A novel alternative splicing form of excitatory amino acid transporter 1 is a negative regulator of glutamate uptake

Ainara Vallejo-Illarramendi, María Domercq and Carlos Matute

Departamento de Neurociencias, Universidad del País Vasco, Leioa, Spain

Abstract

EAAT1 is a major glutamate transporter in the CNS and is required for normal neurotransmission and neuroprotection from excitotoxicity. In the present study, we have identified a novel form of the human EAAT1, named here as EAAT1ex9skip, which lacks the entire exon 9. Quantitative PCR analysis indicates that this variant is expressed throughout the CNS, both in grey matter and axonal tracts, at levels ranging between 10% and 20% of the full-length EAAT1 form. When expressed in HEK293 cells, EAAT1ex9skip mRNA is translated into a truncated protein localized in the endoplasmic reticulum. EAAT1ex9skip has no functional glutamate uptake activity but instead, exerts a dominant negative effect over full-length EAAT1 function. In turn, co-expression of full-length EAAT1 and EAAT1ex9skip variants reduces the insertion of the former into the plasma membrane. Together, these results indicate that the EAAT1ex9skip splice variant is a negative regulator of full-length EAAT1 function in the human brain.

Keywords: EAAT1ex9skip, glutamate aspartate transporter, splice variant.

J. Neurochem. (2005) **95,** 341–348.

High affinity glutamate uptake constitutes the major mechanism by which glutamate homeostasis is preserved in the CNS, protecting neurones and oligodendrocytes from glutamate excitotoxic death due to the excessive activation of glutamate receptors (Choi 1992; Matute *et al*. 1997). Many neurodegenerative conditions, including Parkinson's disease, Alzheimer's disease, cerebral ischemia, traumatic brain injury, epilepsy, Huntington's chorea, amyotrophic lateral sclerosis, schizophrenia and multiple sclerosis, have been linked to disturbed glutamate homeostasis (Danbolt 2001; Matute *et al*. 2001, 2005). Glutamate transporters also play an important role in synapses, where they modulate postsynaptic responses and contribute to glutamate recycling into presynaptic terminals (Danbolt 2001). Glutamate transporters are plasma membrane glycoproteins composed of eight transmembrane domains that are proposed to assemble as homomultimers (Haugeto *et al*. 1996; Eskandari *et al*. 2000; Grunewald and Kanner 2000; Seal *et al*. 2000). To date, five human glutamate transporter subtypes have been cloned (EAAT1–5) (Shashidharan and Plaitakis 1993; Arriza *et al*. 1994; Fairman *et al*. 1995; Arriza *et al*. 1997). In particular, glial EAAT1 and EAAT2 have the greatest impact on glutamate clearance in the CNS (Rothstein *et al*. 1996) and thus, they are essential for normal neurotransmission and protection from excitotoxicity.

Alternative splicing of RNA is a common mechanism that allows for the diversification of a single gene into different protein products (Lopez 1998). Recently, alternative splicing has begun to be considered as one of the most important mechanisms regulating gene expression. This is due to the fact that this highly versatile mechanism can regulate not only the amount of mRNA but primarily, these changes in protein sequence can affect almost all aspects of protein function, such as binding properties, protein stability, enzymatic activity, post-translational modifications and intracellular localization (Stamm *et al*. 2005).

Previous studies report the existence of several human EAAT2 and EAAT3 splice variants (Lin *et al*. 1998; Nagai *et al*. 1998; Matsumoto *et al*. 1999; Meyer *et al*. 1999). Most of these variants have little or no glutamate transport activity,

but they do interfere with translation or post-translational processing of full-length glutamate transporters.

In the present study, we report the identification of a novel, alternatively spliced EAAT1 mRNA, expressed in human brain, that we have named EAAT1ex9skip. While there are previous reports of one splice variant of glutamate aspartate transporter (GLAST), which is the rat EAAT1 homologue (Huggett *et al.* 2000, 2002), this is the first report of a human EAAT1 splice product to date. We have analysed the expression and function of EAAT1ex9skip at RNA and protein level to understand further the role of glutamate transporter splicing variants and their interaction with the full-length proteins. Our experiments show that EAAT1ex9skip is retained in the endoplasmic reticulum (ER) and modulates the functional properties of full length EAAT1 in a dominant-negative way.

Materials and methods

Human brain tissue
Control human tissue used in this study was collected with appropriate autopsy consent procedures (Hospital de Cruces, Bilbao, Spain). Brain regions were dissected according to functional and anatomic regions. At autopsy, tissues were frozen with dry ice and stored at $-80°C$ until use.

RT-PCR analysis of EAAT1 mRNA in human CNS
Total RNA was extracted with Trizol reagent (Invitrogen, Barcelona, Spain), and cDNA was synthesized using random hexamers and Superscript III reverse transcriptase (Invitrogen), following the manufacturer's instructions. Primers used for PCR (Genotek, Sabadell, Spain) were P1: ATGACTAAAAGCAATGGAGAA, P1′: GGTACCTGTGACAAGAC; P2: GAATGGCGGCGCTAGA-TAGT, P2′: CCACATTATTACTGCTACCAG; P3: GTTTAAAAC-CAACTATGAGAAG, P3′: ACCTTGAAGAGGTCCCCAGA; P4: GCTCTGGGGACCTCTTCAAG, P4′: TCTCAGTTTCATTGTCC-TGTGC. PCR was carried out using Taq Platinum Master Mix (Invitrogen) following the manufacturer's instructions. PCR amplification products were resolved on 1% agarose gels, and electrophoretic bands were isolated using Concert Rapid Gel Extraction System (Life Technologies, Barcelona, Spain). The purified amplification product was sequenced in the Genomic Service (Universidad del País Vasco, Spain). Primers P4 and P4′ were used for sense and antisense sequencing, respectively, and sequence analysis was carried out using EditView software.

Quantification of EAAT1ex9skip in different brain regions
Glutamate transporters EAAT1 and the splicing variant EAAT1ex9skip were analysed by real-time quantitative RT-PCR, following the recommendations of User Bulletin 2 (Applied Biosystems, Madrid, Spain). Primers were designed using PrimerExpress software (Applied Biosystems). Analysis of gene expression was performed, starting with 20 ng reverse-transcribed total RNA with 200 nM forward and reverse primers (High Purification Salt Free, Genotek) in a final volume of 20 μL, using the Platinum SYBR Green qPCR SuperMix UDG (Invitrogen) in an ABI PRISM 7000 Sequence Detection System instrument (Applied Biosystems). Primers used were: forward 5′-CGAAGCCATCATGAGACTGGTA-3′ and reverse 5′-TCCCAGCAATCAGGAAGAGAA-3′ for EAAT1 amplification; and forward 5′-TGCCCTCTATGAGGCTTTGG-3′ and reverse 5′-GTCCGGAGGCGATCCCT-3′ for EAAT1ex9skip amplification. Duplicate PCR reactions were carried out for each cDNA sample and the standard deviations of the cycle threshold (Ct) values considered were ≤ 0.38. We verified that the generated fluorescence was not overestimated by contamination resulting from residual genomic DNA amplification (using RT negative controls), primer dimer formation or external DNA contamination (no template controls). PCR products were subjected to a dissociation protocol to ensure that a single amplicon of the expected melting temperature was obtained. To quantify EAAT1 transcript concentrations in tissue samples accurately, normal and variant clones were generated to act as standards in the assay. Concentration of DNA standards was assayed by spectrophotometry and agarose gel analysis and subsequently, serial 10-fold dilutions were made with 60 pg DNA per PCR reaction ($r \geq 0.99$). The amount of each transcript was calculated by plotting the results of the sample against the standard curves. EAAT1ex9skip values are reported as percentage expression of total EAAT1 transcripts. All results are expressed as mean ± SEM.

Cloning and transfection
cDNA amplicons encoding EAAT1 and the EAAT1 exon 9 skipping splice variant were obtained by RT-PCR from human optic nerve RNA using the following primer pair: forward: 5′-CCTGACATG-ACTAAAAGCAATG-3′; reverse: 5′-GTCTACATCTTGGTTT-CACTGT-3′. PCR was carried out with the Taq Platinum Master Mix (Invitrogen) using an annealing temperature of 55°C and an elongation time of 2 min for 35 cycles. The PCR reaction was purified using the GenElute PCR DNA purification kit (Sigma, Madrid, Spain) and ligated into the pTARGET vector (Promega Corporation, Madison, WI, USA). The eGFP vector (Clontech, Madrid, Spain) was co-transfected with EAAT1 and EAAT1ex9skip vectors, then used as an internal control of transfection efficiency. For functional uptake experiments, EAAT1 and EAAT1ex9skip vectors were co-transfected with pTARGET vector to normalize the amount of DNA transfected. Transient transfections into HEK293 cells were carried out using LipofectAMINE Plus reagent (Invitrogen), following the manufacturer's instructions. Cells were grown on polylysine-coated glass coverslips, and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum at 37°C in a CO_2 incubator.

Immunocytochemistry
At 3–4 days after transfection, cells were washed with phosphate-buffered saline (PBS), fixed in 4% paraformaldehyde for 20 min, permeabilized in methanol at $-20°C$ for 10 min and pre-incubated (1 h) with 4% normal goat serum in PBS. Immunostaining for EAAT1 was carried out using an antibody (A522, 1 μg/mL) generously provided by Dr N. C. Danbolt (University of Oslo). The GRP78 antibody was from Santa Cruz Biotechnology (10 μg/mL; sc-1051, Santa Cruz, CA, USA). Incubation with primary antibodies was carried out overnight at 4°C. Binding of primary antibodies was viewed with Alexa Fluor 488 goat anti-rabbit secondary antibody (1 : 200; Molecular Probes, Eugene, OR, USA) and biotinylated horse anti-goat secondary antibody (1 : 200; Vector

Laboratories, Burlingame, CA, USA) followed by Streptavidin-Texas Red (1 : 100; Molecular Probes).

Western blot analysis

Glutamate transporter protein levels were determined in homogenates of cells collected 3–4 days after transfection. Cell lysates were size separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), transferred to nitrocellulose filters (Amersham Pharmacia Biotech, Madrid, Spain), and incubated with antibodies directed against the N-terminal portion of EAAT1 (1 µg/mL; sc-15316, Santa Cruz Biotechnology) and green fluorescence protein (GFP) (0.4 µg/mL; Roche Diagnostics, Barcelona, Spain). Primary antibodies were detected with anti-rabbit or anti-mouse horseradish peroxidase-conjugated secondary antibodies (Sigma). Immunosignals were visualized using SuperSignal West Dura Chemiluminescent Substrate (Pierce, Rockford, IL, USA). Protein band densitometry was carried out using Scion Image for Windows (Scion, Frederick, MD, USA), and GFP immunoreactivity was used to normalize transfection efficiency.

Glutamate uptake assay

Sodium-dependent glutamate uptake was carried out 3–4 days after transfection, using transiently-transfected HEK293 cells. Cells were plated in polylysine-coated, 96-well plates and incubated for 5 min at 37°C with 0.1 nM [^{3}H]glutamic acid (Amersham Pharmacia Biotech, specific activity, SA: 40–50 Ci/mM) and L-glutamate acid (Sigma), with final glutamate concentrations ranging from 1 to 250 µM in a saline buffer (assay buffer: 140 mM NaCl, 5 mM KCl, 2 mM MgCl$_2$, 2 mM CaCl$_2$, 4,5 g/L glucose and 10 mM HEPES, pH 7.4). The reaction was stopped by the addition of 2 mM L-glutamate in ice-cold assay buffer, followed by two rapid washes with ice-cold assay buffer. Net high-affinity uptake was determined by subtracting no-sodium blanks, where NaCl was replaced with choline chloride, from the uptake in the presence of sodium, and expressed as picomoles of glutamate/microgram protein/5 minutes. Uptake data were plotted by non-linear regression using Prism software (GraphPAD Software, San Diego, CA, USA). K_m and V_{max} values were determined using the Eadie–Hofstee transformation. Data are expressed as a mean ± SEM of at least three independent experiments carried out in triplicate.

Statistical analysis

Statistical comparisons were made by two-tailed non-paired Student's t-test using the Microsoft Excel 11.0 software for Macintosh.

Results

Molecular cloning of EAAT1ex9skip

We amplified the EAAT1 coding region from human optic nerve, and we obtained the expected 1629 bp product, together with a smaller sized transcript. To identify the latter, we carried out several PCRs using inner primers that amplified EAAT1 in four fragments (Fig. 1a). We observed that the lower band was located in the 3′ end of EAAT1 cDNA (Fragment 4, Fig. 1b). Isolation and sequencing of the band revealed that this product was an alternatively spliced variant of EAAT1 that lacked exon 9 and hence, we named it

EAAT1ex9skip (GenBank accession number AY954110). Therefore, this is a different splice variant of the rat GLAST1-a isoform which lacks exon 3 (Huggett *et al*. 2000). EAAT1 exon 9 comprises 145 bp and thus, its omission does not alter the RNA reading frame. As a result, the predicted amino acid sequence of EAAT1ex9skip is identical to EAAT1, except for the lack of a 45 amino acid extracellular motif near the carboxy terminal end (Fig. 1c).

EAAT1ex9skip expression in human brain

To establish whether EAAT1ex9skip expression is restricted solely to the human optic nerve, we designed specific primers for this variant and carried out quantitative RT-PCR in three control subjects, using total RNA extracted from different brain regions (cerebellum, corpus callosum, hippocampus, cortex and optic nerve). We first verified that EAAT1ex9skip primers did not detect the full-length EAAT1 cDNA in 45 PCR cycles. We also quantified EAAT1 RNA to normalize EAAT1ex9skip expression in the different samples. This analysis revealed that EAAT1ex9skip mRNA was present in all the areas studied ($p < 0.01$) and that the relative levels of EAAT1ex9skip mRNA range from 8% to 19% of EAAT1 mRNA; the hippocampus and cortex were the regions where it was most and least abundant, respectively (Fig. 2). These data indicate that the EAAT1ex9skip variant is widely expressed throughout the human brain.

Functional expression of EAAT1 and EAAT1ex9skip in HEK293 cells

The coding region of EAAT1 and EAAT1ex9skip forms were cloned from human optic nerve cDNA, and functional characterization was carried out by transient expression in HEK293 cells. Immunocytochemistry with a specific antibody revealed that the full-length EAAT1 protein was predominantly localized in the plasma membrane (Fig. 3a), a finding consistent with previous studies (Huggett *et al*. 2002). In contrast, cells expressing the EAAT1ex9skip variant presented a cytoplasmic immunoreactivity, and mock-transfected cells showed no staining at all. To test whether the EAAT1ex9skip variant is retained in the ER, we double-stained cells for the 78 kDa glucose-regulated protein (GRP78), a chaperone protein located in the ER. GRP78 did not overlap with the full-length EAAT1, although it did co-localize extensively with the EAAT1ex9skip variant (Fig. 3a, right panel). These data indicate that the EAAT1ex9skip splice variant resides in the ER.

The function of these glutamate transporter forms was also examined by measuring Na$^+$-dependent glutamate transport in transiently transfected HEK293 cells. Expression of the full-length EAAT1 protein led to a three to fourfold increase in glutamate transport compared with uptake in cells transfected with vector alone (Fig. 3b). Kinetic parameters V_{max} and K_m were comparable with those determined in
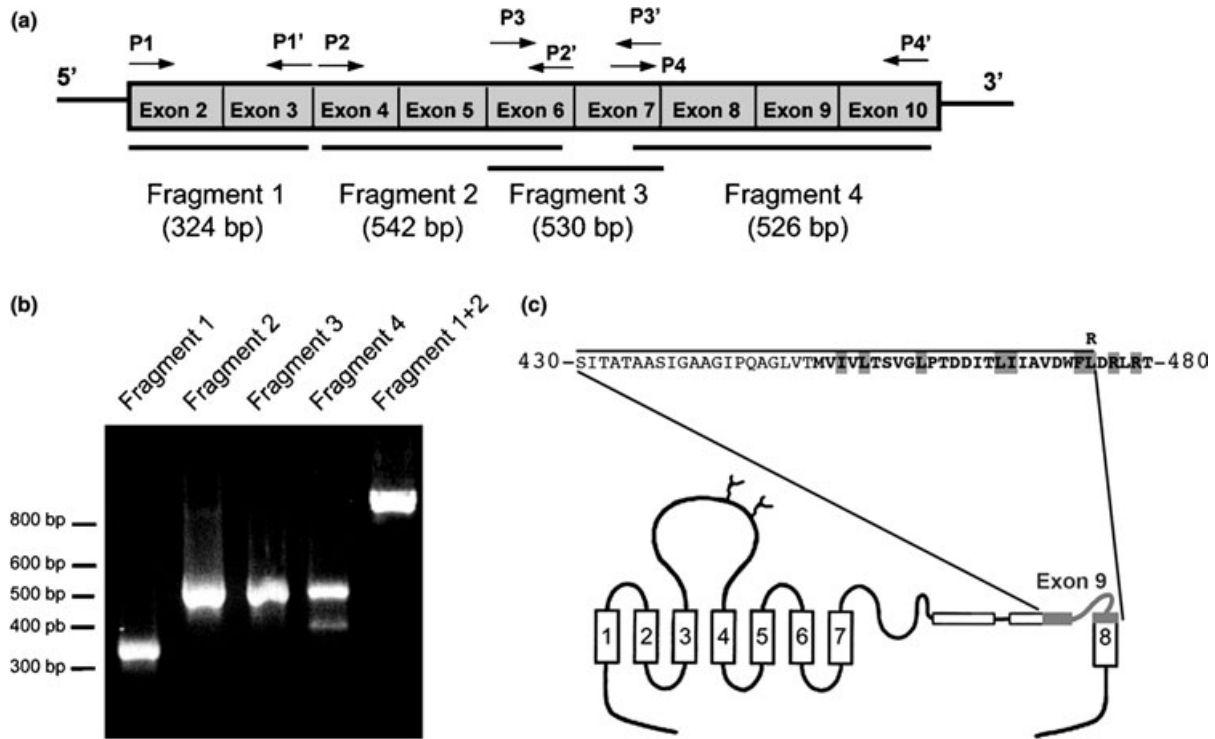
Fig. 1 Identification of EAAT1ex9skip variant. (a) Schematic representation of EAAT1 cDNA coding region. P1–P4 and P1′–P4′ indicate the primers used for amplification (see Materials and methods). (b) PCR analysis of human EAAT1. Amplification with P4–P4′ primers (fragment 4) yields two products in optic nerve RNA. The size of the expected amplicon is 526 bp long and includes exons 8–10, while the shorter band has 391 bp and skips exon 9. (c) Topological model of human EAAT1 (Seal *et al.* 2000). The loss of exon 9 removes the grey region of EAAT1 that represents amino acids 430–474, and substitutes leucine 475 for an arginine residue. Bold residues indicate domain involved in post-translational processing and grey boxes show relevant amino acids.
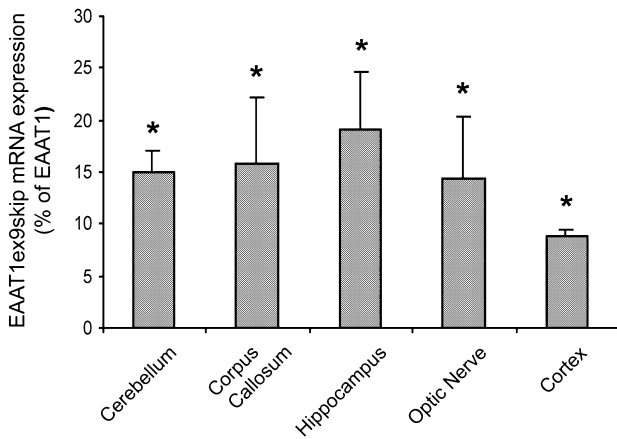

Fig. 2 Quantification of EAAT1ex9skip mRNA in human brain. Histogram shows proportion of EAAT1ex9skip variant in different brain regions of three control subjects, as measured by real-time RT-PCR. The relative contribution of EAAT1ex9skip variant to total EAAT1 transcripts in cerebellum, corpus callosum, hippocampus, optic nerve and cortex was 15.07 ± 2.01, 15.88 ± 6.41, 19.14 ± 5.45, 14.36 ± 6.07 and 8.79 ± 0.74, respectively. Data are expressed as mean ± SEM values; $*p < 0.01$.

previous studies (Arriza *et al.* 1994), hence validating our *in vitro* model. Interestingly, EAAT1ex9skip transfected cells did not show glutamate transport above endogenous levels, a finding which is consistent with the EAAT1ex9skip protein localization in the ER.

Dominant-negative modulation of EAAT1ex9skip variant

Western blot analysis revealed expression of EAAT1 protein in cells transfected with either EAAT1 isoform, whereas no signal was observed in cells transfected with vector alone (Fig. 4a). Under our conditions, EAAT1 antibody recognized bands of approximately 200 kDa, corresponding to multimers (Danbolt 2001). EAAT1 migrated as a major band of approximately 215 kDa and an adjacent, lower-sized band, most likely representing a partially glycosylated species (Schulte and Stoffel 1995). In contrast, the EAAT1ex9skip variant migrated as a single 160 kDa band. This size difference between full-length EAAT1 and EAAT1ex9skip may be due to a lack of glycosylation of the spliced form, in addition to the 45 amino acids omitted; this is suggested by its localization in the ER, which indicates a low degree of
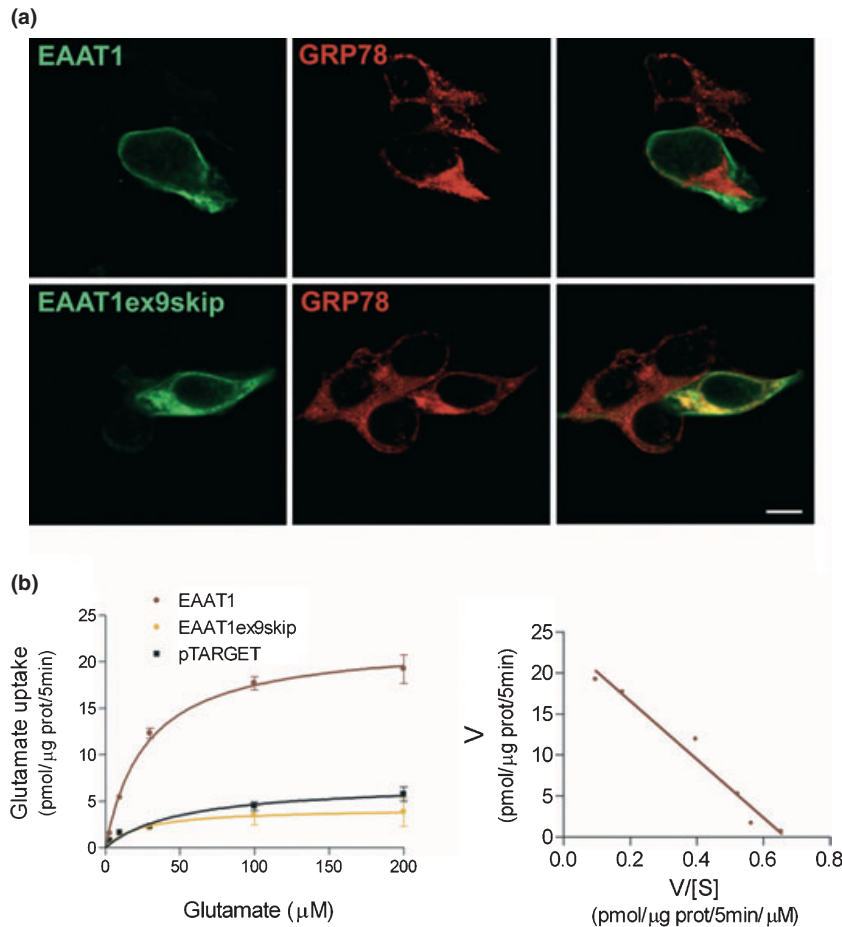
Fig. 3 Expression and functional characterization of EAAT1 isoforms in HEK293 transfected cells. (a) Fluorescence immunostaining for EAAT1 revealed different subcellular localization of EAAT1 and EAAT1ex9skip forms. EAAT1 (green) is expressed at the plasma membrane and does not overlap with staining for GRP78 (red), a protein expressed in the endoplasmic reticulum. In contrast, EAA-T1ex9skip co-localizes with GRP78 expression, indicating that this variant is retained in the endoplasmic reticulum. Representative confocal images of three independent experiments. Scale bar = 10 μm. (b) Glutamate uptake was analysed in transfected cells, using 100 nM of L-[H^3]-glutamic acid in the presence of increasing concentrations of unlabelled substrate, at 37°C for 5 min. EAAT1ex9skip did not show any glutamate transport above endogenous levels. EAAT1 uptake velocity (pM/μg protein/5 min) and K_m (μM) values were 23.5 ± 1.5 and 27.5 ± 2.3, respectively (right panel). Data points represent the average of three independent experiments in triplicate.

protein maturation. Densitometric analysis of immunoreactive bands showed that EAAT1ex9skip variant expression was significantly reduced compared with full-length EAAT1 expression (29.4 ± 8.7%; Fig. 4b). This effect is not due to dissimilar protein translation efficiencies, as cells were also co-transfected with 0.2 μg eGFP vector, and GFP immunoreactivity was used to normalize translation efficiencies.

To test whether this novel EAAT1 splice variant may modulate EAAT1 function, equal quantities of EAAT1 and EAAT1ex9skip plasmids (1 μg each) were co-transfected into HEK293 cells. Both proteins were detected concurrently by western blot (Fig. 4b), although there was noticeably less EAAT1 protein in co-transfected cells compared with cells transfected with EAAT1 plasmid alone. Moreover, co-transfection of EAAT1 with increasing amounts of EAAT1ex9skip resulted in dose-dependent inhibition of glutamate uptake. Taking into consideration endogenous transport in HEK293 cells, the level of inhibition of glutamate uptake reached 44% when both EAAT1 isoforms were co-transfected in the same proportion (Fig. 4c).

Discussion

In this study we identified a novel short form of human EAAT1 which we named EAAT1ex9skip. This form is generated from alternative splicing of exon 9 of the Slc1a3 gene, leading to an in-frame deletion of exon 9 in the EAAT1 mRNA. Using RT-PCR, we found that the EAAT1ex9skip transcript has a widespread distribution and moderate expression levels in post-mortem human brain tissue. Finally,
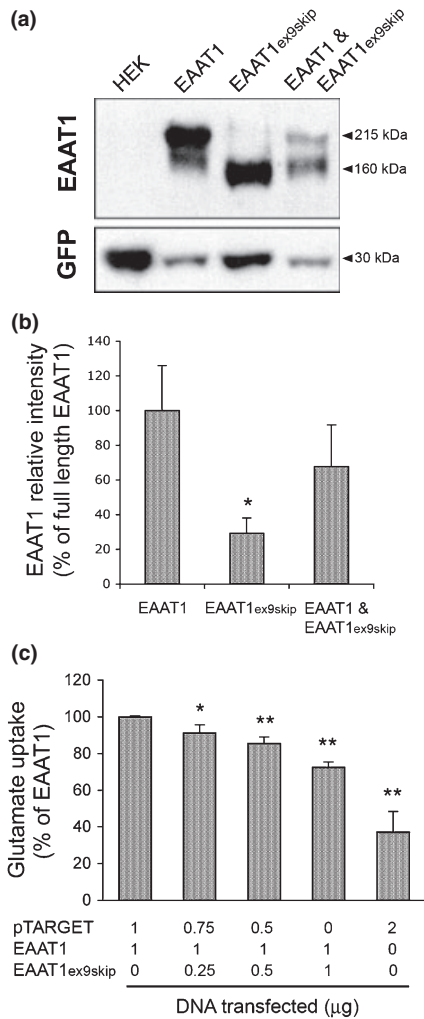
Fig. 4 Dominant-negative effect of EAAT1ex9skip variant over EAAT1 glutamate transporter function. (a) Western blot analysis of HEK293 cells transfected with 1 µg of EAAT1 isoforms plus 0.2 µg green fluorescent protein (GFP) vector, which was used as an internal control of transfection efficiency. (b) Histogram represents the average density of EAAT1, normalized to GFP in transient transfected HEK293 cells ($n = 3$). EAAT1ex9skip protein has a lower expression level compared with full-length EAAT1 ($29.4 \pm 8.7\%$; $*p < 0.05$), and EAAT1 expression was reduced when both EAAT1 forms were co-transfected in similar proportions (67.7 ± 23.9). (c) Modulation of EAAT1 glutamate uptake by EAAT1ex9skip variant. Cells were transfected with 1 µg of EAAT1 cDNA and variable amounts of EAAT1ex9skip cDNA. pTARGET vector was used to normalize for equal amounts of DNA transfected. EAAT1 glutamate transport was inhibited in a dose-dependent manner, reaching 44% inhibition when 1 µg of EAA-T1ex9skip was co-transfected with EAAT1. Data points were obtained by substracting the glutamate uptake in the absence of Na^+. Results presented are means $\pm$ SEM of three independent experiments performed in triplicate. Statistical significance: $*p < 0.05$, $**p < 0.005$.

we have shown by ectopic expression of EAAT1 forms in HEK293 cells that the EAAT1ex9skip transcript is translated into a protein, although at lower levels than full-length

EAAT1. However, it does not retain glutamate uptake activity and it exerts a dominant-negative effect over the full-length EAAT1 protein.

EAAT1 exon 9 encodes 45 amino acids (430–475) near the C-terminal (see Fig. 1c). This region is involved in determining functional differences between EAAT1 and EAAT2 subtypes (Mitrovic *et al.* 1998); it contains arginine motifs involved in the post-translational processing and endoplasmic retention signal of the EAAT2 protein, as well as an upstream leucine-rich motif that is required for the suppression of this signal (Kalandadze *et al.* 2004). Since members in the glutamate transporter family share a high degree of homology in this region, they may also have common mechanisms for proper post-translational maturation. Interestingly, the EAAT1ex9skip variant lacks the upstream leucine-rich motif that lies within residues 451–475 of the full-length EAAT1 transporter and is coded by exon 9. However, the arginine motif (amino acids 477 and 479) is located in exon 10 and is therefore retained by the EAAT1ex9skip variant. As a consequence, this isoform lacks an extracellular domain, which is potentially essential for ER departure.

Consistent with this, we found that EAAT1ex9skip co-localized with the ER chaperone GRP78, as opposed to EAAT1, which is expressed mostly in the plasma membrane. Thus, retention of EAAT1ex9skip in the ER may eventually target this protein for degradation (Hurtley and Helenius 1989). These results, together with previous findings (Kalandadze *et al.* 2004), indicate that glutamate transporters EAAT1 and EAAT2 share similar mechanisms for post-translational maturation, and that EAAT1 exon 9 is required for departure of EAAT1 transporter from the ER and for its forward trafficking through the secretory pathway. Similarly, truncating the C-terminal domain of metabotropic glutamate receptor $mGluR_1$, or the dopamine receptor D_2, results in unmasking an ER retention signal, leading to the retained expression of these alternative splice products in the ER (Chan *et al.* 2001; Prou *et al.* 2001).

The dominant-negative effect that EAAT1ex9skip exerts over EAAT1 could be explained by the oligomeric structure of glutamate transporters (Haugeto *et al.* 1996; Kavanaugh 1998; Eskandari *et al.* 2000). Indeed, the function and protein synthesis of full-length EAAT2 is negatively modulated by splice variants which undergo rapid degradation (Lin *et al.* 1998; Guo *et al.* 2002). However, little is known about the mechanisms implicated in glutamate transporter modulation by splice variants. In line with previous studies, we observed that expression of EAAT1ex9skip was lower than full-length EAAT1, likely due to instability of the truncated protein. Moreover, we found that EAAT1ex9skip reduced EAAT1 expression and function. Altogether, these results suggest that inhibition of EAAT1 by the EAAT1ex9skip variant is due to a protein–protein interaction in the ER that, in turn, leads to a reduction in mature EAAT1 expression in

the plasma membrane. Multimeric complexes formed by the full-length and truncated isoforms are probably retained in the ER because they are incorrectly assembled and thus, targeted for degradation (Hurtley and Helenius 1989; Klausner and Sitia 1990).

An important unresolved issue is whether EAAT splice variants play a physiological or pathological role in the CNS. It has been reported that aberrant RNA processing of EAAT2 is involved in the pathogenesis of amyotrophic lateral sclerosis (Bai and Lipton 1998; Lin *et al*. 1998). However, other studies do not support these findings and favour a physiological function for the EAAT splice variants (Honig *et al*. 2000; Flowers *et al*. 2001). Our data indicate that EAAT1ex9skip is not necessarily related to pathogenic events but rather, plays a physiological role in the CNS, as revealed by its relatively high expression throughout the human CNS. We postulate that the functional significance of EAAT1ex9skip may reside in its ability to modulate EAAT1 expression and activity at the post-translational processing level. Nevertheless, aberrant EAAT1ex9skip expression could potentially lead to abnormal regulation of glutamate homeostasis, which is involved in several neurological disorders (Danbolt 2001).

In summary, we have characterized a novel human EAAT1 splice variant that negatively modulates the expression and function of this glutamate transporter. The fact that this variant is widely expressed in grey and white matter may be relevant to glutamate homeostasis and excitotoxicity, a common event in neurodegenerative and demyelinating diseases.

Acknowledgements

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