Neurobiology of Aging 34 (2013) 1343–1354

Contents lists available at SciVerse ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

Cascade of tau toxicity in inducible hippocampal brain slices and prevention by aggregation inhibitors

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ARTICLE INFO

Article history:
Received 29 June 2012
Received in revised form 10 September 2012
Accepted 23 October 2012
Available online 15 November 2012

Keywords:
Aggregation
Aggregation-inhibitory drugs
Hippocampus
Organotypic slice culture
Tau pathology

ABSTRACT

Mislocalization and aggregation of the axonal protein tau are hallmarks of Alzheimer's disease and other tauopathies. Here, we studied the relationship between tau aggregation, loss of spines and neurons, and reversibility by aggregation inhibitors. To this end we established an in vitro model of tauopathy based on regulatable transgenic hippocampal organotypic slice cultures prepared from mice expressing proaggregant Tau repeat domain with mutation ΔK280 (Tau_{RD}ΔK). Transgene expression was monitored by a bioluminescence reporter assay. We observed abnormal tau phosphorylation and mislocalization of exogenous and endogenous tau into the somatodendritic compartment. This was paralleled by a reduction of dendritic spines, altered dendritic spine morphology, dysregulation of Ca^{++} dynamics and elevated activation of microglia. Neurotoxicity was mediated by Caspase-3 activation and correlated with the expression level of proaggregant Tau_{RD}ΔK. Finally, tau aggregates appeared in areas CA1 and CA3 after three weeks in vitro. Neurodegeneration was relieved by aggregation inhibitors or by switching off transgene expression. Thus the slice culture model is suitable for monitoring the development of tauopathy and the therapeutic benefit of antiaggregation drugs.

1. Introduction

Tau, a microtubule-associated protein in the brain, aggregates abnormally in Alzheimer's disease (AD) and other neurodegenerative tauopathies (Ballatore et al., 2007; Morris et al., 2011). Tau is highly soluble and adopts a natively unfolded structure in solution. In tau fibers of AD (termed paired helical filaments; PHFs), short motifs of tau adopt a ß-conformation which leads to interactions with other tau molecules (von Bergen et al., 2000). The spreading of tau aggregates correlates well with cognitive decline in AD (Braak and Braak, 1994). Abnormal phosphorylation and mislocalization of tau is an early hallmark of neurodegeneration and precedes aggregation (Braak and Braak, 1994; Giannakopoulos et al., 2003; Gomez-Isla et al., 1997). Mutations within the tau repeat domain, like the FTDP-17 mutation ΔK280 (Rizzu et al., 1999) increase tau's propensity for β-structure and promote aggregation in vitro (Barghorn et al., 2000). Mechanisms of tau-mediated cell death are still under debate (Spires-Jones et al., 2011). Caspase activation and caspase-cleaved tau was found in tauopathy models (Gamblin et al., 2003; Rohn et al., 2002) suggesting that apoptosis plays a role in tau-induced cell death. Others found early caspase activation, following tau cleavage thereby initiating tangle formation. After a new tangle has formed, the neuron remained alive and caspase activity seemed to be suppressed (de Calignon et al., 2010). A loss or alteration of dendritic spines has been described in patients with neurodegenerative disorders and is thought to be responsible for cognitive deficits but the underlying mechanisms are poorly understood. To clarify some of these issues we used transgenic hippocampal organotypic slices from pro-aggregant Tau repeat domain with mutation ΔK280 (Tau_{RD}ΔK) mice to study the relationship between tau expression, physiological dysfunction, aggregation, and finally neurotoxicity and their prevention by tau aggregation inhibitors. The hippocampus is highly affected in AD (Braak and Braak, 1991; Smith et al., 2009). Furthermore, the remarkable ability for regeneration makes hippocampal organotypic slices suitable for long-term cultivation (Gahwiler, 1988; Stoppini et al., 1991). Such slices from young animals share many similarities with acute slices, e.g., development of dendritic spines

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http://dx.doi.org/10.1016/j.neurobiolaging.2012.10.024

(De Simoni et al., 2003), but can be kept for weeks and allow extended monitoring and experimental manipulation without the complications of the blood–brain barrier. These preparations are therefore particularly suitable to analyze the time course of pathological events and allow testing of potential drugs. In the present study we show that tau can aggregate in hippocampal slice cultures, that tau aggregation and toxicity occurs in parallel, and that aggregation inhibitors can abolish both aggregation and toxicity. Moreover, alterations and loss of dendritic spines occurred before aggregation and cell death. Our findings suggest that tau pathology starts with missorting of tau into the somatodendritic compartment, resulting in reduction and changes in morphology of dendritic spines. At the same time we observed a reduction of Ca^{++} influx evoked by membrane depolarization in $Tau_{RD}\Delta K$ slices. At later stages tau aggregates and cell death accompanied by caspase-3 activation were observed. Compound bb14, a tau aggregation inhibitor from the rhodanine class, was able to prevent the development of tau pathology, i.e., phosphorylation, missorting, aggregation, and spine loss; it preserved Ca^{++} dynamics and protected neurons against toxicity and cell death.

2. Methods

Transgenic mice expressing the human tau 4-repeat domain with the FDTP-17 mutation ΔK280 ($Tau_{RD}\Delta K$, 129 residues, M-Q244-E372 without K280) and reporter gene firefly luciferase under control of a Tet-operon response element (Mocanu et al., 2008), were crossed with CaMKIIα-tTA mice (Mayford et al., 1996) to generate a regulatable Tet-off system. For histochemical and behavioral details on these double transgenic mice see Sydow et al. (2011). Animals were housed and tested according to standards of the German Animal Welfare Act.

Hippocampal organotypic slice cultures were prepared following Stoppini et al. (1991), with modifications. Briefly, 7–10-day-old mice were decapitated, brains were rapidly removed, and hippocampi dissected at 4 °C. A McIlwain tissue chopper (Gabler, Bad Schwabach, Germany) was used to prepare 400 μm thick transverse slices which were transferred to semiporous cell culture inserts (Millipore, Bedford, MA, USA; 0.4 μm). Inserts containing 6–8 slices were placed in 6-well culture trays containing 1 mL of culture media (50% Minimum Essential Medium (MEM), 25% Hank's Balanced Salt Solution (HBSS), penicillin/streptomycin [all from PAA, Pasching, Austria], 25% horse serum, 4.5 mg/mL glucose [Sigma-Aldrich, St. Louis, MO, USA], pH 7.4). The culture medium was changed on the first day after preparation and afterward every third day. Slices were kept in culture for 3–4 weeks. Suppression of the human tau transgene was achieved by adding doxycycline hydrochloride (Sigma-Aldrich) to the culture media (final concentration 2 μg/mL). The treatment with doxycycline was carried out from DIV 1 unless stated otherwise. During treatment, doxycycline was refreshed every third day, simultaneously with full medium change.

2.1. Immunohistochemistry

Slice cultures were left attached on the Millicell membrane and stained as free-floating sections in 6-well plates. Cultures were first fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS; PAA) for 2 hours at 4 °C. After washing with cold PBS, slices were permeabilized by 0.4% Triton X-100/PBS for 90 minutes at RT. Slices were then blocked with 5% bovine serum albumin (BSA) for 2 hours and afterward incubated with primary antibody diluted in PBS for 2–3 days at 4 °C. After washing with PBS, slices were incubated with secondary antibody for 2 days at 4 °C. After washing, slices were mounted with Permafluor mounting solution (Beckman Coulter, Paris, France), cover-slipped and dried before imaging. The following primary antibodies were used: monoclonal anti-neuronal nuclei (NeuN) antibody (Chemicon International, Temecula, CA, USA) (1:500), pan-tau antibody K9JA (Dako, Hamburg, Germany; Nr. A0024 [1:1000]), microtubule associated protein 2a/b (AP20; Sigma-Aldrich, Germany [1:200]), 12E8 (1:1000) for phosphorylated S262/S356 tau (gift from Dr P. Seubert, Elan Pharma, South San Francisco, CA, USA), PHF1 antibody for phosphorylated S396/404 tau (gift from Dr Peter Davies, Albert Einstein College, Bronx, NY, USA) and anti-Iba1 (Wako Chemicals) (1:1000). All fluorescent (goat anti-rabbit/mouse cyanine 2, 3, and 5)-labeled secondary antibodies were from Dianova (Hamburg, Germany) (1:1000). Thioflavine-S (ThS) (Sigma-Aldrich) staining was done as previously described (Mocanu et al., 2008). Briefly, fixed slices were incubated in 0.05% ThS for 8 minutes, washed twice with 80% ethanol, followed by 3 washing steps with ddH_2O.

2.2. Diolistic labeling for spine detection

The lipophilic tracer 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI; Invitrogen, Carlsbad, CA, USA) was used to investigate dendritic spines in tau transgenic or control slice cultures (Moolman et al., 2004). Gold particles (1.6 μm radius) were coated with DiI by sonication for 20 minutes at RT. DiI coated gold particles were applied under high pressure (650 psi) in living slice cultures using a helium pump (Bio-Rad Laboratories, Munich, Germany). Cultures were immediately fixed in fixation solution and stored at 4 °C for at least 48 hours before imaging using tetramethyl rhodamine isothiocyanate (TRITC)-filter settings. The spine density of apical dendrites of CA1 pyramidal neurons was estimated after 10 and 20 DIV. Apical dendritic branches (>150 μm from cell soma) were imaged by high resolution confocal microscopy (see below). Spine density was determined from Z-stacks using ImageJ (National Institutes of Health, Bethesda, MD, USA). Spines were further classified by the 3-D image analysis software NeuronStudio Version 0.9.92 (Rodriguez et al., 2008) which allows reconstruction of neuronal structures from confocal images and yields classification of spines into thin, stubby, and mushroom.

2.3. Microscopy

Images were acquired with an Olympus laser scanning microscope FV1000 (Olympus, Tokyo, Japan), equipped with a confocal laser scanning unit, argon (Ar; 488 nm) and helium/neon (He/Ne 543 nm and 633 nm). For 2 or 3 channel imaging, images were acquired via sequential scanning. Image stacks were collected for the whole hippocampus at lower magnification and for all hippocampal subfields at higher magnifications. Digital zoom was used for fluorescent dye tracing of single neurons and spines. Maximum projection images were generated from resulting *Z* stacks using ImageJ software.

2.4. Ca^{++} imaging experiments

For Ca^{++} imaging experiments, organotypic slices were used at 15 DIV. Slices were loaded with Fura-2AM (Invitrogen, Carlsbad, CA, USA) at a concentration of 100 μM at 37 °C for 30 minutes. Fura-2 AM stock solution (5 mM dissolved in dimethyl sulfoxide (DMSO)) was added to the culture dish containing 1 mL of medium. After loading, slices were washed with 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)-buffered saline (130 mM NaCl, 5.4 mM KCl; 10 mM HEPES, 25 mM glucose, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$; pH 7.4) for another 30 minutes to allow complete de-esterification of the Fura dye. After washing, cultures were transferred to a submerged imaging chamber of an Examiner A1 microscope (Zeiss). Fura-2 fluorescence was imaged at room