Neurochemistry International 58 (2011) 321–329

Contents lists available at ScienceDirect

Neurochemistry International

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

Effects of fatty acid unsaturation numbers on membrane fluidity and α-secretase-dependent amyloid precursor protein processing

Xiaoguang Yang [a], Wenwen Sheng [b,c], Grace Y. Sun [b,c], James C.-M. Lee [a,*]

[a] Department of Biological Engineering, University of Missouri, Columbia, MO 65211, United States
[b] Department of Pathology and Anatomical Sciences, University of Missouri, Columbia, MO 65211, United States
[c] Department of Biochemistry, University of Missouri, Columbia, MO 65211, United States

ARTICLE INFO

Article history:
Received 5 June 2010
Received in revised form 17 October 2010
Accepted 7 December 2010
Available online 22 December 2010

Keywords:
Fatty acids
Membrane fluidity
$sAPP_\alpha$
Alzheimer's disease

ABSTRACT

Fatty acids may integrate into cell membranes to change physical properties of cell membranes, and subsequently alter cell functions in an unsaturation number-dependent manner. To address the roles of fatty acid unsaturation numbers in cellular pathways of Alzheimer's disease (AD), we systematically investigated the effects of fatty acids on cell membrane fluidity and α-secretase-cleaved soluble amyloid precursor protein ($sAPP_\alpha$) secretion in relation to unsaturation numbers using stearic acid (SA, 18:0), oleic acid (OA, 18:1), linoleic acid (LA, 18:2), α-linolenic acid (ALA, 18:3), arachidonic acid (AA, 20:4), eicosapentaenoic acid (EPA, 20:5), and docosahexaenoic acid (DHA, 22:6). Treatments of differentiated human neuroblastoma (SH-SY5Y cells) with AA, EPA and DHA for 24 h increased $sAPP_\alpha$ secretion and membrane fluidity, whereas those treatments with SA, OA, LA and ALA did not. Treatments with AA and DHA did not alter the total expressions of amyloid precursor protein (APP) and α-secretases in SH-SY5Y cells. These results suggested that not all unsaturated fatty acids but only those with 4 or more double bonds, such as AA, EPA and DHA, are able to increase membrane fluidity and lead to increase in $sAPP_\alpha$ secretion. This study provides insights into dietary strategies for the prevention of AD.

1. Introduction

The accumulation of neurotoxic amyloid-β peptide (Aβ) is a pathologically profound characteristic of Alzheimer's disease (AD) (McGeer et al., 1987; Perlmutter et al., 1990; Frautschy et al., 1998; Dickson, 1999; Stalder et al., 1999; Selkoe, 2000). Aβ is derived from amyloid precursor protein (APP) processing through the amyloidogenic pathway, in which APP is cleaved sequentially by β- and γ-secretases (Vassar, 2004). BACE1 is the major β-secretase for generation of Aβ by neurons (Cai et al., 2001). Alternatively, in the non-amyloidogenic pathway, APP is cleaved by α-secretases between amino acids 16 and 17 within the Aβ domain to produce $sAPP_\alpha$. α-Secretases are members of ADAM (a disintegrin and metalloprotease), including ADAM9, 10, 17 and 19. $sAPP_\alpha$ is neurotrophic and neuroprotective (Thornton et al., 2006) and enhancing APP processing by α-secretases has been suggested as a potential therapeutic strategy for AD (Cheng et al., 2007). Since APP, α-, β- and γ-secretases are membrane protein molecules, APP processing should be governed by the local membrane environment. For example, the activity of β-secretase takes place preferentially in highly molecularly ordered lipid rafts which are cholesterol, saturated phospholipids and sphingolipid-enriched microdomains (Tun et al., 2002; Cordy et al., 2003; Ehehalt et al., 2003; Marlow et al., 2003; Kaether and Haass, 2004; Vetrivel et al., 2004), while the activity of α-secretase is favorable in non-raft domains (Reid et al., 2007). Therefore, APP processing can be manipulated by changing the contents of membrane components, such as cholesterol and sphingolipids (Simons et al., 1998; Kojro et al., 2001; Sawamura et al., 2004; von Arnim et al., 2008).

Since fatty acids are capable of modulating membrane organization and functions (Yehuda et al., 2002; Stillwell et al., 2005; Shaikh and Edidin, 2006, 2008; Pepe, 2007), we hypothesized that the effects of fatty acids (e.g., AA) on membrane fluidity and $sAPP_\alpha$ secretion are dependent on their unsaturation numbers (i.e. the number of double bonds in the hydrocarbon chains). In this

Abbreviations: AA, arachidonic acid; AD, Alzheimer's disease; ADAM, a disintegrin and metalloprotease; ALA, α-linolenic acid; APP, amyloid precursor protein; Aβ, amyloid-β peptide; BCA, bicinchoninic acid; BSA, bovine serum albumin; DHA, docosahexaenoic acid; DMEM, Dulbecco's modified Eagle's medium; DMSO, dimethyl sulfoxide; EPA, eicosapentaenoic acid; FBS, fetal bovine serum; FCVJ, farnesyl-(2-carboxy-2-cyanovinyl)-julolidine; FRAP, fluorescence recovery after photobleaching; LA, linoleic acid; MβCD, methyl-β-cyclodextrin; NF-κB, nuclear factor-κB; OA, oleic acid; PF68, Pluronic F68; PMA, phorbol 12-myristate 13-acetate; PUFA, polyunsaturated fatty acid; RA, all-*trans* retinoic acid; SA, stearic acid; $sAPP_\alpha$, α-secretase-cleaved soluble APP; $sPLA_2$-III, secretory phospholipase A_2 type III.

* Corresponding author at: 240 Agricultural Engineering Building, University of Missouri, Columbia, MO 65211, United States.
Tel.: +1 573 884 3686; fax: +1 573 882 1115.
E-mail address: leejam@missouri.edu (James C.-M. Lee).

0197-0186/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuint.2010.12.004

study, we systematically examined the effects of fatty acids with unsaturation numbers ranging from 0 to 6 double bonds including stearic acid (SA, 18:0), oleic acid (OA, 18:1), linoleic acid (LA, 18:2), α-linolenic acid (ALA, 18:3), arachidonic acid (AA, 20:4), eicosapentaenoic acid (EPA, 20:5) and docosahexaenoic acid (DHA, 22:6) on membrane fluidity and $sAPP_{\alpha}$ secretion in differentiated SH-SY5Y cells. Since these fatty acids are ingredients in daily food, information derived from this study should provide potential dietary strategy for prevention of AD.

2. Materials and methods

2.1. Chemicals and reagents

Dulbecco's modified Eagle's medium (DMEM)/F12 medium (1:1), fetal bovine serum (FBS) and 5-hexadecanoylaminofluorescein were from Invitrogen (Carlsbad, CA). Stearic acid (SA, 18:0), oleic acid (OA, 18:1), linoleic acid (LA, 18:2), α-linolenic acid (ALA, 18:3), arachidonic acid (AA, 20:4), eicosapentaenoic acid (EPA, 20:5), docosahexaenoic acid (DHA, 22:6), *cis*-parinaric acid, albumin from bovine serum (BSA), phorbol 12-myristate 13-acetate (PMA), dimethyl sulfoxide (DMSO) and all-*trans* retinoic acid (RA) were from Sigma–Aldrich (St. Louis, MO). *cis*-2-Eicosenoic acid and *cis*-5,8,11-eicosatrienoic acid were from Cayman (Ann Arbor, MI). Farnesyl-(2-carboxy-2-cyanovinyl)-julolidine (FCVJ) was from Dr. Haidekker's Laboratory (University of Georgia) (Nipper et al., 2008).

2.2. Cell culture

Human neuroblastoma SH-SY5Y cells (1.0×10^6 cells/dish) were seeded into 60 mm dishes and were cultured in DMEM/F12 medium (1:1) containing 10% FBS. For differentiation, SH-SY5Y cells were exposed to 10 μM RA for 6 days. Culture medium was replaced by fresh culture medium every other day. Treatments of cells with different fatty acids including SA, OA, LA, ALA, AA, EPA, DHA, *cis*-2-eicosenoic acid and *cis*-5,8,11-eicosatrienoic acid were in DMEM/F12 medium (1:1) containing 1% BSA for 24 h. All cells were maintained at 37 °C in a 5% CO_2 humidified incubator.

2.3. Fluorescent fatty acid labeled differentiated SH-SY5Y cells

Cells were incubated with 1 μM 5-hexadecanoylaminofluorescein or *cis*-parinaric acid for 40 min and excess fluorescent fatty acids were then removed by washing cells with PBS three times. Fluorescent images were obtained at room temperature using a Nikon TE-2000 U fluorescence microscope with an oil immersion 60× objective lens. Images were acquired using a CCD camera controlled by a computer running a MetaVue imaging software (Universal Imaging, PA).

2.4. Characterization of membrane fluidity by fluorescence microscopy of FCVJ-labeled cells

A fluorescent molecular rotor, farnesyl-(2-carboxy-2-cyanovinyl)-julolidine (FCVJ), was used to measure the relative membrane fluidity in differentiated SH-SY5Y cells. FCVJ was designed to be a more membrane-compatible fluorescent molecular rotor (Haidekker et al., 2001) with the quantum yield strongly dependent on the local free volume. A higher fluorescent intensity of FCVJ reflects the intramolecular-rotational motions being restricted by a smaller local free volume, indicating a more viscous membrane. The hydrocarbon chain of FCVJ is longer to help improve plasma membrane localization and reduce migration into the inner compartments of the cell, cytosolic staining and background fluorescence (Haidekker et al., 2001). Previously, we have verified the application of FCVJ for measuring membrane viscosity by comparing the results obtained using FCVJ with those from the technique of fluorescence recovery after photobleaching (FRAP) (Nipper et al., 2008). In this study, we adapted the protocol from Haidekker et al. (2001) to fluorescently label cells with FCVJ. Briefly, after undergoing different treatments, e.g., SA, OA, LA, ALA, AA, EPA and DHA, SH-SY5Y cells were washed with PBS and incubated in DMEM containing 20% FBS and 1 μM FCVJ for 20 min. Excess FCVJ was removed by washing cells with PBS three times. Fluorescent intensity measurements were performed at room temperature using a Nikon TE-2000 U fluorescence microscope with an oil immersion 60× objective lens. Images were acquired using a CCD camera controlled by a computer running a MetaVue imaging software (Universal Imaging, PA). The fluorescent intensities of FCVJ per cell were measured. Background subtraction was done for all images prior to data analysis.

2.5. Western blot analysis of $sAPP_{\alpha}$ released from differentiated SH-SY5Y cells

After different treatments, e.g., SA, OA, LA, ALA, AA, EPA and DHA, culture medium was collected and the same volume of the cell lysate from each sample was used for western blot analysis using β-actin as internal standard. Medium was centrifuged at 12,000 × *g* for 5 min to remove cell debris, and the same volume of medium from each sample (e.g., 40 μl) was diluted with Laemmli buffer, boiled for 5 min, subjected to electrophoresis in 7.5% SDS-polyacrylamide gels, and transferred to nitrocellulose membranes. Membranes were blocked for 1 h with 5% (w/v) nonfat dry milk in Tris-buffered saline containing 0.1% (v/v) Tween 20 (TBST) and were incubated overnight at 4 °C in 3% (w/v) bovine serum albumin (BSA) with 0.02% (w/v) sodium azide in TBST with a 6E10 monoclonal antibody (1:1000 dilution; Covance, Princeton, NJ) that recognizes residues 1–16 of the Aβ domain of $sAPP_{\alpha}$. Membranes were washed three times during a 15-min period with TBST and incubated with horseradish peroxidase-conjugated goat anti-mouse IgG antibody (1:2000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) in 5% (w/v) nonfat dry milk in TBST at room temperature for 1 h. After washing with TBST for three times, the membrane was subjected to SuperSignal West Pico Chemiluminescent detection reagents from Pierce (Rockford, IL) to visualize bands. The protein bands detected on X-ray film were quantified using a computer-driven scanner and Quantity One software (Bio-Rad).

2.6. Western blot analysis of APP, ADAM9, ADAM10, ADAM17, ADAM19 and BACE1 in differentiated SH-SY5Y cells

After treatments, the protein concentration of the cell lysate was determined by BCA protein assay kit (Pierce Biotechnology, Rockford, IL) according to manufacture's instruction. Equivalent amounts of protein from each sample (e.g., 30 μg) were diluted with Laemmli buffer, boiled for 5 min, subjected to electrophoresis in 7.5% SDS-polyacrylamide gels, and transferred to nitrocellulose membranes. Membranes were blocked for 1 h with 5% (w/v) nonfat dry milk in Tris-buffered saline containing 0.1% (v/v) Tween 20 (TBST) and were incubated overnight at 4 °C in 3% (w/v) BSA with 0.02% (w/v) sodium azide in TBST with 6E10 monoclonal antibody, anti-ADAM9 antibody or anti-ADAM19 antibody (1:1000 dilution; Abcam, Cambridge, MA), anti-ADAM10 antibody (1:1000 dilution; Millipore, Billerica, MA) or anti-ADAM17 antibody (1:1000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA), anti-BACE1 antibody (1:1000 dilution; Sigma–Aldrich, St. Louis, MO). Membranes were washed three times during a 15-min period with TBST and incubated with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit IgG antibody (1:2000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) in 5% (w/v) nonfat dry milk in TBST at room temperature for 1 h. After washing with TBST for three times, the membrane was subjected to SuperSignal West Pico Chemiluminescent detection reagents from Pierce (Rockford, IL) to visualize bands. The protein bands detected on X-ray film were quantified using a computer-driven scanner and Quantity One software (Bio-Rad).

2.7. Quantification of $A\beta_{1-42}$

After treatments, culture medium and cell lysates were collected, supplemented with protease inhibitor cocktail and centrifuged at 12,000 × *g* for 5 min at 4 °C to remove cell debris. An aliquot (100 μl) of supernatant was used for $A\beta_{1-42}$ quantification using an ELISA kit (Invitrogen, Carlsbad, CA) following manufacturer's recommendation. According to the instruction manual, substances including $A\beta_{1-12}$, $A\beta_{1-20}$, $A\beta_{12-28}$, $A\beta_{22-35}$, $A\beta_{1-40}$, $A\beta_{1-43}$, $A\beta_{42-1}$ and APP have no cross-reactivity. The minimum detectable dose of $A\beta_{1-42}$ is <1.0 pg/ml which is similar to a previous study (Prasanthi et al., 2009). The level of $A\beta_{1-42}$ in each sample was measured in duplicates and expressed in pg/ml.

2.8. Statistical analysis

Data are presented as mean ± SD from at least three independent experiments. There are three trials in each experiment. Comparison between two groups was made with students *t* test. Comparisons of more than two groups were made with one-way ANOVA, followed by *Bonferroni's* post hoc tests. Values of $p < 0.05$ are considered to be statistically significant.

3. Results

3.1. Exogenous fatty acids integrated into cellular membranes

In order to study the effects of fatty acids with different unsaturations on cellular membrane fluidity and $sAPP_{\alpha}$ secretion, we first confirmed if fatty acids were able to integrate into membranes of SH-SY5Y cells. Therefore, we incubated differentiated SH-SY5Y cells for 40 min with 5-hexadecanoylaminofluorescein and *cis*-parinaric acid. Both 5-hexadecanoylaminofluorescein and *cis*-parinaric acid are fluorescent fatty acids representing saturated and unsaturated fatty acids, respectively. Fig. 1A and B shows that 5-hexadecanoylaminofluorescein and *cis*-parinaric acid are incorporated into cellular membranes of SH-SY5Y cells, suggesting that exogenous fatty acids used in this study