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Brain Prolyl Endopeptidase Expression in Aging, APP Transgenic Mice and Alzheimer's Disease

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Prolyl endopeptidase (PEP) is believed to inactivate neuropeptides that are present in the extracellular space. However, the intracellular localization of PEP suggests additional, yet unidentified physiological functions for this enzyme. Here we studied the expression, enzymatic activity and subcellular localization of PEP in adult and aged mouse brain as well as in brains of age-matched APP transgenic Tg2576 mice and in brains of Alzheimer's disease patients. In mouse brain PEP was exclusively expressed by neurons and displayed region- and age-specific differences in expression levels, with the highest PEP activity being present in cerebellum and a significant increase in hippocampal but not cortical or cerebellar PEP activity in aged mouse brain. In brains of young APP transgenic Tg2576 mice, hippocampal PEP activity was increased compared to wild-type littermates in the pre-plaque phase but not in aged mice with β-amyloid plaque pathology. This "accelerated aging" with regard to hippocampal PEP expression in young APP transgenic mice might be one factor contributing to the observed cognitive deficits in these mice in the pre-plaque phase and could also explain in part the cognition-enhancing effects of PEP inhibitors in serveral experimental paradigms.

KEY WORDS: Aging; Alzheimer's disease; β-amyloid; prolyl endopeptidase; subcellular localization; transgenic mice.

INTRODUCTION

Prolyl endopeptidase (PEP; EC 3.4.21.26) is a serine peptidase characterized by oligopeptidase activity. Although enzymatic and structural properties of PEP are well established, the biological function of this enzyme remains obscure. PEP is expressed at high levels in the brain (1) and inactivates several neuropeptides including substance P and arginine-vasopression *in vitro* by limited proteolysis (2,3). However, PEP is a cytosolic protein (4), which is mainly localized in the perinuclear space in human neuroblastoma and glioma cell lines (5), indicating intracellular functions for PEP. Indeed, we have recently demonstrated that PEP is associated with the tubulin cytoskeleton and

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Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; bio-GSA, biotinylated lectin from *Griffonia simplicifolia* agglutinin (isolectin B4); GFAP, glial fibrillary acidic protein; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; PB, phosphate buffer; PBS, phosphate-buffered saline; PEP, prolyl endopeptidase (EC 3.4.21.26).

important for physiological functions such as axonal transport and protein secretion (5).

One pathway that may be targeted by PEP is the proteolytical processing of the amyloid precursor protein (APP). β-Amyloid peptides are the major constituents of parenchymal and cerebrovascular deposits present in brains from Alzheimer's disease (AD) patients and are generated by the proteolytic activity of two proteases; β- and γ-secretase (for review see 6,7). PEP inhibition increases the secretion of β-amyloid peptides from neuroblastoma and glioblastoma cell lines into the culture medium (Schulz and Roßner, unpublished observations). This effect is most likely based on the proteolytical protection of the APP C-terminus, which contains a putative PEP cleavage site within the APP reinternalization motif YENPTY (8). In fact, an intact APP reinternalization motif has been shown to be crucial the secretion of β-amyloid peptides (9).

Here we addressed the question whether APP overexpression, increased β-amyloid concentrations or β-amyloid plaque formation in brains of APP transgenic Tg2576 mice and AD patients influence PEP expression or its enzymatic activity.

EXPERIMENTAL PROCEDURES

Animals. The transgenic mice used in this study express the human APP695 with the double mutation K670N, M671L under control of the hamster prion protein promoter (10). The N2 generation of hybrid C57Bl6 × SJL mice was studied at the postnatal age of 8 and 17 months and nontransgenic littermates served as control animals. In each animal group four brains were used for immunohistochemistry and seven brains were evaluated by Western blot analyses and enzymatic activity assays. Together, 44 mouse brains were included in this study.

Human Brain Tissue. The whole procedure of case recruitment, acquisition of patients' personal data, performing the autopsy, and handling the autoptic material has been approved by the responsible Ethical Committee of Leipzig University. The definite diagnosis of AD for all cases used in this study was based on criteria of the National Institute of Neurologic and Communicative Disorders and Stroke (NINDS) and the Alzheimer's Disease and Related Disorders Association (ADRDA; Ref. 11). Tissue samples from four AD patients and four non-demented, age-matched subjects were used for immunohistochemistry and cortical brain tissue from seven AD patients eight control subjects was used for Western blot analyses and enzymatic activity assays.

Tissue Preparation. For Western blot analysis and enzymatic PEP assay, animals were sacrificed by decapitation. The brains were rapidly removed from the skull and parietal cortex, hippocampus and cerebellum were dissected from both hemispheres and snap frozen on dry ice. Tissue homogenates (10% w/v) were prepared in ice-cold extraction buffer (50 mM HEPES pH 7.5; 200 mM NaCl; 1 mM EDTA pH 8.0; 1 mM DTT) using a micromortar (Roth, Karlsruhe, Germany). The soluble cell extract containing PEP was obtained by centrifugation at 18,000 $\times g$ for 10 min.

For histochemistry, animals were anaesthetized with pentobarbital and perfused transcardially with 50 ml phosphate-buffered saline (PBS, 0.1 M; pH 7.4) followed by perfusion with 50 ml 4% formaldehyde in phosphate buffer (PB, 0.1 M; pH 7.4). The brains were removed from the skull and postfixed by immersion in the same fixative overnight. After cryoprotection in 30% sucrose in 0.1 M PB for 2–3 days, the brains were frozen in n-hexane at −68°C and stored at −20°C. Coronal sections (30 μm thick) were cut on a sliding microtome and collected in PBS.

Prolyl Endopeptidase (PEP) enzymatic activity assays. The enzymatic activity of PEP was quantified as described recently (12). Cellular extracts as described above were incubated in assay buffer using the fluorogenic substrate Z-Gly-Pro-NHMec (10 μM; Bachem, Heidelberg, Germany) on a spectrofluorimeter SFM 25 (excitation wavelength 380 nm, emission wavelength 460 nm, Kontron, Neufahrn, Germany).

Antibodies/Lectins. For Western blot analysis a polyclonal rabbit antibody against aa 10–25 of the human N-terminal PEP sequence (S449; see Ref. 12) and a monoclonal actin antibody (Sigma, Deisenhofen, Germany) were used. Primary antibodies were detected with peroxidase-conjugated goat anti rabbit or goat anti mouse antibodies obtained from Dianova (Hamburg, Germany). For immunohistochemical labeling of PEP, conditioned medium of a clonal cell line expressing anti PEP IgM (4D4D6, probiodrug, Halle/S., Germany) was used, followed by its detection with secondary Cy2-conjugated goat anti mouse IgM antibodies (Dinaova, Hamburg, Germany). The Cy3-conjugated mouse monoclonal antibody against glial fibrillary acidic protein (GFAP, clone G-A-5) as well as the polyclonal rabbit anti GFAP antiserum and the biotinylated lectin *Griffonia simplicifolia* agglutinin (isolectin B4; bio-GSA) were provided by Sigma (Deisenhofen, Germany). Streptavidin-Cy3 conjugates were obtained from Dianova.

Western Blot Analysis. PEP was detected by Western blot analysis as described previously (12). To normalize for differences in protein loading or uneven blotting, blots were also incubated with an anti-actin antibody (1:1,000). Primary antibodies were detected by chemiluminescence after incubation with peroxidase-conjugated goat anti rabbit or goat anti mouse antibodies (1:10,000). Blots were digitized (ScanJet 6100C, HEWLETT PACKARD) and images were evaluated by densitometric image analysis using the software package TINA 2.0 (RAYTEST, Straubenhardt, Germany). The sum of grey values over each individual band obtained by densitometry was corrected for actin expression level and data were expressed as percentage change over corresponding control animal value and given as mean ± SEM. Linearity between protein content and optical density readings was proved by running standard curves ranging from 5 to 30 μg protein per lane.

Statistical Analysis. Analysis of variance (ANOVA) followed by the two-tailed student's *t*-test was performed to examine differences in the immunoreactivity for PEP on Western blots between control and APP transgenic animals and between brains from AD patients and non-demented control subjects. Differences between treatments were considered statistically significant when $P < 0.05$.

Immunohistochemistry. PEP protein expressed in brain sections was detected by incubating paraformaldeheyde-fixed tissues with a mouse anti PEP IgM (4D4D6). The specificity of this antibody was proven in wildtype glioma and neuroblastoma cells and in cells expressing PEP antisense mRNA, resulting in reduced PEP

immunoreactivity (5). Furthermore, the intracellular distribution pattern of PEP-EGFP fusion constructs was indistinguishable from the immunochemical labeling signal generated by the 4D4D6 antibody (5). After incubation at 4°C overnight bound PEP antibodies were visualized by incubation with Cy2-conjugated goat anti mouse IgM (20 μg/ml) at room temperature for 45 min. In order to demonstrate co-localization of PEP with glial cells in brains of experimental animals or AD patients, dual flourescent immunolabeling procedures for (i) PEP and microglial cells (bio-GSA binding) and (ii) PEP and astrocytes (GFAP-immunoreactivity) were performed. In any case, PEP was detected first as described above, followed by incubation with bio-GSA (1:100) and streptavidin-Cy3 (20 μg/ml) or with anti-GFAP-Cy3 (1:800). In human brain tissue, GFAP was detected with a digoxygenin-conjugated polyclonal GFAP rabbit-antiserum (10 μg/ml) and Cy3-labeled mouse anti digoxygenin antibodies (20 μg/ml).

Laser Scanning Microscopy. Fluorescent dye-labeled antigens were analysed by laser scanning microscopy (LSM 510, Zeiss, Oberkochen, Germany). For Cy2-labeled antigens, an argon laser with 488 nm excitation and 510 nm emission was used applying a low-range band pass (505–530 nm). For Cy3- labelings cells, a helium–neon-laser with 543 nm excitation and 570 nm emission was used applying a high-range band pass (560–615 nm).

RESULTS

PEP Expression in Mouse Brain

To reveal the distribution and the cellular source of PEP in brain, immunohistochemical labeling was performed in coronal mouse brain sections. PEP was primarily expressed by neurons and detected troughout the brain. PEP immunoreactivity was present in neuronal cytoplasm and axonal and dendritic processes, closely resembling the subcellular localization of PEP in rat primary neurons (Fig. 1a; see also Ref. 5).

PEP expression in different brain regions was compared by Western blot analysis and by an enzymatic PEP activity assay. Densitometric quantification of Western blots revealed the highest PEP expression in cerebellum of adult (8-months-old) mice and lower PEP expression in parietal cortex and hippocampus. In aged, 17-months-old mice, PEP protein levels were unchanged compared to the adult mice, with the exception of the hippocampus, which demonstrated an up-regulation of PEP expression by about 30% (Fig. 1b). These results were mirrored by those derived from the quantification of PEP enzymatic activity in different brain regions. In adult mice, the highest PEP activity was detected in cerebellum (16 mU/mg protein), followed by parietal cortex (11 mU/mg protein) and hippocampus (10 mU/mg protein). In aged mice, PEP enzymatic activity significantly increased in hippocampal tissue, but remained unchanged in the other brain regions studied (Fig. 1c).

The PEP immunohistochemical staining pattern in APP transgenic Tg2576 mice did not differ from that of non-transgenic littermates (Fig. 1a). To show whether PEP expression is induced in glial cells activated in the vicinity of β-amyloid plaques, double immunocytochemical labeling was performed. Although the activation of microglial cells and astrocytes was robust in neocortex and hippocampus of aged Tg2576 mice, we did not detect glial PEP expression (Fig. 1a). However, total PEP protein levels as detected by Western blot analysis (Fig. 1b) and PEP enzymatic activity (Fig. 1c) were increased in the hippocampus of 8-months-old Tg2576 mice as compared to nontransgenic mice. This effect was not present in the hippocampus of aged Tg2576 mice and not found in any other brain region studied.

PEP Expression in Human Brain

In human brain PEP was selectively expressed by neurons as shown by immunohistochemistry. We observed a perinuclear cytoplasmatic labeling and filamentous staining of neurites (Fig. 2a). In brain structures affected by β-amyloid plaque pathology in AD we detected fewer PEP-immunoreactive neurons, which were more intensely stained than in control brain and which appeared to be shrunken (Fig. 2a). In all AD cases investigated, a robust activation of microglial cells and astrocytes was observed in proximity to β-amyloid plaques. However, neither activated microglial cells nor reactive astroyctes expressed PEP as demonstrated by dual immunofluorescent labeling and confocal laser scanning microscopy (Fig. 2a). Total PEP protein levels and enzymatic activity of PEP in parietal cortex were unaltered in AD brain as compared to age-matched control brain specimens (Fig. 2b and c).

DISCUSSION

PEP Expression in Mouse Brain

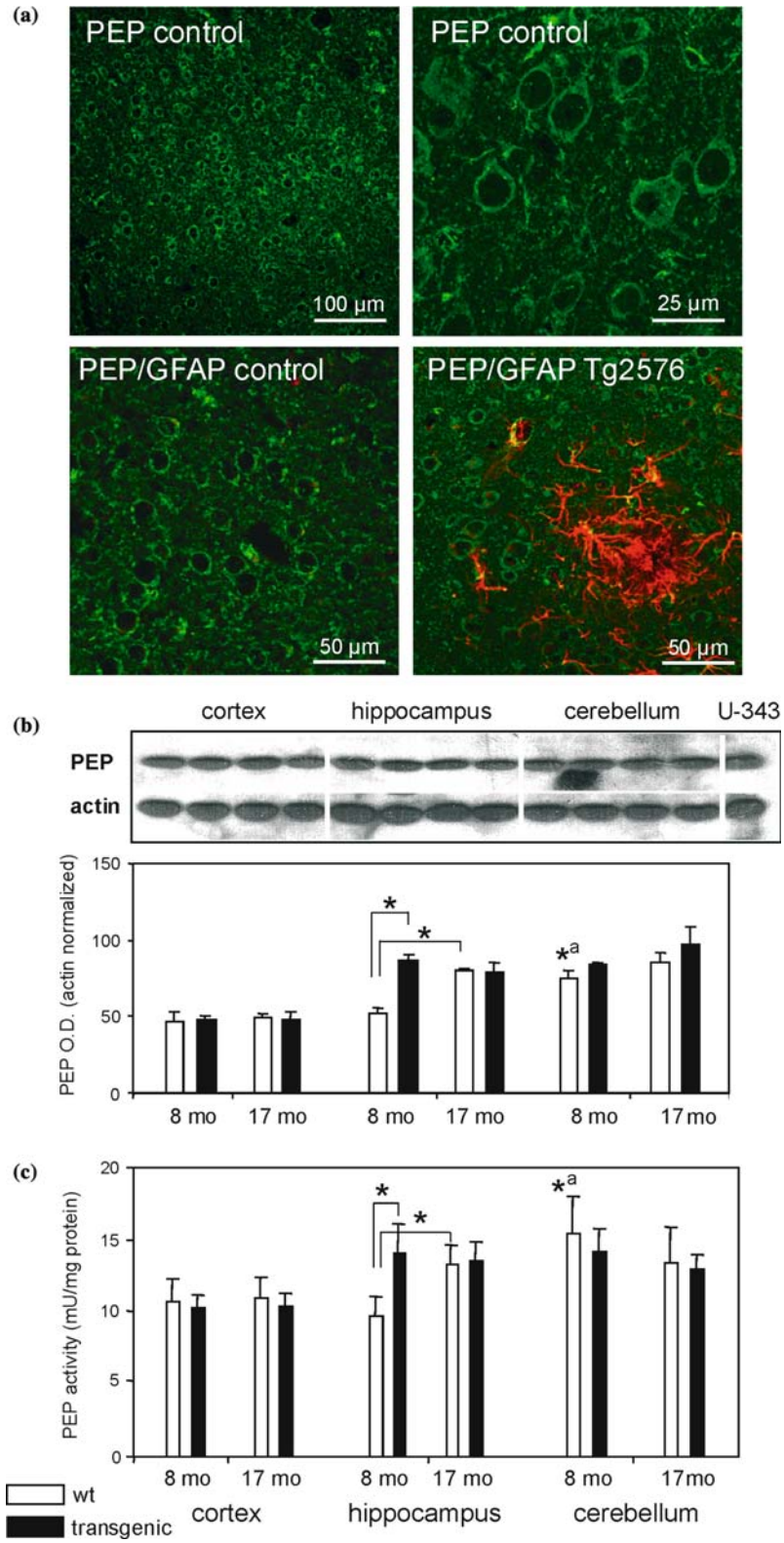
PEP expression and enzymatic activity was analyzed in brains of adult, 8-months-old, and aged, 17-months-old, mice. PEP was localized to neurons and found in the perinuclear region and in neurites. These findings resemble observations made in primary neurons and suggest functions for PEP in axonal transport and/or protein secretion. Indeed, in

an accompanying study we demonstrate the enhanced protein/peptide secretion from neuroblastoma and glioma cell lines under conditions of PEP inhibition (5). The expression and enzymatic activity of PEP in brain of adult mice was highest in cerebellum and significantly lower in cortex and hippocampus. During aging, PEP expression was significantly increased in hippocampus, but not in the other brain regions investigated. Such an age-related increase in PEP expression was also observed in mouse brain by microarray screening (13). Because of the known cognition-enhancing effects of PEP inhibition in several experimental paradigms including scopolamine treatment of rats (14) and middle cerebral artery occlusion (15,16) it is tempting to speculate that the increase in hippocampal PEP activity during aging contributes to the age-related decline in the performance of hippocampus-dependent learning tasks.

The inhibition of PEP activity robustly enhances β-amyloid secretion, most likely by protecting the C-terminal APP reinternalization motif YENPTY (8,9). Therefore, we wanted to reveal whether increased generation of β-amyloid peptides or β-amyloid plaque formation affects by a feedback mechanism the expression or enzymatic activity of PEP in brains of APP transgenic Tg2576 mice. These mice are characterized by 5 to 7-fold overexpression of human APP695 carrying the Swedish mutation K670N, M671L and start to develop β-amyloid plaques by the age of 11 months (10). The amyloid plaque formation is accompanied by a robust activation of microglial cells and astrocytes in proximity to β-amyloid plaques and by the glial expression of proinflammatory cytokines (17–19). PEP expression and its enzymatic activity was analyzed in brains of adult and aged Tg2576 mice and compared to that of nontransgenic littermates. This experimental design allowed to differentiate between the effects on PEP induced by elevated levels of soluble β-amyloid peptides and those produced by β-amyloid plaque formation. We observed increased protein levels and enzymatic activity of PEP in hippocampus of adult Tg2576 mice compared to non-transgenic littermates. The hippocampal PEP activity in adult transgenic mice was as high as in aged control mice, but did not increase further during aging. Therefore, in aged Tg2576 mice, PEP activity in hippocampus was not different from control mice. This "accelerated aging" of Tg2576 mice with regard to PEP activity was not present in cortex or cerebellum and indicates a selective modulation of PEP activity in hippocampus by soluble/oligomeric or fibrillar β-amyloid peptides in the pre-plaque phase. These observations are consistent with those by Laitinen et al. (20), showing no difference in PEP activity of aged, 17-months-old, double transgenic APP-presenilin1 mice compared to wild-type mice. Interestingly, hippocampus-dependent memory deficits precede the appearance of β-amyloid plaques in different APP transgenic mouse lines (21–23). Moreover, memory loss in Tg2576 mice starting at the age of about six months coincides with the appearance of detergent-insoluble β-amyloid aggregates (24). This correlation is inversed in aged transgenic mice and led the authors to conclude that insoluble β-amyloid aggregates are a surrogate marker for small assemblies of β-amyloid that disrupt cognition and occur as intermediates during formation of insoluble β-amyloid aggregates (24). Together with the cognition-enhancing effects of PEP inhibition and the increase in PEP activity during aging our data indicate that PEP may be one among several factors involved the modulation of learning and memory capacities in aging and particularly important under conditions of accelerated β-amyloid generation.

We never observed the expression of PEP by activated glial cells in proximity to β-amyloid plaques, indicating that PEP is a neuron-specific enzyme in brain *in vivo*. However, glial cells isolated from newborn rat brain are capable of expressing PEP in

Fig. 1. (a) In the upper row, the typical neuronal PEP immunofluorescent labeling of wild-type mouse brain is shown at low (left) and higher (right) magnification. The higher magnification image reveals the in the perinuclear and cytoskeletal localization of PEP in parietal cortex of wild-type mouse brain. In the bottom row, PEP (Cy2-labeled; green fluorescence) and GFAP (Cy3-labeled, red fluorescence) immunoreactivities are shown in parietal cortex of 17-months-old wild-type and age-matched APP transgenic Tg2576 mouse brain as indicated. Note the robust astrocytic activation in Tg2576 neocortex and the absence of PEP expression by these reactive astrocytes. (b) Western blot analysis of PEP in brain homogenates from adult (8-months-old) and aged (17-months-old) wild-type and Tg2576 mice as indicated. This panel shows representative examples of Western blots and gives the quantification of optical density readings normalized for actin immunoreactivities. Data are mean ± SEM obtained from seven animals per experimental group and were tested for statistical significance by ANOVA followed by two-tailed student's *t*-test. * Differences are statistically significant at $P < 0.05$. (c) Enzymatic activity of PEP in brain homogenates from adult (8-months-old) and aged (17-months-old) wild-type and Tg2576 mice as indicated. Data are mean ± SEM from seven animals per experimental group and were tested for statistical significance by ANOVA followed by two-tailed student's *t*-test. *: Differences as indicated are statistically significant at $P < 0.05$. *[a]: PEP activity in cerebellum of 8-months old control mice is significantly higher than in parietal cortex and hippocampus of the same brains.


(a)
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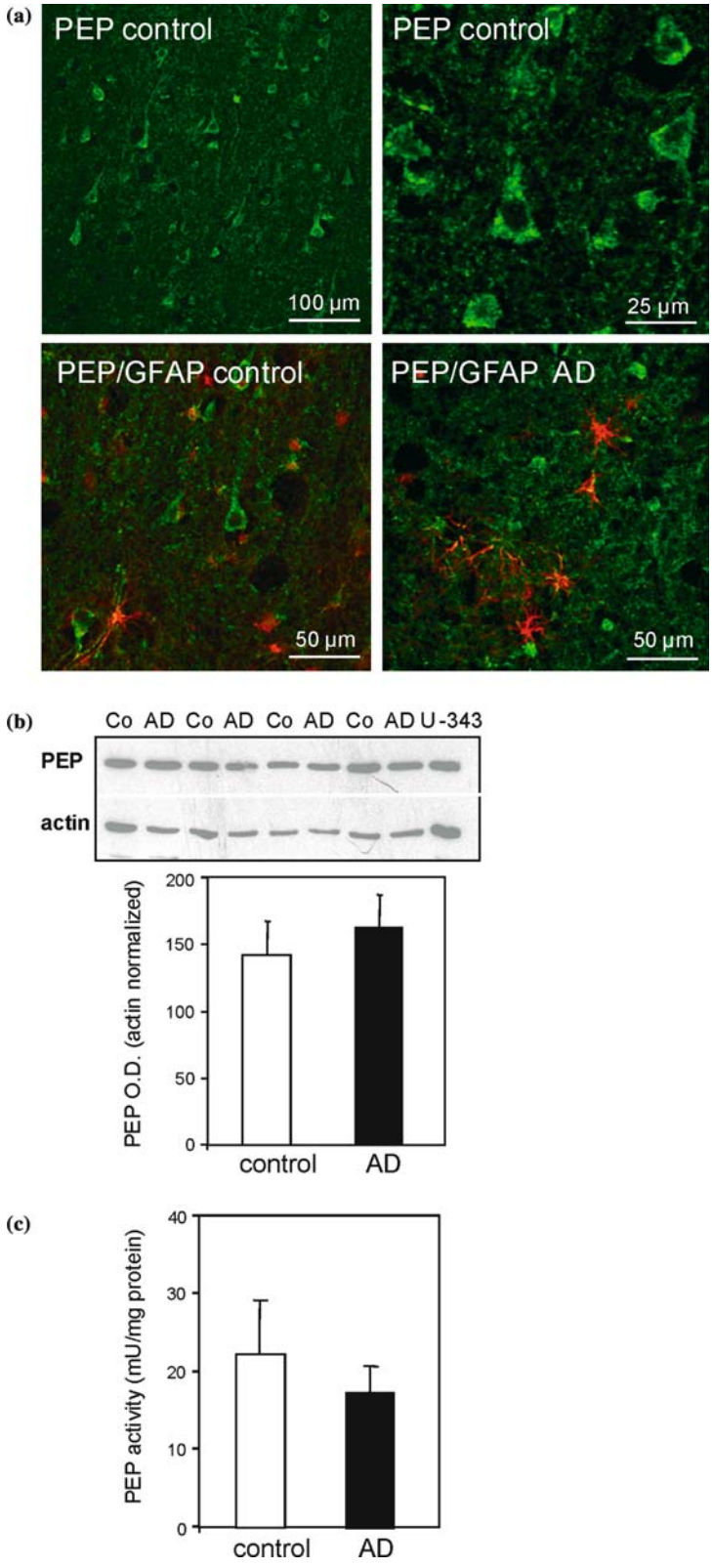
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(c)
PEP activity (mU/mg protein)
40
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20
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0
control
AD

Fig. 2. (a) PEP immunoreactivity in brain of a non-demented human control subject and in AD brain as indicated. PEP is neuronally expressed as shown at low magnification in parietal cortex (upper left). The higher magnification image (upper right) reveals the in the perinuclear and cytoskeletal localization of PEP in pyramidal neurons of parietal cortex in control brain. In bottom row, double immunofluorescent labelings for PEP (Cy2-labeled; green fluorescence) and GFAP (Cy3-labeled; red fluorescence) are shown for control (left) and AD (right) human parietal cortex. Note the intense PEP labeling in fewer neurons, which display shrunken morphology. PEP is not expressed by reactive astrocytes in AD brain. (b) Western blot analysis of PEP in brain homogenates from non-demented human control subjects and AD patients as indicated. This panel shows representative examples of Western blots and gives the quantification of optical density readings normalized for actin content. Data are mean ± SEM from seven AD patients and eight control subjects and were tested for statistical significance by ANOVA followed by two-tailed student's *t*-test. Co... control (c) Enzymatic activity of PEP in brain homogenates from control subjects and AD patients as indicated. Data are mean ± SEM from seven AD patients and eight control subjects and were tested for statistical significance by ANOVA followed by two-tailed student's *t*-test.

culture as shown in enzymatic activity assays by Mentlein et al. (25) and by immunocytochemistry in our laboratory (5).

PEP Expression in Human Brain

As observed for the mouse brain, PEP was exclusively expressed by neurons of the parietal cortex as shown by immunohistochemistry. The neuronal labeling was most prominent in the perinuclear region and in cellular processes including those of pyramidal layer V neurons. In brain samples from AD patients, the enzymatic activity of PEP was similar to that of control cases. However, at the immunohistochemical level we detected fewer PEP-immunoreactive neurons in the AD brain, which were more intensely stained than in control brain and which appeared to be shrunken. Of course, based on these observations, it is not possible to state whether the increased PEP expression by individual neurons contributes to AD pathology. However, the neuroprotective effects of PEP inhibition on one side and the frequent co-occurance of robust PEP expression and morphological characeristics of neurodegeneration in the AD brain on the other side indicate that increased PEP expression may play a role in AD-associated neurodengeration as also suggested in by Laitinen et al. (20). The brains of AD patients are also characterized by a robust activation of microglial cells and astrocytes in proximity to β-amyloid plaques. These glial cells are thought to be important players in both neurodegeneration and neuroprotection. In their activated state, glial cells express a variety of proteins including pro- and anti-inflammatory cytokines and growth factors, which are not expressed by resting glial cells. However, as already demonstrated brains of Tg2576 mice, the spectrum of proteins induced in reactive glial cells in proximity to β-amyloid plaques does not include PEP in the AD brain.

CONCLUSIONS

In this study we demonstrated the increased PEP expression in hippocampus of adult Tg2576 mice before the appearance of β-amyloid plaques but in parallel with the development of memory deficits. In AD brain, neurons which robustly express PEP show morphological characteristics of neurodegeneration. The observations presented here indicate that the reported neuroprotective and cognition-enhancing effects of PEP inhibition might be particularly relevant under conditions of increased APP expression and enhanced generation of β-amyloid peptides. Therefore, we believe that PEP inhibitors are potentially beneficial for the treatment of neurodegnerative diseases including AD.

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REFERENCES

1. Goossens, F., DeMeester, I., Vanhoof, G., and Scharpe, S. 1996. Distribution of prolyl oligopeptidase in human peripheral tissues and body fluids. Eur. J. Clin. Chem. Clin. Biochem. 34:17–22.
2. Mentlein, R. 1988. Proline residues in the maturation and degradation of peptide hormones and neuropeptides. FEBS Lett. 234:251–256.
3. Wilk, S. 1983. Prolyl endopeptidase. Life Sci. 33:2149–2157.
4. Dresdner, K., Barker, L. A., Orlowski, M., and Wilk, S. 1982. Subcellular distribution of prolyl endopeptidase and cation-sensitive neutral endopeptidase in rabbit brain. J. Neurochem. 38:1151–1154.
5. Schulz, I., Zeitschel, U., Rudolph, T., Gerhartz, B., Bigl, V., Demuth, H.-U., and Roßner, S. 2005. Subcellular localization

suggests novel functions for prolyl endopeptidase in protein secretion. J. Neurochem. 94:970–979.

6. Roßner, S., Ueberham, U., Schliebs, R., Perez-Polo, J. R., and Bigl, V. 1998. The regulation of APP metabolism by cholinergic mechanisms and neurotrophin receptor signaling. Progr. Neurobiol. 56:541–569.
7. Selkoe, D. J. and Schenk, D. 2003. Alzheimer's disease: molecular understanding predicts amyloid-based therapeutics. Annu. Rev. Pharmacol. Toxicol. 43:545–584.
8. Schulz, I., Rosche, F., Manhart, S., Rahfeld, J.-U., Heiser, U., Hoffmann, T., Wermann, M., Bär, J., and Demuth, H.-U. 2005. NPXY-motif containing C-terminal C31 fragment of amyloid precursor protein – A putative novel intracellular substrate of prolyl endopeptidase. Submitted.
9. Soriano, S., Lu, D. C., Chandra, S., Pietrzik, C. U., and Koo, E. H. 2001. The amyloidogenic pathway of amyloid precursor protein (APP) is independent of its cleavage by caspases. J. Biol. Chem. 276:29045–29050.
10. Hsiao, K., Chapman, P., Nilsen, S., Eckman, C., Harigaya, Y., Younkin, S., Yang, F., and Cole, G. 1996. Correlative memory deficits, Aβ elevation and amyloid plaques in transgenic mice. Science 274:99–102.
11. McKhann, G., Drachmann, D., Folstein, M., Katzman, R., Price, D., and Stadlan, E. M. 1984. Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA work group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. Neurology 34:939–944.
12. Schulz, I., Gerhartz, B., Neubauer, A., Holloschi, A., Heiser, U., Hafner, M., and Demuth, H. U. 2002. Modulation of inositol 1,4,5-triphosphate concentration by prolyl endopeptidase inhibition. Eur. J. Biochem. 269:5813–5820.
13. Jiang, C. H., Tsien, J. Z., Schultz, P. G., and Hu, Y. 2001. The effects of aging on gene expression in the hypothalamus and cortex of mice. Proc. Natl. Acad. Sci. USA 98:1930–1934.
14. Toide, K., Iwamoto, Y., Fujiwara, T., and Abe, H. 1995. JTP-4819: A novel prolyl endopeptidase inhibitor with potential as a cognitive enhancer. J. Pharmacol. Exp. Ther. 274:1370–1378.
15. Shinoda, M., Matsuo, A., and Toide, K. 1996. Pharmacological studies of a novel prolyl endopeptidase inhibitor, JTP-4819, in rats with middle cerebral artery occlusion. Eur. J. Pharmacol. 305:31–38.
16. Shishido, Y., Furushiro, M., Tanabe, S., Shibata, S., Hashimoto, S., and Yokokura, T. 1999. Effects of prolyl endopeptidase inhibitors and neuropeptides on delayed neuronal death in rats. Eur. J. Pharmacol. 372:135–142.
17. Benzing, W. C., Wujek, J. R., Ward, E. K., Shaffer, D., Ashe, K. H., Younkin, S. G., and Brunden, K. R. 1999. Evidence for glial-mediated inflammation in aged APPsw transgenic mice. Neurobiol. Aging 20:581–589.
18. Mehlhorn, G., Hollborn, M., and Schliebs, R. 2000. Induction of cytokines in glial cells surrounding cortical β-amyloid plaques in transgenic Tg2576 mice with Alzheimer pathology. Int. J. Devl. Neurosci. 18:423–431.
19. Apelt, J. and Schliebs, R. 2001. β-Amyloid-induced glial expression of both pro-and anti-inflammatory cytokines in cerebral cortex of aged transgenic Tg2576 mice with Alzheimer plaque pathology. Brain Res. 894:21–30.
20. Laitinen, K. S., van Groen, T., Tanila, H., Venalainen, J., Mannisto, P. T., and Alafuzoff, I. 2001. Brain prolyl oligopeptidase activity is associated with neuronal damage rather than beta-amyloid accumulation. Neuroreport 12:3309–3312.
21. Holcomb, L., Gordon, M. N., McGowan, E., Yu, X., Benkovic, S., Jantzen, P., Wright, K., Saad, I., Mueller, R., Morgan, D., Sanders, S., Zehr, C., O'Campo, K., Hardy, J., Prada, C. M., Eckman, C., Younkin, S., Hsiao, K., and Duff, K. 1998. Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. Nat. Med. 4:97–100.
22. Dineley, K. T., Xia, X., Bui, D., Sweatt, J. D., and Zheng, H. 2002. Accelerated plaque accumulation, associative learning deficits, and up-regulation of alpha 7 nicotinic receptor protein in transgenic mice co-expressing mutant human presenilin 1 and amyloid precursor proteins. J. Biol. Chem. 277:22768–22780.
23. Van Dam, D., D'Hooge, R., Staufenbiel, M., Van Ginneken, C., Van Meir, F., and De Deyn, P. P. 2003. Age-dependent cognitive decline in the APP23 model precedes amyloid deposition. Eur. J. Neurosci. 17:388–396.
24. Westerman, M. A., Cooper-Blacketer, D., Mariash, A., Kotilinek, L., Kawarabayashi, T., Younkin, L. H., Carlson, G. A., Younkin, S. G., and Ashe, K. H. 2002. The relationship between Abeta and memory in the Tg2576 mouse model of Alzheimer's disease. J. Neurosci. 22:1858–1867.
25. Mentlein, R., von Kolszynski, M., Sprang, R., and Lucius, R. 1990. Proline-specific proteases in cultivated neuronal and glial cells. Brain Res. 527:159–162.