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AD synapses contain abundant Aβ monomer and multiple soluble oligomers, including a 56-kDa assembly

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Abstract

Much evidence indicates that soluble amyloid beta (Aβ) oligomers are key mediators of early cognitive loss, but the localization and key peptide species remain unclear. We have used flow cytometry analysis to demonstrate that surviving Alzheimer's disease (AD) synapses accumulate both Aβ and phosphorylated tau (p-tau). The present experiments use peptide-specific X-map assays and Western blot analyses to identify the Aβ peptide species in synaptosome-enriched samples from normal human subjects, neurologic controls, and AD cases. Aβ40 peptide levels did not vary, but both Aβ42 and Aβ oligomers were increased in soluble AD extracts, with oligomer levels 20-fold higher in aqueous compared with detergent extracts. In Western blot analysis, a ladder of sodium dodecyl sulfate (SDS)-stable oligomers was observed in AD cases, varying in size from monomer, the major peptide observed, to larger assemblies up to about 200 kDa and larger. Multiple oligomers, including monomer, small oligomers, a 56-kDa assembly, and amyloid precursor protein (APP) were correlated with the Aβ level measured in flow cytometry-purified synaptosomes. These results suggest that multiple amyloid precursor protein processing pathways are active in AD synapses and multiple soluble oligomeric assemblies may contribute to synaptic dysfunction.

Keywords: Synaptosome; Flow cytometry; Parkinson's disease; Synaptophysin; PSD-95; A11 antibody; OC antibody

1. Introduction

A number of studies have documented that levels of soluble amyloid beta (Aβ) peptides are superior to amyloid deposits as correlates of cognitive decline in Alzheimer's disease (AD; McLean et al., 1999; Näslund et al., 2000). Accordingly, the original amyloid cascade hypothesis has evolved to propose that soluble oligomeric Aβ assemblies precede deposition and are the proximal cause of synaptic dysfunction and early impairment in AD (see Walsh and Selkoe, 2007 for review). However, the size of the key assembly state and relevant downstream pathways remain the subject of intense study. Among natural low-n assemblies, dimers and/or trimers in particular have been isolated from AD brain and shown to impair cognition in vitro (Cleary et al., 2005; Klyubin et al., 2008; Shankar et al., 2008; Townsend et al., 2006). In the Tg2576 mouse model a larger assembly, (Aβ*56), possibly a multimer of smaller oligomers, was associated with cognitive decline in Tg2576 mice (Lesné et al., 2006).

Soluble Aβ peptides are associated with synaptic loss (Lue et al., 1999), and multiple studies have shown that

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soluble oligomers bind to dendritic spines in primary cultures (Lacor et al., 2004, 2007). Recent evidence also suggests that brief passive immunotherapy has acute and extended benefits on synaptic density and plasticity (Rozkalne et al., 2009; Spires-Jones et al., 2009). Consistent with synaptic Aβ release, interstitial Aβ levels are increased by synaptic activity (Cirrito et al., 2005, 2008), and have been shown to correlate with neurological status in patients with brain injury (Brody et al., 2008). Reasoning that study of surviving synaptic terminals is critical for understanding the sources for synaptic Aβ production and release as well as pathways leading to loss of synapses, we have analyzed human synaptosomal preparations by flow cytometry analysis and have shown that Aβ accumulates in synaptic terminals in multiple regions of AD brain. Phosphorylated tau (p-tau) also accumulates in Aβ-bearing synapses, and the colocalization of Aβ and p-tau is accompanied by increased synaptosome size, modest losses of the postsynaptic density scaffold protein, PSD-95, and increased cholesterol and ganglioside GM1 (Gylys et al., 2004b, 2007, 2008). With flow cytometry, the synaptosomal Aβ signal is best detected by an N-terminal antibody (10G4) that does not discriminate between peptides; the present study correlates the flow cytometry signal with a series of peptide and conformation-specific antibodies along with a series of Aβ peptide-specific assays on the Luminex platform. We report here that monomeric Aβ is prominent among multiple sodium dodecyl sulfate (SDS)-stable soluble Aβ species, including a 56-kDa assembly, in synaptic terminals from AD cortex.

2. Methods

2.1. Materials

The monoclonal anti-Aβ antibody 10G4 has been described previously (Mak et al., 1994). Polystyrene microsphere size standards were purchased from Polysciences, Inc. (Warrington, PA, USA), and rhodamine-conjugated anti-mouse antibody from Chemicon (San Diego, CA, USA). The following monoclonal antibodies were purchased: anti-SNAP-25 (Sternberger Monoclonals, Inc., Lutherville, MD, USA), anti-PSD 95 (Upstate Biotechnology, Lake Placid, NY, USA), 6E10 antibody (Signet Labs, Dedham, MA, USA), anti-synaptophysin from Abcam (Cambridge, MA, USA), 4G8 antibody (Covance, Denver, PA, USA), and anti-amyloid precursor protein (APP) 3E9 (MBL, Naka-ku Nagoya, Japan). A11 was the kind gift of C. Glabe (UC, Irvine, CA, USA), and OC antibody was received from R. Kayed (UTMB, Galveston, TX, USA). The rabbit anti-Aβ42 and anti-Aβ-40 antibodies were from T. Golde (Mayo Clinic, Jacksonville, FL, USA).

2.2. Human brain specimens

Brain samples, primarily superior parietal (A7) cortex were obtained at autopsy from the Alzheimer's Disease Research Centers at USC and UCLA; for some experiments frontal (A9) or parietal (A39) samples were substituted. Samples were obtained from a total of 14 cases (10 female, 4 male); 7 were diagnosed clinically and histopathologically with AD, and 3 were neurological control cases. The control cases included 2 Parkinson's disease (PD) and 1 tauopathy case. The 4 cognitively normal aged controls were confirmed histopathologically. The mean age of AD cases was 86.3, and 84.6 for normal and control cases. The mean postmortem interval for AD cases was 8.2 hours, and for normal and control cases was 7.0 hours.

2.3. P-2 preparation

Samples (~0.3–5 g), were minced and slowly frozen on the day of autopsy in 10% dimethyl sulfoxide and 0.32 M sucrose and stored at −70 °C until homogenization. The crude synaptosome (P-2) fraction was prepared as described previously (Gylys et al., 2003), briefly, the homogenate was first centrifuged at 1000*g* for 10 minutes; the resulting supernatant was centrifuged at 10,000*g* for 20 minutes to obtain the crude synaptosomal pellet. Aliquots of P-2 are routinely cryopreserved in 0.32 M sucrose and banked at −70 °C until the day of the experiment.

2.4. Immunolabeling of P-2 fraction

P-2 aliquots were immunolabeled for flow cytometry analysis according to a method for staining of intracellular antigens (Schmid et al., 1991). Pellets were fixed in 0.25% buffered paraformaldehyde (1 hour, 4 °C) and permeabilized in 0.2% Tween 20/phosphate-buffered saline (PBS) (15 minutes, 37 °C). Antibodies were labeled directly with Alexa Fluor 488 or 647 reagents according to kit directions (Zenon Alexa Fluor Labeling Kit, Invitrogen, Carlsbad, CA, USA). The labeled antibody mixture was added to P-2 aliquots (5 μL of P-2 pellet/sample, ~5–8 μg/μL) and incubated at room temperature for 30 minutes. Pellets were washed 2 times with 1 mL 0.2% Tween 20/PBS, and resuspended in PBS buffer (0.75 mL) for flow cytometry analysis. The synaptosomal pellet was dispersed for all washes and for incubations with fixative, detergent, and antibody, then collected by centrifugation (1310*g* at 4 °C).

2.5. Flow cytometry

Data were acquired using the BD-FACSCalibur and the BD SORP LSRII analytic flow cytometers (Becton-Dickinson, San Jose, CA, USA) equipped with argon 488 nm, helium-neon 635 nm, and helium-cadmium 325-nm lasers. Five thousand particles were collected and analyzed for each sample. Debris was excluded by establishing a size threshold set on forward light scatter. Alexa 488 and Alexa 647 fluorochromes were detected by the FL1 and Ssc-W photomultiplier tube detectors, respectively. Analysis was performed using FCS Express software Version 3 (DeNovo Software, Ontario, Canada).