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Tau kinase inhibitors protect hippocampal synapses despite of insoluble tau accumulation

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A better understanding of the cellular and molecular pathomechanisms of Alzheimer's disease (AD) is a prerequisite for the development of efficient treatments. We have used a novel assay system based on virus-transduced organotypic hippocampal slice cultures that mimics important aspects of tau-driven AD pathology in a short time frame. Human tau P301L, when expressed in pyramidal neurons of hippocampal slice cultures, was increasingly phosphorylated at several disease-relevant epitopes, leading to progressive neuronal dystrophy and formation of RIPA-insoluble tau. AD-like tau hyperphosphorylation was reduced by the tau kinase inhibitors lithium and SRN-003-556, but RIPA-insoluble tau remained unaffected after treatment with any of these substances. Only SRN-003-556 was able to protect hippocampal neurons from synaptic damage that was presumably caused by a toxic soluble tau fraction. These data provide first mechanistic insights towards the functional benefits of SRN-003-556 that have been observed *in vivo*.

Keywords: Alzheimer's disease; Model system; Hyperphosphorylation; Pathomechanism; Neuroprotection

Introduction

Tau pathology has been postulated as a common path of neurodegeneration in a variety of chronic neurological diseases, hence termed tauopathies, including AD (Forman et al., 2004; Mandelkow and Mandelkow, 1998; Sergeant et al., 2005). Recent data from animal models recapitulating key features of AD suggest that tau pathology mediates neuronal death and cognitive deficits downstream of toxic amyloid species accumulation (Gotz et al., 2001; Oddo et al., 2006). Therefore, targeting tau pathology presents a valid therapeutic strategy in chronic neuropathies.

Various transgenic animals have been designed to model age-dependent tau pathology and to analyse the relation between tau pathology and neurodegeneration (LaFerla and Oddo, 2005; Spires and Hyman, 2005). In conclusion, the exact mechanism leading from accumulation of toxic tau species to neuronal death is still not entirely evident. Moreover, it has become questionable whether the most common pathological hallmark of tauopathies—neurofibrillary tangles (NFT)—are toxic intermediates themselves or if they are the result of a protective mechanism that attempts to reduce toxic soluble tau species (Santacruz et al., 2005; Spires et al., 2006). Nevertheless, it remains commonly accepted that tau phosphorylation is one of the earliest signs of neurodegeneration (Braak and Braak, 1995; Davies, 2000; Ramsden et al., 2005). Le Corre et al. (2006) succeeded recently in a proof-of-concept study in tau P301L transgenic mice (JNPL3) to reduce motor deficits and tau hyperphosphorylation by using a novel tau kinase inhibitor, the K252a analogue SRN-003-556. Most interestingly, the number of neurofibrillary tangles was not reduced in these experiments, once again pointing towards a non-toxic or perhaps even protective function of NFTs. Furthermore, the exact role of hyperphosphorylation in tauopathies still needs to be elucidated.

Because tau can be phosphorylated on multiple sites, depending on age and development, and because a whole plethora of kinases might perform these reactions, the unambiguous identification of relevant tau kinases and their respective target sites has been proven to be tremendously difficult. Probably even more important is the question whether only the phosphorylation of specific tau residues triggers neuronal degeneration. Therefore, we have developed an assay that reflects the *in vivo* situation in terms of cellular composition and intercellular architecture, kinase profiles, and developmental stage more accurately than cell lines.

Results and discussion

We used Herpes simplex virus type-1 vectors for transduction of organotypic hippocampal slice cultures from 8-day-old rats. The expression of a control transgene (eGFP) was mainly apparent in CA3 neurons, extending to some degree into CA1, targeting a disease-relevant area of the hippocampus (Fig. 1a). eGFP was exclusively found in neurons but not in glial cells, as revealed by the localisation and morphology of transduced cells (Figs. 1a, b). Following transduction with a mutant tau variant that causes hereditary tauopathy, tau P301L, we detected recombinant human tau in neuronal cell bodies of the CA3 region as well as in neuronal processes

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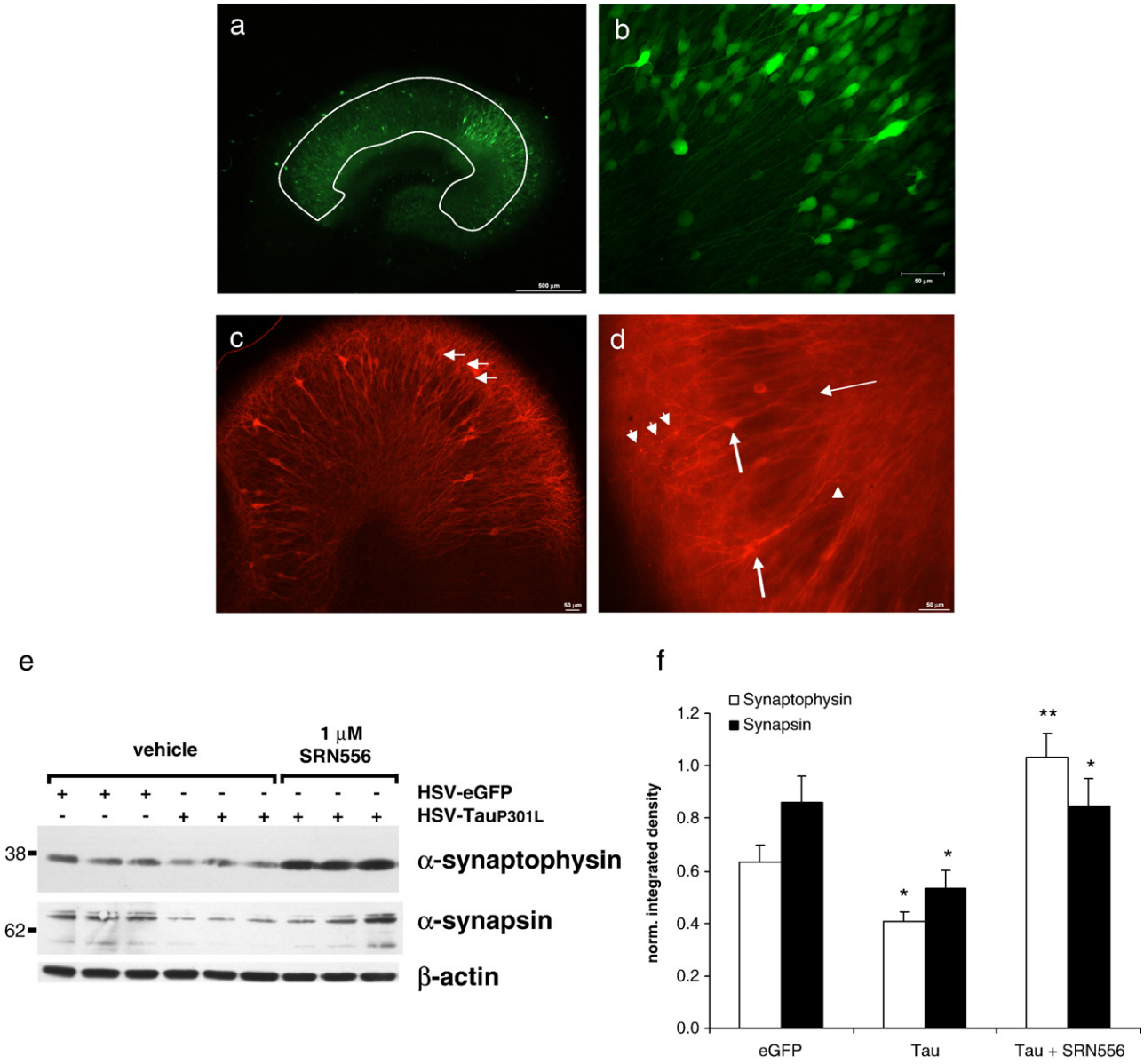

Fig. 1. Expression of eGFP and human tau P301L in hippocampal slice cultures leads to synaptic dystrophy. (a) Fluorescence micrograph of a Herpes simplex virus-1 infected hippocampal slice 6 days post infection. eGFP expression is apparent in neurons of the CA3 region and to some degree in CA1 (indicated by a white line). (b) Higher magnification of eGFP-expressing pyramidal neurons in the CA3 region 6 days post infection. (c) Immunostaining of tau P301L transduced slices with rabbit anti human tau 4 days post infection reveals intensive staining of pyramidal neurons in the CA3 region. In some cells, dendrite labelling (arrows) was detectable without evident tau accumulation in the cell body. (d) Micrograph at higher magnification reveals dendritic staining of CA3 neurons. Some dendrites, which protrude into the stratum oriens, show a punctate, fragmented staining pattern (arrowheads). Respective cell bodies are indicated with arrows. (e) Western blot analysis of RIPA-lysates from slice cultures infected with eGFP or human tau P301L viral vectors, incubated in the presence of SRN-003-556 or vehicle, on day 6 post transduction. Membranes were analysed with monoclonal antibodies anti synaptophysin, rabbit anti synapsin, and with anti β-actin (from top to bottom). Synaptic marker proteins synaptophysin and synapsin were reduced upon tau P301L-expression, but not upon eGFP expression. Their levels were restored upon addition of 1 μM SRN-003-556 on days 1 to 6 post infection.

(Figs. 1c, d). In some neurons, dendrite labelling was detectable before evident tau accumulation in the cell body (Fig. 1d, arrows). No tau staining was obtained in eGFP-transduced slices, thus confirming the specificity of the antibody for human tau (data not shown).

Tau expression levels increased continuously from day 2 until day 8 post infection as judged by Sandwich ELISA (Fig. 3e, human tau).

While most dendrites of tau P301L expressing neurons still looked healthy under these conditions, others, projecting from prominently labelled cell bodies (Fig. 1c, arrows), showed almost fragmented swellings and seemed to undergo dystrophic changes from day 4 onwards (Fig. 1d, arrowheads). This staining pattern was reminiscent of Wallerian degeneration (Beirowski et al., 2005) and may suggest that those neurons that accumulate tau P301L are the first to enter early stages of degeneration.