BRAIN RESEARCH 1347 (2010) 132–141

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BRAIN RESEARCH

Research Report

Chronic restraint stress alters the expression and distribution of phosphorylated tau and MAP2 in cortex and hippocampus of rat brain

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ARTICLE INFO

Article history:
Accepted 24 May 2010
Available online 1 June 2010

Keywords:
Chronic restraint stress
Tau
Phosphorylation
MAP2

ABSTRACT

Microtubule-associated proteins (MAPs) play a critical role in maintaining normal cytoskeletal architecture and functions. In the present study, we aim to explore the effects of the emotional stressor, chronic restraint stress, on the expression levels and localization of tau and MAP2. We found that after chronic restraint stress, soluble hyperphosphorylated tau was greatly increased, whereas MAP2 was decreased. Moreover, immunohistochemistry analysis demonstrated that phosphorylated tau and MAP2 displayed the similar subcellular distribution pattern after chronic restraint stress. Robust hyperphosphorylated tau immunolabeling was observed both in cortex and hippocampus of stressed animals and mainly located to perikaryal/dendritic elements. After stress, the MAP2 was mainly distributed in the perikaryal compartments, discontinuous dendrites and neuropil. Moreover, the distribution pattern of MAP2 in hippocampus significantly changed. Immunofluorescence double labeling indicated that hyperphosphorylated tau increased in the regions where there displayed an decrease of MAP2. These results suggest that the involvement of repeated restraint stress may not only induce phosphorylation state of tau and distribution of phosphorylated tau, but also alter the content and neuronal distribution of MAP2. Tau and MAP2 are most important MAPs for neuronal cells, the subcellular distribution change of them might be link to functional change of neurons after emotional stress.

1. Introduction

Several types of microtubule-associated protein (MAP) have been involved in eukaryotes, including microtubule motors, microtubule plus-end-binding proteins, centrosome-associated proteins, enzymatically active MAPs, and structural MAPs. The MAP2/Tau family of structural MAPs, which along with the MAP1A/1B family form one of the 'classical', well-characterized families of MAPs. In mammals, the family consists of the neuronal proteins MAP2 and Tau and the non-neuronal protein MAP4. Tau and MAP2 are best known for their microtubule stabilizing activity and for proposed roles

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doi:10.1016/j.brainres.2010.05.074

regulating microtubule networks in the axons and dendrites of neurons. Contrary to this simple, traditional view, accumulating evidence suggests a much broader range of functions, such as binding to filamentous actin (F-actin), recruitment of signaling proteins, and regulation of microtubule-mediated transport (Dehmelt and Halpain, 2005) .

Tau, which is predominately located in axons in mature neurons, is also implicated in Alzheimer's disease (AD) and other dementias. Abnormal hyperphosphorylated tau protein in AD brain not only forms the neurofibrillary tangles (NFTs), but also sequesters normal tau, MAP1 and MAP2, and disrupts microtubules (Alonso et al., 1994, 1997). In contrast, MAP2 as well as MAP1 and tubulin deposit in Lewy bodies, a cytopathologic marker of Parkinson's disease. In addition, many observations confirm the notion of a role for increase in non-phosphorylated MAP2 and MAP1B at hippocampus in somatodendritic and cytoarchitectural abnormalities associated to schizophrenia (Benitez-King et al., 2004). Moreover, studies have indicated that the increase of hyperphosphorylated tau is concomitant with the decrease of MAP2, and hyperphosphorylated tau accumulation in neuropil may displace MAP2 (Ashford et al., 1998). Therefore, both tau and MAP2 were involved in machinery of neurodegeneration.

Epidemiological researches have suggested that a linkage between stress and neurodegeneration (Bissette, 2009; Friedl et al., 2009). The categorized stressors include "physiological" and "emotional" stressors (Dayas et al., 2001). Animal studies have showed that tau phosphorylation could be induced by physiological stressors such as food deprivation, forced swimming in cold water (Hartig et al., 2005, 2007; Yoshida et al., 2006). Recent studies also showed an elevated phosphorylated tau in emotional stress models (Rissman et al., 2007). However, the expression and distribution of MAPs in brain after stress are still not well elucidated. Restraint is a typical emotional stressor, which is related to the pathogenesis of depression (Yoshida et al., 2006). Acute restraint stress induces reversible increase of soluble phosphorylated tau in mouse brain, whereas chronic restraint stress induces both soluble and insoluble phosphorylated tau (Rissman et al., 2007).

In this study, we used the chronic restraint rat model, to investigate the effect of chronic restraint stress on the expression and distribution of phosphorylated tau and MAP2. We demonstrate that chronic restraint stress induced an increase in soluble phosphorylated tau and altered the distribution patterns of both phosphorylated tau and MAP2. The findings indicate that emotional stressor is able to cause phosphorylation of tau and abnormal distribution of tau and MAP2 in neuron.

2. Results

2.1. *Chronic restraint stress induces tau phosphorylation*

We initially determined whether the increase of hyperphosphorylated tau was observable in response to chronic restraint stress. Western analysis was used to detect phosphorylated tau at Ser202 site in the cortex and hippocampus extracts. We found that phosphorylated tau was significantly increased in soluble fraction extracted by RAB (Fig. 1), but did not change in RAB insoluble fraction which was subsequently extracted by RIPA buffer containing detergent (Fig. 2A). In addition, we did not find phosphorylated tau in the FA fraction which is considered as the NFT tau (Fig. 2B). These results indicate that after repeated restraint stress for 14 consecutive days, phosphorylated tau in rat brain was significantly increased in soluble form but not insoluble form which could be considered as pathological (paired helical filaments, PHF)-like tau.

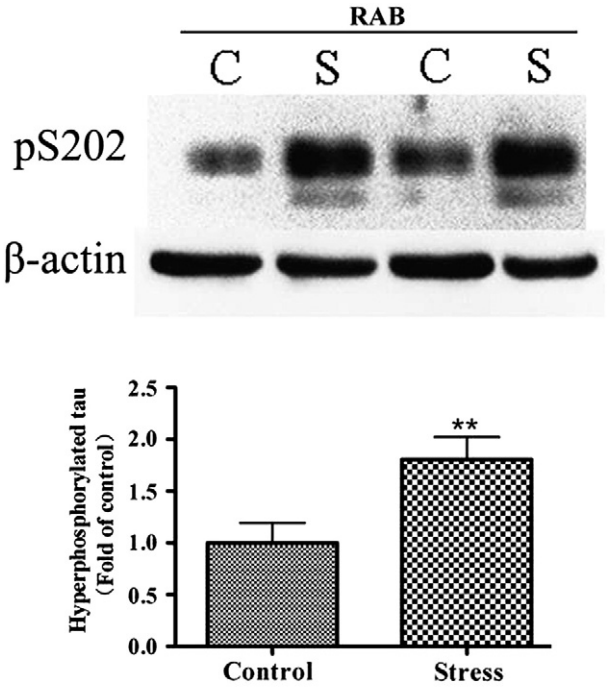

Fig. 1 – Chronic restraint stress increases soluble phosphorylated tau. Western blot analysis of cortex/hippocampus phosphorylated tau at Ser202 site under control (C) and chronic restraint stress (S) conditions. Quantitative analysis, expressed as mean ± S.E.M percentage of integrated intensity values of controls, reveals that stressed animals manifest comparably robust in phosphorylated tau at Ser202 site. Differs significantly from unstressed controls ($P < 0.01$), $n = 6$ rats per condition. β-Actin was used as a loading control.**

2.2. *The distribution patterns of phosphorylated tau changes after repeated restraint stress*

Our previous finding indicates that the distribution of phosphorylated tau in rat brain after cold water stress was markedly different compared with controls (Feng et al., 2005). To probe the localization of phosphorylated tau in chronic restraint stressed animals and control animals, immunohistochemistry method was employed with a anti-phospho-tau pS202 which recognizes tau phosphorylated at Ser202 site. The staining showed an increase of phosphorylated tau in the cortex and hippocampus of stressed animals compared with control ones (Figs. 3 and 4).