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Featured Article

Elevated phospholipase D isoform 1 in Alzheimer's disease patients' hippocampus: Relevance to synaptic dysfunction and memory deficits

Balaji Krishnan*, Rakez Kayed, Giulio Taglialatela

UTMB Mitchell Center for Neurodegenerative Diseases, Department of Neurology, University of Texas Medical Branch, Galveston, TX, USA

Abstract

Introduction: Phospholipase D (PLD), a lipolytic enzyme that breaks down membrane phospholipids, is also involved in signaling mechanisms downstream of seven transmembrane receptors. Abnormally elevated levels of PLD activity are well-established in Alzheimer's disease (AD), implicating the two isoforms of mammalian phosphatidylcholine cleaving PLD (PC-PLD1 and PC-PLD2). Therefore, we took a systematic approach of investigating isoform-specific expression in human synaptosomes and further investigated the possibility of therapeutic intervention using preclinical studies.

Methods: Synaptosomal Western blot analyses on the postmortem human hippocampus, temporal cortex, and frontal cortex of AD patient brains/age-matched controls and the 3XTg-AD mice hippocampus (mouse model with overexpression of human amyloid precursor protein, presenilin-1 gene, and microtubule-associated protein tau causing neuropathology progressing comparable to that in human AD patients) were used to detect the levels of neuronal PLD1 expression. Mouse hippocampal long-term potentiation of PLD1-dependent changes was studied using pharmacological approaches in *ex vivo* slice preparations from wild-type and transgenic mouse models. Finally, PLD1-dependent changes in novel object recognition memory were assessed following PLD1 inhibition.

Results: We observed elevated synaptosomal PLD1 in the hippocampus/temporal cortex from postmortem tissues of AD patients compared to age-matched controls and age-dependent hippocampal PLD1 increases in 3XTg-AD mice. PLD1 inhibition blocked effects of oligomeric amyloid β or toxic oligomeric tau species on high-frequency stimulation long-term potentiation and novel object recognition deficits in wild-type mice. Finally, PLD1 inhibition blocked long-term potentiation deficits normally observed in aging 3XTg-AD mice.

Discussion: Using human studies, we propose a novel role for PLD1-dependent signaling as a critical mechanism underlying oligomer-driven synaptic dysfunction and consequent memory disruption in AD. We, further, provide the first set of preclinical studies toward future therapeutics targeting PLD1 in slowing down/stopping the progression of AD-related memory deficits as a complementary approach to immunoscavenging clinical trials that are currently in progress.

Keywords: Phospholipase D; Alzheimer's disease; Memory; Synaptic; Hippocampus; Electrophysiology; Novel object recognition; Aβ; Tau

1. Introduction

Alzheimer's disease (AD), the most common form of dementia, is the fifth leading cause of death in America [1]. Therapeutic strategies, based on the last 3 decades of research, have largely targeted accumulations of oligomeric amyloid β (oAβ) [2] and oligomeric tau species (otau) [3]. Clinical trials that target these aggregates alone have not enjoyed as much success [4,5]. Thus, recent studies have also started to identify other players in the early events, such as synapse dysfunction [6,7].

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*Corresponding author. Tel.: 409 772 8069; Fax: 409 747 0015.
E-mail address: bakrishn@utmb.edu

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In the present study, we systematically addressed the role of phospholipase D (PLD) in early events leading to synaptic vulnerability. PLD enzyme superfamily members are transphosphatidylases that conduct headgroup exchange on phosphatidic acid at the terminal phosphodiester bond [8]. In addition, PLD signaling is important for several physiological processes including membrane trafficking, cytoskeletal reorganization, and autophagy that is regulated upstream by several transmembrane receptors including G-protein-coupled receptors, receptor tyrosine kinases, and integrin receptors [9,10]. In fact, our previous studies show that PLD isoforms can act as convergent second messengers for dopaminergic, serotonergic, and glutamatergic neurotransmission [11–13] affecting synaptic function and memory processes.

Mammalian phosphatidylcholine cleaving PLD (two isoforms: PLD1 and PLD2) cleaves phosphatidylcholine to phosphatidic acid/choline via phosphodiesterase action, and brain-associated PLD function can affect membrane curvature, exocytosis, endocytosis, vesicle release, and neurite outgrowth, all of which are important in synaptic function [9]. Our previous studies also implicated PLD isoforms in rodent associative memory mechanisms [11–13].

Evidence for aberrant increase in PLD activity in postmortem AD brains, compared to controls, arrived nearly 3 decades ago [14]. Mouse studies implicating PLD2 (the constitutive isoform) in Aβ-driven synaptic dysfunction [15] were not validated by confirming that an increased expression of PLD2 in postmortem AD brains, indeed, contributes to the neuropathology of AD-like memory deficits. Attention to the possibility of PLD1 (the inducible isoform) contributing to AD synaptic dysfunction was completely disregarded.

A role for PLD1 in AD, done exclusively in cell culture, proposed a protective role for PLD1 against Aβ synthesis [16,17], which is confounded by a study in humans that shows that there is upregulated astroglial PLD1 expression in AD brains [18] but does not result in any neuroprotection. Thus, a clearer role for PLD isoforms in AD requires an approach that starts with addressing the relative levels of neuronal PLD1 and PLD2 expression in normal and diseased states in the human brain.

Here, we report, for the first time, a systematic study demonstrating the relative synaptosomal expression levels of the two phosphatidylcholine cleaving PLD isoforms in postmortem control and AD brains. Furthermore, we extended our observation to study whether inhibition of PLD isoforms via specific small molecule inhibitors [8] prevents synaptic dysfunction and memory defects of Aβ and tau oligomers in wild-type mice using electrophysiology and behavior, respectively. In addition, we also addressed whether the convergent synaptic deficits induced by overexpression of human genes for amyloid precursor protein (APP) and tau in the 3XTg-AD mice (mouse model with overexpression of human APP, presenilin-1 gene, and microtubule-associated protein tau) [19] were attenuated by inhibiting PLD1 signaling.

2. Materials and methods

2.1. Human subjects and autopsy brain tissues

Postmortem de-identified brain tissues were obtained through materials transfer agreement from Oregon Brain Bank at Oregon Health and Science University, Portland, OR. After obtaining informed consent, enrolled donor subjects were clinically evaluated in accordance with Oregon Brain Bank at Oregon Health and Science University's, Portland, OR, institutional review board–approved protocols. A neuropathological assessment was performed at autopsy for amyloid plaques and neurofibrillary tangles, per standardized CERAD criteria and Braak staging. Participants were classified as AD (n = 11) when possessing Mini–Mental State Examination score below 10. Control participants (n = 8) performed normally (Mini–Mental State Examination of 28–30). Donor subject samples were de-identified before arrival at UTMB. The clinical data of the subjects used in the study are provided in Table 1.

2.2. Western blot analysis

Isolation of synaptosomal fractions from the frozen brain sections (hippocampus, frontal cortex, and temporal cortex) obtained from different groups as mentioned previously was

Table 1
Diagnosis of the de-identified subjects based on Braak staging of plaques and tangles localization [20,21] and MMSE

Diagnosis	Subject number	Age	Sex	Braak stage	MMSE	PMI
Ctrl	767	86	F	2	-	8
Ctrl	785	82.7	M	1	-	<14
Ctrl	1957	>89	F	4	30	8
Ctrl	1977	92	F	4	-	4
Ctrl	1874	43	F	0	-	10
Ctrl	1876	28	F	0	-	15.5
Ctrl	1899	79	F	2	25	14
Ctrl	2229	71	F	2	-	14.5
AD	1538	84	M	5	26	5.5
AD	1749	79	?	6	-	6
AD	1756	68	M	6	7	11.5
AD	1766	63	F	6	18	3.5
AD	1776	>89	F	6	6	6.3
AD	1777	67	F	6	9	20.5
AD	1910	63	F	6	-	44
AD	1985	?	?	-	-	-
AD	2201	86	M	6	-	16
AD	2316	83	M	5	-	11
AD	2317	88	M	6	-	4

Abbreviations: AD, Alzheimer's disease; Ctrl, control; F, female; M, male; MMSE, Mini–Mental State Examination; PMI, post-mortem interval.

NOTE. PMI provides the postmortem interval for brain removal after death of the subject in hours. As indicated, samples from either sex were used for the study, where Braak stage of 5 and more with MMSE (for those available) 24 and below are diagnosed as AD, whereas the others were selected as the control group.