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Alzheimer amyloid beta inhibition of Eg5/kinesin 5 reduces neurotrophin and/or transmitter receptor function

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ABSTRACT

The mechanism by which amyloid beta (Aβ) causes neuronal dysfunction and/or death in Alzheimer's disease (AD) is unclear. Previously, we showed that Aβ inhibits several microtubule-dependent kinesin motors essential for mitosis and also present in mature neurons. Here, we show that inhibition of kinesin 5 (Eg5) by Aβ blocks neuronal function by reducing transport of neurotrophin and neurotransmitter receptors to the cell surface. Specifically, cell-surface NGF/NTR(p75) and NMDA receptors decline in cells treated with Aβ or the kinesin 5 inhibitor monastrol, or expressing APP. Aβ and monastrol also inhibit NGF-dependent neurite outgrowth from PC12 cells and glutamate-dependent Ca++ entry into primary neurons. Like Aβ, monastrol inhibits long-term potentiation, a cellular model of NMDA-dependent learning and memory, and kinesin 5 activity is absent from APP/PS transgenic mice brain or neurons treated with Aβ. These data imply that cognitive deficits in AD may derive in part from inhibition of neuronal Eg5 by Aβ, resulting in impaired neuronal function and/or survival through receptor mislocalization. Preventing inhibition of Eg5 or other motors by Aβ may represent a novel approach to AD therapy.

1. Introduction

Genetic and biochemical studies have identified the amyloid beta (Aβ) peptide as playing a key role in the pathogenesis of Alzheimer's disease (AD), but the mechanism by which Aβ and other AD-related proteins, such as tau and ApoE, cause neuronal degeneration is still being elucidated (Hardy, 2009; Lee, 1996; Lee and Trojanowski, 2006; Mandelkow and Mandelkow, 1998; Potter and Wisniewski, 2012). For example, neuronal function depends critically on the correct localization and function of neurotransmitter and neurotrophin receptors, which are disrupted in AD, but the mechanism of this disruption is unknown (Abisambra et al., 2010; Almeida et al., 2005; Liu et al., 2010; Snyder et al., 2005; Tong et al., 2004). Previous findings suggested that receptor dysfunction may be linked to microtubule defects. For example, APP over-expression or Aβ treatment disrupts the function and structure of the cellular microtubule (MT) network, requires tau for its pathogenic effects (Abisambra et al., 2010; Boeras et al., 2008; Borysov et al., 2011; Geller and Potter, 1999; Granic et al., 2010; Hamano et al., 2005; Liu et al., 2008, 2009; Pigino et al., 2001; Rapoport et al., 2002; Roberson et al., 2007; Shah et al., 2009; Tezapsidis et al., 2003) and causes mislocalization of low-density lipoprotein receptor in cultured neurons (Abisambra et al., 2010). Furthermore, Aβ directly binds to and inhibits certain microtubule-dependent kinesin motors, including Eg5/kinesin 5/kif11 (Borysov et al., 2011), which are necessary for mitotic spindle structure and function (Heald and Walczak 1999; Hsu and Satya-Prakash, 1985; Mailhes et al., 2004; Mazumdar et al., 2004; Walczak and Heald, 2008). For example, studies of

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Michaelis–Menten kinetics revealed that Aβ competitively inhibits Eg5/kinesin 5 but has no effect on the classic KH1 kinesin motor or on CENP-E (Borysov et al., 2011). Furthermore, Aβ inhibits the binding of Eg5 to microtubules (Borysov et al., 2011). The fact that the several Aβ-inhibited motors Eg5/kinesin 5, Kif11, and MCAK are also present and functional in mature neurons (Baas, 1998; Takemura et al., 1996) and that Aβ expressed in transgenic mice carrying human AD-causing mutant amyloid precursor protein (APP) reduces the activity of kinesin 5/Eg5 in mouse brain to undetectable levels (Borysov et al., 2011) suggested to us that MT motor inhibition by Aβ might cause much of the neuronal dysfunction of AD by disrupting microtubule-dependent movement of key cellular constituents. To test this hypothesis, we asked whether Aβ inhibition of kinesin 5/Eg5 disrupts the localization of neurotrophin and neurotransmitter receptors to the cell surface, leading to impaired neuronal function. Specifically, cell surface levels of NGF/NTR(p75) and N-methyl-D-aspartate (NMDA) receptors were found to be greatly reduced in cells treated with Aβ or expressing APP, or treated with monastol, an Eg5/kinesin 5 inhibitor (Kapoor et al., 2000). Both Aβ and monastrol consequently inhibit NGF-dependent neurite outgrowth from PC12 cells and reduce glutamate-dependent Ca++ entry into primary neurons. Furthermore, Eg5/kinesin 5 activity is absent from primary neurons treated with Aβ, as it is in APP/PS transgenic mice brain, as mentioned previously (Borysov et al., 2011). Finally, like Aβ, monastrol inhibits long-term potentiation, a cellular model of NMDA-dependent learning and memory. These data imply that cognitive deficits in Alzheimer's disease may derive in part from inhibition of neuronal Eg5/kinesin 5 by Aβ, resulting in impairment of neuronal function through neurotransmitter and neurotrophin receptor mislocalization.

2. Methods

2.1. Antibodies

The following primary antibodies were used: anti-NMDA NR1 (extracellular) antibody (Alomone labs, Jerusalem, Israel; 3 μg/mL); anti-NMDAR2B (Millipore, Billerica, MA; 2 μg/mL); anti-extracellular p75 (gift from Dr Moses Chao; Huber and Chao, 1995); anti-alpha tubulin, wheat germ agglutinin conjugates (WGA, Invitrogen, Grand Island, NY). Goat anti-rabbit AlexaFluor 488, 594, and goat anti-mouse AlexaFluor 488 (Invitrogen, Molecular Probes, Grand Island, NY) antibodies were diluted according to the manufacturer for immunohistochemistry. WGA was conjugated with AlexaFluor 633. Anti-human Eg5 was from Abcam, Cambridge, MA; (ab37009). CellMask plasma membrane stain (Invitrogen) was also used.

2.2. Animals

Nontransgenic (NTG) mice were used for preparing primary neuron cultures. Day 18 pregnant mice were anesthetized with 0.1 mg/g Nembutal, and the whole brains of E18 embryos were immediately removed for processing. Primary neurons were obtained from the cortex and hippocampus. Brain slices for electrophysiology were obtained from 18-day NTG mice as described previously. All animal studies were approved by the University of South Florida's Institutional Animal Care and Use Committee and abided by that Committee's Policies on Animal Care and Use in accordance with the Guide for the Care and Use of Laboratory Animals, the Animal Welfare Regulations Title 9 Code of Federal Regulations Subchapter A, "Animal Welfare", Parts 1–3, and the Public Health Service Policy on Humane Care and Use of Laboratory Animals.

2.3. Cell culture

H4 cells (American Type Culture Collection [ATCC]) were cultured in Opti-MEM (Life Technologies, Grand Island, NY), 10% FBS fetal bovine serum (FBS), 1% penicillin and/or streptomycin, H4APP cells (from Todd Golde) were supplemented with additional 0.1% hygromycin. PC12 cells were plated at low density on collagen-coated plastic in RPMI plus 1% horse serum and nerve growth factor (NGF) (50 ng/mL). Neurites were noticeable at 1–3 days. Medium was changed 3 times per week. Neurons were prepared as described in Padmanabhan et al. (2006) with modifications. Timed pregnant C57/black mice were obtained from Harlan (Tampa, FL), the animals were anesthetized with pentobarbital, the embryos were dissected out, and their cortices and hippocampi triturated in. The dissociated cortex was centrifuged and the cells resuspended in neurobasal primary media (Invitrogen) with B27 supplement, penicillin and/or streptomycin and L-glutamine (all Life Technologies), and plated onto poly-L-lysine-coated (Sigma, St. Louis, MO) culture plates. Unattached cells were removed after 4 hours. The cells were replenished with half fresh media every fourth day.

2.4. Aβ internalization

FITC-Aβ1-42 (Anaspec, Fremont, CA; HiLyte Flour 488-labeled; 1 μM) was added to 1-week-old neuronal cultures and the cells were incubated overnight. Medium containing FITC-Aβ was removed, the cells washed with PBS, and fresh medium added before image acquisition. Cells were primarily neurons by morphology.

2.5. Immunolabeling of cell surface receptors

The immmunolabeling experiments were performed on 3-week-old cultures in poly-L-lysine coated 6-well-plates and 8-chamber slides. After treatment with either monastrol (50–100 μM; Sigma), Aβsc or Aβ42 (1 μM) for 24 hours (or 48 hours for PC12 cells) the cells were washed twice with PBS. Cells were fixed in 4% paraformaldehyde for 5 minutes at room temperature. After 2 rinses in PBS the nonspecific binding was blocked with 10% normal goat serum, 0.2% Triton X-100, 0.02% NaN3 in Tris-buffered saline (or in blocking buffer without detergent for non-permeabilized cell experiments) for 45 minutes at room temperature, then incubated for 2 hours at room temperature with primary antibody diluted in blocking buffer. After three, 5 minutes of wash in PBS, slides were incubated with secondary antibody diluted (1:1000) in blocking buffer for 45 minutes at room temperature in the dark and washed 2 times in PBS. Slides were stained with Hoechst (1 mg/mL in PBS) for 3 minutes to reveal cellular nuclei, washed for 3 minutes three times in PBS, and mounted using GelMount (Fisher Scientific, Waltham MA).

2.6. Eg-5 immunoprecipitation

Endogenous mouse Eg5 and/or kinesin 5 protein was immunoprecipitated with anti-human Eg5 (Abcam, ab37009, 1:5 dilution), and subjected to enzymatic kinetics assay based on Kinesin ELIPA Biochem kit (Cytoskeleton Inc) as described (Borysov et al., 2011).

2.7. Live and/or dead assay

Primary neurons were grown for 3 weeks before treated with either monastrol (100 μM; Sigma), Aβsc or Aβ42 (1 μM) for 48 hours. Live dead assay (Molecular Probes) was used according to the manufacturer's instructions and the reaction was measured by a Bio-Tek Synergy 2 plate reader. Data reported were obtained from 6 independent experiments. *p*-Values were obtained from paired *t* test analyses.