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Alzheimer brain-derived amyloid β-protein impairs synaptic remodeling and memory consolidation

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ABSTRACT

Aggregation of the amyloid β-protein (Aβ) is believed to play a central role in initiating the molecular cascade that culminates in Alzheimer-type dementia (AD), a disease which in its early stage is characterized by synaptic loss and impairment of episodic memory. Here we show that intracerebroventricular injection of Aβ-containing water-soluble extracts of AD brain inhibits consolidation of the memory of avoidance learning in the rat and that this effect is highly dependent on the interval between learning and administration. When injected at 1 hour post training extracts from 2 different AD brains significantly impaired recall tested at 48 hours. Ultrastructural examination of hippocampi from animals perfused after 48 hours revealed that Aβ-mediated impairment of avoidance memory was associated with lower density of synapses and altered synaptic structure in the dentate gyrus and CA1 fields. These behavioral and ultrastructural data suggest that human brain-derived Aβ impairs formation of long-term memory by compromising the structural plasticity essential for consolidation and that Aβ targets processes initiated very early in the consolidation pathway.

1. Introduction

Over the course of the past 5 decades, the incidence of Alzheimer's disease (AD) has increased at an alarming rate, such that the sheer number of sufferers with this disorder is now placing an unsustainable burden on health care systems throughout the world (Alzheimer's Association, 2011; Ferri et al., 2005; Prince, 2009). The initial harbingers of disease are often difficult to differentiate from the cognitive changes that frequently accompany old age (Small et al., 1999), with deficits in episodic memory typifying the early stages of AD and usually occurring before impairment of semantic and nondeclarative memory (Grober et al., 2008; Mormino et al., 2009; Rosenbaum et al., 2005; Small, 2000). The pathological changes seen in the medial temporal lobe of individuals with mild cognitive impairment and early AD are in accord with the initial symptoms of the disease (Scheff et al., 2006, 2007) and support the hypothesis that, at least initially, AD results from disruption of normal hippocampal function and memory consolidation pathways (Coleman and Yao, 2003; Mesulam, 1999).

The molecular changes leading to perturbation of synaptic plasticity in AD are not well understood, but substantial data indicate that the amyloid β-protein (Aβ) might be responsible for these effects (Klein et al., 2001; Selkoe, 2002). However, the forms of Aβ that mediate memory impairment and the toxic pathways activated by Aβ remain unresolved. Numerous studies have shown that nonfibrillar, water-soluble Aβ from a variety of sources are potent synaptotoxins (Cleary et al., 2005; Klyubin et al., 2008; Lambert et al., 1998; Walsh et al., 2002). In earlier studies we used the most disease-relevant form of nonfibrillar Aβ, Aβ extracted from the water-soluble phase of AD brain (Freir et al., 2011b; Shankar et al., 2008). Postmortem studies indicate that elevated levels of water-soluble Aβ are specific for AD (Kuo et al., 1996; Lue et al., 1999; Mc Donald et al., 2010; McLean et al., 1999; Tabaton et al., 1994) and in vitro studies show that such material robustly inhibits long-term potentiation, facilitates long-term depression, and reduces the density of dendritic spines (Barry et al., 2011; Freir et al., 2011b; Li et al., 2009; Shankar et al., 2008).

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Most previous work involving intracerebroventricular (icv) injection of either synthetic or cell culture-derived Aβ focused on spatial reference memory or working memory (Cleary et al., 2005; Lesne et al., 2006; Nakamura et al., 2001; Poling et al., 2008; Reed et al., 2011), and in some of our previous studies we employed a passive avoidance paradigm (Freir et al., 2011a; Shankar et al., 2008). A major advantage of the latter approach is the fact that rapid acquisition ensures the synchronization of subsequent memory-associated neuroplastic events and as a consequence allows examination of long-term memory consolidation (Foley et al., 2000; Nakamura et al., 2001; Seymour et al., 2008). In preliminary experiments we found that administration of Aβ from a single human brain perturbed consolidation of avoidance learning (Shankar et al., 2008). Memory consolidation involves time-dependent synaptic remodeling (Murphy and Regan, 1998; Platano et al., 2008) and is underpinned by changes in protein synthesis and posttranslation modification (Davis and Squire, 1984; Lamprecht and LeDoux, 2004). Hence, we sought to determine if Aβ-containing extracts from other AD brains could also disrupt the memory of learned behavior, that is, if this is a generalizable phenomenon typical of AD brain-derived Aβ, and if it is accompanied by alterations in synaptic form and number. In an effort to gain insight into the molecular pathways through which Aβ exerts its effects we also investigated the expression and processing of certain AD-associated proteins some of which are known to change within the first few hours immediately after avoidance learning (Conboy et al., 2005; O'Sullivan et al., 2007).

Here we demonstrate that Aβ-containing extracts from 2 different AD brains effectively diminished consolidation of avoidance learning and that this was highly dependent on the interval between learning and Aβ administration. Though further work is required to understand the molecular pathways on which Aβ acts, we show that this impairment is accompanied by a significant decrease in the total density of synapses in both the dentate gyrus and CA regions of the hippocampus. We also present evidence of altered synaptic ultrastructure, including, but not limited to, increases in synaptic pitting and the number of synaptic vacuoles. Together these results suggest that water-soluble Aβ species isolated from human brain inhibit the synaptic remodeling necessary for memory consolidation while also perturbing normal synapse structure. These findings are consistent with the fact that synapse loss is the best known pathologic marker of AD severity (Davies et al., 1987; Masliah et al., 2001; Scheff et al., 2007) and suggest that water-soluble forms of Aβ might also mediate this process in the human brain.

2. Methods

2.1. Reagents and antibodies

2.1.1. Reagents

All reagents were obtained from Sigma-Aldrich Ltd (Dublin, Republic of Ireland) unless specified otherwise.

2.1.2. Antibodies

Rabbit polyclonal antibodies to Aβ (AW8), the N-terminus of Amyloid precursor-like protein (APLP)-1 (W1NT), C-terminus of APLP1 (W1CT) and of APLP2 (W2CT) were from the Laboratory for Neurodegenerative Research and have been described previously (Mc Donald et al., 2010; Sala Frigerio et al., 2010). Antibodies to: $Aβ_{40}$ (2G3) and $Aβ_{42}$ (21F12), beta-amyloid cleaving enzyme (BACE)1 (3D5), tau (5E2), prion protein (PrP) (ICSM-35), and the C-terminus of amyloid precursor protein (APP) (C1/1.6) were generous gifts from Drs P. Seubert (Elan Pharmaceuticals), R. Vassar (Northwestern University), K. Kosik (University of California Santa Barbara), P. Mathews (Nathan Kline Institute), and J. Collinge (University College London) and have been described before (BACE1 3D5: Zhao et al., 2007; APP C1/1.6: Boland et al., 2010; PrP ICSM-35: Freir et al., 2011b; tau 5E2: Kowall and Kosik, 1987; $Aβ_{40}$ 2G3 and $Aβ_{42}$ 21F12: Mc Donald et al., 2010). All other antibodies were purchased from the commercial sources indicated. Antibodies were directed to: the N-terminus of APP (22C11, mouse monoclonal, Millipore/Chemicon, Billerica, MA, USA); full length APLP2 (D2-II, rabbit polyclonal, 171617, Calbiochem, EMD Chemicals Inc, Gibbstown, NJ, USA); dynamin-1 (PA1-660, rabbit polyclonal, Thermo Scientific Pierce [Affinity BioReagents], Rockford, IL, USA); phospho (p)-tau (AT8, mouse monoclonal, Thermo Scientific Pierce [Affinity BioReagents]); cAMP response element-binding protein (CREB) (48H2, rabbit monoclonal, Cell Signaling Technology Inc, Danvers, MA, USA); p-CREB (Ser133, rabbit polyclonal, Cell Signaling Technology Inc); post-synaptic denisty protein 95 (PSD-95) (mouse monoclonal, Thermo Scientific Pierce [Affinity BioReagents]); synaptic vesicle protein 2 (SV-2) (SV-2a [E-15], goat polyclonal, Santa Cruz Biotechnology, Inc, Santa Cruz, CA, USA), synaptophysin (mouse monoclonal, Millipore, Temecula, CA, USA); synaptopodin (rabbit polyclonal, 163002, SynapticSystems GmbH, Gottingen, Germany) and Microtubule-associated protein (MAP)2 (HM-2, mouse monoclonal, Sigma-Aldrich Ltd). The secondary antibodies used were: sheep anti-mouse horse-radish peroxidase (HRP) and donkey anti-rabbit-HRP (both from Amersham, GE Healthcare Life Sciences, Little Chalfont, UK). CREB and pCREB controls were prepared from SK-N-MC cells treated with or without 3-isobutyl-1-methylxanthine and forskolin (Cell Signaling Technology Inc). Further details about antibodies are provided in Table 1.

Table 1
List of antibodies used in the study

Protein	Antibody name	Monoclonal/polyclonal	Dilution
Aβ	AW8	Rabbit polyclonal	1:80
$Aβ_{40}$	2G3	Mouse monoclonal	1 μg/mL
$Aβ_{42}$	21F12	Mouse monoclonal	1 μg/mL
APP	C1/1.6	Mouse monoclonal	1:1000
APP	22C11	Mouse monoclonal	0.5 μg/mL
APLP1	W1NT	Rabbit polyclonal	1:500
APLP1	W1CT	Rabbit polyclonal	1:500
APLP2	D2-II	Rabbit polyclonal	1:1000
APLP2	W2CT	Rabbit polyclonal	1:500
BACE1	3D5	Mouse monoclonal	1 μg/mL
Dynamin-1	PA1-660	Rabbit polyclonal	1:5000
Tau	5E2	Mouse monoclonal	0.2 μg/mL
p-tau	AT8	Mouse monoclonal	0.2 μg/mL
PrP	ICSM-35	Mouse monoclonal	0.5 μg/mL
CREB	48H2	Rabbit monoclonal	1:1000
pCREB	1B6	Rabbit polyclonal	1:500
PSD-95	Anti-PSD-95	Mouse monoclonal	0.5 μg/mL
SV-2	E-15	Goat polyclonal	1:1000
Synaptophysin	Anti-synaptophysin	Mouse monoclonal	1:10,000
Synaptopodin	Anti-synaptopodin	Rabbit polyclonal	1:1000
MAP2	HM-2	Mouse monoclonal	1:2000

Key: Aβ, amyloid β; APLP, amyloid precursor-like protein; APP, amyloid precursor protein; BACE-1, beta-amyloid cleaving enzyme-1; CREB, cAMP response element-binding protein; MAP2, microtubule-associated protein; p, phosphorylated; PrP, prion protein; PSD-95, post-synaptic density protein 95; SV-2, synaptic vesicle protein 2.

2.2. Preparation of Aβ-containing soluble human brain TBS extracts

Human brain tissue was obtained and used in accordance with the University College Dublin Human Research Ethics Committee guidelines (under approval LS-E-10-10-Walsh). Two brain samples were used for this study: brain 1 from a 85-year-old man (Drs R. Dykoski and J. Cleary, Minneapolis VA Health Care System, Minneapolis, MN, USA) with a clinical and pathological diagnoses of AD and brain 2 from a 78-year-old woman (from Asterand plc,