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TAU PATHOLOGY-MEDIATED PRESYNAPTIC DYSFUNCTION

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Abstract—Brain tauopathies are characterized by abnormal processing of tau protein. While somatodendritic tau mislocalization has attracted considerable attention in tauopathies, the role of tau pathology in axonal transport, connectivity and related dysfunctions remains obscure. We have previously shown using the squid giant synapse that presynaptic microinjection of recombinant human tau protein (htau42) results in failure of synaptic transmission. Here, we evaluated molecular mechanisms mediating this effect. Thus, the initial event, observed after htau42 presynaptic injection, was an increase in transmitter release. This event was mediated by calcium release from intracellular stores and was followed by a reduction in evoked transmitter release. The effect of htau42 on synaptic transmission was recapitulated by a peptide comprising the phosphatase-activating domain of tau, suggesting activation of phosphotransferases. Accordingly, findings indicated that htau42-mediated toxicity involves the activities of both GSK3 and Cdk5 kinases.

Key words: tauopathy, IP3 receptor, ryanodine receptor, phosphatase-activating domain of tau, GSK3, Cdk5.

INTRODUCTION

Present knowledge indicates that all brain tauopathies involve the generation of aberrantly phosphorylated, truncated, and misfolded tau neurotoxic species (Rao et al., 2014; Kovacs, 2015). Synaptic dysfunction and abnormalities in axonal transport are early pathogenic events in tauopathies that precede the formation of neurofibrillary tangles (NFTs) and neuronal cell death (Majid et al., 2014; Polydoro et al., 2014; Jadhav et al., 2015). Normally, a substantial amount of cellular tau is sorted into axons (Rao et al., 2014; Jadhav et al., 2015), and there is compelling evidence to suggest that the missorting of tau into the somatodendritic compartment plays a pathological role in tauopathies (Zempel and Mandelkow, 2014). Nevertheless, pathological axonal tau localizations are also prominent (Rao et al., 2014; Tai et al., 2014; Jadhav et al., 2015). Furthermore, it has been recently proposed that pathological-tau spreading may occur trans-synaptically from pre- to the postsynaptic sites (de Calignon et al., 2012). In addition, misfolded tau species may be internalized at the axon terminals and be transported retrogradely (Wu et al., 2013). It is therefore evident that the presynaptic issues represent a prominent parameter in the tauopathies. Presently, the mechanisms linking axonal tau pathology to synaptic dysfunction remain elusive; in part because of the synaptic size limitations that are characteristic of mammalian forms preventing direct access to the synaptic machinery.

To address the possibility that tau accumulation and/or mislocalization at the presynapse triggers synaptic dysfunction we evaluated acute effects of "human wild type" tau protein using the squid synapse preparation. Our previous results demonstrated that recombinant human tau isoform (full length h-tau42) induces a short-lasting increase in spontaneous transmitter release, followed by a rapid decrease and failure of synaptic transmission (Moreno et al., 2011). Microinjected htau42 became phosphorylated at the pathological AT8 antibody epitope. Intriguingly, endogenous tau levels are within 1–2 μM ranges *in vivo* and perfusion of 25 μM of wild-type htau42 in squid axoplasm did not affect axonal transport (Morfini et al., 2007). These observations suggest that the loss of synaptic function which is characteristic of Alzheimer's disease and other tauopathies involve an abnormal presynaptic distribution of tau, rather than an overall increase in cellular tau levels (Yuan et al., 2008).

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Abbreviations: Cdk5, cyclin-dependent protein kinase 5; ER, endoplasmic reticulum; FAT, fast axonal transport; FTD, frontotemporal dementia; GSK3, glycogen synthase kinase 3; PAD, phosphatase-activating domain.

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In the present study, we found evidence indicating that microinjection of htau42 in synaptic terminals abnormally increases levels of cytosolic calcium, presumably from intracellular stores. Additional experiments indicate that the phosphatase-activating domain (PAD (Kanaan et al., 2011)) comprising aminoacids 2–18 of htau42 is necessary and sufficient to produce disruption of synaptic transmission. Pharmacological experiments indicate that the toxic effect of htau42 on synaptic function involves the activities of cyclin-dependent protein kinase 5 (Cdk5) and glycogen synthase kinase 3 (GSK3) (LaPointe et al., 2009). Taken together, these results identify multiple pathogenic events associated with tau-mediated synapto-toxicity at the molecular level, therefore providing novel therapeutic targets to address synaptic dysfunction in tauopathies.

EXPERIMENTAL PROCEDURES

Recombinant tau proteins

Wild-type human tau htau42 (isoform with four tubulin-binding motifs and two extra exons in the N-terminal domain which contains 441 a.a.), its variant htau 3RC (a protein which contains three tubulin-binding motifs and the carboxyl terminal region) and the 2R fragment which has 62 amino acids were isolated as previously described (Perez et al., 2001) (see Fig. 2). PAD peptide and scrambled PAD peptide from (GenScript). Fig. 2A shows a schematic representation of the different tau constructs.

Other reagents

SB216763 (Tocris), 2-Aminoethoxydiphenylborane (2-APB), Xestospongin C, and dantrolene (Sigma–Aldrich), ING-135 was a generous gift from Dr. Kozikowski, TFP5 was described before in (Shukla et al., 2013), TNT-1 and tau5 antibodies were also previously described (LaPointe et al., 2009). The drug concentration for each of the reagents was chosen based on the IC_{50}/EC_{50}, and the effective doses reported in the literature in different preparations, as specified in Table 1. Dose-related target specificity for the different reagents is also presented in Table 1.

Electrophysiology and microinjections

The squid stellate ganglia isolation from the mantle and the electrophysiological techniques used have been described previously (Llinas et al., 1985). In short two glass micropipette electrodes impaled the largest (most distal) presynaptic terminal digit at the synaptic junction site while the postsynaptic axon was impaled by one microelectrode at the junctional site. One of the pre-electrodes was used for pressure microinjection of the different protein/peptides or other compounds (as described in each experiment) and also supported voltage clamp current feedback, while the second monitored membrane potential. The exact location of injection, the diffusion and steady-state distribution of the different treatments/fluorescent dye mix (0.001% dextran fluorescein) were monitored using a fluorescence microscope attached to a Hamamatsu camera system (ARGUS 100, Middlesex, NJ, USA). Microinjections were normalized by determining that the amount of fluorescence that reached the presynaptic terminals was comparable. Recordings were done when fluorescence values (measured as arbitrary units) were 20–25× over background (using the same power gain). In all experiments a good correlation was observed between the localization of the fluorescence and the electrophysiological findings.

Statistical methods

Linear mixed models were used to evaluate the outcome 'Peak to Peak Interval' over 7 times [between 0 and 60 min, for all treatments]. The p values presented were adjusted for multiple testing [by FDR method] at the 60 min. The analysis was performed in log-scale, due to the skewness of the outcome data. We used fixed effects for treatment, time, time square and their interactions with treatment. Both interactions [time and time square by treatment were significant-$p < 0.0001$, for both]. We used random effects for intercept and slope for time, for each experiment. Fixed effects were used to estimate the treatment effects over time. Random effects were used to account for the repeated measures for each animal over time, and natural variability between animals. In this model, absence of postsynaptic spike, due to amplitude reduction of postsynaptic potential to sub-threshold level was also included.

When spikes were generated, their amplitude was evaluated using the same linear mixed model. No significant amplitude differences were observed between any of the groups, therefore these data are not reported throughout the manuscript.

RESULTS

The initial set of experiments (Fig. 1A–D) addressed mechanisms triggered by injection of wild-type recombinant htau42 (80 nM) in the synaptic compartment. Evoked pre- and post-synaptic action potentials were recorded in current-clamp mode following our standard protocol (Llinas et al., 1985). The synapse was activated by direct depolarization of the presynaptic terminal every 5 min (low-frequency protocol). Microinjected htau42 was monitored by fluorescence microscopy using a fluorescent dye/protein mix and correlated with its effect on synaptic release (see Experimental procedures). Once the fluorescent material injected into the axon reached the presynaptic terminal htau42 produced a recordable inhibition of synaptic transmission within 45 ± 15 min post-injection (Fig. 1A, B), as reported before (Moreno et al., 2011).

IP3 receptors are involved in hTau-mediated synapto-toxicity

We have previously shown that shortly after (5 ± 1.5 min) microinjected recombinant htau42 protein reached the presynaptic terminal a transient increment in post-synaptic noise was observed, indicative of an increment