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Brief communication

Modulation of insulin signaling rescues BDNF transport defects independent of tau in amyloid-β oligomer-treated hippocampal neurons

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ABSTRACT

Defective brain insulin signaling contributes to the cognitive deficits in Alzheimer's disease (AD). Amyloid-beta oligomers (AβOs), the primary neurotoxin implicated in AD, downregulate insulin signaling by impairing protein kinase B/AKT, thereby overactivating glycogen synthase kinase-3β. By this mechanism, AβOs may also impair axonal transport before tau-induced cytoskeletal collapse and cell death. Here, we demonstrate that a constitutively active form of protein kinase B/AKT prevents brain-derived neurotrophic factor (BDNF) transport defects in AβO-treated primary neurons from wild type ($tau^{+/+}$) and tau knockout ($tau^{-/-}$) mice. Remarkably, inhibition of glycogen synthase kinase-3β rescues BDNF transport defects independent of tau. Furthermore, exendin-4, an anti-diabetes agent, restores normal BDNF axonal transport by stimulating the glucagon-like peptide-1 receptor to activate the insulin pathway. Collectively, our findings indicate that normalized insulin signaling can both prevent and reverse BDNF transport defects in AβO-treated neurons. Ultimately, this work may reveal novel therapeutic targets that regulate BDNF trafficking, promote its secretion and uptake, and prolong neuronal survival during AD progression.

1. Introduction

Insulin signaling is required for neuronal survival and sustains critical brain functions such as learning and memory (Kleinridders et al., 2014). When defective, insulin signaling contributes to Alzheimer's disease (AD) (De Felice et al., 2014; Yang and Song, 2013). Amyloid-β oligomers (AβOs), a neurotoxin implicated in AD, induce a variety of cellular insults, including synaptic failure, dysregulation of intracellular signaling cascades, hyperphosphorylation of tau, and disruption of fast axonal transport (FAT) (Ferreira and Klein, 2011). FAT of proteins and organelles is essential for neuronal polarity, synaptic maintainance, and survival (Hinckelmann et al., 2013). Notably, FAT defects are associated with several neurodegenerative diseases, including the early stages of AD (Goldstein, 2012; Kim et al., 2011).

AβOs lead to the loss of cell surface insulin receptors (IR), deregulating downstream effectors in the insulin pathway (De Felice et al., 2009, 2014). How insulin signaling regulates FAT in healthy and diseased neurons is poorly characterized. Previously, we demonstrated that insulin signaling and insulin mimetics can prevent AβO-induced FAT defects (Bomfim et al., 2012); however, it is unknown how effectors downstream of the insulin receptor, such as protein kinase B/AKT and glycogen synthase kinase-3β (GSK-3β), modulate FAT. GSK-3β, a tau kinase, hyperphosphorylates tau (p-tau), promoting its aggregation and dissociation from microtubules, leading to cytoskeletal collapse and FAT disruption (Morris et al., 2011). Alternatively, GSK-3β may disrupt transport by inhibiting motor proteins in the absence of tau-dependent microtubule destabilization (Weaver et al., 2013). We sought to determine if insulin-related signaling could prevent AβO-induced disruption of brain-derived neurotrophic factor (BDNF) transport in a tau-independent manner. Impaired BDNF transport compromises hippocampal synaptogenesis and learning enhancement, and reduced levels of BDNF correlate with AD progression (Scharfman and Chao, 2013). By live-imaging BDNF in cultured hippocampal neurons from tau $^{+/+}$ and tau $^{-/-}$ mice, we demonstrated that AβO-induced FAT defects were prevented or reversed by modulating AKT and glycogen synthase kinase-3β (GSK3β). Similarly, we showed that the anti-diabetes drug, exendin-4, rescued FAT defects via the GLP-1 pathway independent of tau.

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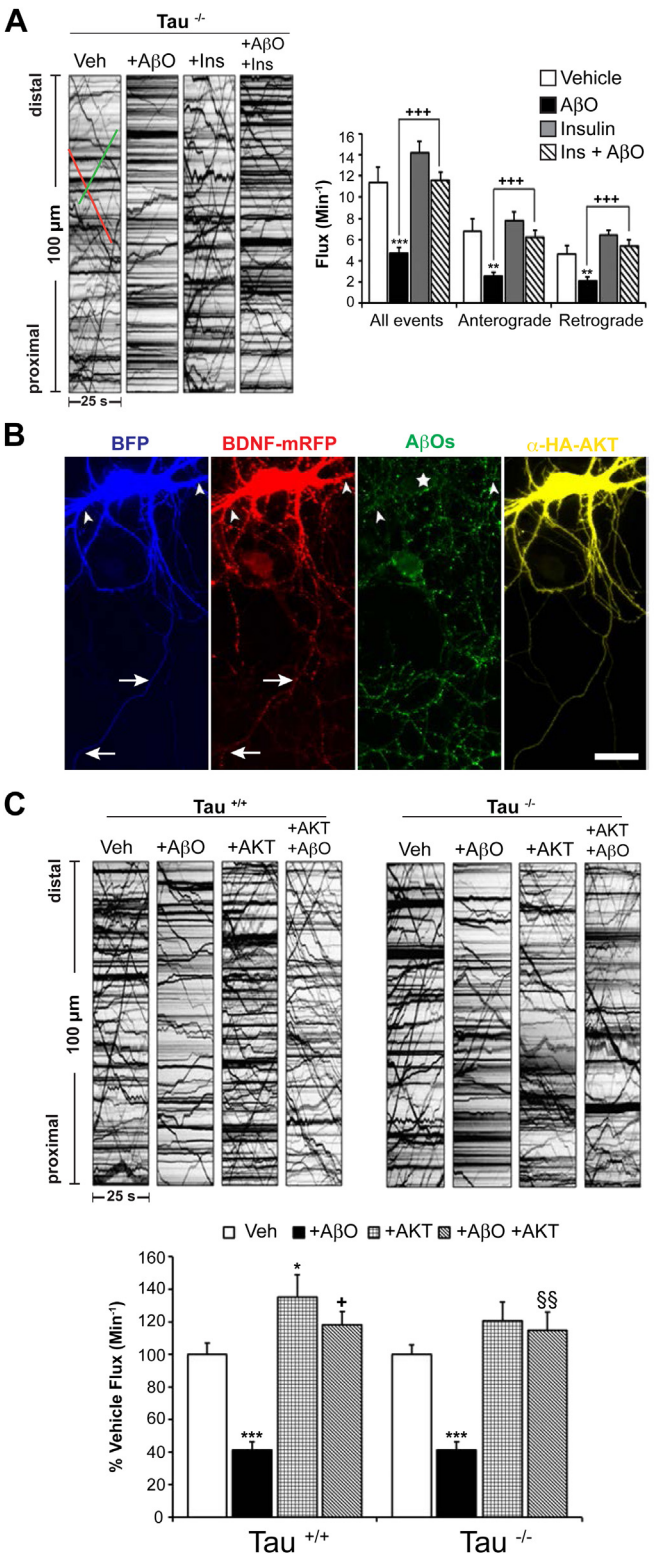

Fig. 1. Insulin and constitutively active AKT prevent AβO-induced transport defects in a tau independent manner. (A) Data and representative kymographs of BDNF transport in control and 500 nM AβO-exposed tau$^{-/-}$ neurons in the presence insulin. Green lines indicate anterograde transport, and red lines indicate retrograde transport. (B) Expression of soluble BFP and BDNF-mRFP in an AβO-treated tau$^{-/-}$ neuron (from left to right). Retrospective immunocytochemistry confirms AKT expression and that AβOs remain oligomeric after 18 hours in culture. Arrows indicate axon; arrowheads indicate dendrites. Scale bar = 25 µm (C) Data and representative kymographs of BDNF transport in control and 500 nM AβO-exposed tau$^{-/-}$ neurons expressing constitutively active AKT in tau$^{+/+}$ and tau$^{-/-}$ neurons. A minimum of 20 cells from 3 different cultures were analyzed per condition; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared with tau $^{+/+}$ vehicle; $+p < 0.05$, $+++p < 0.001$ compared with tau$^{+/+}$ + AβOs; §§$p < 0.01$ compared with tau$^{-/-}$ + AβOs. Representative BDNF vesicle transport is shown in Supplementary Fig. 1. See Supplementary Table 1 for complete transport statistics. Abbreviations: AβO, amyloid-beta oligomer; BDNF, brain-derived neurotrophic factor. (For interpretation of the references to color in this Figure, the reader is referred to the Web version of this article.)

2. Methods

2.1. Hippocampal cell cultures and expression of transgenes

Primary neurons were prepared exactly as described previously in Kaech and Banker (2006) and cultured in primary neuron growth media (Lonza). The astrocyte feeder layer for the neuronal coculture was generated using neural progenitor cells as described in Miranda et al. (2012). Plasmids were transfected into neurons at 9–12 days in vitro using Lipofectamine 2000 (Invitrogen), as per the manufacturer. Transgene expression was allowed to proceed for 24 hours before imaging. pcDNA3 Myr HA-AKT1 was purchased from Addgene.org (Plasmid #9008; Ramaswamy et al., 1999).

2.2. Aβ-oligomer preparation and drug treatments

Soluble Aβ oligomers (AβOs) were prepared exactly by the method of Lambert et al. (1998). AβOs (0.5 µM) were added to cultures 18 hours before imaging. To confirm AβO binding to dendrites and verify qualitatively that AβOs remained oligomeric after 18 hours in culture, cells were stained with an Aβ oligomer–specific antibody (NU-1, 1:1000; from W. Klein, Northwestern University). One hour before AβO or vehicle exposure, cells were incubated with 1.0 µM insulin (Sigma) or equivalent volumes of vehicle (PBS). For the GSK-3β inhibitor VIII (0.5 µM; Calbiochem), tideglusib (2.5 µM; Sigma), and exendin-4 (0.3 µM; Tocris) experiments, 18 hours following AβO or vehicle exposure, cells were incubated with drug or equivalent volumes of vehicle (DMSO) for 1–5 hours before imaging.

2.3. Imaging, image analysis, and immunocytochemistry

BDNF-mRFP transport was analyzed using a Leica DMI 6000B microscope equipped with a cooled CCD camera controlled by *MetaMorph* (Universal Imaging) according to Kwinter et al. (2009). Vesicle flux, velocity, and run lengths were obtained through tracing kymographs in *MetaMorph*. All values were compiled for analysis in Microsoft Excel, and significance difference between treatments was analyzed by *t* test with equal or unequal variances at a 95% confidence interval. To assess ca-AKT-HA plasmid expression, neurons were stained with anti-HA (1:500; Boehringer Mannheim) and processed as previously described (Kwinter et al., 2009).

3. Results

3.1. Insulin and the insulin-signaling intermediate AKT prevent AβO-induced BDNF transport defects independent of tau

AβOs cause IR internalization, preventing IR substrate-1 phosphorylation, and ultimately perturbing downstream effectors in the insulin-signaling pathway. Insulin pretreatment protects against AβO-induced insulin receptor internalization (De Felice et al., 2009; Zhao et al., 2008) and insulin application before AβO treatment prevents BDNF transport defects in hippocampal neurons from