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Research Paper

Local Somatodendritic Translation and Hyperphosphorylation of Tau Protein Triggered by AMPA and NMDA Receptor Stimulation

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ABSTRACT

Tau is a major component of the neurofibrillary tangles (NFT) that represent a pathological hallmark of Alzheimer's disease (AD). Although generally considered an axonal protein, Tau is found in the somato-dendritic compartment of degenerating neurons and this redistribution is thought to be a trigger of neurodegeneration in AD. Here, we show the presence of tau mRNA in a dendritic ribonucleoprotein (RNP) complex that includes $Ca2^{+}$-calmodulin dependent protein kinase (CaMK)IIα mRNA and that is translated locally in response to glutamate stimulation. Further, we show that Tau mRNA is a component of mRNP granules that contain RNA-binding proteins, and that it interacts with Myosin Va, a postsynaptic motor protein; these findings suggest that tau mRNA is transported into dendritic spines. We also report that tau mRNA localized in the somato-dendritic component of primary hippocampal cells and that a sub-toxic concentration of glutamate enhances local translation and hyperphosphorylation of tau, effects that are blocked by the gluatamatergic antagonists MK801 and NBQX. These data thus demonstrate that alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA) stimulation redistributes tau to the somato-dendritic region of neurons where it may trigger neurodegeneration.

1. Introduction

Intracellular inclusions of hyperphosphorylated tau protein (neurofibrillary tangles, NFT) and extracellular deposits of amyloid β (Aβ) are prominent neuropathological features in Alzheimer disease brains. The propagation of NFTs from the entorhinal cortex to the neocortex, followed by neuron and synapse loss, corresponds closely with the clinical progression of Alzheimer's disease (AD) signs, from impaired memory to dementia; this suggests that the formation and propagation of NFT may be involved in the symptomatology of AD.

In the healthy brain, tau is an exclusively axonal protein, engaged in the assembly and stability of microtubules. In contrast, in the AD brain, tau is hyperphosphorylated and forms fibrils that appear as neuropil threads in dendrites and as NFTs in the somatodendritic compartment and axons. NFT formation is preceded by a pre-tangle stage where non-fibrillar and hyperphosphorylated tau accumulates in the soma and dendrites of neurons (Uchihara et al., 2001; Götz et al., 1995). These observations indicate that tau hyperphosphorylation in somatodendrites precedes tau fibrillation and the appearance of neurofibrilar lesions. Thus, understanding the mechanism of tau missorting is an indispensible prerequisite for the development of therapeutic interventions against AD and other tauopathies.

Various mechanisms of tau transport into the somatodendritic compartment have been proposed. For example, while retrograde movement of tau is prevented by a barrier at the initial segment of the axon, hyperphosphorylated tau can transgress this barrier and redistribute to other parts of the neuron (Li et al., 2011). Besides the recent description that amyloid β (Aβ) oligomers induce hyperphosphorylated tau missorting into hippocampal dendrites (Zempel et al., 2013), little is known regarding the pathophysiological stimuli that lead to the accumulation of hyperphosphorylated tau in dendrites.

Recent studies have ascribed a role for tau in the physiological regulation of synaptic function. For example, Ittner et al. reported that tau is required for Fyn-mediated *N*-methyl-D-aspartate (NMDA) receptor activation in the postsynaptic density (PSD) (Ittner et al., 2010), and tau has been shown to be essential for the induction of long term depression (LTD) (Kimura et al., 2013) as well as Brain-derived neurotrophic factor (BDNF)-dependent morphological plasticity (Chen et al., 2012). Other studies demonstrated that neuronal activation leads to the translation of proteins that participate in synapse formation and plasticity (*e.g.* Calcium/calmodulin dependent protein kinase II: CaMKIIα, Glutamate receptor: GluR, and Arc) in dendritic spines (Bramham and

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Wells, 2007; Steward and Halpain, 1999; Steward and Worley, 2002; Steward and Schuman, 2003); the mRNAs encoding these molecules are carried to the dendrite by messenger ribonucleoprotein (mRNP) granules, a complex of mRNAs and mRNA-binding proteins. Given this, and evidence for the involvement of tau in the modulation of synaptic function (Kimura et al., 2013), we hypothesized that tau mRNA is transported into dendrites and spines and locally translated upon stimulation as well as the other synaptic proteins. We here verified this hypothesis by demonstrating that stimulation of glutamatergic receptors results in the transport of tau mRNA to dendrites where it is subsequently translated.

2. Materials and Methods

2.1. Animal Studies

Animal studies were performed under the Guidelines for Animal Experiments at Nihon University.

2.2. Antibodies and Reagents

Anti-tau (rabbit) (Cat# sc-1996 RRID:AB_632468), anti-Microtubule associate protein 2 (MAP2) (rabbit) (Cat# sc-20172 RRID:AB_2250101) and anti-β-actin (rabbit) (Cat# sc-10731 RRID:AB_2223515) antibodies were from Santa Cruz Biotechnology. Rabbit anti-Staufen1 (Cat# ab73478 RRID:AB_1641030) and mouse anti-MAP2 (Cat# ab11267 RRID:AB_297885) antibodies were from Abcam. Anti-Fragile X mental retardation protein (FMRP) (Cat# MAB2160 RRID:AB_2283007) and anti-tau1 (Cat# MAB3420 RRID:AB_94855) antibodies (both from mouse) were from Millipore. Mouse anti-AT8 antibody (Cat# MN1020 RRID:AB_223647) was purchased from Thermo Scientific. Anti-Myosin Va (Cat# 3402S RRID:AB_2148475) and anti-S6 antibodies (Cat# 2217 also 2217L, 2217S RRID:AB_331355) (both raise in rabbit) were from Cell Signaling Technology. Alexa Fluor 488-conjugated goat anti-mouse IgG (Cat# A-11001 RRID:AB_2534069), Alexa Fluor 488-conjugated goat anti-rabbit IgG (Cat# A-11034 also A11034 RRID:AB_2576217), Alexa Fluor 555-conjugated goat anti-mouse IgG (Cat# A-21422 also A21422 RRID:AB_141822), and Alexa Fluor 555-conjugated goat anti-rabbit IgG (Cat# A-21428 also A21428 RRID:AB_141784) were purchased from Thermo Fisher Scientific. Glutamate, 4′,6-diamidino-2-phenylindole (DAPI), cycloheximide, 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[*f*]quinoxaline-7-sulfonamide (NBQX), and D-(-)-2-amino-5-phosphonopentanoic acid (D-AP5) were obtained from Wako Pure Chemical Industries. MK801 and (*S*)-3,5-dihydroxyphenylglycine (DHPG) were purchased from Sigma-Aldrich. 2-chloro-4-((2,5-dimethyl-1-(4-(trifluoromethoxy) phenyl)-1H- imidazol-4-yl)ethynyl)pyridine (CTEP) was purchased from Selleck Chemicals. Fluorescein 12-UTP and T7 RNA polymerase were purchased from Roche. Alkaline phosphatase (Calf intestine) was from Toyobo.

2.3. Immunoprecipitation, Western Blotting and RT-PCR

Immunoprecipitation was carried out using Dynabeads Protein G (Life Technologies) following the manufacturer's protocol. Antibodies (2 μg of each) were bound to the beads and incubated with cell extracts, cell fractions or sucrose gradient fractions (4 h, 4 °C). Beads were recovered, washed with PBS containing 0.1% BSA, and the immune complex eluted (20 mM Tris–HCl [pH 7.5], 140 mM NaCl, and 2% SDS). Proteins were analyzed by Western blotting and co-immunoprecipitated mRNAs were extracted for use as templates in RT-PCR assays. For Western blot analysis, proteins were separated on 10% SDS-PAGE gels, transferred to polyvinylidene difluoride (PVDF) membranes and incubated with relevant primary antibody and corresponding secondary horseradish peroxidase-conjugated antibody (GE Healthcare Life Sciences); protein signals were detected using Enhanced Chemi Luminescence (ECL) (GE Healthcare Life Sciences) and semi-quantified by densitometry. Where necessary, antibodies were stripped using Restore PLUS Western blot stripping buffer (Thermo Scientific).

For RT-PCR, RNAs were extracted with SDS-phenol-chloroform and dissolved in water. First-strand cDNA was synthesized with Moloney Murine Leukemia Virus (MMLV) reverse transcriptase (Takara) using an oligo (dT) primer; double-strand cDNA was synthesized using specific primer pairs (Supplementary Table 1). The RT-PCR products were stained with ethidium bromide and analyzed using a gel documentation system (BioRad GelDoc XR Plus ImageLab).

2.4. Preparation of Synaptosomal Fractions

Synaptosomal fractions were prepared from cerebral cortical and hippocampal tissues from 5-week old male CB57BL/6J mice (Charles River Laboratories International, Inc.). All solutions used contained 1 mM $MgSO_4$, 15 mM *N*-tris[hydroxymethyl]-methyl-2-aminoethanesulfonic acid (Na-TES, pH 7.4), RNase inhibitor (0.2 unit/μl, Takara) and protein inhibitor (complete cocktail without EDTA, Roche). After homogenization of tissues in 9 volumes of 0.3 M sucrose solution (first with a loose-type, then with a tight-type Dounce homogenizer; Wheaton), homogenates were passed through a series of nylon mesh filters (111, 70 and 52 μm; Spectrum) and centrifuged (900 ×*g*, 10 min, 4 °C). After washing with 0.3 M sucrose, pellets were suspended in 18% Ficoll/0.3 M sucrose solution and centrifuged (7500 ×*g*, 40 min, 4 °C, Beckman Coulter SW40Ti rotor). Supernatants were then diluted with 2 volumes of the Ficoll/sucrose solution and centrifuged (13,000 ×*g*, 20 min, 4 °C SW40Ti rotor) and the resulting pellet was resuspended in 0.3 M sucrose solution containing 1% NP-40 to yield synaptosomal fractions. Proteins and RNAs were analyzed by Western blotting and RT-PCR, respectively. When glutamate treatments were used, primary culture of E18 CB57BL/6J mouse hippocampal neurons were grown on polylysine-coated 5-cm dishes (Iwaki) and maintained for 16 days *in vitro*. Synaptosmal preparations were prepared after treatment of cells with glutamate.

2.5. Preparation of Neuronal mRNP Granule-enriched Fractions

Cerebral cortices and hippocampi from 34-week old wild-type and tau-knockout CB57BL/6J mice were prepared by the Institute of Immunology, Utsunomiya (Japan). Briefly, brains were washed with ice-cold PBS and homogenized in TKM buffer (50 mM triethanolamine [pH 7.8], 50 mM KCl, 5 mM $MgCl_2$, 0.25 M sucrose, 1 mM PMSF, protein inhibitor [complete cocktail without EDTA, Roche], 1 mM DTT and RNase inhibitor [0.2 unit/μl, Takara]). Homogenates were centrifuged (1000 ×*g*, 10 min, 4 °C) and 0.5 ml of each supernatant was loaded onto a 15–45% sucrose gradient (9 ml) with a 0.75 ml cushion (45% sucrose) before centrifugation at 36,000 rpm (2 h, 4 °C, Beckman Coulter SW40Ti rotor). The gradient was fractionated, and the distribution of RNAs and ribosomal protein in each fraction was monitored by absorbance at 254 nm and Western blotting of S6 ribosome protein, respectively.

2.6. Primary Culture, Glutamate Treatment and Immunocytochemistry

Hippocampal neurons were prepared from embryonic (E18) CB57BL/6J mice. Cells were grown in polylysine-coated glass chambers (Lab-Tek) and maintained for 16–17 days in neurobasal medium containing 0.5 mM L-glutamine and 2% B27 Supplement (Gibco). When differentiated, neurons were exposed to glutamate (0.5 mM) for 5 min and the medium was promptly replaced with fresh neurobasal medium and incubated for a further 25 min, before fixation with 4% paraformaldehyde (15 min, room temperature), permeabilization (1% Triton-X100), and blocking with DMEM containing fetal bovine serum. Specimens were then incubated with appropriate primary antibody (2 h, RT). After washing with PBS, specimens were incubated with corresponding secondary antibody (Alexa Fluor 488-conjugated goat anti-rabbit antibody, Alexa Fluor 555-conjugated goat anti-mouse antibody, Alexa

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