid transporters in the brain play an important role in maintaining the concentrations below neurotoxic levels (Kanai and Hediger, 1996). System Xₐₙ⁻ and system xC⁻ are acidic amino acid transporters, the mRNAs of which are referred to as EAAT (excitatory amino acid transporter) (Palacín et al., 1998), and xCT (Sato et al., 1999), respectively. System Xₐₙ⁻ recognizes L-glutamic acid (L-Glu), L-aspartic acid (L-Asp), and D-aspartic acid (D-Asp). The corresponding Michaelis-Menten constants for the Na⁺-dependent and Na⁺-independent process were 36.3 μM and 155 μM, respectively. [³H]L-Asp and [³H]D-Asp uptake by TR-AST5 had an Na⁺-dependent and Na⁺-independent manner. This study demonstrated that GLT-1, system xC⁻, and GLAST were expressed in TR-AST, which has the characteristics of type 2 astrocytes and is able to transport acidic amino acids.

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Abbreviations: 4F2hc, heavy chain of 4F2 cell surface antigen; ALS, amyotrophic lateral sclerosis; CNS, central nervous system; EAAT, excitatory amino acid transporter; GFAP, glial fibrillary acidic protein; GLAST, L-glutamate/L-aspartate transporter; GLT-1, L-glutamate transporter; SV, simian virus; TR-AST, conditionally immortalized rat type 2 astrocyte cell line; tsA58 TG rat, transgenic rat harboring temperature-sensitive SV40 large T-antigen gene.
firmed their origin, antigenic phenotypes, and developmental changes in both types of astrocytes (Miller et al., 1989). Of these two types of astrocytes, type 1 astrocytes appear at the embryonic stage, while type 2 astrocytes start to appear at the postnatal stage on day 8 to 10 (Miller et al., 1989). Moreover, the number of type 2 astrocytes increases with the development of the CNS (Miller et al., 1985). Recently, Kondo and Raff reported that type 2 astrocyte cells have an ability to revert to multipotential neural stem cells in vitro (Kondo and Raff, 2000). These observations suggest that type 2 astrocytes could play an important role in maintaining the mature CNS. To date, there have been several reports on the biochemical aspects of type 2 astrocytes, such as their endogenous amino acid levels (Levi and Patrizio, 1992), excitatory amino acid receptors (Fan et al., 1999; Gallo et al., 1989), and glutamine synthetase level (Juurlink and Hertz, 1991). However, there are few reports about acidic amino acid transport in type 2 astrocytes because many studies have been performed without classifying the cell type involved.

The purpose of this study was to investigate the acidic amino acid transport in type 2 astrocyte in vitro. To achieve this aim, we established conditionally immortalized rat type 2 astrocyte cell lines, from the mature brain of transgenic rats expressing the mature CNS. To date, there have been several reports on the biochemical aspects of type 2 astrocytes, such as their endogenous amino acid levels (Levi and Patrizio, 1992), excitatory amino acid receptors (Fan et al., 1999; Gallo et al., 1989), and glutamine synthetase level (Juurlink and Hertz, 1991). However, there are few reports about acidic amino acid transport in type 2 astrocytes because many studies have been performed without classifying the cell type involved.

The purpose of this study was to investigate the acidic amino acid transport in type 2 astrocyte in vitro. To achieve this aim, we established conditionally immortalized rat type 2 astrocyte cell lines, from the mature brain of transgenic rats harboring temperature-sensitive SV40 large T-antigen gene (tsA58 TG rats), which have recently been developed as a source of conditionally immortalized cell lines (Takahashi et al., 1999).

**Materials and Methods**

**Animals**

The origin and characteristics of the male tsA58 TG rats (lines # 1507-2 and 1519-8) have been previously described (Takahashi et al., 1999). The investigations using rats described in this report conformed to the guidelines of the Animal Care Committee, Graduate School of Pharmaceutical Sciences, Tohoku University.

**Reagents**

L-[2, 3-3H]Glutamic acid ([3H]L-Glu, 24.0 Ci/mmol) and D-[2, 3-3H]aspartic acid ([3H]D-Asp, 18.0 Ci/mmol) were purchased from NEN Life Science Products Inc. (Boston, MA). L-[2, 3-3H]Aspartic acid ([3H]L-Asp, 22.0 Ci/mmol) was obtained from Amersham Pharmacia Biotech (Buckinghamshire, England). [Carboxyl-33C]Inulin (14C]inulin, 2.64 mCi/g) was purchased from ICN (Irvine, CA). Rabbit anti-glial fibrillary acidic protein (GFAP) antibody was purchased from Sigma (St Louis, MO), Mouse A2B5 antibody and dispase were obtained from Boehringer Mannheim (Mannheim, Germany). Mouse anti-SV40 large T-antigen antibody (Ab-2) was obtained from Oncogene Research Products (Cambridge, MA). All other chemicals were of reagent grade and available commercially.

**Establishment and characteristics of cell lines**

Astrocyte cells were cultured using a modification of the procedure of Nakahata et al. (Nakahata et al., 1996). A cerebrum digestion of adult tsA58 TG rats was suspended in Dulbecco’s modified Eagle medium (Nissui Pharmaceutical Co., Tokyo, Japan) supplemented with 100 U/ml benzyl penicillin, 100 µg/ml streptomycin (Sigma, St Louis, MO) and 10% fetal bovine serum (Moregate, Bulimba, Australia) and cultured at 37°C in a humidified atmosphere of 5% CO2 and 95% air during first 48–72 h in order to attach cells on culture plates. Cells were subsequently cultured at 33°C to promote the cell proliferation caused by temperature-sensitive SV40 large T-antigen. Following two passages, cells were seeded at low density (~100 cells per dish) to establish cell colonies. The colony, which had the characteristic morphology of type 2 astrocytes, was surrounded by a stainless-steel cylinder, i.e. a penicillin cup, and selectively trypsinized in the cylinder. After performing this procedure twice, cells were cloned. Cell lines exhibited contact-inhibition after 9–days culture following a logarithmic growth with doubling-times of 23–32 h at 33°C. Cell lines could be passed over 100 times without any morphological changes.

**Immunocytochemistry**

Cells were fixed using paraformaldehyde. The specimen was incubated with rabbit anti-GFAP (1:100, for 1 h at room temperature) or mouse A2B5 antibody (1:100, for 16 h at 4°C) as the primary antibody. Following incubation with the secondary antibody conjugated with horseradish peroxidase, staining was carried out using 0.05 mM 3,3-diaminobenzidine (Wako Pure Chemicals, Osaka, Japan) in the presence of H2O2.

**Uptake study**

Cells (1.2 × 10^6 cells) were cultured on PRIMARIA 24-well plates (Becton Dickinson, Bedford, MA) at 33°C for 24 h and washed with 1 ml ECF buffer consisting of 122 mM NaCl, 25 mM NaHCO3, 3 mM KCl, 1.4 mM CaCl2, 1.2 mM MgSO4, 7H2O, 0.4 mM K2HPO4, 10 mM D-glucose, and 10 mM HEPES (pH 7.4, 290 mOsm/kg) at 37°C. Na+-free ECF buffer was prepared by replacement of NaCl and NaHCO3 with choline chloride and choline bicarbonate. Uptake was initiated by applying 200 µl ECF buffer containing 1.25 µCi [3H]L-Glu, [3H]L-Asp, or [3H]D-Asp and 0.25 µCi [14C]inulin to estimate the volume of water adhering at 37°C. After a predetermined time period, uptake was terminated by removing the applied solution and cells were immersed in ice-cold ECF buffer. Following this, the cells were solubilized in 750 µl 1% Triton-X100/phosphate-buffered saline (PBS). Radioactivity was measured in a liquid scintillation counter equipped with an appropriate crossover correction for [3H] and [14C] (LS6500, Beckman, Fullerton, CA) and protein content was determined using a DC protein assay kit (Bio-Rad, Hercules, CA) with bovine serum albumin as a standard. The amino acid uptake was expressed as the cell-to-medium ratio using the following equation:

\[
\text{Cell/medium ratio} = \frac{(\text{H dpm per cell protein (mg))}}{(\text{H dpm}}
\]
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Reverse transcription-polymerase chain reaction (RT-PCR) analysis

Total cellular RNA was prepared from PBS washed cells using Trizol reagent (Gibco BRL, Rockville, MD). Single-strand cDNA was made from 1 μg total RNA by reverse transcription (RT) using an oligo dT primer. Polymerase chain reaction (PCR) was performed using GeneAmp (PCR system 9700, Perkin-Elmer, Norwalk, CT). Sequences of sense primers, antisense primers, and annealing temperatures were as follows: the sense primer was 5′-TAC CCG AGT GGA ACT TTG ATG-3′, the antisense primer was 5′-TAA AGT TGG TGT GGC AGC CTG-3′, and the annealing temperature was 55°C, for glutamine synthetase; the sense primer was 5′-GAC AGC CAC CTC AGC GCC GA-3′, the antisense primer was 5′-GAG GTG CCA CAA GCT TGG CC-3′, and the annealing temperature was 64°C, for GLAST; the internal sense primer was 5′-CTC ACT GAC TGT GGT TGG TG-3′, the internal antisense primer was 5′-GAG GTG CCA CAA GCT TGG CC-3′, and the annealing temperature was 55°C for GLT-1; the sense primer was 5′-CCT GGC ATT TGG ACAG CTA CAT-3′ and the antisense primer was 5′-TCA GAA TTG CTG TGA GCT TGC A-3′, and the annealing temperature was 60°C for 4F2hc. The xCT primers were designed from the sequence of mouse xCT (Sato et al., 1999) since rat xCT has not been cloned. PCR reactions were performed by denaturing at 94°C for 0.5 min, annealing for 0.5–1 min, and extension at 72°C for 1 min. The PCR products were separated by electrophoresis on a 2 or 2.5% agarose gel and analyzed using an imager (EPIPRO 7700, Aisin, Aichi, Japan) after staining with ethidium bromide. These PCR products were then cloned into a plasmid vector using a p-GEM® Easy Vector System I (Promega, Madison, WI) and sequenced from both directions by DNA sequencer (model 4200 Li-COR, Lincoln, NE).

Data analysis

For kinetic studies, the Michaelis-Menten constant (Km) and maximal uptake rate (Vmax) of L-Glu uptake were calculated from the following equation using the nonlinear least-squares regression analysis program, MULTI (Yamaoka et al., 1981). 

\[ V = \frac{V_{max} \times [S]}{(K_m + [S])} \]

where V and [S] are the uptake rate of L-Glu at 5 min and the concentration of L-Glu, respectively. Statistical significance among means of two groups was determined by Student’s t-test and those of more than two groups were determined by one-way analysis of variance (ANOVA) followed by the modified Fisher’s least squares difference method. Unless otherwise indicated, all data represent means±S.E.M.

Results

Establishment of TR-AST

Five cell lines were established from five tsA58 TG rats. Fig. 1A shows the cell morphology and Figs. 1B and C illustrate the positive immunocytochemical staining for anti-GFAP antibody and A2B5 antibody, respectively, suggesting type 2 astrocytes. In addition, they expressed glutamine synthetase as a result of RT-PCR (Fig. 2), which activity is present in type 2 astrocyte (Juurlink and Hertz, 1991). The SV40 large T-antigen was detected at 94 kDa and conditionally reduced by 67.3% and 76.3% at 37°C and 39°C culture for 2 days, respectively (data not shown). These cell lines were named conditionally immortalized rat type 2 astrocyte cell lines (TR-AST).

Messenger RNA expression of acidic amino acid transporters

The mRNA expression of acidic amino acid transporters was analyzed using RT-PCR. GLAST (EAAT1) mRNA was amplified from TR-AST3 and 5 at 209 bp (Fig. 3A). GLT-1 (EAAT2) mRNA was amplified from all TR-AST1-5 at 458 bp by RT-PCR (Fig. 3B). System xC–, which consists of xCT and 4F2hc, was amplified at the expected 182 bp and 141 bp, respectively (Figs. 3C and D) in TR-AST1-5. Rat brain mRNA, used as a positive control, was ampli-

Fig. 1. Phase microscopic pictures of TR-AST4 (A) and immunostaining for anti-GFAP (B) and A2B5 antibody (C). Scale bars: 100 μm.
RT-PCR analysis revealed that TR-AST5 has expression of GLAST, GLT-1, and xCT-4F2hc. Therefore, acidic amino acid transport property in TR-AST5 was characterized and compared to TR-AST4, which did not express GLAST. In TR-AST5, the Na⁺-dependence of [³H]L-Glu, [³H]L-Asp, and [³H]D-Asp uptake was 31.0±2.0%, 67.0±2.5%, and 83.0±2.6%, respectively (Table I). The presence of 1 mM L-Asp and D-Asp significantly inhibited the [³H]L-Glu uptake by 33.9±1.2% and 19.9±2.8%, respectively (Table I). Moreover, L-Glu uptake by TR-AST5 showed saturable Na⁺-dependent and Na⁺-independent manners (Fig. 4). The corresponding $K_m$ values were $36.3±13.7$ μM and $155±28$ μM (mean±S.D.), and $V_{max}$ values were $0.248±0.071$ nmol/(min·mg protein) and $0.803±0.049$ nmol/(min·mg protein) (mean±S.D.), respectively. In TR-AST4, the Na⁺-dependence of [³H]L-Glu, [³H]L-Asp and [³H]D-Asp uptake by TR-AST4 was $32.2±2.8\%$, $33.1±6.6\%$, and $71.6±3.7\%$, respectively (Table I). [³H]L-Glu uptake was inhibited by $94.8±0.9\%$ in the presence of 1 mM L-Glu (Table I). L-Asp and D-Asp, at a concentration of 1 mM, also significantly reduced the [³H]L-Glu uptake by $42.9±1.9\%$ and $33.2±5.8\%$, respectively, whereas L-arginine, at a concentration of 1 mM, had no effect (Table I).

### Discussion

In the present study, we investigated the acidic amino acid transport properties of type 2 astrocytes using TR-ASTs, which have the characteristics of type 2 astrocytes (Figs. 1 and 2). [³H]L-Glu uptake by TR-AST5 showed saturable Na⁺-dependent and Na⁺-independent processes (Fig. 4). RT-PCR analysis revealed the mRNA expression of GLT-1, xCT, and 4F2hc in all TR-ASTs (Figs. 3B, C, and D), while GLAST mRNA was expressed only in TR-AST3, 5 (Fig. 3A). To the best of our knowledge, this is the first evidence that type 2 astrocyte expresses GLT-1.

### Table I. Characteristics of Acidic Amino Acid Uptake by TR-AST4 and TR-AST5

<table>
<thead>
<tr>
<th>Substrates</th>
<th>Na⁺</th>
<th>Inhibitors</th>
<th>Percent of control (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>TR-AST4</td>
</tr>
<tr>
<td>[³H] L-Glu</td>
<td>+</td>
<td>None (control)</td>
<td>100±4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None (Na⁺-free)</td>
<td>67.8±2.8 b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-Glutamic acid</td>
<td>5.25±0.85 c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-Aspartic acid</td>
<td>57.1±1.9 c)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D-Aspartic acid</td>
<td>66.8±5.8 b)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>L-Arginine</td>
<td>89.8±4.9</td>
</tr>
<tr>
<td>[³H] L-Asp</td>
<td>+</td>
<td>None (control)</td>
<td>100±2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None (Na⁺-free)</td>
<td>66.9±6.6 b)</td>
</tr>
<tr>
<td>[³H] D-Asp</td>
<td>+</td>
<td>None (control)</td>
<td>100±6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>None (Na⁺-free)</td>
<td>28.4±3.7 a)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. (n=3-7). a, b, c: p<0.05, 0.01, 0.001 significantly different from respective control (100%). N.D.: Not determined.
Acidic Amino Acid Transport at Type 2 Astrocyte

Fig. 4. Kinetic analysis for L-Glu uptake by TR-AST5. [3H]L-Glu uptake by TR-AST5 was performed in the presence (■) and absence (○) of Na+. Each point represents the mean±S.E.M. (n=4). The dotted line shows the Na+-dependent L-Glu uptake.

TR-AST4 exhibited the uptake activities for [3H]L-Asp and [3H]D-Asp in an Na+-dependent manner (Table I). Moreover, [3H]L-Glu uptake by TR-AST4 was inhibited by 43% and 33% in the presence of 1 mM L-Asp and D-Asp, respectively (Table I). Considering all this evidence, the Na+-dependent [3H]L-Glu uptake by TR-AST4 is likely to be mediated by GLT-1 since GLAST was not detected in TR-AST4 (Fig. 3A). On the other hand, TR-AST5 expressed GLAST, GLT-1, and xCT-4F2hc (Fig. 3) and had L-Glu uptake with both Na+-dependent and Na+-independent manners (Table I). Moreover, [3H]L-Glu uptake by TR-AST5 was inhibited by 1 mM of L-Glu, L-Asp, and D-Asp (Table I). Because the primers for GLT-1 were not suitable for quantification of mRNA, the kinetic analysis was examined to evaluate transport properties by uptake clearance and affinity for L-Glu uptake in the presence and absence of Na+. The K_m values and V_max values for an Na+-dependent and Na+-independent process were 36.3±13.7 µM and 155±28 µM, and 0.24±0.071 nmol/(min·mg protein) and 0.80±0.049 nmol/(min·mg protein), respectively (Fig. 4). The K_m values for GLAST (77 µM; Storck et al., 1992) and GLT-1 (2 µM; Pines et al., 1992) were similar to that of Na+-dependent process. The K_m value for system xC- (160 µM; Sato et al., 1999) was identical to that of Na+-independent process. The uptake clearance according to V_max/K_m in an Na+-dependent and Na+-independent process was 6.83 µl/(min·mg protein) and 5.18 µl/(min·mg protein), respectively. Therefore, the L-Glu uptake clearance in TR-AST5 for an Na+-dependent process was similar to that of an Na+-independent process. Taken together, TR-AST5 had the expression of GLT-1, GLAST, and system xC- mRNA and exhibited the corresponding Na+-dependent and Na+-independent L-Glu uptake, although kinetic study could not distinguish GLT-1 and GLAST because their K_m values are similar (Palacín et al., 1998).

GLT-1 is essential for maintaining brain homeostasis since epilepsy is observed in the knock-out mouse of GLT-1 (Tanaka et al., 1997). Moreover, it selectively disappears in the condition of amyotrophic lateral sclerosis (ALS) (Rothstein et al., 1995). Thus, we hypothesize that type 2 astrocytes express GLT-1 and contribute to brain homeostasis by removing excess amounts of excitatory neurotransmitters. In contrast to our observations, Kondo et al. have reported that the dominant acidic amino acid transporter in cultured astrocytes is GLAST, not GLT-1 (Kondo et al., 1995). This contradictory observation may be due to the difference in cell type. We used type 2 astrocyte cell lines, while they used newborn mouse astrocytes, which are thought to be rich in type 1 astrocytes. Further study is needed to clarify the different role in type 1 and type 2 astrocytes toward an acidic amino acid transport. In EAAC1 (EAAT3) and EAAT4, which belong to the same family with GLAST and GLT-1, it is reported that their transport activity is modulated by interacting proteins, such as GTRAP3-18 for EAAC1 (Lin et al., 2001), and GTRAPs 41 and 48 for EAAT4 (Jackson et al., 2001), respectively. These findings suggest that similar systems may exist for GLAST and GLT-1. Thus, the clarification of their regulation mechanism would be helpful for our understanding of the different role which each astrocyte cell type plays in acidic amino acid transport.

GLAST mRNA was detected in TR-AST3 and 5, while GLT-1 was detected in all TR-ASTs (Figs. 3A and B). Rothstein et al. have shown the regional distribution of GLAST expression by immunohistochemical analysis in adult rats, i.e., cerebellum, hippocampus, cortex, and striatum (Rothstein et al., 1994). Since TR-ASTs were established from the whole cerebrum of adult rats, it is possible that each TR-AST is established from a different part of the cerebrum. This suggests that GLAST is expressed in heterogeneous type 2 astrocytes in vivo or regulated the expression in developing cell lines.

Contributions by system xC- toward the L-Glu uptake by TR-AST4 and TR-AST5 were shown to occur in an Na+-independent manner (Table I). Cho and Bannai have shown that system xC- is essential for supplying the components of glutathione (GSH) in cultured astrocytes (Cho and Bannai, 1990). Although the astrocytes used in their study were not classified as either type 1 or type 2, it would seem that type 2 astrocytes were involved.

The existence of several types of L-Glu transporters is likely to be needed for the maintenance of brain homeostasis. L-Glu could be removed from the synaptic space by type 2 astrocytes via GLT-1, system xC-, and, in part, GLAST. Several types of transporters could act as a backup system when one transporter is disrupted, e.g., lack of GLT-1 in ALS. System xC- functions not only as an “L-Glu remover” for the synaptic space, but also as an “L-cystine supplier” for astrocytes. Intercellular L-cystine could be reduced to L-cysteine (L-Cys) and used as a component of GSH (Cho and Bannai, 1990) with L-Glu. In addition, L-Glu is metabolized to L-glutamine (L-Gln) by glutamine...
from astrocytes and shown that L-Gln undergoes efflux via
al. have cloned the neutral amino acid transporter, ASCT2,
tribute to the turnover of L-Glu in type 2 astrocytes and may
the pathways, which have not been reported in type 2 astrocytes (but re-
ized to L-Gln by glutamine synthetase (B). Broken line indicates

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synthetase which is present in type 2 astrocytes (Juurlink
and Hertz, 1991); indeed, TR-ASTs expressed its mRNA (Fig.
2). The fate of GSH and L-Gln in type 2 astrocytes is
not clear yet, but they have been studied using unclassified
astrocytes. Sagara et al. have studied the GSH efflux mech-
anism in cultured astrocytes (Sagara et al., 1996). Bröer et
al. have cloned the neutral amino acid transporter, ASCT2,
from astrocytes and shown that L-Gln undergoes efflux via
ASCT2 (Bröer et al., 1999). Further study is needed to
determine whether these hypotheses are rational in type 2
astrocytes. A putative mechanism of L-Glu turnover in type
2 astrocytes is shown in Fig. 5.

In conclusion, we have identified the acidic amino acid
transport mechanism in conditionally immortalized rat type
2 astrocyte cell lines, TR-ASTs, which were established
from tsA58 TG rats. TR-ASTs express GLT-1 mRNA, sup-
porting the view that type 2 astrocytes express GLT-1. TR-
AST5 showed expression of GLT-1, GLAST, as well as
system xc− and L-Glu uptake activities in both Na+-depend-
ent and Na+-independent manners. These transporters con-
tribute to the turnover of L-Glu in type 2 astrocytes and may
help in the detoxification of excitatory toxicity caused by
acidic amino acids in the CNS in the mature brain.

Fig. 5. Putative mechanism for L-Glu turnover in type 2 astrocytes. L-
Glu uptake occurs via system XAG and system xc−. Thereafter, L-Glu is ex-
changed with L-cystine via system xc− and undergoes re-uptake (A), and is
metabolized to L-Gln by glutamine synthetase (B). Broken line indicates
Acidic Amino Acid Transport at Type 2 Astrocyte


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