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Age-dependent axonal degeneration in an Alzheimer mouse model

Oliver Wirths [a], Joachim Weis [b], Rakez Kayed [c], Takaomi C. Saido [d], Thomas A. Bayer [a,*]

[a] *Department of Psychiatry, Division of Neurobiology, Saarland University, Building 90, D-66421 Homburg/Saar, Germany*
[b] *Institute of Neuropathology, Technical University, Pauwelsstr. 30, Aachen, Germany*
[c] *Department of Molecular Biology and Biochemistry University of California, Irvine, CA, USA*
[d] *The Laboratory for Proteolytic Neuroscience, RIKEN Brain Science Institute, Wako, Saitama 351-0198, Japan*

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Abstract

Some neurodegenerative diseases including Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) exhibit prominent defects in axonal transport. These defects can manifest as axonal swellings or spheroids, which correspond to axonal enlargements and aberrant accumulation of axonal cargoes, cytoskeletal proteins and lipids. Recently, a controversial scientific debate focussed on the issue whether Aβ serves as a trigger for aberrant axonal transport in the pathophysiology of AD. Prominent axonopathy has been shown to be induced by overexpression of proteins involved in several neurodegenerative diseases. Neurofilament, apolipoprotein E, Niemann-Pick protein and Tau transgenic mice with axonal trafficking deficits have been reported. Furthermore, motor deficits are frequently observed in patients with AD, which has been attributed to the typical tauopathy in post-mortem brain tissue. In the present report, we analyzed axonal neuropathology in the brain and spinal cord of a transgenic mouse model with abundant intraneuronal Aβ42 production and provide compelling evidence for axonal degeneration. The APP/PS1ki mice showed characteristic axonal swellings, spheroids, axonal demyelination and ovoids, which are myelin remnants of degenerated nerve fibers in an age-dependent manner. Abundant accumulation of intraneuronal N-modified Aβ, Thioflavin S-positive material and ubiquitin was found within the somatodendritic compartment of neurons. We conclude that the intraneuronal accumulation of Aβ-amyloid peptides is followed by axonal degeneration, and thus might be a causative factor for the axonal changes seen in AD.

Keywords: Axonal degeneration; Axonopathy; Alzheimer; Amyloid; Intraneuronal Aβ; Transgenic mice; Axonal transport

1. Introduction

Alzheimer's disease (AD) is a severe neurodegenerative disorder characterized by two neuropathological hallmarks, extracellular senile plaques and neurofibrillary tangles. While tangles mainly consist of an abnormally phosphorylated Tau protein, the senile plaques are aggregates of the 39–43 amino acid Aβ peptide, which originates from the larger amyloid precursor protein (APP). Most AD cases occur sporadically; mutations in the genes for amyloid precursor protein, presenilin 1 (PS1) or presenilin 2 (PS2) cause a small percentage of familial early-onset AD cases. These mutations have been shown to increase the amount of the pathologic Aβ42-isoform, which rapidly aggregates into amyloid fibrils (for review, see [2]). Early axonal transport deficits have been reported in a variety of neurodegenerative diseases [38,42]. In AD patients, axonal alterations in the brain manifest in the form of dystrophic neurites decorating amyloid plaques. These neurites correspond to axonal swellings, which mostly contain abnormal accumulations of axonal proteins, such as neurofilaments or hyperphosphorylated Tau. APP plays a role in axonal structure and function and undergoes fast anterograde axonal transport [2,15], presumably by a kinesin-I-mediated mechanism [13]. It has been previously reported that APP, as well as the β-site APP-cleaving enzyme (BACE) and PS1 are transported in the same axonal compartment [12], however, this has been questioned very

* Corresponding author. Tel.: +49 6841 16 24290; fax: +49 6841 16 24378.
E-mail address: thomas.bayer@uniklinik-saarland.de (T.A. Bayer).

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recently [17]. So far, only a few studies have reported AD-related pathological alterations in the spinal cord. Whereas the occurrence of neurofibrillary tangles in the spinal cord of AD patients is well established [31,33], there is only scarce evidence for the presence of amyloid plaques in this location in human patients [21]. However, motor deficits including gait disturbances have been reported in AD patients, raising the question whether underlying axonal alterations contribute to this particular feature of the disease [22,32]. In addition, phenotypic overlaps exist between AD and ALS in Guam-Parkinsonism-ALS-dementia complex [33].

Transgenic mice have been proven to be a valuable model system to study the pathological alterations in AD. In this study, we analyzed APP expression, Aβ deposition and axonal alterations in the brain and spinal cord of a recently described transgenic mouse model expressing mutant APP on a homozygous mutant PS1 knock-in background. These mice have been shown to develop amyloid plaques at 2 months of age with robust antecedent intraneuronal accumulation of Aβ peptides in hippocampal and cortical areas. Furthermore, they exhibit a significant CA1/2 neuronal loss at 10 months of age (>50%), a pathological feature that has not been convincingly proven in most other APP transgenic mouse models [4]. In the present report, we describe severe axonopathy and intraneuronal Aβ accumulation in a bigenic mouse model of AD.

2. Material and methods

2.1. Transgenic mice and tissue preparation

The generation of APP/PS1ki mice has been described previously [4]. Human mutant APP751, harbouring the Swedish and London mutations is expressed under the control of the murine Thy-1 promoter, whereas murine PS1 with two FAD-linked mutations (PS1M233T and PS1L235P) is expressed under the control of the endogenous mouse PS1 promoter. All mice named as PS1ki were homozygous for PS1 knock-in mutations, in comparison to the APP/PS1ki mice, which harboured one additional hemizygous APP751SL transgene. In total, female APP/PS1ki mice at 2 months ($n=5$), 6 months ($n=6$), 10 months ($n=12$) and 14 months ($n=4$), as well as an appropriate amount of age-matched PS1ki, APP single transgenic and wild type control animals of the same gender have been used in this study (generous gift of Dr. Laurent Pradier, Sanofi-Aventis, France). All animals were handled according to French and German guidelines for animal care.

2.2. Human post-mortem samples

Post-mortem spinal cord samples of a patient with early-onset AD (male, 44 years of age with spastic paraplegia and dementia) were obtained with written informed consent and in accordance with local law (generous gift of Charles Duyckaerts). The patient with pathologically confirmed AD harboured a mutation in the PS1 gene (Phe 386 Ser, in exon 11). The same mutation was also found in two additional affected family members.

2.3. Balance beam

To evaluate balance and general motor abilities, a 1 cm wide dowel beam is attached to two support columns 44 cm above a padded surface. At either end of the 51 cm long beam, a 14 cm × 10 cm wooden platform is attached. During the single day of testing, each animal is given three trials. For each trial, animals are placed at the centre of the beam and released. The time required for the animal to fall from the beam is recorded and the scores of the three individual trials are averaged. If an animal is able to remain on the beam for the whole duration of the 60-s trial or escapes to one of the attached platforms, the maximum time of 60 s is recorded [1]. Statistical differences were tested using one-way ANOVA followed by Bonferroni's Multiple Comparison Test.

2.4. Immunohistochemistry and histology

Mice were anaesthetized and transcardially perfused with ice-cold phosphate-buffered saline (PBS) followed by 4% paraformaldehyde (PFA). Spinal cord samples were carefully dissected and post-fixed in 4% buffered formalin at 4 °C. Immunohistochemistry was performed on 4 μm paraffin sections as described previously [41]. Antibodies against Ubiquitin, Synaptophysin, Tau (DAKO), phosphorylated Tau AT8 (Innogenetics), phosphorylated Tau PS199 (Biosource), NF-68 (Sigma), NF-200 and phosphorylated NF-M and NF-H (both Chemicon), antiserum 23850 against human APP, as well as antiserum 692 (generous gift of G. Multhaup) against human Aβ were used [4]. We further used antibodies against N-modified Aβ forms including AβN1[D], AβN1[rD] against racemized aspartate, AβN3[pE] against pyroglutamate at position 3, Aβ17-x which detects the p3 fragment [30], as well as the oligemer-specific antibody OC [14]. Biotinylated secondary anti-rabbit and anti-mouse antibodies (1:200) were purchased from DAKO. Staining was visualized using the ABC method, with a Vectastain kit (Vector Laboratories, Burlingame, CA) and diaminobenzidine as chromogen. Counterstaining was carried out with hematoxylin. For immunofluorescenct double-labelling, FITC- and TexasRed-conjugated secondary antibodies (Vector Laboratories) were used.

Visualization of aggregated forms of Aβ was performed with 1% Thioflavin S (Sigma) including 1 μg/ml of 4′6-diamidine-2′-phenylindole dihydrochloride (DAPI, Sigma) [4].

2.5. Ultrastructural analysis

For electron microscopy, animals were transcardially perfused with ice-cold (PBS), pH 7.4, containing 4%