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β-Amyloid impairs axonal BDNF retrograde trafficking

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Abstract

The neurotrophin, brain-derived neurotrophic factor (BDNF), is essential for synaptic function, plasticity and neuronal survival. At the axon terminal, when BDNF binds to its receptor, tropomyosin-related kinase B (TrkB), the signal is propagated along the axon to the cell body, via retrograde transport, regulating gene expression and neuronal function. Alzheimer disease (AD) is characterized by early impairments in synaptic function that may result in part from neurotrophin signaling deficits. Growing evidence suggests that soluble β-amyloid (Aβ) assemblies cause synaptic dysfunction by disrupting both neurotransmitter and neurotrophin signaling. Utilizing a novel microfluidic culture chamber, we demonstrate a BDNF retrograde signaling deficit in AD transgenic mouse neurons (Tg2576) that can be reversed by γ-secretase inhibitors. Using BDNF-GFP, we show that BDNF-mediated TrkB retrograde trafficking is impaired in Tg2576 axons. Furthermore, Aβ oligomers alone impair BDNF retrograde transport. Thus, Aβ reduces BDNF signaling by impairing axonal transport and this may underlie the synaptic dysfunction observed in AD.
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Keywords: β-Amyloid; Alzheimer; BDNF; Neurotrophin; Axonal transport; Microfluidic chamber

1. Introduction

Alzheimer disease is defined pathologically by the accumulation of extracellular Aβ plaques and intracellular neurofibrillary tangles, which accompany synaptic and neuronal loss in the AD brain. While Aβ plaque accumulation is a clear risk factor associated with AD, cognitive decline precedes plaque pathology. Studies now suggest that soluble and/or oligomeric Aβ can cause synaptic deficits and correlate more closely with cognitive dysfunction than Aβ plaque load (Lue et al., 1999; McLean et al., 1999; Naslund et al., 2000).

Coincidentally, Aβ induces synaptic deficits similar to those observed in $BDNF^{-/-}$ and $TrkB^{-/-}$ mice. For example, soluble Aβ oligomers dramatically impair hippocampal long-term potentiation (LTP) and AD transgenic mice exhibit synaptic loss, impaired hippocampal LTP, and impaired hippocampal-dependent learning (Hsiao et al., 1996; Hsia et al., 1999; Mucke et al., 2000; Walsh et al., 2002; Cleary et al., 2005; Lesne et al., 2006). Thus, Aβ may impair brain function by disrupting BDNF signaling, which mediates neuronal plasticity involved in learning and memory (Tong et al., 2004; Garzon and Fahnestock, 2007). Supporting this notion, BDNF levels are reduced in pre-clinical stages of AD (Peng et al., 2005). BDNF enhances excitatory synaptic transmission, long-term synaptic plasticity, and hippocampal LTP, an analogue of learning (Kang and Schuman, 1995; Levine et al., 1995; Figurov et al., 1996; Akaneya et al., 1997; Gottschalk et al., 1998; Messaoudi et al., 1998). Reduced BDNF signaling leads to hippocampal and cortical synaptic plasticity deficits. Mice lacking either BDNF

Abbreviations: AD, Alzheimer disease; Aβ, β-amyloid; APP, amyloid precursor protein; BDNF, brain-derived neurotrophic factor; CNS, central nervous system; LTP, long-term potentiation; PI3K, phosphoinositide-3-kinase; PS1, presenilin-1; Rab7, Ras-related GTP-binding protein 7; Tg2576, AD transgenic mouse line; WT, wild-type.

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or TrkB exhibit impaired arborization, deficits in synaptic sprouting, decreased synapse number, impaired hippocampal LTP, impaired hippocampal-dependent learning, and cortical degeneration (Korte et al., 1995; Patterson et al., 1996; Causing et al., 1997; Martinez et al., 1998; Minichiello et al., 1999; Pozzo-Miller et al., 1999; Mizuno et al., 2000; Xu et al., 2000; Genoud et al., 2004).

When BDNF binds to TrkB, the phosphoinositide-3-kinase (PI3K), Ras/MAPK, and PLCγ/PKC pathways are activated. Signaling involves both local activation and long-distance retrograde transport of the BDNF/TrkB signal (Du and Poo, 2004). For axonal retrograde transport, TrkB is internalized into signaling endosomes that require the dynein motor protein to activate downstream signaling cascades (Grimes et al., 1996; Watson et al., 1999, 2001; Delcroix et al., 2003; Heerssen et al., 2004). The PI3K pathway plays a critical role in retrograde trafficking (Nielsen et al., 1999) and because Aβ interferes with BDNF-mediated PI3K activation (Tong et al., 2004), but not TrkB phosphorylation, we hypothesized that Aβ affects axonal retrograde transport and the downstream propagation of TrkB signaling.

Here, we directly tested this hypothesis. We have developed a microfluidic chamber that allows central nervous system (CNS) axons to be fluidically isolated to a compartment in which molecules, such as BDNF, can be selectively applied (Taylor et al., 2005). Using this chamber, we identified impaired TrkB retrograde processing in the axons of AD transgenic mouse (Tg2576) neurons. Furthermore, we found that Aβ oligomers cause these transport deficits. Therefore, in AD, transport deficits might underlie neuronal dysfunction and synaptic loss.

2. Materials and methods

2.1. Assembly of microfluidic culture chambers

The chamber was fabricated in PDMS using rapid prototyping and soft lithography similar to previously published procedures (Taylor et al., 2003). Glass coverslips (24 mm × 40 mm, No. 1, Corning Inc., New York, NY), sonicated in 95% EtOH (30 min), and dried in a sterile hood, were immersed in sterile aqueous solution (0.5 mg/ml poly-L-lysine (Sigma, St. Louis, MO)) in PBS (24 h, 5% CO_2, 37 °C incubator), rinsed, and dried in a sterile hood overnight. The chambers were noncovalently assembled by conformal contact. The chambers consist of two parallel microfluidic channels, both connected by inlet and outlet wells. These two channels or compartments are separated by a solid barrier region with >150 microgrooves embedded in the bottom of the connecting barrier. A slight volume difference between the two channels (40 μl) was used to generate a fluidic resistance within the microgrooves, facilitating the isolation of BDNF to axons.

2.2. Cell culture of primary neurons

All procedures were performed under an IUCAC approved protocol. Primary cortical neuron cultures were derived from embryonic rat (E18) or mice (E16) as previously described (Loo et al., 1993; Cribbs et al., 1996). Briefly, dissected tissue was dissociated with trypsin, triturated, and plated in microfluidic chambers fitted with poly-L-lysine coated glass coverslips in serum-free neurobasal supplemented with B27 (Invitrogen, Carlsbad, CA). Cells were plated at a density of 5×10^6 cells/ml and maintained in cultures until used.

Tg2576 neurons were derived from crossing Tg2576 mice containing the APP (amyloid precursor protein) double mutation K670N, M671L driven by the hamster prion promoter to B6SJL F1 mice (Jackson Labs, Bar Harbor, ME). The presence or absence of the human APP gene was demonstrated in individual pups by DNA genotyping. After DNA extraction, PCR analysis was performed using primers APP1502: 5′-CTG ACC ACT CGA CCA GGT TCT GGG T-3′ and APP1503: 5′-GTG GAT AAC CCC TCC CCC AGC CTA GAC CA-3′ as previously described (Hsiao et al., 1996). Non-transgenic littermates of the Tg2576 mice (APP) were used as the source of wild-type (WT) neurons. Neuronal purity was assessed by immunostaining with a mouse monoclonal β-III-tubulin (1:1000, Chemicon, Temecula, CA) and rabbit polyclonal GFAP (Glial Fibrillary Acidic Protein) (1:4000, DAKO, Denmark). Glial contamination was 4% ($n = 6$). To block Aβ production, neurons were treated with γ-secretase inhibitor IX (1 μM, Calbiochem, San Diego, CA) overnight.

2.3. Purification of BDNF-GFP

Endotoxin-free BDNF-GFP plasmid (generous gift from Dr. Masami Kojima, was introduced by nucleofection (Amaxa, Gaithersburg, MD)) into HEK cells followed by selection in DMEM containing 10% FBS and G418 (1 mg/ml, plasmid contains a neomycin cassette). BDNF-GFP was isolated from stably transfected pre-pro-BDNF-GFP HEK293 cells as follows: after cells reached confluency, secreted pro-BDNF-GFP from the media was removed and concentrated with Amicon YM-30 centrifugal filters (5000 g, 2 h) (30,000 MW cutoff, Millipore, Billerica, MA). Pro-BDNF was converted to mature BDNF-GFP by treatment with plasmin (Sigma, St. Louis, MO) as previously described (Pang et al., 2004). Mature BDNF-GFP was further purified by size exclusion chromatography (Amicon YM-50) where the flow-thru contained the protein of interest. BDNF-GFP is indistinguishable from BDNF both biochemically and biologically (Hartmann et al., 2001; Kohara et al., 2001), and we confirmed that our purified BDNF-GFP was biologically active. To assess biological activity, BDNF-GFP (20 ng/ml) was added to rat primary neurons and the BDNF-mediated activation of CREB (phosphorylation) was examined and compared to commercially available BDNF (50 ng/ml, Peprotech, Rocky Hill, NJ). The BDNF-GFP