RESEARCH ARTICLE

Excitatory Amino Acid Transporter EAAT-2 in Tangle-bearing Neurons in Alzheimer's Disease

Dietmar Rudolf Thal

Department of Neuropathology, University of Bonn Medical Center, Germany.

The excitatory amino acid transporter EAAT-2 is physiologically expressed in astrocytes. This study demonstrates that distinct subclasses of neurons exhibited EAAT-2 immunoreactivity in cases with Alzheimer's disease (AD). EAAT-2 was identified in the following types of neurons: Cortical pyramidal cells, fascia dentata granule cells, neurons of the basal nucleus of Meynert, the substantia nigra, the paraventricular nucleus of the hypothalamus, oral and central raphe nuclei, locus coeruleus, parabrachial nucleus, and neurons of the reticular formation of the brain stem. All EAAT-2-positive neurons displayed cytoskeletal abnormalities with abnormal τ-protein and often showed condensed and shrunken nuclei. None of the control cases without AD-related pathology showed EAAT-2-immunoreactive neurons. These results indicate that AD-related neurodegeneration is associated with the expression of the glutamate transporter EAAT-2 in altered neurons. Since an aberrant expression of EAAT-1 in neurons has recently been described, the finding of a neuronal expression of EAAT-2 strongly supports the hypothesis that abnormalities in the glutamate transport play an important role in the pathogenesis of AD.

Brain Pathol 2002;12:405-411.

Introduction

Excitatory amino acid transporters (EAATs) are proteins which are essential for the uptake of glutamate and aspartate from the synaptic cleft by astrocytes and neurons (8, 15, 24, 29). EAAT-1 (GLAST) and EAAT-2 (GLT-1) are located in astrocytes, (14, 24, 27, 29). EAAT-3 (EAAC-1) and EAAT-4 have been described in neurons (14, 20, 22).

The deposition of the amyloid β-protein (Aβ) as well as the presence of neurofibrillary tangles (NFTs), neuritic plaques (NPs), and neuropil threads (NTs) are histopathological hallmarks of Alzheimer's disease (AD) (3, 6, 7). Recently, it has been shown that EAAT-1 is expressed in NFT-bearing neurons in AD cases (28). The glial transport of excitatory amino acids (EAAs) in AD is deficient (18) and the number of EAAT-2 expressing cortical astrocytes is decreased (16). The protein levels of cortical EAAT-1 and cortical EAAT-2 in a given AD brain vary from normal to reduced levels (1). Aβ is capable of reducing the glutamate uptake in astrocytes in vitro (9) and EAAT-2 is discussed to play an important role in Aβ-protein precursor (AβPP) processing (16, 18, 19). In doing so, AD-related changes in the astroglial EAAT-2 expression appear to be associated with the presence of Aβ (16), whereas it is not clear whether changes in EAAT-2 expression are also related with neurofibrillary pathology. Since EAA transport seems to play an important role for NFT formation as well as for Aβ pathology, it is important for the understanding of the pathogenetic mechanisms to further clarify the role of EAATs in the pathogenesis of AD.

This study is aimed to examine whether EAAT-2 is involved in the generation of AD-related neuronal changes. By studying samples from the neocortex, the allocortex, the basal ganglia, the diencephalon, the thalamus, the brain stem, and the cerebellum, it is shown that neurons are capable of expressing EAAT-2 under pathological conditions, ie, in distinct types of NFT-bearing neurons in AD cases.

Material and Methods

Brains from 21 autopsy cases from both genders, aged 61 to 89 years, were investigated (Table 1). The cases had usually been examined one to 4 weeks prior to death by different clinicians according to standardized protocols. The protocols included the assessment of cognitive function and recorded the ability to care for and dress self, eating habits, bladder and bowel continence, speech patterns, writing and reading, short-term and long-term memory, and orientation within the hospital setting. These data were used to retrospectively assess CDR-scores for each patient (11) (Tab. 1). For this purpose, the data from the clinical protocols were transformed into CDR-levels according to the standard CDR-protocol (11). For case numbers 8 and 12, sufficient clinical recordings which allowed the determination of CDR scores were not available. AD was diagnosed according to the recently published consensus criteria (33). Cases with AD-related neurofibrillary and/or Aβ pathology which were clinically diagnosed as being

Corresponding author:
Dr D. R. Thal, Institut für Neuropathologie, Universität Bonn, Sigmund Freud Str. 25, D-53105 Bonn, Germany.

Case Number	Age	Gender	AβMTL-Phase	NFT-Stage	CERAD	CDR	Neuropathology
1	66	f	0	0	0	0	I
2	62	m	0	0	0	0	-
3	69	f	0	0	0	0	-
4	61	m	0	0	0	0	-
5	77	m	0	2	0	0	ADRP
6	61	f	1	0	0	0	ADRP, I
7	71	m	2	1	0	0	ADRP, CAA
8	64	m	2	1	0	n.a.	n.a.
9	69	m	2	1	0	0	ADRP, CAA
10	73	m	2	3	0	0	ADRP, I
11	87	m	2	3	1	0	ADRP, CAA
12	66	m	3	2	0	n.a.	n.a.
13	85	m	3	4	1	2	AD, I, CAA
14	86	f	3	6	2	3	AD, AG, I, CAA
15	83	m	4	4	3	3	AD, I, CAA
16	89	f	4	4	3	2	AD, CAA
17	87	f	4	4	1	3	AD, CAA
18	83	m	4	5	3	3	AD, CAA-MI
19	89	f	4	5	3	3	AD, CAA
20	78	f	4	5	2	3	AD, CAA
21	68	f	4	6	2	1	AD, CAA

Table 1. List of cases. Age in years. Gender: m = male, f = female. AβMTL-Phase = phase of β-amyloidosis in the MTL (31). NFT-stage = stage of the expansion of AD-related neurofibrillary pathology according to Braak and Braak (3). CERAD = neuritic plaque score according to the CERAD-criteria (21) as indicated with the Gallyas silver method. CDR = Clinical Dementia Rating Score (11) (0 = not demented, 0.5 = questionable dementia or mild cognitive impairment, 1 = mild dementia, 2 = moderate dementia, 3 = severe dementia). Neuropathology: - = no pathological changes and clinically no cognitive deficits; AG = few argyrophilic grains in the MTL; ADRP = AD-related NFT- and/or Aβ-pathology in brains of non-demented patients (clinical dementia rating [CDR] score = 0); AD = clinically (CDR score≥1) and histopathologically verified Alzheimer's disease; CAA = Cerebral Amyloid Angiopathy; MI = microinfarcts; I = small infarcts; n.a. = CDR-scores are not available and the neuropathological observation shows no other changes than AD-related pathology.

cognitively normal (CDR = 0) were categorized as putatively non-demented cases with AD-related pathology (ADRP cases). ADRP cases as defined in our study also include Aβ-only and NFT-only cases. The control cases showed no dementia (CDR = 0) and were free of AD-related histopathological changes.

The brains were fixed in a 4% aqueous solution of formaldehyde for 3 weeks. The right hemisphere was cut coronally into 1 cm thick slices after removal of the brain stem at the mid-brain level. The brain stem and the cerebellum were cut perpendicular to the Meynert-brain-stem axis into 5 mm thick slices. Two blocks of the anterior MTL and of the MTL at the level of the lateral geniculate body were embedded in PEG and cut at 100 μm. One block of the superior frontal gyrus, the superior parietal lobe, the area 17, 18 and 19, the cingulate gyrus, the anterior MTL including the entorhinal region, the middle and posterior medial temporal lobe containing the hippocampus, the basal ganglia, the basal nucleus of Meynert, the septum, the hypothalamus, the thalamus, the midbrain, the pons, the medulla oblongata, and the cerebellum were embedded in paraffin, and 10 μm thick sections were cut. One paraffin section of a given region as well as one PEG section of the PEG-embedded MTL-blocks was stained with the Gallyas silver method for the detection of NFTs, NTs and NPs (2). Likewise, sections were stained with the Campbell-Switzer silver method for the detection of Aβ-deposits (2, 12). The sensitivity of the Campbell-Switzer silver technique for the detection of Aβ-deposits is equal to that of the immunocytochemistry with anti-$A\beta_{17\text{-}24}$ (4G8) (31, 32). For purposes of topographical orientation, sections were stained with aldehyd-fuchsin-darrow red for lipofuscin pigment and Nissl material.

The distribution of NFTs and NTs was assessed and diagnosis of stages in the development of neurofibrillary changes (NFT-stage) was performed using published criteria and were achieved without knowledge of clinical and pathological data or age and gender of the individuals (3, 33). The CERAD neuritic plaque score was determined by estimating the mean neuritic plaque frequency in the hippocampus and in the frontal, parietal, temporal, and occipital cortex as stained with the

Case Number	Neuropathology	EAAT-2-positive Neurons
1	-	-
2	-	-
3	-	-
4	-	-
5	ADRP	-
6	ADRP	-
7	ADRP	NBM
8	n.a.	-
9	ADRP	-
10	ADRP	NBM,PVN
11	ADRP	-
12	n.a.	-
13	AD	NBM,PVN
14	AD, AG	CA1, temporal neocortex, NBM, RF, LC, PBN, Raphe, SN
15	AD	CA1, temporal neocortex, entorhinal cortex, RF, LC, PBN, Raphe, SN, RN
16	AD	CA1, NBM, PVN, RF, LC, PBN, Raphe
17	AD	RF,Raphe,SN
18	AD, CAA-MID	CA1, CA4, Fascia dentata, temporal neocortex
19	AD	PVN,LC,PBN,Raphe,RF
20	AD	PVN,LC,PBN,Raphe,RF
21	AD	-

Table 2. List of cases exhibiting EAAT-2 positive neurons. Neuropathology: - = no pathological changes and clinically no cognitive deficits, AG = few argyrophilic grains in the MTL, ADRP = AD-related NFT- and/or Aβ-pathology in brains of non-demented patients, AD = clinically and histopathologically verified Alzheimer's disease, CAA-MI = CAA with multiple infarcts, n.a. = CDR-scores are not available and the neuropathological observation shows no other changes than AD-related pathology. The regions in which EAAT-2 positive neurons exist are listed. NBM = basal nucleus of Meynert; PVN = paraventricular nucleus of the hypothalamus; RF = reticular formation; LC = locus coeruleus; PBN = parabrachial nucleus; Raphe = oral and central raphe nuclei; SN = substantia nigra; RN = red nucleus. The neocortical pyramidal neurons, the neurons of the basal nucleus of Meynert, of the paraventricular nucleus of the hypothalamus, of the locus coeruleus, of the oral raphe nuclei, of the substantia nigra, and the neurons of the reticular formation receive among others glutamatergic input from cortical neurons (4, 23).

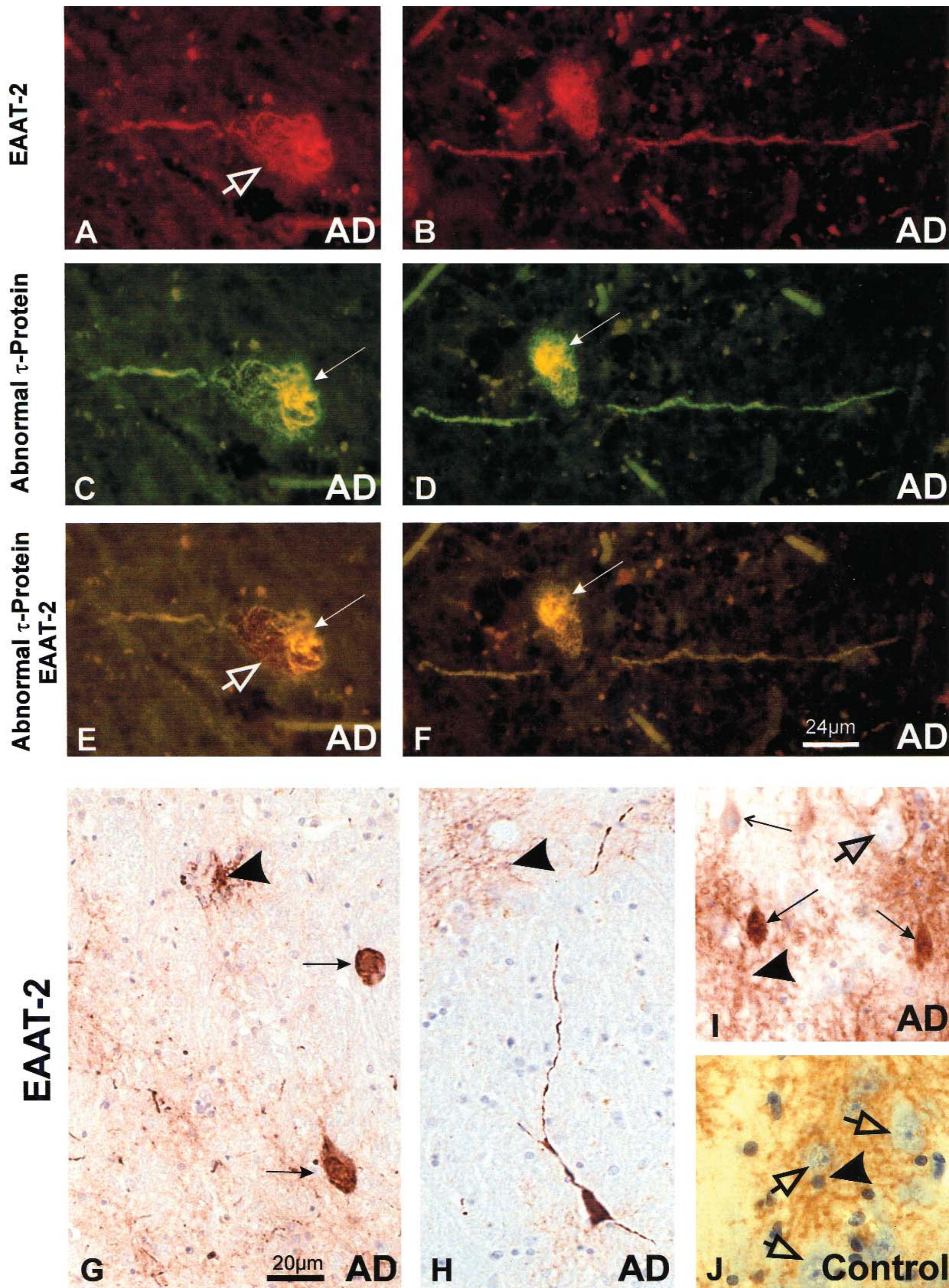
Gallyas silver method according to published criteria (21, 33). The phases of β-amyloidosis in the MTL (AβMTL-phase) were carried out using Campbell-Switzer stained sections as recently published (31).

The presence of Aβ was observed immunocytochemically with an antibody directed against $A\beta_{17-24}$ (Signet, Dedham, Mass: 4G8, 1/5000, 48 hours at 4°C) following formic acid pretreatment. EAAT-2 was detected with a monoclonal antibody (Novocastra, Newcastle, UK: 1H8 [20], 1/40, 24 hours at 22°C, microwave pretreatment) and with a polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, Calif: polyclonal goat [EAT-2], 1/50, 24 hours at 22°C, microwave pretreatment). The primary antibodies were detected with a biotinylated secondary antibody and the ABC complex (Vectastain: Vector Laboratories, Burlingame, Calif), and this was visualized with 3,3-diaminobenzidine (DAB) (10). Immunostained paraffin sections were counterstained with hematoxylin.

In cases numbers 16, 17, and 20, double label immunocytochemistry was done with the monoclonal mouse IgG-antibody against EAAT-2 and a monoclonal mouse IgM antibody against abnormal τ-protein directed against an abnormal configuration epitope of the τ-protein (13), (TG-3 monoclonal IgM-antibody [generous gift from Dr. P. Davies], 1/10, 24 hours at 22°C). Anti-EAAT-2 was detected with a carbocyanine 2-labeled secondary antibody specifically directed against mouse IgG (Dianova, Hamburg, Germany) whereas anti-TG-3 was detected with a carbocyanine 3-labeled secondary antibody specifically directed against mouse IgM (Dianova, Hamburg, Germany). The anti-mouse IgG antibody used for double immunofluorescence did not crossreact with mouse IgM and vice versa.

Results

In 2 non-demented ADRP-cases and in 8 of 9 AD-cases, distinct types of neurons showed EAAT-2-immunoreactivity in the perikaryon as well as in the neurites (Figure 1A, B, H; Table 2). Neuronal EAAT-2 expression was never observed in controls without AD-related pathology (Figure 1J). Positive EAAT-2-immunoreactivity in neurons could be detected with


EAAT-2
Abnormal τ-Protein
Abnormal τ-Protein
EAAT-2
A
AD
B
AD
C
AD
D
AD
E
AD
F
24μm
AD
EAAT-2
G
20μm
AD
H
AD
I
AD
J
Control

both anti-EAAT-2 antibodies used in this study. EAAT-2 occurred in neocortical and allocortical pyramidal cells, fascia dentata granule cells, melanin-containing neurons of the substantia nigra, neurons of the basal nucleus of Meynert, the paraventricular nucleus of the hypothalamus, the oral and central raphe nuclei, the reticulo-tegmental nucleus of the pons, the parabrachial nuclei, the locus coeruleus, and in the neurons of the reticular formation of the brain stem (Figure 1A, B, G-I; Table 2). The localization of the EAAT-2-positive neurons varied from case to case. In case number 18, CA1 pyramidal cells exhibited EAAT-2 but the brain stem was unaffected whereas in case number 20, brain stem nuclei contained EAAT-2-positive neurons and CA1-pyramidal cells appeared free of intraneuronal EAAT-2 (Table 2). Neuritic plaques with dystrophic neurites exhibiting Gallyas-positive neurofibrillary material did not show EAAT-2 immunoreactivity. Small infarcts in one control, 2 ADRP, and 4 AD cases were not associated with local EAAT-2 induction. In the ADRP-cases, EAAT-2-positive neurons were only seen in the basal nucleus of Meynert and in NFT-bearing neurons of the paraventricular nucleus of the hypothalamus (Table 2).

The EAAT-2 positive material in neurons was located in the cytoplasm and in the neurites (Figure 1). In some neurons the complete cytoplasm exhibited EAAT-2-immunoreactivity (Figure 1H, I) and in other neurons EAAT-2-positive material was closely associated with NFTs (Figure 1G). The nuclei of the EAAT-2 positive neurons were shrunken and condensed in comparison to adjacent neurons not exhibiting EAAT-2 (Figure 1I). A nucleolus was not detectable in these neurons. Double labeling of EAAT-2 and abnormal τ-protein (TG3) indicated that only those neurons exhibited EAAT-2 immunoreactivity which also showed intracellular accumulation of abnormal τ-protein. Astroglial expression of EAAT-2 was seen in AD, ADRP, and control cases (Fig. 1G-J).

Discussion

The present study shows that distinct types of neurons display cytoplasmic EAAT-2-immunoreactive material under pathological conditions in AD brains. No evidence for a neuronal localization of EAAT-2 has been noted or reported in healthy individuals as well as in patients with amyotrophic lateral sclerosis (7, 16, 20, 25). In the normal human brain, EAAT-2 is exclusively expressed in astrocytes (20, 24). The intraneuronal detection of EAAT-2, therefore, represents a pathological event. The induction of EAAT-2 in neurons under pathological conditions as well as the recently reported aberrant neuronal expression of EAAT-1 (28) indicate the principal potential of neurons to express astrocyte-specific proteins under pathological conditions.

Both EAAT-2 antibodies used in this study detect neuronal EAAT-2 and the monoclonal anti-EAAT-2 antibody has been shown to specifically detect EAAT-2 in the human brain without crossreacting with EAAT-1 (20). Neuronal EAAT-2 was only seen in distinct types of neurons with neurofibrillary changes whereas other types of neurons with neurofibrillary changes and neuritic plaques in the same section did not exhibit EAAT-2-immunoreactivity. These findings strongly indicate that the immunoreaction observed is not the result of crossreactivity with the abnormal τ-protein, but shows a pathological expression of EAAT-2 in affected neurons. Whether the neuronal EAAT-2 is identical with astroglial EAAT-2 or whether it represents a splice variant of EAAT-2 cannot be answered by studying formalin-fixed tissue as used here.

The neuronal expression of EAAT-2 is restricted to neurons containing abnormal τ-protein, and, therefore, appears to be associated with AD-related neuronal pathology. Most of the EAAT-2-positive neurons display shrunken and condensed nuclei indicating that these neurons are severely altered. This association points to a causal relationship between the pathological expression

Figure 1. (Opposing page) EAAT-2 exhibiting neurons in AD. **A-F:** Double label immunocytochemistry shows EAAT-2 exhibiting neurons of the substantia nigra (**A, B**) being also positive for abnormal τ-protein (TG-3) (**C, D, E, F**). The abnormal τ-protein in the cell body and the dendrites which co-localizes EAAT-2 is indicated by white arrows in **C-F**. EAAT-2 positive material in the perikaryon which is not co-localized with abnormal τ-protein is shown by the lucent white arrows (**A, E**). **G-I**: EAAT-2 positive neurons in AD-cases in the central raphe nucleus (**G**), the reticular formation of the midbrain (**H**), and in the Ammon's horn sector CA1 (**I**). EAAT-2 positive neurons exhibit EAAT-2 in the perikaryon as well as in the dendrites (**A, B, H**). In most of the EAAT-2 positive neurons the EAAT-2 positive material shows a dense staining in the entire cell body. Some neurons show EAAT-2 positive material which is tightly associated with NFTs (arrows in **E-G**). The nucleus is not detectable (**G, H, I;** arrows with black arrowhead). Few less densely stained neurons have a shrunken and condensed nucleus. The nucleolus is not seen in these cells (**I**; arrow with thin arrowhead). Not affected neurons do not exhibit any EAAT-2 immunoreactivity and have a nucleus with a nucleolus (**I**; short arrow with lucent arrowhead). In all AD cases EAAT-2 expressing astrocytes were seen as well (arrowheads **G-I**). For comparison, in the control case (**J**) CA1 neurons are not stained with anti-EAAT-2 (lucent arrowheads), whereas astrocytes exhibited EAAT-2 (arrowheads in **J**) (Case number: 17 [**A-F**], 14 [**G, H**], 18 [**I**], 1 [**J**]; anti-EAAT-2 [**A, B, G-J**]; anti abnormal τ-protein [**C, D**]; anti-EAAT-2/ anti abnormal τ-protein [**E, F**]; Scale bar: 24 µm [**A-F**], 20 µm [**G-J**]).

of EAAT-2 in neurons and the formation of AD-related cytoskeletal alterations. Since high glutamate levels appear critical for the formation of abnormal τ-protein and the generation of paired helical filaments (5, 26), it is tempting to speculate that neuronal expression of EAAT-1 as reported by Scott et al (28) and of EAAT-2 as reported here can be induced by abnormal glutamatergic stimulation probably due to the reduced astroglial glutamate uptake (18).

Aβ is capable of inhibiting astroglial glutamate uptake in vitro (9). These data would favor the hypothesis that the AD-related neuronal expression of EAAT-2 is the result of AD-related pathological processes. Another argument for this point of view is that most cases with early AD-related changes (NFT stage I and II cases) do not express neuronal EAAT-2 although there are already neurons in the transentorhinal and entorhinal region which exhibit Gallyas-positive neurofibrillary changes. Alternatively, a dysfunction of the glutamate transport in astrocytes or in neurons may initiate the evolution of neurofibrillary changes or Aβ-deposition. Arguments favoring this point of view are: *i)* the generation of abnormal phosphorylated τ-protein can be induced by excitotoxic stimulation (5, 26), and *ii)* regions which develop Aβ-deposits receive input from glutamatergic or aspartergic neurons (4, 23, 30). To clarify which hypothesis is wrong and which is right, it is essential to study an animal model with functional modified glial and/ or neuronal glutamate transport.

Cerebral infarction is unlikely to play a role for the induction of EAAT-2 in neurons for the following reasons: *i)* small infarcts are also seen in control cases without EAAT-2 positive neurons and EAAT-2 immunoreactive neurons occur in AD cases without infarcts or microinfarcts; and *ii)* EAAT-2 positive neurons do also exhibit abnormal τ-protein, ie, a pathology characteristic for AD-related neurodegeneration but not for ischemia/hypoxia induced neurodegeneration.

Only distinct types of neurons (Table 2) are susceptible for the pathological expression of EAAT-2. Although the types of neurons exhibiting EAAT-2 vary strikingly from case to case, all of them receive glutamatergic input from cortical association and/or projection fibers (23). This also argues in favor of a critical role of abnormal glutamatergic stimulation for the expression of EAAT-2 in neurons. Interindividual regional differences in abnormal glutamatergic stimulation may account for the observed variation between individual AD brains.

The treatment of AD patients with the anti-excitatoxic drug memantine leads to an improvement of the clinical status (17, 34). Since there is a pathological expression of EAAT-1 (28) and EAAT-2 in NFT-bearing neurons these findings may display a morphological correlate of the abnormalities in the glutamate transport. Therefore, anti-excitotoxic drugs should either reduce the occurrence of EAAT-1 and EAAT-2 positive neurons in comparison to untreated patients or may slow down the expansion of AD-related pathology or both. These parameters could be used in autopsy confirmed treatment studies to verify the success of a given treatment.

In summary, the astrocyte-specific glutamate transporter EAAT-2 can be expressed by neurons under pathological conditions and this neuronal induction of EAAT-2 occurs only in severely altered neurons exhibiting abnormal τ-protein. Since an aberrant expression of EAAT-1 in neurons has recently been reported (28) the finding of an aberrant expression of EAAT-2 in neurons strongly supports the hypothesis that abnormalities in the glutamate transport play an important role in the pathogenesis of AD.

Acknowledgments

The author gratefully acknowledges the technical assistance Mrs M. Babl, I. Szasz, Mr H. Friedrich, T. Chatsopoulos, R. Baldauf and S. Janik. I thank Prof Heiko Braak and Prof Otmar D. Wiestler for reading the manuscript, Dr P. Davies for providing the TG-3 antibody, and Dr P. Müller for providing autopsy material. This study was supported by DFG-grant No. TH 624/4-1.

References

1. Beckstrom H, Julsrud L, Haugeto O, Dewar D, Graham DI, Lehre KP, Storm-Mathisen J, Danbolt NC (1999) Interindividual differences in the levels of the glutamate transporters GLAST and GLT, but no clear correlation with Alzheimer's disease. *J Neurosci Res* 55:218-229.
2. Braak H, Braak E (1991) Demonstration of amyloid deposits and neurofibrillary changes in whole brain sections. *Brain Pathol* 1:213-216.
3. Braak H, Braak E (1991) Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol* 82:239-259.
4. Cotman CW, Monaghan DT, Ottersen OP, Storm-Mathisen J (1987) Anatomical organization of excitatory amino acid receptors and their pathways. *Trends Neurosci* 10:273-280.
5. De Boni U, McLachlan DR (1985) Controlled induction of paired helical filaments of the Alzheimer type in cultured human neurons, by glutamate and aspartate. *J Neurol Sci* 68:105-118.
6. Esiri MM, Hyman BT, Beyreuther K, Masters CL (1997) Ageing and Dementia. In: *Greenfields Neuropathology*, vol 2, Graham DI, Lantos PL (eds) Arnold, London, pp 153-233.

7. Fray AE, Ince PG, Banner SJ, Milton ID, Usher PA, Cookson MR, Shaw PJ (1998) The expression of the glial glutamate transporter protein EAAT2 in motor neuron disease: an immunohistochemical study. *Eur J Neurosci* 10:2481-2489.
8. Greenamyre JT, Porter RH (1994) Anatomy and physiology of glutamate in the CNS. *Neurology* 44:S7-13.
9. Harris ME, Wang Y, Pedigo NW, Jr., Hensley K, Butterfield DA, Carney JM (1996) Amyloid beta peptide (25-35) inhibits Na+-dependent glutamate uptake in rat hippocampal astrocyte cultures. *J Neurochem* 67:277-286.
10. Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. *J Histochem Cytochem* 29:577-580.
11. Hughes CP, Berg L, Danziger WL, Coben LA, Martin RL (1982) A new clinical scale for the staging of dementia. *Br J Psychiatry* 140:566-572.
12. Iqbal K, Braak H, Braak E, Grundke-Iqbal I (1993) Silver labeling of Alzheimer neurofibrillary changes and brain β-amyloid. *J Histotechnol* 16:335-342.
13. Jicha GA, Lane E, Vincent I, Otvos L, Jr., Hoffmann R, Davies P (1997) A conformation- and phosphorylation-dependent antibody recognizing the paired helical filaments of Alzheimer's disease. *J Neurochem* 69:2087-2095.
14. Kanai Y, Hediger MA (1992) Primary structure and functional characterization of a high-affinity glutamate transporter. *Nature* 360:467-471.
15. Kanai Y, Smith CP, Hediger MA (1993) The elusive transporters with a high affinity for glutamate. *Trends Neurosci* 16:365-370.
16. Li S, Mallory M, Alford M, Tanaka S, Masliah E (1997) Glutamate transporter alterations in Alzheimer disease are possibly associated with abnormal APP expression. *J Neuropathol Exp Neurol* 56:901-911.
17. Marx J (2000) Alzheimer's congress. Drug shows promise for advanced disease. *Science* 289:375-377.
18. Masliah E, Alford M, DeTeresa R, Mallory M, Hansen L (1996) Deficient glutamate transport is associated with neurodegeneration in Alzheimer's disease. *Ann Neurol* 40:759-766.
19. Masliah E, Alford M, Mallory M, Rockenstein E, Moechars D, Van Leuven F (2000) Abnormal glutamate transport function in mutant amyloid precursor protein transgenic mice. *Exp Neurol* 163:381-387.
20. Milton ID, Banner SJ, Ince PG, Piggott NH, Fray AE, Thatcher N, Horne CH, Shaw PJ (1997) Expression of the glial glutamate transporter EAAT2 in the human CNS: an immunohistochemical study. *Brain Res Mol Brain Res* 52:17-31.
21. Mirra SS, Heyman A, McKeel D, Sumi SM, Crain BJ, Brownlee LM, Vogel FS, Hughes JP, van Belle G, Berg L (1991) The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part II. Standardization of the neuropathologic assessment of Alzheimer's disease. *Neurology* 41:479-486.
22. Nagao S, Kwak S, Kanazawa I (1997) EAAT4, a glutamate transporter with properties of a chloride channel, is predominantly localized in Purkinje cell dendrites, and forms parasagittal compartments in rat cerebellum. *Neuroscience* 78:929-933.
23. Paxinos G (1990) *The human nervous system*. Academic Press, San Diego.
24. Pines G, Danbolt NC, Bjoras M, Zhang Y, Bendahan A, Eide L, Koepsell H, Storm-Mathisen J, Seeberg E, Kanner BI (1992) Cloning and expression of a rat brain L-glutamate transporter. *Nature* 360:464-467.
25. Rothstein JD, Van Kammen M, Levey AI, Martin LJ, Kuncl RW (1995) Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Ann Neurol* 38:73-84.
26. Sautiere PE, Sindou P, Couratier P, Hugon J, Wattez A, Delacourte A (1992) Tau antigenic changes induced by glutamate in rat primary culture model: a biochemical approach. *Neurosci Lett* 140:206-210.
27. Schmitt A, Asan E, Puschel B, Kugler P (1997) Cellular and regional distribution of the glutamate transporter GLAST in the CNS of rats: nonradioactive in situ hybridization and comparative immunocytochemistry. *J Neurosci* 17:1-10.
28. Scott HL, Pow DV, Tannenberg AE, Dodd PR (2002) Aberrant expression of the glutamate transporter excitatory amino acid transporter 1 (EAAT1) in Alzheimer's disease. *J Neurosci* 22:RC206.
29. Storck T, Schulte S, Hofmann K, Stoffel W (1992) Structure, expression, and functional analysis of a Na(+)-dependent glutamate/aspartate transporter from rat brain. *Proc Natl Acad Sci U S A* 89:10955-10959.
30. Thal DR, Rub U, Orantes M, Braak H (2002) Phases of Aß-deposition in the human brain and its relevance for the development of AD. *Neurology* 58:1781-1800.
31. Thal DR, Rub U, Schultz C, Sassin I, Ghebremedhin E, Del Tredici K, Braak E, Braak H (2000) Sequence of Abeta-protein deposition in the human medial temporal lobe. *J Neuropathol Exp Neurol* 59:733-748.
32. Thal DR, Sassin I, Schultz C, Haass C, Braak E, Braak H (1999) Fleecy amyloid deposits in the internal layers of the human entorhinal cortex are comprised of N-terminal truncated fragments of Abeta. *J Neuropathol Exp Neurol* 58:210-216.
33. The National Institute on Aging (1997) Consensus recommendations for the postmortem diagnosis of Alzheimer's disease. The National Institute on Aging, and Reagan Institute Working Group on Diagnostic Criteria for the Neuropathological Assessment of Alzheimer's Disease. *Neurobiol Aging* 18:S1-2.
34. Winblad B, Poritis N (1999) Memantine in severe dementia: results of the 9M-Best Study (Benefit and efficacy in severely demented patients during treatment with memantine). *Int J Geriatr Psychiatry* 14:135-146.