BRAIN RESEARCH 1383 (2011) 300–306

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BRAIN RESEARCH

Research Report

Effects of streptozotocin-induced diabetes on tau phosphorylation in the rat brain

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ARTICLE INFO

Article history:
Accepted 21 January 2011
Available online 31 January 2011

Keywords:
Hyperglycemia
Tau hyperphosphorylation
Glycogen synthase kinase-3
Protein phosphatase-2A

ABSTRACT

Brain protein kinase B (Akt) and glycogen synthase kinase-3 (GSK-3) activities are adaptable to changes of peripheral blood glucose level *in vivo*. GSK-3 phosphorylates microtubule-associated protein tau at multiple sites, which can be antagonized by protein phosphatase-2A (PP-2A). The imbalance among these enzymes might have potential connections with diabetes mellitus (DM) and Alzheimer's disease (AD). In this study hyperglycemia rat DM model was achieved by streptozotocin (STZ) treatment. The phosphorylation of tau in the rat hippocampus was detected with specific antibodies. Insulin and Li_2CO_3 administration were also employed to find out the regulatory efforts of the kinases. We observed that rat hippocampus tau was hyperphosphorylated at Ser^{396}/Ser^{404} (*PHF-1* sites) in STZ-induced DM model, accompanied by lowered phosphorylation levels of Akt, GSK-3 and PP-2A. Lithium, a specific GSK-3 inhibitor, nearly reversed all phosphorylation of tau at above sites in 30 days. Insulin administration restored the blood glucose level in DM rats but suppressed PP-2A activity, resulting in the *PHF-1* sites of tau not being dephosphorylated. These findings strongly suggest that STZ-induced hyperglycemia may cause disorder of Akt/GSK-3/PP-2A regulations in rat brain and further lead to abnormal phosphorylation of hippocampus tau.

1. Introduction

Diabetes mellitus (DM) is a common metabolic disease in human beings with characteristic symptoms of hyperglycemia and impaired insulin secretion or insulin resistant. It has been reported that about 60–70% of DM patients also present the diabetic neuropathy manifestations in either peripheral or central nervous systems (Manschot et al., 2008; Vinik, 2004). Many clinical studies indicated an association of DM with higher risks of cognitive impairment and neurodegenerative

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Abbreviations: AD, Alzheimer's disease; Akt, protein kinase B; DM, diabetes mellitus; GSK-3, glycogen synthase kinase-3; PHF, paired helical filament; PP-2A, protein phosphatase-2A; STZ, streptozotocin

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doi:10.1016/j.brainres.2011.01.084

diseases, such as Alzheimer's disease (AD). Furthermore, the co-occurrence of DM and AD is not rare in elderly individuals with chronic metabolic disorders (Areosa and Grimley, 2002; MacKnight et al., 2002; Janson et al., 2004; Stewart and Liolitsa, 1999). However, little is known about the underlying mechanisms of how DM and AD are coupled.

Hyperphosphorylation of brain microtube-associated protein tau has been proved key in AD etiopathogenesis (Alonso et al., 2008; Gong and Iqbal, 2008). Insulin, an important signaling molecule, is capable of regulating the activity of some kinases that are responsible for tau phosphorylation (Yan et al., 2003). Therefore, both abnormal insulin signaling and pathological glucose fluctuations may contribute to the tau hyperphosphorylation, particularly in those patients with diabetes. *In vivo*, insulin controls the intracellular and plasma glucose level through a multi-enzyme signaling cascade including Akt and GSK-3 (Taniguchi et al., 2006). Akt is a positive regulator in this pathway, which is activated by phosphorylation of Thr^{308} and Ser^{473} by insulin signaling. GSK-3 activity is inhibited when Ser^{21} of its α-isoform or Ser^{9} of its β-isoform (GSK-3β) is phosphorylated by Akt (Asano et al., 2007; Patel et al., 2004). Thus, down-regulation of insulin signaling leads to the dephosphorylation and consequent activation of GSK-3. GSK-3β phosphorylates tau at most sites in relation to tauopathies of AD (Mattson, 2001), which therefore could be the hinge of DM and AD. Based on these facts, insulin resistance or deficit could be a key factor that over-activates GSK-3 for tau hyperphosphorylation in DM patients. However, enhanced tau phosphorylation in rat brain was observed at conditions with either STZ-induced insulin deficiency or insulin administration-induced hyperinsulinemia in two different reports; Very interestingly, elevated blood glucose values were observed in both cases (Clodfelder-Miller et al., 2006; Freude et al., 2005). In mild cognitive impairment and AD patients, one fact that cannot neglect is the abnormal brain glucose utilization/transportation (Fellgiebel et al., 2004). Therefore, a possible casual relation between the glucose abnormality and tau phosphorylation may exist in AD. Jope group reported that Akt and GSK-3 were very sensitive to the circulating glucose level (Clodfelder-Miller et al., 2005). Moreover, in pancreatic islet β-cells, glucose was found to be able to affect PP-2A activity, a powerful phosphatase that dephosphorylates many kinases and almost all tau sites reported to date (Arendt et al., 1998; Kowluru, 2005; Wang et al., 2007). Therefore, it is quite possible that abnormal alteration of glucose level in DM patients plays a role in promoting brain tau phosphorylation by its influence on the kinases/phosphatase activities. Indeed it was reported that changes in the PP2A Bβ regulatory subunit during insulin deficiency seemed to be related to hyperphosphorylation in STZ treated mice (Planel et al., 2007).

To investigate if hyperglycemia affects brain tau phosphorylation via Akt/GSK3/PP-2A interplay, we embarked a long-term study (up to 30 days) with STZ-induced DM rat models. Phosphorylation of tau at multiple sites, accompanied by elevated GSK-3 activity and suppressed Akt/PP-2A actions, was detected in rat hippocampus in the current model. Insulin administration showed limited effects on the tau phosphorylation at *PHF-1* sites in STZ-treated rats due to its simultaneous suppression of both GSK-3 and PP-2A activities. Therefore our study showed that STZ-induced diabetes interfered Akt/GSK-3/PP-2A activities and further contributed to abnormal phosphorylation of hippocampus tau.

2. Results

2.1. *Persistent hyperglycemia in rats was induced by STZ*

To determine if the hyperglycemia animal model was built successfully, the fasting blood glucose (FBG) of rats was measured. A sharp increase of FBG level to 24.56±3.40 mM in STZ group was observed from the third day, which is about 5 times over the control group (5.08±0.58 mM). Moreover, intra-peritoneal injection of STZ successfully induced a persistent hyperglycemia up to 30 days in rats (14.82±0.94 mM). As summarized in Table 1, both insulin and lithium administration showed some immediate effects of lowering blood glucose level in STZ-treated rats in 3 days, although the FBG readings were still much higher than the control group at that time. In 30 days, daily injection of 5 IU/mg insulin eventually restored FBG to normal level. Although Li_2CO_3 feeding helped to decrease blood glucose level, FBG still remained at relatively high level (9.24±0.83 mM). Meanwhile, the level of plasma insulin was quantified by radioimmunoassay in all groups. As expected, STZ caused dramatic insulin deficit in rats. High insulin concentration was detected in STZ+insulin group due to the exogenetic insulin administration.

2.2. *Increased phosphorylation of tau at Tau-1 and PHF-1 sites in rats with STZ-induced hyperglycemia*

After 30 days' treatments, the level of tau phosphorylation was investigated in rat hippocampus by Western-blot using

Table 1 – Summary of four experimental groups.

Groups	Control group (Con)	STZ group (STZ)	STZ+insulin group Ins	STZ+Li_2CO_3 group (Li)
Number of rats	5	5	5	5
STZ injection	No	Yes	Yes	Yes
Insulin injection	No	No	Daily	No
Diet	Normal diet	Normal diet	Normal diet	Li_2CO_3 mixed diet
FBG 3 days (mM)	5.08±0.58	$24.56\pm3.40^{**}$	$16.98\pm2.29^{\#}$	21.02±2.11
FBG 30 days (mM)	5.42±0.50	$14.82\pm0.94^{**}$	$5.60\pm0.50^{\#\#}$	$9.24\pm0.83^{\blacktriangle}$
Plasma insulin (IU/L)	16.48±3.67	$6.12\pm1.27^{**}$	$22.76\pm4.32^{\#\#}$	$7.92\pm1.56^{\blacktriangle\blacktriangle}$

STZ and FBG are abbreviations of streptozotocin and fasting blood glucose respectively. All results are presented as mean±SD. $^{**}p<0.01$ *vs* control group; $^{\#}p<0.05$ *vs* STZ group, $^{\#\#}p<0.01$ *vs* STZ group; $^{\blacktriangle}p<0.05$ *vs* Ins group, $^{\blacktriangle\blacktriangle}p<0.01$ *vs* Ins group.