β -secretase-cleaved amyloid precursor protein in Alzheimer brain: a morphologic study

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Abstract

β -amyloid ($A\beta$) is the main constituent of senile plaques seen in Alzheimer's disease. $A\beta$ is derived from the amyloid precursor protein (APP) *via* proteolytic cleavage by proteases β - and γ -secretase. In this study, we examined content and localization of β -secretase-cleaved APP (β -sAPP) in brain tissue sections from the frontal, temporal and occipital lobe. Strong granular β -sAPP staining was found throughout the gray matter of all three areas, while white matter staining was considerably weaker. β -sAPP was found to be localized in astrocytes and in axons. We found the β -sAPP immunostaining to be stronger and more extensive in gray matter in Alzheimer disease (AD) cases than controls. The axonal β -sAPP staining was patchy and unevenly distributed for the AD cases, indicating impaired axonal transport. β -sAPP was also found surrounding senile plaques and cerebral blood vessels. The results presented here show altered β -sAPP staining in the AD brain, suggestive of abnormal processing and transport of APP.

Keywords: Alzheimer's disease • amyloid precursor protein • senile plaques • immunohistochemistry

Introduction

Alzheimer's disease (AD) is characterised by the presence of intracellular neurofibrillary tangles (NFTs), neurofibrillary threads, extracellular amyloid plaques and accumulation of amyloid in cerebral blood vessels. The major component of the amyloid is a 39-43 amino acids peptide called β -amyloid ($A\beta$). $A\beta$ derives from the amyloid precursor protein (APP), which is a widely expressed transmembrane protein [1]. Although the function

of APP is not known, it has been proposed to have a role in regulation of neurite extension, synapse formation, neuronal development and neuroprotection [2-4].

$A\beta$ is produced from APP *via* proteolytic cleavage by proteases called β - and γ -secretase. The β -secretase cleavage by the aspartatic protease BACE [5] initiates $A\beta$ formation by cleaving at the amino terminus of the $A\beta$ domain, generating both the β -secretase cleaved APP ectodomain (β -sAPP) and the membrane-bound carboxy-terminal fragment C99 [6]. A subsequent cleavage of C99 by γ -secretase produces the mature $A\beta$ peptide. In an alternate, non-amyloidogenic processing pathway,

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APP is instead cleaved within the A β domain to produce the soluble α -secretase cleaved APP (α -sAPP) ectodomain and the membrane-bound carboxyl-terminal fragment C83, which through γ -secretase action yields the non-toxic soluble p3 peptide. All of the known mutations linked to AD have been shown to alter production and secretion of the A β peptide (reviewed in [7]), and APP processing is therefore considered a key event in AD pathogenesis. Investigation of β -sAPP localisation and levels in brain may yield important clues to regulation of β -amyloid formation, but also to the normal function of APP. In this study, we have morphologically characterised the distribution of β -sAPP in brain through immunostaining of the frontal, temporal and occipital lobes with a β -sAPP specific antibody. It is, to our knowledge, the first report of β -sAPP localisation in brain tissue.

Material and methods

Tissue

Post-mortem brain tissue from sporadic AD patients and controls was obtained from available material in the Huddinge Brain Bank, Sweden. We selected 9 individuals: three with neuropathological diagnoses of definite AD, three cases with possible AD and three controls. The mean age did not differ significantly between the groups. The AD patients fulfilled the clinical NINCDS criteria for probable and possible AD respectively [8] and the CERAD criteria for definitive and possible AD [9]. The controls consisted of patients without a history of dementia or other neurological/psychiatric diseases.

Tissue processing

Briefly, 4% formaldehyde-fixed were taken from frontal associative cortex (BA 9), medial temporal gyrus (BA 21) and primary occipital cortex (BA 17), embedded in paraffin and cut with a microtome to sections of 6 μ m thickness and mounted onto coated slides.

Immunohistochemistry

Following deparaffinization, masked epitopes were exposed by microwave treatment for 10 min in citrate buffer (pH 6.0). The sections were washed and incubated in DAKO protein block solution (DAKO) for 1 h, then with the polyclonal β -sAPP specific β 939 antibody at a concentration of 0.5 μ g/ml in TBS overnight. The affinity-purified β 939 antibody reacts specifically with the N-terminal fragment of APP generated by β -secretase cleavage of APP (β -sAPP) as previously shown by Sennvik *et al* [10]. The antibody does not react with full length APP nor α -sAPP. The sections were washed in TBS for 10 min and incubated in biotinylated anti-rabbit antibody (DAKO) at a concentration of 5 μ g/ml in TBS for 30 min, washed in TBS for 10 min. Bound antibody was detected using the DAKO catalysed signal amplification system with diaminobenzidine as chromogen. Some of the sections were counterstained with either alkaline Congo Red or with hematoxyline. The Congo Red staining, which is used to detect the presence of amyloid, was visualised using a Nikon polarizing filter set and fluorescence microscopy. For control staining, the primary antibody was omitted. Immunostaining without subsequent amplification resulted in insubstantial staining.

Fig. 1 Microphotographs of frontal cortex sections immunostained for β -sAPP. a) Alzheimer's disease (AD) and b) control brain. Gray matter stains strongly for β -sAPP, while white matter staining is light. In AD brain most cortical layers are evenly stained. There is also characteristically more intensive staining at the border between gray and white matter in AD brain. In control brain, the most intense staining is found in superficial layers. Astrocyte-like cells stain strongly for β -sAPP in c) AD and d) control brain. There are no major differences in the shape or the cellular features between astrocyte-like cell in AD and control brain. The white matter of e) AD and f) control brain stained by antibodies against β -sAPP. Typical granular staining in the axon-like structures indicates axonal transport of the β -sAPP. In AD brain, e) axon-like structures may be empty (arrowheads) or the intraaxonal immunostaining of β -sAPP (arrows) may be patchy. In controls, f) the white matter displays evenly distributed immunostaining. g) β -sAPP positive staining in one neuronal cell in the AD brain. Magnification: a) and b) are taken with a 4x objective, c)-g) with a 100x objective immersed in oil. c)-g) are also counterstained with hematoxyline. Roman numbers indicate cortical layers. * indicates perivascular space in white matter.

Results

Both control and AD brains showed extensive granular β -sAPP immunostaining throughout the gray matter in frontal, temporal and occipital lobe (Fig. 1a and 1b). The staining patterns were similar for all the investigated regions, although the staining intensity was lower in the occipital lobe. Generally, severe AD cases stained more strongly for β -sAPP in gray matter than mild AD cases, which in turn stained more strongly than controls.

Granular white matter immunostaining was found in all three regions, albeit to a much lesser extent compared to gray matter (Fig. 1a and 1b). Again, the occipital lobe showed the weakest staining. As particular areas around blood vessels, within the white matter of the AD cases stained strongly, control cases were seen to have overall weaker white matter staining.

The most conspicuous finding within gray matter was staining of the astrocyte-like formation, characterized by distinctive ramification around a centrally placed cell soma. Counterstaining with hematoxyline showed the cell bodies to be of typical astrocyte origin (Fig 1c and 1d). While staining intensity varied among the astrocytes, it was the most prominent in the subpial glial compartment of layer I and in the gray matter throughout layer II-IV. For the AD cases, the staining in the layers V-VI was notably more intense than in the controls, particularly on the border of gray and white matter (Fig 1a). In fact, β -sAPP immunostaining of the cortical layers in AD cortex replaced the characteristic scattered pattern of astrocytes with a more confluent one, where individually stained astrocytes could not be easily distinguished.

Both controls and AD cases showed strong and discrete stellate patterns of β -sAPP in white matter, most often with a single or an assembly of glial cells at the core. The granular white matter immunostaining was found to be evenly distributed and ribbonlike. On sections counterstained with hematoxyline, immunopositive granules were shown to be localised in axon-like structures (Fig 1e and 1f). For these structures, staining was strongest in controls, as granules there were numerous and regularly distributed. In the AD cases, the staining was often patchy and the distribution was increasingly irregular (Fig 1e). Perineuronal staining could frequently be seen in both controls and

AD cases. In the AD cases, there was also occasionally intraneuronal staining (Fig 1g).

Pathological hallmarks of AD such as amyloid deposits and tangles could not be visualized and distinguished in brain based on β -sAPP immunostaining alone. However, when counterstaining with Congo Red, accentuated β -sAPP staining could be seen around amyloid plaque cores in the outer plaque ring (Fig 2a). Increased staining could also be seen in the non-core plaques where β 939 staining paralleled Congo-positive fibrillary plaque formation (Fig 2b). Immunostaining was further obtained in the vicinity of the congofilic-positive subarachnoidal and penetrating blood vessels (Fig 2c) and extracellularly around tangle formation (Fig 2d). In none of these instances did the β -sAPP staining overlap with the Congo-positive structures.

Discussion

In this report, we have described the distribution of β -sAPP in control and AD brain. The sections stained strongly and extensively for β -sAPP in the gray matter of the frontal cortex and the medial temporal gyrus. The staining was slightly weaker in the primary occipital lobe, especially for the control cases. It has been suggested that the amyloid deposition and tangle formation spread during the disease progression from archicortical (entorhinal and hippocampus) regions towards neocortical regions (predominantly temporal and parietal), resulting in a temporal and spatial variation in the distribution of neuropathological changes within the brain [11, 12]. The occipital lobe is one of the brain regions least affected in AD. Since β -secretase cleavage is a prerequisite for A β formation, the weaker occipital β -sAPP staining was not unexpected.

The staining pattern of β -sAPP reported here correlates well with the reported expression pattern of BACE in the mouse brain [13], showing high levels in deep cortical layers. Although the staining patterns were similar in control and AD brain, the intensity differed. This difference is due to pathological features in AD brain; the increase in astrocyte density and/or the larger amount of core and non-core neuritic plaques and amyloid-bearing vessels, all of which were found to be associated with β -sAPP.

Fig. 2 β -sAPP staining around Congo positive structures in AD frontal cortex. a) Characteristic senile plaque with Congo-positive core. β -sAPP immunostaining surrounds the core. b) β -sAPP immunostaining in the core-less, neuritic-like plaque. c) β -sAPP immunostaining around Congo-positive vessels. d) β -sAPP immunostaining around tangle-bearing, Congo-positive neuron. The β -sAPP staining does not overlap with the Congo-positive amyloid structure. All microphotographs are taken under polarizing and light microscopy. Magnification: 40x objective. Double staining was performed with alkaline Congo Red.

Several other groups have reported discrete granular and globular immunostaining of APP in brain, particularly concentrated to neurites and neuropil threads [14, 15]. The isoform APP751, which contains the Kunitz protease inhibitor region, has been shown in neuropil and astrocyte cell bodies, but only in a subgroup of senile plaques [16]. APP

has been shown to be present in senile plaques, cerebrovascular amyloid and granular amyloid deposits [17, 18], but there are also reports showing APP to be present only in a subset of senile plaques [19, 20]. APP within the cell has been detected in the cytoplasm [16, 21], in the axon [22-26], but also in neuronal vesicles and on neuronal surfaces

[21]. As these reports generally include both α -sAPP, β -sAPP and full length APP, in the denotation of APP, it is uncertain to what extent they corroborate or contradict our results.

The immunostaining pattern in gray matter corresponded closely to astrocyte cell distribution. In concordance with the known distribution of astrocytes, the majority of stained cells were found in the subpial glial layer and layers II-VI of the gray matter, while only a few stained cells were found in layer I or in the white matter. The strong staining of the upper layers in particular, suggests β -sAPP involvement in neuronal plasticity. Since the upper layers are the phylogenetically youngest, plasticity of the neuronal connections is higher than in underlying layers.

Although neuron-like cells were seldom stained directly, the major source of the β -sAPP is most likely neuronal, since white matter staining was much less extensive. It is also possible that the immunostaining in gray matter could be the result of β -sAPP uptake by glial cells, for instance during a scavenging process. sAPP has been shown to bind to the scavenger receptor and to be internalised in transfected CHO cells [27]. High expression levels of the scavenging receptor have been detected near amyloid plaques in AD brain.

In white matter we found a granular linear staining pattern approximate to an axon-like distribution in both controls and AD case. Hematoxyline counterstaining revealed the linearly distributed granules to be intraaxonal, indicating presence and transport of β -sAPP in the axon. The normal presence of β -sAPP in the axon is supported by several previous studies [22–26] and the axonally transported APP can be processed in the axolemma or the axon [28, 29]. Newly synthesised APP is believed to be delivered to the synaptic terminal by fast anterograde axonal transport and then transported to the dendrites by a transcytotic mechanism [23]. Antibodies against both N-terminal and C-terminal parts of APP have been found to stain the axon [24]. Immunostaining for the N-terminus of APP shows a segmental pattern along axons and dendrites [30].

It has been reported that APP functions as a membrane receptor protein, linking kinesin1 to vesicles moved by fast anterograde axonal transport [31] and that overexpression of the APP homologue APPL in *Drosophila* disrupts axonal transport. The

function of APP as a membrane receptor would account for its normal presence in the axon. The A β region and the cytoplasmic C-terminus of APP seems to be required for the axonal entry and accumulations [25, 32]. An axonal membrane compartment containing both APP, β -secretase and presenilin-1 was recently identified, suggesting that amyloidogenic processing of APP can occur during axonal transport [23]. Another recent report [32] shows that overexpression of APPL causes axonal vesicle accumulation leading to neuronal apoptosis, and that AD pathogenic mutations further enhance that effect. AD associated presenilins showed to be a substrate for such proteolytic systems leading to neuronal sensitization to apoptosis.

White matter β -sAPP staining in AD brain was generally stronger than in controls, possibly due to influence of the vascular pathology and unspecific microgliosis in the white matter. The axonal structures, however, contained a reduced amount of immunopositive granules. This reduction of β -sAPP may reflect disturbed axonal transport, processing or accumulation of β -sAPP in the remaining axons. Loss of axonal network or neurodegenerative changes in the soma may have a further negative influence on axonal β -sAPP transport. Impaired axonal transport might also explain the general uneven staining in AD white matter.

For the AD cases we found immunostaining surrounding, but excluded from the core amyloid plaques. β -sAPP was also found in the vicinity of cerebral blood vessels in gray and white matter, for control and AD brains alike. The implications of these findings are unclear, but β -sAPP staining is localised in the same regions around the plaques as GAP-43 [33], which suggests that β -sAPP has a regulatory function in neuronal sprouting.

The results presented here show altered β -sAPP staining in the AD brain, suggestive of abnormal processing and transport of APP.

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