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Glutamate transporters in platelets: EAAT1 decrease in aging and in Alzheimer's disease

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Abstract

Platelets release glutamate upon activation and are an important clearance system of the amino acid from blood, through high-affinity glutamate uptake, similar to that described in brain synaptosomes. Since platelet glutamate uptake is decreased in neurodegenerative disorders, we performed a morphological and molecular characterization of platelet glutamate transporters. The three major brain glutamate transporters EAAT1, EAAT2 and EAAT3 are expressed in platelets, with similar molecular weight, although at lower density than brain. A Na⁺-dependent-high-affinity glutamate uptake was competitively inhibited by known inhibitors but not by dihydrokainic acid, suggesting platelet EAAT2 does not play a major role in glutamate uptake at physiological conditions. We observed decreased glutamate uptake V_{max} , without modification of transporter affinity, in aging, which could be linked to the selective decrease of EAAT1 expression and mRNA. Moreover, in AD patients we found a further EAAT1 reduction compared to age-matched controls, which could explain the decrease of platelet uptake previously described. Platelet glutamate transporters may be used as peripheral markers to investigate the role of glutamate in patients with neuropsychiatric disorders.

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Keywords: Human peripheral marker; EAAT2; EAAT3; Molecular characterization; Excitotoxicity; Glutamate uptake

1. Introduction

Glutamate is the major excitatory neurotransmitter in the central nervous system (CNS) and is involved in the pathogenesis of acute and chronic neurological disorders, when its extracellular concentration rises to toxic levels (excitotoxicity) [7,14,36]. Clearance of the excessive neurotoxic concentrations of glutamate is ensured by a high affinity glutamate uptake system, present in neurons and glial cells [32,39]. Defects in glutamate uptake were described in autaptic brain samples in neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS) [5,45,47] and Alzheimer's disease (AD) [37].

Three different glutamate transporters were cloned from rat brain [42,52] and from rabbit intestine [28]. Their human counterparts have 95% homology in amino acid sequence and were respectively called EAAT1, EAAT2 and EAAT3 [2]. More recently, two new transporters, which appear linked to chloride fluxes, were cloned from human cerebellum [16] and retina [1]. The first three transporters are involved in high affinity, sodium-dependent glutamate uptake and knock-out models indicate a specific role of each transporter in preventing excitotoxicity and neuronal degeneration [46,54]. Moreover, reduced expression of GLT1 (EAAT2) in global transient ischemia animal model [56] and in ALS patients [30,47] seems responsible for the elevation of extracellular glutamate concentration and neuronal death. Molecular and/or functional characterizations of glutamate transporters were performed in different cell culture models from CNS and peripheral tissues, in order to investigate the regulation of their expression, translocation

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and activity by various endogenous and exogenous factors [11–13,21,35,38,49].

Platelets were extensively studied as models of synaptic apparatus, since they release and reuptake neurotransmitters such as serotonin and dopamine [3,57], express serotonin [51] and glutamate receptors [23,50] and they served as clinical markers for neuropsychiatric disorders such as depression. Moreover, in AD they were recently proposed as CNS model to study molecular amyloid-precursor protein (APP) alterations [9]. A high affinity glutamate uptake, with kinetic characteristics similar to brain synaptosomes, was also shown in platelets, which appear the major site of clearance of glutamate from blood [34]. NMDA receptors were described in platelets [22,50] and in megakaryocytes [24,50], suggesting their importance in regulating platelet activation and aggregation. We recently described a glutamate release from platelets during aggregation, underling that peripheral glutamate signaling seems to play a role in the regulation of platelet functions [33]. Moreover, we demonstrated a significant decrease in high affinity glutamate uptake in platelets from idiopathic Parkinson's disease patients, correlated to the severity of disorder [19], and from AD [17] and ALS patients [18]. However, the mechanisms of decreased platelet glutamate uptake in neurodegenerative disorders remain unknown. In the present study, we characterized platelet glutamate transporters and compared platelet expression to that of rat brain, human lympho-monocytes, human myeloid leukemia (GFD-8) cell line and human neuroblastoma (SH-SY5Y) cell line after retinoic acid differentiation. This molecular and biochemical characterization may serve as the basis to investigate the physiological modulation of glutamate transporters and their possible alterations in disorders of CNS. We also investigated possible modifications of platelet glutamate transporter expression in aging and in AD patients.

2. Materials and methods

2.1. Platelet and white blood cell preparation

After overnight fast, 30 ml of blood were collected, from antecubital vein of consenting donors, placed in plastic tubes containing K_2 -EDTA (0.17 M) and immediately processed for platelet preparation. Platelet-rich plasma (PRP) was separated by centrifugation at $400 \times g$ for 15 min, at $15^\circ C$. The top three-fourths of PRP were removed and centrifuged at $3600 \times g$ for 15 min to obtain a platelet pellet.

Lympho-monocytes were prepared from the cellular pellet, obtained with PRP, by 1:20 (v/v) dilution with erythrocyte lysis buffer (1.5 M NH_4Cl , 0.1 M $KHCO_3$, 1 mM Na_2 -EDTA) and centrifugation at $500 \times g$ for 10 min, at $4^\circ C$.

For immunocytochemistry and electron microscopy studies, platelets were immediately processed, for routine uptake studies platelets were stored at $-80^\circ C$ in 1 ml 0.32 M sucrose containing 5% dimethyl sulfoxide (Sigma–Aldrich,

St. Louis, MO, USA) to maintain membrane integrity. Tripan blue and May-Grunwald Giemsa stains showed 80% vital platelets with intact organelles after thawing. Preliminary biochemical experiments were also performed in fresh platelet preparations and no difference was observed compared to frozen platelet preparations. Platelet and lympho-monocyte pellets for Western blot and RT-PCR studies were stored at $-80^\circ C$ until use.

2.2. Cell culture preparations

Human myeloid leukemia (GFD-8) cell line was maintained in RPMI 1640 medium, supplemented with 10% heat inactivated fetal calf serum (FCS), penicillin (100 U/ml), streptomycin (100 g/ml) and granulocyte and macrophage stimulation factor (GM-CSF 0.1 ng/ml). Human neuroblastoma cells (SH-SY5Y) were maintained in Dulbecco's modified Eagle's medium (DMEM), supplemented with 10% heat inactivated fetal calf serum (FCS), penicillin (100 U/ml), streptomycin (100 g/ml), L-glutamine (2 mM), sodium pyruvate (1 mM) and $1 \times$ non-essential amino acids. The cells were differentiated for 48 h with $10 \mu M$ *all-trans* retinoic acid [40]. Cell lines were maintained in 5% CO_2 humidified incubator at $37^\circ C$. GFD-8 subconfluent cultured cells were collected in cold phosphate buffered saline (PBS) containing protease inhibitors (2 μg /ml Antipaine, 2 μg /ml Leupeptine, 2 μg /ml Aprotinine, 100 μg /ml phenyl methyl sulphonyl fluoride, PMSF) and pelleted for Western blot studies. SH-SY5Y and GFD-8 subconfluent cultured cells were centrifuged at $200 \times g$ for 10 min, at $4^\circ C$ and dry pellets were stored at $-80^\circ C$ for RT-PCR analysis.

2.3. Immunoelectron microscopy and immunofluorescence

Platelets were fixed in pH 7.4 PBS containing 10% paraformaldehyde and 0.1% glutaraldehyde for 2 h. Incubation with primary antibody (anti-C-terminal-EAAC1 1:800, -GLAST 1:100 and -GLT-1 1:200; by Chemicon Temecula, CA, USA) in PBS and 1% BSA was performed overnight at $4^\circ C$. Platelets were washed for three times in PBS, centrifuged at $500 \times g$ for 10 min and then incubated with the secondary antibody in PBS and 1% BSA for 1 h at room temperature. The immunofluorescence (IF) secondary antibody was bound to fluorescein isothiocyanate (FITC 1:20, Sigma, Milano, Italy), while the immunoelectron microscopy (IEM) secondary antibody was bound to colloidal gold. It is not uncommon that after such a light fixation procedure and overnight incubation, the primary antibody can recognize epitopes localized on cytoplasmic side of the membrane.

2.4. Western blot analysis

For protein extraction, platelets, lympho-monocytes, GFD-8 cells and rat brain tissue samples were sonicated in ice-cold PBS containing 50% (w/v) sucrose, 1 mM EDTA,

5 mM EGTA, 20 µg/ml Aprotinine, 20 µg/ml Leupeptine, 10 mM Antipaine, 0.1 mM PMSF. Aliquots of cell and tissue extracts were subjected to 7.5% SDS–polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose. To separate cytoplasmic and membrane fractions, platelet pellets were suspended in 1 M HEPES buffer containing 100 mM EGTA, 1 M MgCl₂, 20 µg/ml Aprotinine, 20 µg/ml Leupeptine, 10 mM Antipaine, 0.1 mM PMSF at 4 °C for 30 min, and then centrifuged at 5×10^4 g for 60 min at 4 °C (minor modifications by Colciaghi, [9]). Proteins obtained from sub-fractions were also subjected to SDS–PAGE, as previously described. Blots were blocked and incubated for 2 h at room temperature on an orbital shaker in 5% non-fat dried milk in TBS-T buffer (50 mM Tris–HCl pH 7.6, 200 mM NaCl, 0.1% Tween 20) with affinity-purified anti-C-terminal-GLT-1, -GLAST and -EAAC1 antibodies diluted 1:800, 1:1000 and 1:800, respectively. Rabbit anti-actin antibody (1:2500; Sigma-Aldrich, St. Louis, MO, USA) was used as internal standard. Peroxidase-linked anti-guinea pig, -goat and -rabbit IgG secondary antibody (1:5000; Chemicon, Temecula, CA, USA or Amersham Pharmacia, MI, Italy) was incubated for 1 h at room temperature on an orbital shaker in 5% non-fat dried milk in TBS-T buffer. The presence of signals was detected by chemiluminescent reagents such as ECL Plus kit (Amersham Pharmacia, MI, Italy). Competition with the immunizing peptide for each transporter and immunoreactivity tests with secondary antibodies, without all three primary antibodies, were performed and no immunoreactivity was observed. Imaging densitometer (Bio-Rad, Hercules, CA, USA) was employed to scan Western blot signals.

2.5. RT-PCR analysis

Total RNA was isolated from 10^7 platelets, white blood cells, SH-SY5Y and GFD-8 cells by using RNeasy Total RNA Kits (Qiagen, MI, Italy). Primers used for RT-PCR of EAAT1, EAAT2 and EAAT3 (GenBank: U03504, U03505, U03506) were as follows: 5'EAAT1 primer (5'-GAAACGCTTGTGGGTGCTG-3') corresponding to position 649–667 and 3'EAAT1 primer (5'-GTGGCA-GAAGAGGAGGTCC-3') corresponding to position 1112–1133; 5'EAAT2 primer (5'-ACGAGGAGGCCAAC-GCAACAAG-3') corresponding to position 638–659 and 3'EAAT2 primer (5'-GATGCCCCCGTGGATGATGAG-G-3') corresponding to position 1002–1133; 5'EAAT3 primer (5'-CGTCTGGGCTTGATTGTCTTT-3') corresponding to position 651–672 and 3'EAAT3 primer (5'-TGC-ACCAACGGGTAACACGA-3') corresponding to position 1088–1107. These studies were performed by using “ready to go” beads (Amersham-Pharmacia, MI, Italy). Respectively, 300 ng for EAAT1, and 200 ng for EAAT2 and EAAT3 of RNA samples were amplified with Perkin-Elmer Cetus DNA Thermal cycler for 33 cycles for EAAT1 and 30 cycles for EAAT2 and EAAT3. The RNA samples were incubated at 42 °C for 30 min and then at 94 °C for 5 min.

The amplification profile involved denaturation at 94 °C for 60 s, primer annealing at 59 °C for 2 min (EAAT1), at 62 °C (EAAT2) and 60 °C (EAAT3) for 1 min and extension at 72 °C for 2 min. PCR products (32 µl) were resolved by 2% agarose gel electrophoresis. Beta-actin sense primer (5'-TGACGGGGTCACCCACACTGTGCCCATCTA-3') and antisense primer (5'-CTAGAAGCATTTCGGGTGGA-CGATGGAGGG-3') were used to amplify the correspondent house keeping gene from platelet cDNA. Imaging densitometer (Bio-Rad, Hercules, CA, USA) was employed to scan cDNA signals.

2.6. Glutamate uptake studies

To investigate the sodium-dependent glutamate uptake in platelets, we followed the original method of Mangano and Schwarcz [34], with minor modifications [19]. Briefly, platelets were suspended and washed in 0.32 M sucrose; 50 µl aliquots were added to 425 µl Tris-citrate buffer pH 7.0 and samples were preincubated for 10 min at 37 °C. To investigate the Na⁺-dependent high affinity uptake, blank samples were preincubated with similar Tris-citrate buffer which had sodium chloride substituted by equimolar choline chloride and sodium-citrate by equimolar potassium-citrate. Uptake was started by the addition of 25 µl [³H]glutamate (specific activity = 57 Ci/mmol, Du Pont, MA, USA) 1–200 µM final concentration, and stopped after 30 min by the addition of 3 ml ice-cold Tris buffer containing 1 mM cold glutamate. Platelets were separated by centrifugation and washed pellets were solubilized in Opti-Fluor (Packard Instrument Company, CT, USA) and counted in a beta-counter with 60% efficiency. All assays were routinely performed in triplicate. Net high affinity uptake was determined by subtracting no-sodium blanks from the uptake in the presence of sodium and expressed as pmol glutamate/mg protein/30 min. Na⁺-independent low affinity uptake was calculated as difference between uptake at 37 °C and at 4 °C, both in the presence or absence of sodium. The effect of L-aspartic acid, L-glutamic acid, dihydrokainic acid (DHK), L(-)-threo-3-hydroxyaspartic acid (THA) and L-serine-O-sulphate (SOS) on the platelet uptake at 5 µM glutamate was also determined. 25 µl aliquots of different drugs were added to 50 µl platelet sample and 400 µl Tris-citrate buffer pH 7.0, to obtain final concentrations between 1 and 1000 µM, both in the presence or absence of sodium. Glutamate uptake was then performed as described. The effect of uptake inhibitors was determined comparing the high affinity uptake in presence or absence of L-aspartic acid, L-glutamic acid, DHK, THA and SOS.

2.7. Controls and patients

Sixty control subjects (29 females and 31 males), without any evidence of neuropsychiatric disorders or cognitive dysfunction (blood donors of San Gerardo Hospital or University for Aged People), were selected for this study. The age

range and mean age of controls were respectively 20–85 and 66 ± 5 years. Ten AD patients (six females and four males, with age range 68–89 years and mean age 78 ± 4 years) were selected from a previous casuistry [17] and all patients re-

ceived neuropsychological tests including the Mini-Mental State Examination (MMSE) and the Global Deterioration Scale (GDS). Controls and patients under anti-platelet medication were excluded from the study.

Fig. 1. EAAT1 (A), EAAT2 (B) and EAAT3 (C) indirect immunofluorescent staining show immunoreactivity in platelets and in lymphomonocytes (arrow) (original magnification $20\times$); antibody binding to platelet plasma-membrane is confirmed by indirect immunogold electron microscopy performed also with anti-EAAT2 (E) antibody (original magnification $58,000\times$). Similar pictures are obtained with EAAT1 and EAAT3 antibodies (data not shown). No immunoreactivity was observed when platelets were incubated without primary antibodies (D).

2.8. Statistical analysis

All results are expressed as mean $\pm$ S.E.M. One-way analysis of variance (ANOVA), followed by Dunnett test for independent samples, was used to assess the significance of differences between means of investigated groups. Statistical analysis of correlations was performed with Pearson's correlation test.

3. Results

As shown in Fig. 1, all three investigated human glutamate transporters (EAAT1, EAAT2 and EAAT3) were detected in human platelets, by immunofluorescence and immunoelectronmicroscopy, although at lower density than in brain tissue. Pre-incubation of antibodies with synthetic peptides derived from glutamate transporters completely blocked the immunoreaction. Moreover, secondary antibodies did not reveal any immunoreactivity when incubated without primary antibodies for transporters (Fig. 1D). Immunoreactivity for all three transporters was also detected on the plasma membrane of lympho-monocytes. To characterize the three transporters at the molecular level, Western blot analysis was performed on lysates of human platelets, lymphocytes, GFD-8 cell line and rat brain. As shown in Fig. 2, the antibodies for the three glutamate transporters detected specific bands on Western blot, similar to those reported in the literature for rat and human brain [29,46]. The glutamate transporters bands were completely abolished when antibodies were pre-adsorbed with 50 μ M synthetic GLAST, GLT-1 and EAAC1 oligopeptides (data not shown). Moreover, preliminary Western blot studies, performed on

Fig. 2. Fifty micrograms of total protein were run into 7.5% SDS-polyacrylamide gels for all peripheral samples, while 2 μ g were used for the rat brain. (A) EAAT1 (1:1000 α GLAST) shows a band at 65 kDa in platelets (PL), lympho-monocytes (PB) and GFD-8 cell line (G), as in rat brain (R). (B) EAAT2 (1:800 α GLT-1) shows a band at 73 kDa in platelets and lympho-monocytes, as in rat brain, while GFD-8 cell line does not express EAAT2 protein. (C) EAAT3 (1:800 α EAAC1) displays a band at 69 kDa for rat brain (R), platelets (PL), lympho-monocytes (PB) and GFD-8 (G).

Fig. 3. Platelets (PL), lympho-monocytes (PB) and GFD-8 (G) show the same cDNA products for EAAT1 (A), EAAT2 (B) and EAAT3 (C) at 485, 384 and 457 bp, respectively. mRNA species tested in platelets and lympho-monocytes are similar to mRNA species of GFD-8 and SH-SY5Y (SY), human neuroblastoma cell lines (C). M = molecular weight marker VIII (Roche, Basel, Switzerland). Primers sequences were obtained by using oligo[®]primers analysis software, Version 6 (Wojciech Rychlik, PhD; NBI, Plymouth, MN, USA).

platelet membrane and cytoplasmic fractions, indicated a different distribution of three transporters in subcellular fractions: EAAT1 is prevalent in membrane (65%), EAAT2 in cytoplasmic fraction (70%) and EAAT3 is quite ubiquitous (55% in membrane and 45% in cytoplasmic fraction). RT-PCR was also performed in SH-SY5Y, GFD-8, platelet and lympho-monocyte mRNA extracts. SH-SY5Y cell line was used as human central model. All samples showed the same cDNA pattern for EAAT1, EAAT2 and EAAT3 at 485, 384 and 457 bp, respectively (Fig. 3). These cDNA products are not due to DNA contamination because after enzymatic digestion with DNase, before RT-PCR were performed, all samples showed the specific cDNA. All RT-PCR products were analyzed for beta-actin that showed a signal at 661 bp. Functional activity of platelet glutamate transporters was also investigated in a large group of controls. We observed kinetic characteristics of a Na^+ -dependent, high affinity glutamate uptake ($V_{\text{max}} = 160 \pm 10$ pmol/mg protein/30 min, $K_m = 22.8 \pm 5.4$ μ M; values are expressed as mean $\pm$ S.E.M.) and a Na^+ -independent but temperature-dependent low affinity glutamate uptake ($V_{\text{max}} = 440 \pm 70$ pmol/mg protein/30 min; $K_m = 124.1 \pm 41.1$ μ M). To investigate the specific involvement of each transporter in glutamate uptake, we tested the effect of various compounds on glutamate uptake, using different concentrations of DHK, THA, L-aspartic acid, L-glutamic acid and SOS. DHK, an EAAT2 selective inhibitor, did not decrease significantly the uptake of 5 μ M glutamate, even at the highest concentrations (1 mM). On the contrary, not selective inhibitors such as L-aspartic acid ($\text{IC}_{50} = 5 \pm 1.8$ μ M), L-glutamic acid ($\text{IC}_{50} = 6.6 \pm 2.7$ μ M) and THA ($\text{IC}_{50} = 3 \pm 1.2$ μ M) and SOS ($\text{IC}_{50} = 110 \pm 22$ μ M), an EAAT1 and EAAT3 inhibitor, effectively inhibited

platelet glutamate uptake, although $SOS K_i$ value was higher compared to those of other inhibitors but similar to that previously observed in human motor cortex [2]. The IC_{50} values of each inhibitor were calculated from at least four independent experiments and expressed as mean $\pm$ S.E.M.

To assess age-related modifications of glutamate transport we compared 30 normal subjects with the age range of 20–55 years, mean 41 ± 4 years (12 women and 18 men) and 30 older subjects with the age range of 65–85 years, mean 74 ± 3 years (17 women and 13 men). High affinity glutamate uptake in platelet from older subjects

was significantly decreased compared to the younger group ($V_{max} = 130 \pm 10$ pmol/mg protein/30 min versus 170 ± 15 pmol/mg protein/30 min, $P < 0.05$), although no linear correlation was observed between glutamate uptake and age. Western blot and RT-PCR studies, performed in a subgroup of these subjects, showed a specific decrease of EAAT1 expression ($P < 0.05$) and mRNA levels ($P < 0.01$) (Fig. 4), while EAAT2 and EAAT3 proteins and mRNAs were unchanged, with wide variability observed in tested subjects. Finally, we showed a further significant decrease of EAAT1 expression ($P < 0.05$) and mRNA levels ($P < 0.01$) in

Fig. 4. Platelet EAAT1 expression and mRNA are decreased in aging, where we described a reduced glutamate uptake (graphic at the top right side). Ten younger subjects (white column) and 10 older subjects (black column) were compared by Western blot (A) and RT-PCR (B) studies. Data are expressed as percentage of younger subjects $\pm$ S.E.M. * $P = 0.03$, ** $P < 0.01$ at ANOVA. An example of EAAT1 expression is shown in picture A (the upper bands), while beta-actin is the lower band; in picture B is shown the decrease of EAAT1 cDNA (the lower signals), beta-actin is the upper band.

Fig. 5. EAAT1 decrease in platelets from AD patients (black column) compared to older subjects (white column). (A) EAAT1 Western blot signal at 66 kDa was normalized to beta-actin protein signal (42 kDa); also RT-PCR (B) for EAAT1 (485 bp) was performed using beta-actin primers (661 bp) as internal standard. Data are expressed as percentage of controls $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$ at ANOVA. These molecular alterations could explain the glutamate uptake reduction we previously observed in platelets from AD patients compared to age-matched subjects and patients with multi-infarct dementia.

platelets from AD patients compared to age-matched normal subjects (Fig. 5). In these AD patients, we previously observed a decrease of platelet glutamate uptake [17].

4. Discussion

By morphological and molecular studies, we demonstrated for the first time the presence of the three major glutamate transporters in human platelets. Since cells were not permeabilized, before performing IF and IEM experiments, EAAT1, EAAT2 and EAAT3 glutamate transporters detected with these methods were mainly present on platelet and lympho-monocytes membranes. However, our subcellular studies confirmed the presence of glutamate transporters both on platelet membrane and cytoplasmic fraction. Moreover, molecular characterization revealed that human platelets and lympho-monocytes express molecular form (Fig. 2) and mRNA (Fig. 3) for EAAT1, EAAT2 and EAAT3 similar to those described in CNS. Since DHK, a specific inhibitor for EAAT2, did not significantly affect glutamate uptake, while SOS, an inhibitor for EAAT1 and EAAT3, blocked glutamate uptake, although with lower potency compared to THA, L-aspartic acid and L-glutamic acid, EAAT2 does not seem to play a major role in platelet glutamate uptake activity in physiological conditions, while both other transporters seem to be operative. These re-

sults do not exclude EAAT2 involvement in physiological platelet uptake, since it could be a platelet transporter with a slower kinetic rate and it could begin to work at higher glutamate concentrations, when EAAT1 and EAAT3 could be endangered. The EAAT2 subcellular distribution (70% in cytoplasmic fraction) could also justify its minor involvement in physiological glutamate uptake of platelet; EAAT2 could be available in platelet membranes after translocation induced by particular stimuli, as described for EAAT3 in CNS-derived cell lines [48]. It must be also underlined that GFD-8 cell line did not express any EAAT2 immunoreactivity, but clearly showed EAAT2 mRNA. The lack of EAAT2 expression in precursor cells (megacariocytes) suggests that EAAT2 mRNA is translated only after complete differentiation into platelets. Since these organelles have no nucleus, they can translate only mRNA species of their precursor cells, when they need a new protein.

Moreover, our biochemical and molecular studies in platelets from older subjects suggest a physiological down-regulation of glutamate uptake and EAAT1 transporter with aging (Fig. 4). Since glutamate uptake is decreased in platelets from patients with Parkinson, AD and ALS [17–19], we are now investigating the specific role of glutamate transporters in all these patients. Our preliminary results in 10 AD patients compared to age-matched controls showed a further significant decrease of EAAT1 protein and mRNA compared to old normal subjects (Fig. 5), although no correlation was observed with MMSE. It is remarkable that in AD patients not only EAAT2 [55] but also EAAT1 [49] display an aberrant expression in neurons, indicating an important role also for this latter glutamate transporter in AD. These data support the hypothesis that abnormalities in glutamate transport play an important role in the pathogenesis of AD. Furthermore, since different mechanisms may contribute to modify glutamate transporters (free radicals, lipidperoxidation products and amyloid adducts) and uptake (abnormal APP processing and loss of ATP), we suggest that beta-amyloid accumulation in AD patients may decrease glutamate uptake through oxidative-damage of transporters. Moreover, in different non-neuronal tissues, oxidative processes were also described as biomarkers of AD [26,43]. On the basis of these results we suppose that systemic glutamate transporter modifications might explain the decrease of glutamate uptake, that we previously described in platelets from AD patients, and we propose this peripheral model as marker of CNS excitotoxicity. In fact in platelet, as in SNC, APP metabolism alterations [4,9,20,41], mitochondrial dysfunctions [25] and oxidative processes have been described [26].

As recent studies have also shown that glutamate transporter expression and activity may be regulated by different endogenous or exogenous factors [6,8,10,13,15,27,31,35,44,48,53], we are now studying the possible role of physiological stimuli, such as PKC activation, or oxidative stress on glutamate transporter activation/inactivation or translocation in platelets. The final goal of these studies is the

possibility of using platelets as peripheral model to investigate the role of glutamate, its transporters and its effect pathway in neurodegenerative disorders, with the clear advantage of being easy to obtain, suitable for pharmacological and clinical studies and, compared to human brain tissue studies, of being independent from postmortem or end-stage changes.

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