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Phospholipid mass is increased in fibroblasts bearing the Swedish amyloid precursor mutation

Eric J. Murphy [a,*], Hsueh-Meei Huang [b], Richard F. Cowburn [c], Lars Lannfelt [d], Gary E. Gibson [b]

[a] *Department of Pharmacology, Physiology, and Therapeutics and Department of Chemistry, School of Medicine and Health Sciences, University of North Dakota, 501 N. Columbia Road, Room 3700, Grand Forks, ND 58202-9037, United States*

[b] *Department of Neurology and Neuroscience, Weill Medical College of Cornell, Burke Medical Research Institute, White Plains, NY 10605-2593, United States*

[c] *Karolinska Institute, Neurotec Department, Division of Experimental Geriatrics, Novum, Kaspac, S 141 57 Huddinge, Sweden*

[d] *Uppsala University Hospital, Box 609, Department of Public Health and Geriatrics, S 751 25 Uppsala, Sweden*

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Abstract

Phospholipid changes occur in brain regions affected by Alzheimer disease (AD), including a marked reduction in plasmalogens, which could diminish brain function either by directly altering signaling events or by bulk membrane effects. However, model systems for studying the dynamics of lipid biosynthesis in AD are lacking. To determine if fibroblasts bearing the Swedish amyloid precursor protein (swAPP) mutation are a useful model to study the mechanism(s) associated with altered phospholipid biosynthesis in AD, we examined the steady-state phospholipid mass and composition of fibroblasts, including plasmalogens. We found a 15% increase in total phospholipid mass, accounted for by a 24% increase in the combined total of phosphatidylethanolamine and plasmanylethanolamine mass and a 19% increase in the combined total of phosphatidylcholine (PtdCho) and plasmanycholine (PakCho) mass in the swAPP mutant bearing fibroblasts. Cholesterol mass was unchanged in these cells. The changes in phospholipid mass did not alter the cellular molar composition of the phospholipids nor the cholesterol to phospholipid ratio. While plasmalogen mass was not altered, the ratio of choline plasmalogen (PlsCho) mass to PtdCho + PakCho mass was decreased 16% and there was a 14% reduction in the proportion of PlsCho as a percent of total phospholipids in the swAPP mutant bearing fibroblasts. This change in choline plasmalogen is consistent with the reported decreases in plasmalogen proportions in affected regions of AD brain, suggesting that these cells may serve as a useful model to determine the mechanism underlying changes in plasmalogen biosynthesis in AD brain.

Keywords: Fibroblasts; Swedish amyloid mutation; Phospholipids; Plasmalogens; Alzheimer disease

1. Introduction

Cultured fibroblasts isolated from Alzheimer disease (AD) patients are a useful model to study biochemical changes that are characteristic of AD in a manner that is independent of diet, pharmacological intervention, or neurodegeneration. Fibroblasts have been derived from all major AD family groups including those with mutations in genes encoding for amyloid precursor protein (APP), presenilin-1 (PS-1), presenilin-2 (PS-2), and from patients with classical sporadic AD. Multiple biochemical processes differ between AD and control fibroblasts: calcium homeostasis [7,23,30,31,41,63]; signal transduction [27,39,40,71,80]; elevated inositol(1,4,5)tris-phosphate production in response to agonists [40]; mitochondrial function [14,15,30]; protein kinase C and G-protein activity [21,35,71]. Cellular signal transduction is also altered as demonstrated by the different properties of the β-adrenoceptor and forskolin-stimulated adenylyl cyclase activities in fibroblasts bearing presenilin-1 mutations or the Swedish amyloid precursor protein (swAPP) double mutation [79].

A number of biochemical alterations found in AD brain are also present in fibroblasts isolated from patients with AD. For instance, α-keto-glutarate dehydrogenase activity is reduced in brains and fibroblasts from AD patients [3,28]. Protein kinase

* Corresponding author. Tel.: +1 701 777 3450; fax: +1 701 777 4490.
E-mail address: emurphy@medicine.nodak.edu (E.J. Murphy).

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C isozymes are differentially cleaved by increased proteolysis in APP mutation bearing fibroblasts [21], which may account for the decrease in protein kinase C activity measured in fibroblasts derived from sporadic AD patients [35]. Cytochrome *c* oxidase (complex IV) activity is reduced in fibroblasts from sporadic AD patients [15] and in affected regions of AD brain [12,13]. Fibroblasts bearing either PS-1 or PS-2 mutations [69] or the APP mutation [42] secrete higher levels of amyloid-β-peptide (Aβ) than do fibroblasts from non-AD subjects, which is consistent with the accumulation of Aβ in AD brain. In AD brain, there is an accumulation of lipid peroxidation products, such as 4-hydroxynonenal (4-HNE) and acrolein [8,50,56,65,68] and a similar increase in 4-HNE and malondialdehyde occurs in fibroblasts carrying APP and PS-1 gene mutations [10]. These alterations are important because increased lipid peroxidative products modify many proteins and alter function. For example, glial peroxidative damage to the glutamate transporter GLT-1 in AD brain [45] may result in elevated extracellular glutamate levels in the synapse. Fibroblasts isolated from patients with sporadic AD have reduced glutamate uptake in the presence of HNE [2,47]. Bombesin-induced Ca^{2+} release from the ER (BRCS) is inositol trisphosphate-mediated and is greatly enhanced in fibroblasts from AD patients [41] and in neurons and fibroblasts from PS-1-transgenic mice [11,46,76]. Thus, these similarities between AD brain and AD derived fibroblasts demonstrate the utility of fibroblasts from AD patients to address the mechanisms underlying AD pathology.

Abnormal brain phospholipid metabolism is implicated in AD. Numerous alterations in brain lipids occur in AD, including changes in: phospholipids [19,20,33,36,74,77,81,82], phospholipid fatty acid composition [75], cholesterol [77,81] and phospholipid catabolites [4,62]. Phospholipid levels are decreased in frontal lobe white matter and hippocampus from brains of patients with AD [77]. Furthermore, the mole composition of ethanolamine glycerophospholipids is decreased, while mole percentage of phosphatidylserine is increased in synaptosomes prepared from affected regions of AD brain [82]. Reductions in mole percentage of ethanolamine plasmalogen may account for this observed decrease in ethanolamine glycerophospholipids [33,34,36] as well as for the decrease in critical temperature required for proper membrane formation seen in affected regions from AD brain [32,34]. Unfortunately, post-mortem tissue is not conducive to study phospholipid biosynthesis, especially plasmalogen biosynthesis, which depends upon reactions in intact and functional peroxisomes and microsomes [72]. Because fibroblasts derived from AD patients have been used to examine multiple biochemical abnormalities in AD, these cells may also serve as a model system to study altered phospholipid metabolism in AD.

Few studies have been done on phospholipid metabolism in fibroblasts from AD patients. We reported that there were no changes in phospholipid composition or mass, including plasmalogens, in fibroblasts bearing presenilin-1 mutations [60]. In the current study, we measured the steady-state phospholipid mass of fibroblasts bearing the swAPP mutation and control fibroblasts to determine if this fibroblast might be a useful model in which to study altered plasmalogen biosynthesis in the future.

2. Materials and methods

2.1. Cell cultures

Human skin fibroblast cell lines were established at the Department of Geriatric Medicine of the Karolinska Institute from skin biopsies taken from affected and non-affected individuals in the Swedish family with the APP670/671 mutation. Six lines were established from individuals with the APP670/671 mutation, of which four lines were from symptomatic carriers and two lines from asymptomatic individuals [30]. The genotype, clinical diagnosis, age of the patient, mini mental status exam of APP mutant fibroblast donors was documented. Cells were maintained as previously described [29] in Dulbecco's modified Eagle medium containing low glucose (1 g/l) supplemented with 10% fetal bovine serum in a humidified 5% CO_2 incubator at 37 °C [29,60]. Prior to shipping, T-75 flasks were filled with medium and shipped by overnight express delivery to the University of North Dakota. Upon arrival the medium was removed until the flasks contained 25 ml of medium. Cells were then maintained in an incubator for 5 days in an atmosphere of 5% CO_2 and 95% humidity. The swAPP mutation bearing fibroblasts ($n = 6$) and control fibroblasts ($n = 6$) were isolated from six individuals, thus the n of six represents six individuals per group. All measurements were made between passages 8 and 20.

2.2. Cellular lipid extraction

Cellular lipids were extracted as described previously [16,58,60]. Briefly, cells were rinsed twice with phosphate buffered saline to remove all traces of medium, the washes were removed, and then the cells were frozen by floating the flask on liquid N_2. The flasks were removed from the liquid N_2 and 2-propanol (2 ml) was added to the frozen cells. The cells were removed from the flask by scraping and the 2-propanol containing the cells was transferred to a tube containing hexane (6 ml). The flask was rinsed with 2-propanol (2 ml) to remove any remaining cells and the rinse was added to the tube containing the previous aliquot of 2-propanol resulting in an extraction solvent of *n*-hexane:2-propanol (3:2, v/v). Extracts were stored in tubes containing a nitrogen atmosphere at −80 °C until analyzed.

2.3. Sample preparation and protein analysis

The cellular debris and denatured proteins were pelleted by centrifugation and the lipid containing solvent was removed and saved for analysis. Residual organic solvent was evaporated from the protein pellet overnight at room temperature and the pellet was dissolved in 0.2 M KOH (2 ml) overnight at 60 °C in tightly capped test tubes. Protein concentration was measured using a modified dye-binding assay [6]. Absorbance was converted to milligrams using a standard curve based on bovine serum albumin.

2.4. Phospholipid and cholesterol analysis

Prior to high performance liquid chromatography (HPLC), sample volume was reduced under a stream of nitrogen and then filtered through a 0.2 μm Nylon filter. Samples were then reduced in volume to dryness and redissolved in 0.3 ml of solvent. Phospholipids were separated using a gradient of *n*-hexane:2-propanol (3:2, v/v) and *n*-hexane:2-propanol:water (3:2, v/v + 5.5%) as previously described [18,59]. The HPLC system consisted of a Selectosil column (5 μm, 4.6 mm × 250 mm, Phenomenex, Torrance, CA), a Beckman 126 pump (Fullerton, CA), and a Beckman 166 UV/Vis detector. The ethanolamine glycerophospholipid (EtnGpl) and choline glycerophospholipid (ChoGpl) fractions were quantitatively divided in half. One half was used to determine plasmalogen composition [59], while the remaining half was used to quantify lipid phosphorus [67]. The neutral lipid fraction from the phospholipid separation was saved and cholesterol mass determined using an iron binding assay [5]. Phospholipid mass was determined by measuring lipid phosphorus in each fraction [67].