Hippocampal Drebrin Loss in Mild Cognitive Impairment

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Key Words

Alzheimer's disease • Mild cognitive impairment • Synaptic protein • Synaptophysin • Synaptotagmin • Drebrin • Hippocampus

Abstract

Alterations in the relative abundance of synaptic proteins may contribute to hippocampal synaptic dysfunction in Alzheimer's disease (AD). The extent to which perturbations in synaptic protein expression occur during the earliest stages of cognitive decline remains unclear. We examined protein levels of presynaptic synaptophysin (SYP) and synaptotagmin (SYT), and postsynaptic drebrin (DRB), a marker for dendritic spine plasticity, in the hippocampus of people with an antemortem clinical diagnosis of no cognitive impairment (NCI), mild cognitive impairment (MCI) or mild/moderate AD. Although normalized SYP and SYT levels were preserved, DRB was reduced by approximately 40% in the hippocampus of MCI and AD compared to NCI subjects. This differential alteration of synaptic markers in MCI suggests a selective impairment in hippocampal postsynaptic dendritic plasticity in prodromal AD that likely heralds the onset of memory impairment in symptomatic disease.

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Introduction

Hippocampal synapse loss is a prominent feature of Alzheimer's disease (AD) [1]. Decreases in synaptic density correlate better than amyloid plaques or neurofibrillary tangles with the degree of cognitive impairment in AD, suggesting that synaptic dysfunction contributes to the clinical presentation of the disease [2]. The efficacy of synaptic transmission depends upon the intricate coordination of multiple specialized proteins involved in synaptic vesicle trafficking (e.g. targeting and docking, membrane fusion/exocytosis and endocytosis) and pre- and postsynaptic structure and plasticity [3, 4]. Therefore, perturbations in synaptic protein stoichiometry may play a role in synaptic dysfunction in AD. Several studies have shown that the expression levels of select synaptic proteins such as synaptophysin (SYP) [5], synaptotagmin (SYT) [6] and drebrin (DRB) [7] are decreased in the hippocampus in late-stage AD, but whether similar alterations occur in people clinically diagnosed with mild cognitive impairment (MCI), a putative prodromal stage of AD [8], remains unclear. To address this question, we performed quantitative immunoblotting experiments to measure the levels of SYP (a presynaptic vesicle marker), SYT (a synaptic protein critical for Ca²⁺-dependent neurotransmitter

Table 1. Clinical, demographic, and neuropathological case characteristics by diagnostic category

	Clinical diagnosis				p value	Pairwise comparison
	NCI (n = 12)	MCI ^a (n = 9)	AD ^a (n = 11)	total (n = 32)		
Age at death, years	83.4 ± 5.9 (75–93)	86.4 ± 4.2 (80–93)	86.6 ± 7.1 (75–98)	85.3 ± 5.9 (75–98)	0.4 ^b	–
Number of males	3 (25%)	4 (44%)	6 (55%)	13 (40%)	0.4 ^c	–
Years of education	16.8 ± 3.7 (12–25)	16.4 ± 2.2 (12–20)	15.6 ± 4.6 (7–22)	16.3 ± 3.6 (7–25)	1.0 ^b	–
Number with ApoE ε4 allele	0	1 (11%)	5 (45%)	6 (19%)	0.0094 ^c	NCI < AD
MMSE score	28.1 ± 1.9 (23–30)	26.6 ± 3.4 (21–30)	14.1 ± 8.1 (0–24)	22.8 ± 8.2 (0–30)	<0.000 ^b	(NCI, MCI) > AD
Post-mortem interval, h	4.7 ± 3.3 (1.8–12.4)	3.9 ± 1.8 (2.3–7.6)	4.6 ± 1.5 (2.7–7.3)	4.5 ± 2.3 (1.8–12.4)	0.5 ^b	–
Distribution of Braak scores						
0	0	0	0	0	<0.000 ^b	(NCI, MCI) < AD
I/II	6	0	0	6		
III/IV	6	8	1	15		
V/VI	0	1	10	11		
NIA-Reagan diagnosis (likelihood of AD)						
No AD	5	0	0	5	<0.0001 ^b	(NCI, MCI) < AD
Low	2	3	0	5		
Intermediate	5	5	2	12		
High	0	1	9	10		
CERAD diagnosis						
No AD	6	1	0	7	0.0009 ^b	NCI < AD
Possible	0	1	0	1		
Probable	5	4	2	11		
Definite	1	3	9	13		

Figures are means ± SD with ranges in parentheses unless indicated otherwise.

^a 7 of the 9 MCI cases were amnesic MCI; 7 of the 11 AD cases were mild/moderate AD (i.e. MMSE score ≥10). ^b Kruskal-Wallis test, with Bonferroni correction for multiple comparisons. ^c Fisher's exact test, with Bonferroni correction for multiple comparisons.

release), and DRB (a postsynaptic dendritic spine marker) in postmortem hippocampus obtained from subjects classified antemortem as no cognitive impairment (NCI), MCI or mild/moderate AD. These findings were correlated with performance on antemortem cognitive tests and postmortem neuropathologic variables.

Materials and Methods

Subjects

Hippocampi were obtained from 32 individuals enrolled in the Rush Alzheimer's Disease Center Religious Orders Study (n = 16) [9] or the University of Kentucky Alzheimer's Disease Center (n = 16) [10] who underwent annual detailed clinical evaluations and brain donations at death [9–11]. Hippocampal tissue containing the CA subfields and the dentate gyrus was harvested at the level of the lateral geniculate nucleus and frozen on dry ice. Cases were matched for age (85.3 ± 5.9 years, mean ± SD), years of education (16.3 ± 3.6), postmortem interval (4.5 ± 2.3 h) and gender (40% males) and were categorized antemortem as NCI [n = 10; Mini Mental State Exam (MMSE) score = 28.1 ± 1.9], MCI insufficient to meet criteria for dementia (n = 9; MMSE score = 26.6 ± 3.4), or mild/moderate AD (n = 11; MMSE score =

14.1 ± 8.1) (table 1) [9]. Seven of the 9 MCI cases were amnesic MCI based on impairments in episodic memory alone [8]. A neuropathologist classified each case based on CERAD (Consortium to Establish a Registry for Alzheimer's Disease) [12], Braak staging [13], and NIA-Reagan [14] diagnostic criteria (table 1).

Quantitative Immunoblotting

Immunoblotting was performed using a previously reported procedure [11]. Briefly, frozen hippocampi were homogenized with protease inhibitors and centrifuged at 1,000 rpm for 10 min at 4°C. S1 fraction proteins (25 µg/sample) were separated by SDS-PAGE, transferred to Immobilon P membranes (Millipore, Mass., USA), blocked in Tris-buffered saline (pH 7.4)/0.1% Tween-20/5% milk, and incubated overnight at 4°C with mouse anti-SYP (clone SY38; 1:1,000; MP Biomedicals, Calif., USA), mouse anti-SYT (clone 41; 1:2,000; BD Transduction Labs, Calif., USA), and mouse anti-DRB (clone M2F6; 1:2,000; MBL International, Mass., USA) in blocking buffer. Membranes were also incubated with mouse anti-tubulin (clone KMx-1; 1:50,000; Chemicon, Calif., USA) as the loading control; hence, for quantitative analysis, synaptic protein levels were normalized to tubulin levels in each sample [11]. Blots were incubated for 1 h with horseradish peroxidase-conjugated goat anti-mouse IgG secondary antibody (1:8,000; Pierce, Ill., USA) and reactivity was quantified using Kodak 1D image analysis software (Perkin-Elmer, Mass., USA). Each sample was analyzed on three different Western blots in independent experiments.

Table 2. Summary of hippocampal DRB, SYP, and SYT levels by diagnosis category

	Clinical diagnosis (mean $\pm$ SD)				p value ^a	Pairwise comparison
	NCI (n = 12)	MCI (n = 9)	AD (n = 11)	total (n = 32)		
DRB	0.43 $\pm$ 0.20 (0.18–0.84)	0.24 $\pm$ 0.13 (0.06–0.49)	0.25 $\pm$ 0.13 (0.07–0.45)	0.32 $\pm$ 0.18 (0.06–0.84)	0.045	NCI > (MCI, AD)
SYP	3.64 $\pm$ 1.33 (1.41–6.38)	3.32 $\pm$ 1.55 (0.92–5.69)	3.80 $\pm$ 1.89 (1.51–6.86)	3.60 $\pm$ 1.56 (0.92–6.86)	0.84	–
SYT	0.88 $\pm$ 0.39 (0.35–1.62)	0.91 $\pm$ 0.45 (0.34–1.64)	0.94 $\pm$ 0.61 (0.41–2.32)	0.91 $\pm$ 0.47 (0.34–2.32)	0.97	–

Figures in parentheses indicate ranges.

^a Kruskal-Wallis test, with Bonferroni correction for multiple comparisons.

Statistical Analysis

Synaptic protein levels were compared among groups using Kruskal-Wallis testing with Bonferroni correction for multiple comparisons. Relationships between synaptic protein levels and clinical and pathologic variables were assessed by Spearman correlation. The level for statistical significance was set at 0.05 (two-tailed).

Results

Hippocampal Synaptic Protein Levels in MCI and AD

There were no significant differences in mean hippocampal SYP or SYT levels across the clinical groups (fig. 1; table 2). By contrast, mean DRB protein levels were decreased by approximately 40–45% in MCI and AD compared to NCI (fig. 1; table 2). SYP, SYT, or DRB levels were not correlated with either antemortem performance on the MMSE, episodic memory tests referable to hippocampal function (e.g. logical memory immediate recall) [9], or postmortem Braak, CERAD, or NIA-Reagan neuropathological scores.

Discussion

Synapse loss in the hippocampus correlates with the severity of clinical symptoms in AD [1], yet the pathogenic mechanisms underlying synaptic dysfunction remain unclear. Previous reports have demonstrated preferential reductions in specific synaptic proteins in late-stage AD, including SYP [5], SYT [6], and DRB [7]. The present quantitative immunoblotting study revealed that DRB levels were also selectively reduced in MCI and early-stage AD, whereas SYP and SYT levels were stable across diagnostic groups, indicating that alterations in the expression of synaptic regulatory elements occur early in the disease process. These findings are similar to

Fig. 1. Representative immunoblot showing hippocampal DRB, SYT, SYP, and β -tubulin (β -TUB) immunoreactivity in samples from NCI, MCI, and AD subjects.

those found in the temporal neocortex, where levels of DRB were reduced in MCI, SYP was decreased only in late-stage AD, and SYT was stable during the progression of AD [11].

Functionally, SYP is a key player in membrane trafficking events preceding exocytosis, whereas SYT is a Ca^{2+} microsensor that modulates activity-dependent exocytosis [15]. Hence, hippocampal presynaptic efficacy may be relatively preserved in the prodromal stages of AD. Alternatively, the stability of these presynaptic markers may reflect the ability of the hippocampus to display biochemical plasticity in MCI [16].

DRB, on the other hand, is an F-actin-binding protein localized to postsynaptic dendritic spines at excitatory synapses [17], where it plays a role in synaptic plasticity by regulating spine morphogenesis [17], organizing postsynaptic densities [18], and targeting receptors [19]. Intriguingly, DRB-dependent clustering of actin was shown to be critical for the postsynaptic targeting of the protein PSD-95, which is also involved in excitatory postsynaptic plasticity [18] and reduced in the MCI hippocampus [10].

The lack of association between DRB levels alone and cognitive performance may signify that alterations of multiple synaptic markers are required to precipitate clinically relevant disturbances in cognition function. Taken together, these findings suggest that the hippocampus displays postsynaptic pathology and decreased synaptic plasticity in the prefatory stages of AD, which over time contributes to the onset of frank dementia.

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