ELSEVIER

Neurobiology of Aging 29 (2008) 357–367

NEUROBIOLOGY OF AGING

www.elsevier.com/locate/neuaging

Activation of protein kinase C modulates BACE1-mediated β-secretase activity

Lizhen Wang, Hoon Shim, Chengsong Xie, Huaibin Cai *

Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, MD 20892-3707, United States

Received 12 July 2006; received in revised form 21 September 2006; accepted 7 November 2006
Available online 6 December 2006

Abstract

β-Site APP cleavage enzyme 1 (BACE1) is the β-secretase responsible for generating amyloid-β (Aβ) peptides in Alzheimer's disease (AD). Previous studies suggest that activation of protein kinase C (PKC) modulates the β-secretase-mediated cleavage of APP and reduces the production of Aβ. The mechanism of PKC-mediated modulation of β-secretase activity, however, remains elusive. We report here that activation of PKC modulated β-secretase activity through either suppressing the accumulation or promoting the translocation of BACE1 protein in a cell type-dependent manner. We found that activation of PKC suppressed the accumulation of BACE1 protein in fibroblasts through an enhancement of intracellular protease activities. In neurons, activation of PKC did not alter the expression level of BACE1, but led to more BACE1 translocated to the cell surface, resulting in a decreased cleavage of APP at the β1 site. Together, Our findings provide novel mechanisms of PKC-mediated modulation of β-secretase activity, suggesting that alteration of the intracellular trafficking of BACE1 may serve as a useful therapeutic strategy to lower the production of Aβ in AD.
Published by Elsevier Inc.

Keywords: BACE1; APP; PKC; Protein degradation; Protein translocation; Amyloid β; β-Secretase; Fibroblast; Neuron

1. Introduction

A wide array of studies have demonstrated that genetic mutations linked to Alzheimer's disease (AD) invariably increase the production and deposition of amyloid β (Aβ) peptides, strongly supporting the idea that excessive accumulation of Aβ peptides contributes to the pathogenesis of AD (Hardy and Higgins, 1992). Aβ peptides are derived from amyloid precursor protein (APP) by sequential endoproteolytic cleavages of β- and γ-secretases (Selkoe, 1994). Alternatively, APP is cleaved by α- and γ-secretases to generate the non-pathogenic p3 fragments (Selkoe, 1994). β-APP cleaving enzyme 1 (BACE1) is the β-secretase responsible for generation of Aβ peptides (Vassar et al., 1999). BACE1 cleaves APP at both β1 and β11 sites and generates β1 and β11 carboxyl terminal fragments (CTF) of APP (Cai et al., 2001; Vassar et al., 1999). Genetic deletion of BACE1 abolishes the generation of APP β1 and β11 CTFs, prevents the generation and deposition of Aβ peptides, and rescues cognitive impairments and other neuropathological abnormalities, such as dystrophic neurites, astrogliosis, and microgliosis in transgenic mice overexpressing mutant APP (Laird et al., 2005; Ohno et al., 2004). Although cognitive deficits, emotional alterations, and premature lethality are reported in BACE1-deficient mice (Harrison et al., 2003; Laird et al., 2005; Ohno et al., 2004), it seems that partial inhibition of BACE1 expression, which does not cause obvious side effects, is sufficient to reduce amyloid accumulation and improve the cognitive function of APP transgenic mice (Laird et al., 2005; Singer et al., 2005). Thus, we decided to investigate the intracellular signaling transduction pathways that partially suppress the expression and function of BACE1 for their potential therapeutic values in AD.

Previous studies suggest that activation of protein kinase C (PKC)-mediated intracellular signal transduction pathways

* Corresponding author at: Building 35, Room 1A116, 35 Convent Drive, Bethesda, MD 20892-3707, United States. Tel.: +1 301 402 8087; fax: +1 301 480 2830.
E-mail address: caih@mail.nih.gov (H. Cai).

0197-4580/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.neurobiolaging.2006.11.001

affects α and β-secretase-mediated cleavage of APP, resulting in a reduction of Aβ peptides (Buxbaum et al., 1992; Hung et al., 1993; Savage et al., 1998). However, the mechanism of PKC-mediated inhibition of β-secretase activity is unknown. To address this question, we examined the role of PKC in BACE1 protein expression and its β-secretase activity in mouse fibroblasts and primary cultured cortical neurons. We found that the level of BACE1 protein in mouse fibroblasts was down regulated by phorbol myristate acetate (PMA), a PKC activator, through an enhancement of intracellular protease-mediated protein degradation pathways. The expression of BACE1 protein in primary cultured mouse cortical neurons, however, was not affected by PMA treatment. Instead, application of PMA increased the presentation of BACE1 as well as APP proteins at the plasma membrane, resulting in a reduction of APP β1 CTF in neurons. Therefore, that the PMA-induced increase of BACE1 degradation in fibroblasts and BACE1 translocation to the cell surface in neurons underlines novel mechanisms of PKC-mediated suppression of β-secretase activity at APP β1 site.

2. Materials and methods

2.1. Generation of mouse fibroblasts

Fibroblast cultures were established from postnatal day 1 wild type and BACE1$^{-/-}$ pups. Briefly, the skins were dissected, minced, plated on culture dishes and incubated at 37 °C in medium containing Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, Carlsbad, CA) and 10% FBS (Invitrogen) for 7 days. The resulting monolayer of primary fibroblasts were dissociated by trypsin digestion and expanded, and then immortalized with transfection of large T antigen.

2.2. Cell culture and treatment

Fibroblast cell lines were cultured in DMEM (Invitrogen) supplemented with 10% FBS, penicillin (100 units/ml), streptomycin (100 μg/ml) (Invitrogen) and maintained at 37 °C in a humidified 5% CO_2 atmosphere. The cells (10^6) were seeded in 6 well plates and stimulated 18 h later with PMA (Sigma, St. Louis, MO) for various times. Where indicated, the cells were preincubated with appropriate inhibitors 30 min before PMA stimulations and the inhibitors were maintained during the stimulations. PMA and all inhibitors, except for chloroquine (Sigma), were solubilized in Dimethyl sulfoxide (DMSO). Chloroquine was solubilized in water.

2.3. Western blotting

Cells were washed twice with ice-cold phosphate-buffered saline (PBS) and lysed in a buffer containing 50 mM Tris–HCl, pH 7.4, 1% nonidet P-40 (NP-40), 0.4% sodium dodecyl sulphate (SDS) and a protease inhibitor mixture (Roche Diagnostic, Alameda, CA). Whole cell lysates were prepared from the supernatant fraction after centrifugation at 12,000 × *g* for 10 min at 4 °C. Protein concentrations were determined by the bicinchoninic acid method (Pierce, Rockford, IL) using bovine serum albumin (BSA) as standard. For Western blotting, 10 μg of total protein were separated by NuPage 4–12% BisTris-polyacrylamide gel electrophoresis (Invitrogen) using MES running buffer (Invitrogen) for BACE1 and full-length APP or by Novex 16% Tricine gel electrophoresis (Invitrogen) for APP CTFs. Separated proteins were then transferred to polyvinylidene difluoride membranes (PVDF) and incubated with antibody specific for BACE1 (Cai et al., 2001), or the C-terminal of APP (Sigma) at a 1:2000 dilution. Bound antibodies were detected by the enhanced chemiluminiscent method. Membranes were stripped to prepare them for a second round of probing with β-actin or β-tubulin antibodies (Chemicon, Temecula, CA; 1:5000 dilution).

2.4. Surface biotinylation

Fibroblasts and neurons were grown in 35-mm^2 dishes and treated with vehicle (DMSO) or PMA for the indicated times. Cell surface biotinylation was performed as described previously (O'Brien et al., 1997). Briefly, cells were cooled on ice, washed two twice with ice-cold labeling buffer containing: 125 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 1 mM NaH_2PO_4, 10 mM dextrose, 2.5 mM $CaCl_2$, 1.25 mM $MgCl_2$, and 5% CO_2, and then incubated with labeling buffer containing 1 mg/ml Sulfo-NHS-LC-Biotin (Pierce) for 20 min on ice. Unreacted biotinylation reagent was washed with ice-cold labeling buffer and quenched by two successive 20 min washes in labeling buffer containing 100 mM glycine on ice, followed by two washes in ice-cold TBS (50 mM Tris, pH 7.5, 150 mM NaCl). Cultures were harvested in modified RIPA buffer (1% Triton X-100, 0.5% SDS, 0.5% deoxycholic acid, 50 mM $NaPO_4$, 150 mM NaCl, 2 mM EDTA, 50 mM NaF, 10 mM sodium pyrophosphate, 1 mM sodium orthovanadate, and protease inhibitor complex). The lysates were cleared by centrifugation for 15 min at 14,000 × *g* at 4 °C. The resulting supernatant was incubated with 100 μl of 50% NeutraAvidin agarose (Pierce) for 3 h at 4 °C. The NeutraAvidin agarose was washed five times with RIPA buffer. Bound proteins were eluted with SDS sample buffer by boiling for 15 min.

2.5. β-Secretase activity assay

The quantification of β-secretase activity in fibroblast cell lines or primary cultured neurons was carried out according to manufacturer's instructions with minor modifications (R&D Systems, Minneapolis, MN). Briefly, fibroblast cells or neurons were washed in ice-cold PBS and incubated in extraction buffer for 1 h on ice. Cells were homogenized in extraction buffer, and centrifuged at 10,000 × *g* for 1 min. Supernatant (50 μl) was added to each well in microplate and mixed with 50 μl 2× reaction buffer and 5 μl substrate. The plates were