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Role of calpain and caspase in β-amyloid-induced cell death in rat primary septal cultured neurons

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Abstract

The invariant characteristic features associated with Alzheimer's disease (AD) brain include the presence of extracellular neuritic plaques composed of amyloid β (Aβ) peptide, intracellular neurofibrillary tangles containing hyper-phosphorylated tau protein and the loss of basal forebrain cholinergic neurons. Studies of the pathological changes that characterize AD and several other lines of evidence indicate that in vivo accumulation of $A\beta_{1-42}$ may initiate the process of neurodegeneration observed in AD brains. However, the cause of degeneration of the basal forebrain cholinergic neurons and their association to Aβ peptides or phosphorylated tau protein have not been clearly established. In the present study, using rat primary septal cultures, we have shown that $A\beta_{1-42}$, in a time (1–48 h) and concentration (0.01–20 μM)-dependent manner, induce toxicity in cultured neurons. Subsequently, we have demonstrated that Aβ toxicity is mediated via activation of cysteine proteases, i.e., calpain and caspase, and proteolytic breakdown of their downstream substrates tau, microtubule-associated protein-2 and αII-spectrin. Additionally, Aβ-treatment was found to induce phosphorylation of tau protein along with decreased levels of phospho-Akt and phospho-Ser^9glycogen synthase kinase-3β. Exposure to specific inhibitors of caspase or calpain can partially protect cultured neurons against Aβ-induced toxicity but their effects are not found to be additive. These results, taken together, suggest that Aβ peptide can induce toxicity in rat septal cultured neurons by activating multiple intracellular signaling molecules. Additionally, evidence that inhibitors of caspase and calpains can partially protect the cultured basal forebrain neurons raised the possibility that their inhibitors could be of therapeutic relevance in the treatment of AD pathology.

Keywords: Alzheimer's disease; Akt kinase; Glycogen synthase kinase-3β; Neurodegeneration; Neuroprotection; Tau phosphorylation

1. Introduction

Diverse proteolytic enzymes are known to be involved in mediating both acute and chronic neuronal cell death. A family of cysteine proteases referred to as caspases has been mechanistically implicated as a key executor of the cell death mediated via apoptosis. To date, 14 caspases have been identified that can be subdivided into three broad categories; initiator caspases, pro-inflammatory caspases and executioner caspases. Activation of caspase-3, which acts as a common downstream effector, has been suggested to play an important role in execution of the apoptotic cascade during development and degenerative disorders (Chan and Mattson, 1999; Fan et al., 2005; Wang, 2000). In addition to caspases, calcium-dependent cysteine proteases calpains have been shown to influence

Abbreviations: Aβ, beta-amyloid peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; DEVD, Z-DEVD-FMK; DIV, days in vitro; ECL, enhanced chemiluminescence; EM, electron microscopy; GSK-3β, glycogen synthase kinase-3β; HBSS, Hank's balanced salt solution; MAP2, microtubule-associated protein-2; MTT, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide; PBS, phosphate-buffered saline; PI3K, phosphatidylinositol 3-kinase; PFA, paraformaldehyde; SBDP, αII-spectrin breakdown products.

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apoptotic cascade by regulating the proteolytic cleavage of target proteins. These proteases are ubiquitously expressed usually in two different isoforms (μ- and m-calpains) and their activity is known to be regulated by a highly specific endogenous inhibitor named calpastatin (Nixon, 2003; Raynaud and Marcilhac, 2006).

A growing body of recent studies has suggested that calpains and caspases may work independently (Lankiewicz et al., 2000; Wang, 2000) or co-operatively (Fifre et al., 2006; Nath et al., 1996; Neumar et al., 2003; Newcomb-Fernandez et al., 2001) to influence cell death mechanism observed in a variety of experimental paradigms. Cross-talk between the two cysteine proteases is suggested by the evidence that: i) both enzymes can cleave many common substrates including cytoskeletal and regulatory proteins with varying functional consequences; ii) caspases can indirectly influence calpain activity by regulating cleavage of calpastatin; and iii) calpains can mediate cleavage of a number of caspases which can lead to either activation or inhibition of their activity (Nixon, 2003; Raynaud and Marcilhac, 2006). At present, however, very little is known about the spatial and temporal activation of these two proteases and their possible interaction in neurodegenerative diseases where apoptosis has been considered to play a major role in the loss of neurons.

Alzheimer's disease (AD), the most common cause of dementia affecting elderly people, is a progressive neurodegenerative disorder characterized by the presence of intracellular neurofibrillary tangles and extracellular neuritic plaques in affected regions of the brain (Lopez and DeKosky, 2003; Selkoe and Schenk, 2003). Neuritic plaques contain a compact deposit of β-amyloid (Aβ) peptides surrounded by dystrophic neurites and activated glial cells, whereas neurofibrillary tangles consist of hyperphosphorylated form of a microtubule associated protein tau (Brion et al., 2001; Iqbal et al., 2005). Loss of synapses and neurons that occur primarily in cortex, hippocampus and certain subcortical regions is also considered to be one of the cardinal features of AD (Lopez and DeKosky, 2003; Selkoe and Schenk, 2003). Of all the vulnerable areas, the basal forebrain cholinergic neurons, which project to the hippocampus and neocortex, are reported to be most severely affected in AD brains. It is suggested that loss of cortical and hippocampal cholinergic innervations contribute to the progressive cognitive deficits observed in AD patients (Auld et al., 2002; Francis et al., 1999; Kar et al., 2004; Schliebs, 2005). Accompanying these structural lesions, there is also evidence that activity of caspases and calpains are increased in vulnerable regions of the AD brain even before synaptic loss, tau phosphorylation and neuronal degeneration. The active forms of caspases and calpains are found to co-localize with neurofibrillary tangles, senile plaques and dystrophic neurites thus raising the possibility that these proteases may be involved in the cytoskeletal disorganization and degeneration of neurons in AD (Cotman et al., 2005; Cribbs et al., 2004; Nixon, 2003; Saito et al., 1993; Vanderklish and Bahr, 2000). However, at present, neither the cause of preferential decimation of the basal forebrain cholinergic neurons nor their association to caspases or calpains has been established.

Earlier studies by our group and others have shown that Aβ-related peptides by inducing phosphorylation of tau protein can lead to the destabilization of microtubules, impaired axonal transport and eventual death of basal forebrain cholinergic neurons (Alvarez et al., 2002; Le et al., 1997; Zheng et al., 2002). However, very little is known about the intracellular pathways involved in Aβ-mediated tau phosphorylation or toxicity in these neurons. Additionally, no information is available whether caspases and calpains can act together or independently to regulate the toxic effects of Aβ peptides on cholinergic neurons. In the present study, using primary rat septal cultured neurons, we report that Aβ peptide, at μM concentrations, can induce loss of neurons via activation of specific intracellular signaling pathways involving caspases and calpains. Our results also indicate that calpains and caspases act together and inhibition of either protease can protect neurons against Aβ-mediated toxicity thus suggesting a possible therapeutic implication of these proteases inhibitors in attenuating the loss of forebrain cholinergic neurons observed in AD pathology.

2. Experimental

2.1. Materials

Time-pregnant Sprague–Dawley rats obtained from Charles River (St. Constant, QC, Canada) were maintained according to guidelines approved by the University of Alberta Animal Care Committee and the Canadian Council for Animal Care. $A\beta_{1-42}$ and its reverse sequence $A\beta_{42-1}$ were purchased from American Peptide (Sunnyvale, CA), cell permeable calpain inhibitor calpastatin was from Calbiochem (La Jolla, CA) and cell permeable caspase-3 inhibitor Z-DEVD-FMK was from R&D Systems (Minneapolis, MN). Polyacrylimide electrophoresis gels (4–20%) were purchased from Invitrogen (Burlington, Canada) and enhanced chemiluminescence (ECL) kit was from Amersham (Mississauga, Canada). Anti-phospho-Ser^{9}glycogen synthase kinase-3β (GSK-3β), anti-phospho-Ser^{473}Akt, anti-caspase-3, anti-caspase-9 and anti-Akt antisera were purchased from Cell Signaling (Mississauga, Canada), whereas anti-GSK-3β was from BD Transduction Labs (Mississauga, Canada). Anti-phospho-tau AT180(Thr231) and AT270 (Thr^{181}) were from Innogenetics, Belgium. Anti-calpain-1, anti-β-actin and Hoechst 33258 were from Sigma-Aldrich (Mississauga, Canada), whereas anti-non-erythroid αII-spectrin was from BioMol (Plymouth Meeting, PA). Anti-microtubule associated protein-2 (MAP2), anti-total-tau and all secondary antibodies were purchased from Santa Cruz Biotechnology (San Diego, CA). Cell culture reagents such as N2, B27, trypsin were purchased from Invitrogen (Carlsbad, CA), whereas all other reagents were from Sigma-Aldrich or Fisher Scientific (Nepean, Canada).

2.2. Rat septal neuronal culture

Primary septal cultures were prepared from 17-day-old fetus of time-pregnant Sprague–Dawley rats as described before (Zheng et al., 2002). In brief, septal area was dissected in calcium- and magnesium-free Hank's balanced salt solution (HBSS) supplemented with 15 mM HEPES, 10 U/ml penicillin and 10 μg/ml streptomycin. Collected tissues were washed with HBSS and then digested at 37 °C with 0.25% trypsin in HBSS for 15 min. Cell suspension was then prepared by repeated aspirations through a Pasteur pipette. Following centrifugation at 800 × *g* for 10 min, the medium was removed and cells were resuspended in a chemically defined serum-free NeuroBasal medium supplemented with 1% N_2, 2% B27, 50 μM L-glutamine, 15 mM HEPES, 10 U/ml penicillin and 10 μg/ml streptomycin. Neurons were then