Archives of Biochemistry and Biophysics 636 (2017) 42–49

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

JNK signaling pathway regulates sorbitol-induced Tau proteolysis and apoptosis in SH-SY5Y cells by targeting caspase-3

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ARTICLE INFO

Keywords:
Tau proteolysis
Hyperosmotic stress
MAPK
Caspase-3
Apoptosis

ABSTRACT

Growing evidence suggests that Diabetes Mellitus increases the risk of developing Alzheimer's disease. It is well known that hyperglycemia, a key feature of Diabetes Mellitus, may induce plasma osmolarity disturbances. Both hyperglycemia and hyperosmolarity promote the altered post-translational regulation of microtubule-associated protein Tau. Interestingly, abnormal hyperphosphorylation and cleavage of Tau have been proven to lead to the genesis of filamentous structures referred to as neurofibrillary tangles, the main pathological hallmark of Alzheimer's disease. We have previously described that hyperosmotic stress induced by sorbitol promotes Tau proteolysis and apoptosis in SH-SY5Y cells via caspase-3 activation. In order to gain insights into the regulatory mechanisms of such processes, in this work we explored the intracellular signaling pathways that regulate these events. We found that sorbitol treatment significantly enhanced the activation of conventional families of MAPK in SH-SY5Y cells. Tau proteolysis was completely prevented by JNK inhibition but not affected by either ERK1/2 or p38 MAPK blockade. Moreover, inhibition of JNK, but not ERK1/2 or p38 MAPK, efficiently prevented sorbitol-induced apoptosis and caspase-3 activation. In summary, we provide evidence that JNK signaling pathway is an upstream regulator of hyperosmotic stress-induced Tau cleavage and apoptosis in SH-SY5Y through the control of caspase-3 activation.

1. Introduction

Tau belongs to the microtubule-associated proteins family. It is mainly expressed in neurons of both central (CNS) and peripheral (PNS) nervous system, where it regulates the organization and integrity of microtubules network by binding to tubulin monomers, among other functions [1]. In a set of neurodegenerative disorders referred to as tauopathies, Tau dissociates from microtubules and eventually auto-assembles into insoluble filamentous structures that accumulate in a variety of brain regions. The most prevalent tauopathy is Alzheimer's disease (AD). In neurons from AD patients, Tau aggregates into neurofibrillary tangles (NFT), that have been considered to be a key component of the pathological cascade leading to neurodegeneration [2,3]. It has been largely documented that post-translational modifications, mainly hyperphosphorylation and proteolysis, alter Tau biology and facilitate its aggregation [4,5]. Aberrant Tau fragmentation has been attributed to caspases, calpains and the proteasome system [6–9]. However, the precise molecular mechanisms underlying Tau cleavage remain elusive.

In the last years, it has been suggested that Diabetes Mellitus may increase the risk of developing AD [10]. Although epidemiological studies highlight a strong association between Type-2 Diabetes Mellitus and the development of both cognitive dysfunction and different types of dementia [11], there is not yet a clear knowledge about the pathophysiological events that link these two types of disorders associated with elderly patients. Brain homeostasis is commonly disrupted in a broad spectrum of neurological pathologies, including AD. Maintenance of brain homeostasis requires, among others, a proper osmotic equilibrium. It is well known that hyperglycemia, a common feature of both Type-1 and Type-2 Diabetes Mellitus, may induce plasma osmolarity disturbances [12]. This occurs through the polyol pathway, an accessory metabolic pathway that converts glucose to fructose bypassing glycolysis, with the generation of sorbitol as an intermediate metabolite [13,14]. The polyol pathway has been proven to be active throughout

Abbreviations: AD, Alzheimer's disease; CNS, central nervous system; HHS, hyperglycemic hyperosmolar state; NFT, neurofibrillary tangles; PNS, peripheral nervous system

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https://doi.org/10.1016/j.abb.2017.11.004
Received 29 June 2017; Received in revised form 19 October 2017; Accepted 5 November 2017
Available online 07 November 2017
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the human body, and thus in brain [15,16]. Taken into account that the main feature of sorbitol is its ability to induce hyperosmotic stress, one possible mechanism by which Type-2 Diabetes Mellitus links AD may be through disruption of environmental osmolarity. In fact, osmolarity disturbances have been proven to be responsible for a wide range of neurodegenerative events associated to AD, such as neuronal apoptosis [17,18] and both Tau hyperphosphorylation [17] and proteolysis [18].

In response to disturbances in fluid and electrolyte homeostasis, neurons integrate osmolar signals at the molecular level in order to initiate compensatory and adaptive responses. One of the essential mechanisms to do this is through regulatory protein kinases that coordinate intracellular signaling pathways. In mammals, the mitogen-activated protein kinases family (MAPK) comprises four conventional components: p38 MAPK, JNK, ERK1/2 and ERK5 [19]. MAPK regulate diverse cellular programs including apoptosis, proliferation, survival or differentiation [19]. It is well established that several members of the MAPK family become activated in response to various types of cellular stresses, including hyperosmotic shock [19–21]. Besides this finding, it has been found an increased expression of active MAPK kinases co-localizing with abnormal Tau deposits, including NFT, in individual neurons in AD [22,23]. Furthermore, it has been suggested that the sequential activation of ERK1/2 and caspase-3 mediates the neurotoxic effects of β-amyloid, a key molecule involved in the initiation and propagation of AD, in rat hippocampal neurons through Tau cleavage [24].

We have previously described that hyperosmotic stress induces both Tau proteolysis and apoptosis in human neuroblastoma SH-SY5Y cells via activation of caspase-3 [18]. In order to gain insights into the regulatory mechanisms of these processes, and taken into account the findings above described, in this work we have investigated the role of MAPK signaling pathways in sorbitol-induced Tau proteolysis and apoptosis in this cell line. We provide evidence that JNK inhibition leads to an efficient prevention of caspase-3 activation, which in turns blocks sorbitol-induced Tau cleavage and apoptosis observed in this model.

2. Materials and methods

2.1. Reagents and antibodies

D-sorbitol was purchased from Sigma-Aldrich, (St. Louis, MO, USA). Tissue culture reagents were from BioWhitaker (Walkersville, MD, USA). Tissue culture flasks and dishes were from Fisher Scientific (Madrid, Spain). Complete protease inhibitor cocktail tablets were from Roche Molecular Biochemicals (Indianapolis, IN, USA). SB203580 and PD98059 inhibitors were from Calbiochem (San Diego, CA, USA). BIRB0796 was from Axon Medchem (Groningen, The Netherlands). PD184352 was from Selleck Chemicals (Houston, USA). JNK inhibitor I, (L)-Form, Cell-permeable was from Calbiochem, Merck (Darmstadt, Germany). Caspase-3 fluorogenic substrate *N*-acetyl-Asp-Glu-Val-Asp-7-amido-4-methylcoumarin was from BD Biosciences (San José, CA, USA). Anti-Tau antibody was generated by injecting rabbits with glutathione S-transferase (GST)-tagged Tau [25]. Phospho-p38 MAPK (Thr180/Tyr182) (product number 9211), p38 MAPK (product number 9212), phospho-SAPK/JNK (Thr183/Tyr185) (product number 9251), SAPK/JNK (product number 9252), MAPKAPK-2 (product number 3042), phospho-p44/42 MAPK (ERK1/2, Thr202/Tyr204) (product number 9101), p44/42 MAPK (ERK1/2) (product number 9102) and cleaved Caspase-3 (Asp175) (product number 9661) antibodies were from Cell Signaling Technology (Beverly, MA, USA). Anti-*c*-Jun (pS63) (product number 558036) was from BD Biosciences. Anti-actin antibody (product number A2066 from Sigma-Aldrich) was used to verify the equal loading of protein across lanes. Goat anti-rabbit IgG (H + L) secondary antibody HRP-conjugated and goat anti-mouse IgG (H + L) secondary antibody HRP-conjugated were from Pierce (Rockford, IL, USA). Other reagents were obtained from different commercial sources and were of the highest purity available.

2.2. Cell cultures and treatments

Undifferentiated SH-SY5Y human neuroblastoma cells were grown, seeded and stimulated with 0.5 M sorbitol as it was described in Ref. [18]. When required, cells were pre-incubated for 1 h with MAPK inhibitors (SB201580, BIRB0796, PD98059, PD184352 or JNK inhibitor I), prior to stimulation with sorbitol for 1 h.

2.3. Western blot analysis

Treated and control cells were lysed as it was described in Ref. [18]. After lysis, cell debris was removed by centrifugation at 20000*g* for 5 min at 4 °C and protein concentrations in the supernatants were determined using the Bio-Rad protein assay, according to the instructions of the manufacturer. Equal amounts of protein (10–30 μg) were separated on 10%–12% SDS-PAGE and blotted onto nitrocellulose membranes (Bio-Rad Laboratories, Hercules, CA, USA). The membranes were then blocked with 5% non-fat dry milk Tris-buffered saline (pH 7.5) containing 0.5% Tween-20 and incubated with appropriate primary antibodies (anti-Tau 0.2 μg/ml, anti-actin 0.25 μg/ml, anti-Phospho-ERK1/2 dilution 1:1000, anti-ERK1/2 dilution 1:1000, anti-Phospho-p38 MAPK dilution 1:1000, anti-p38 MAPK dilution 1:1000, anti-Phospho-SAPK/JNK dilution 1:1000, anti-SAPK/JNK dilution 1:1000, anti-*c*-Jun (pS63) 0.25 μg/ml, anti-cleaved Caspase-3 dilution 1:1000 and anti-Actin dilution 1:1000) and horseradish peroxidase (HRP)-conjugated secondary antibodies (goat anti-rabbit 0.2 μg/ml and goat anti-mouse 0.5 μg/ml) in antibody buffer [5% non-fat dry milk in Tris-buffered saline (pH 7.5) containing 0.05% Tween-20 (TBS-T)]. After required washes with TBS-T, proteins were analysed using an enhanced chemiluminescence detection system (SuperSignal West Pico Chemiluminescent Substrate, Pierce, Rockford, IL, USA) and exposed to hyperfilm-ECL (Amersham, Pharmacia Biotech, Freiburg, Germany). Films were scanned and the intensity of each band was analysed using the ImageJ program. Results were expressed as percentage of control.

2.4. Caspase-3 activity assay

Caspase-3 activity was determined by using the fluorogenic peptide substrate *N*-acetyl-Asp-Glu-Val-Asp-7-amido-4-methylcoumarin as it was described in Ref. [18].

2.5. Analysis of nuclear DNA content by flow cytometry

The ploidy determination of cells was estimated by flow cytometry DNA analysis as previously described [26]. DNA content per nucleus was then analysed in a FC500 flow cytometer (Beckman Culter, Hialeah, FL, USA).

2.6. Statistical analysis

Each experiment was repeated at least three times, with good agreement among the results of individual experiments. All data are expressed as the mean ± standard error of the mean. Results were analysed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. P values of less than 0.05 were considered significant.

3. Results

3.1. Hyperosmotic stress promotes the phosphorylation of conventional families of MAPK

We have recently described that hyperosmotic stress induced by sorbitol stimulates Tau proteolysis and apoptosis in SH-SY5Y cells.

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