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Altered phosphorylation but no neurodegeneration in a mouse model of tau hyperphosphorylation

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Abstract

The role of hyperphosphorylation of tau in Alzheimer's disease is still unsolved. Here we describe a novel transgenic mouse model, expressing a pseudohyperphosphorylated (PHP) variant of the longest human CNS tau isoform in forebrain neurons. We report that pseudohyperphosphorylation decreases phosphorylation at T205 while other sites (T212, S262) are less or not affected compared to mice expressing wildtype tau. Despite the differences in phosphorylation, the subcellular distribution of tau is not affected and mice do not develop highly aggregated states of tau. PHP tau expressing mice do not show any evidence for neurodegeneration as determined from morphometric measurements of neocortical regions, caspase activation, analysis of mitochondrial dysfunction, or determination of spine densities. In agreement, no differences in learning and memory are observed. The data indicates that moderate levels of modified tau alone are not sufficient to induce tau aggregation or neurodegeneration in transgenic mice. With our model it becomes possible to study the effects of hyperphosphorylation at conditions which may prevail in an early preaggregation state of the disease.

Keywords: Tau protein; Pseudohyperphosphorylation; Transgenic mouse; Tauopathy; Degeneration

1. Introduction

Patients with Alzheimer's disease (AD) show neuron loss as well as changes in the morphology of axons and dendritic branches in many brain regions (Spires and Hyman, 2004). Common abnormalities also include loss of dendritic spines. In AD and related diseases with a tau pathology, the microtubule-associated protein tau exhibits an increased and stable phosphorylation at multiple sites ("hyperphosphorylation"). In AD, abnormal phosphorylation of tau appears to be one of the earliest signs of the disease, preceding the aggregation of tau (Braak et al., 1994). Levels of hyperphosphorylated tau correlate with the severity of the disease in AD (Holzer et al., 1994) suggesting a decisive role of tau hyperphosphorylation in disease progression.

We have previously generated tau constructs in which ten selected serine/threonine residues, known to be phosphorylated to a high extent in AD brain, were substituted

Abbreviations: AD, Alzheimer's disease; CA, cornu ammonis; CC, corpus callosum; DG, dentate gyrus; FA, formic acid; FTDP-17, frontotemporal dementia and parkinsonism linked to chromosome 17; GSK3β, Glycogen Synthase Kinase-3β; HC, hippocampus; MBR, microtubule binding region; NC, neocortex; NFTs, neurofibrillary tangles; PHFs, paired helical filaments; PHP, pseudohyperphosphorylated; PRR, proline rich region; WT, wildtype.

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with glutamate to create a stable pseudohyperphosphorylation (Eidenmüller et al., 2000). We could show that pseudohyperphosphorylated (PHP) tau mimics key structural and functional aspects of hyperphosphorylated tau protein (Eidenmüller et al., 2000; Maas et al., 2000; Eidenmüller et al., 2001). Thus, PHP tau provides a useful model to test the effect of disease-like permanent and high stoichiometric tau phosphorylation. When expressed at high levels, PHP tau induced neurotoxicity in neural cell lines, primary neuronal cultures and hippocampal slices (Fath et al., 2002; Shahani et al., 2006; Leschik et al., 2007) suggesting that these tau modifications are sufficient for disease-related changes.

In contrast to culture experiments, transgenic *Caenorhabditis elegans* overexpressing human PHP tau did not show neuron loss although defects in development could be observed (Brandt et al., 2009). Since phosphorylation mechanisms in *C. elegans* markedly differ from mammals as evidenced by a very high phosphorylation of human tau in the worm (Brandt et al., 2009), the effect of tau hyperphosphorylation needs to be examined also in a more suitable animal model.

While many mouse models have been generated that develop an AD pathology (reviewed in Götz et al., 2004; Lee et al., 2005; Games et al., 2006; McGowan et al., 2006), we aimed to create a model that concentrates on a single aspect of pathology, i.e. a permanent and stable hyperphosphorylation of tau at selected sites, to enable a differential study of the effects of hyperphosphorylation. Thus, we produced transgenic mice expressing human wt or PHP tau under the control of the CaMKII promoter that drives protein expression restricted to forebrain neurons, the brain region that is affected in AD. Here, we report that the mice express moderate levels of the transgene in addition to endogenous tau. Pseudohyperphosphorylation alters the extent of phosphorylation of tau at additional, non-modified sites. These modifications do not result in protein accumulation or aggregation. Furthermore, we report that despite the differences in phosphorylation, mice do not develop any signs of degeneration even at old ages nor exhibit spine loss. In agreement, no behavioral changes were observed.

2. Methods

2.1. Materials

Chemicals were purchased from Sigma (Deisenhofen, Germany) unless stated otherwise. The following primary antibodies were used, and specificity and sources are given in parentheses: Tau antibodies: monoclonal phosphorylation-independent tau antibody Tau-5 (mouse; Labvision, Westinghouse, CA), monoclonal phosphorylation-independent human tau antibody HT-7 (mouse; Pierce, Rockford, IL); phosphorylation-dependent and site-specific tau antibodies: pT205 (Thr-205; rabbit; Biosource, Camarillo, CA), pT212 (Thr-212; rabbit; Biosource, Camarillo, CA), pS262 (Ser-262; rabbit; Biosource, Camarillo, CA); other antibodies: monoclonal tubulin antibody DM1A (mouse; Sigma, Munich, Germany), mouse polyclonal cleaved caspase-3 (Asp175) antibody (rabbit; Cell Signaling, Beverly, MA), monoclonal FLAG® epitope antibody M5 (mouse; Sigma, Munich, Germany), monoclonal neuronal nuclei antibody NeuN (mouse; Chemicon, Temecula, CA), monoclonal porin antibody (mouse; Molecular Probes, Eugene, OR), monoclonal ATP synthase subunit d antibody (mouse; Molecular Probes, Eugene, OR). As secondary antibodies, cyanine 3 (Cy3)-coupled anti-mouse antibody (Dianova, Hamburg, Germany) and peroxidase-conjugated anti-mouse and anti-rabbit antibodies (JacksonImmunoResearch, West Grove, PA) were used.

2.2. Constructs and generation of heterozygous transgenic mice

Human wt tau and PHP tau constructs of the longest human brain isoform (441 aa) in pHSVpUC-vector served as starting material (Fath et al., 2002). Tau was N-terminally tagged with the sequence MDKDDDDK (FLAG). The FLAG-tau sequences were amplified by PCR and ligated into the vector pNN265 that was cut before by EcoRV. The identity of the PCR products was verified by sequencing. Tau constructs together with 5′ intron/exon signal and 3′ poly-A signal were cut out by NotI and subcloned into the vector pMM403, which contains the CaMKII promoter. Vector sequences were removed prior to microinjection. Purified, linearized DNA (0.7 μg/ml) was microinjected into B6D2F2 mouse zygotes. Mice were genotyped by PCR analysis of lysates from tail biopsies using transgene-specific primers (forward 5′-GGACTACAAAGACGATGACGAT-3′, reverse 5′-GTGGTTCCTTCTGGGATCTCCG-3′). Injection of the human wt tau construct in 57 pronuclei produced 9 positive founders, from which 4 transmitted the gene when bred with C57Bl/6. Injection of the PHP tau construct in 88 mice produced 10 positive founders, from which 3 animals produced positive littermates after breeding with C57Bl/6. All animals were maintained and sacrificed according to National Institutes of Health guidelines and German animal care regulations. Unless stated otherwise, young mice are considered to be between 2 and 5 months and old mice between 17 and 21 months of age.

2.3. Stainings

Mice were perfused transcardially with 4% paraformaldehyde in phosphate buffer (PFA in PBS), pH 7.4. Brains were removed, postfixed over night at 4 °C in 4% PFA in PBS, and stored in PBS at 4 °C. For rapid Golgi-staining, brains were stained according to (Bubenaite, 1929) with minor modifications. In short, PFA-fixed brains were incubated in 2.5% (w/v) $K_2Cr_2O_7$ at 34 °C for 3 days. The brains were shortly washed in 1% (w/v) $AgNO_3$ and incubated for 4 days at 34 °C in 1% (w/v) $AgNO_3$. Coronal