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Neuronal activity and secreted amyloid β lead to altered amyloid β precursor protein and presenilin 1 interactions

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ABSTRACT

Deposition of amyloid β (Aβ) containing plaques in the brain is one of the neuropathological hallmarks of Alzheimer's disease (AD). It has been suggested that modulation of neuronal activity may alter Aβ production in the brain. We postulate that these changes in Aβ production are due to changes in the rate-limiting step of Aβ generation, APP cleavage by γ-secretase. By combining biochemical approaches with fluorescence lifetime imaging microscopy, we found that neuronal inhibition decreases endogenous APP and PS1 interactions, which correlates with reduced Aβ production. By contrast, neuronal activation had a two-phase effect: it initially enhanced APP–PS1 interaction leading to increased Aβ production, which followed by a decrease in the APP and PS1 proximity/interaction. Accordingly, treatment of neurons with naturally secreted Aβ isolated from AD brain or with synthetic Aβ resulted in reduced APP and PS1 proximity. Moreover, applying low concentration of $Aβ_{42}$ to cultured neurons inhibited de novo Aβ synthesis. These data provide evidence that neuronal activity regulates endogenous APP–PS1 interactions, and suggest a model of a product-enzyme negative feedback. Thus, under normal physiological conditions Aβ may impact its own production by modifying γ-secretase cleavage of APP. Disruption of this negative modulation may cause Aβ overproduction leading to neurotoxicity.

Introduction

Alzheimer's disease (AD) is a progressive neurological disorder of the elderly that is associated with synaptic loss and displays amyloid plaques and neurofibrillary tangles as characteristic pathological hallmarks. The main components of amyloid plaques are Aβ peptides of different lengths generated from the amyloid β precursor protein (APP) by β-secretase and PS1/γ-secretase.

Recent study linked regional differences in neuronal activity and cerebral metabolism with amyloid deposition in the brain of transgenic mice (Bero et al., 2011); however, the underlying mechanism remains unclear. It has been suggested that neuronal activity may control APP processing, but reports in the literature how Aβ production is affected provide conflicting results. Studies using slice cultures and cultured cortical neurons show a positive correlation of Aβ secretion with neuronal activity, and suggest that neuronal activation favors cleavage of APP by β-secretase over α-secretase (Hoe et al., 2009; Kamenetz et al., 2003; Lesne et al., 2005). By contrast, other studies report that stimulation of NMDA receptors in primary cultured neurons inhibits Aβ release, induces trafficking of α-secretase ADAM10 to the postsynaptic membrane, and increases α-secretase mediated APP cleavage within the Aβ region (Marcello et al., 2007). The role PS1/γ-secretase plays in activity-controlled Aβ generation, however, remains poorly understood. Moreover, although the role of Aβ in Alzheimer's disease pathology is well established, it remains unclear whether Aβ at low, physiological concentrations may have a function in the brain.

Thus, the goal of the present study is to elucidate how neuronal activity affects endogenous Aβ production, to determine if APP and PS1/γ-secretase interaction is involved, and to evaluate whether Aβ may play a regulatory role in its own production. Using biochemical and morphological methods, we found that neuronal activation increases while neuronal inhibition decreases Aβ production and APP–PS1 proximity in primary neurons. Moreover, we show changes

Abbreviations: Aβ, amyloid β; APP, amyloid β precursor protein; AD, Alzheimer's disease; BACE1, β-secretase; DIV, days in vitro; FLIM, fluorescence lifetime imaging microscopy; FRET, fluorescence resonance energy transfer; mAb, monoclonal antibody; NDS, normal donkey serum; PS1, presenilin 1; PTX, picrotoxin; TTX, tetrodotoxin; SEC, size exclusion chromatography.

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in APP–PS1 interactions over time, and report a novel observation that elevated Aβ generated after initial neuronal activation has a negative feedback effect on APP–PS1 interactions, diminishing further Aβ generation. This suggests that at physiological concentrations Aβ may play a negative autoregulatory role in the normal brain.

Materials and methods

Antibodies and transfection

Rabbit anti-APP C-terminus (APP CT, Sigma-Aldrich, St. Louis, MO) and mouse monoclonal antibodies (mAbs) to PS1 loop domain (Millipore, Temecula, CA) were used to assess APP–PS1 proximity by FLIM. Aβ specific mAbs 82E1 (IBL, Fujioka, Japan), 6E10 (Covance, Princeton, NJ) and mAb to actin (AC-40, Sigma-Aldrich) were used for immunoblotting. Rabbit anti-GAD65, anti-GluR2 mAb (both from Millipore, Temecula, CA) and chicken anti-MAP2 (Abcam, Cambridge, MA) were used to assess GABAergic and glutamatergic neurons in culture. Rabbit polyclonal R1282 antibody used for Aβ immunoprecipitation was a gift from Dr. Dennis Selkoe (Brigham and Women's Hospital, Boston). The capture antibody (BNT77, against $A\beta_{11-28}$) and the detection antibodies (BA27 for $A\beta_{40}$ and BC05 for $A\beta_{42}$) used for Aβ-ELISA were from Takeda (Osaka, Japan). Alexa Fluor 488-conjugated or Cy3-conjugated species-specific anti-IgG secondary antibodies used for immuno-detection were from Molecular Probe (Eugene, OR) and Jackson ImmunoResearch Laboratories (West Grove, PA), respectively. The carboxyl-terminal GFP-tagged truncated APP construct, APP C99-GFP, was generated as previously described (Kinoshita et al., 2002). Transfection of this plasmid into primary neurons was performed using lipofectamine 2000 (Invitrogen, Carlsbad, CA).

Primary neuronal cultures

Mixed cortical and hippocampal primary neurons were generated from embryonic days 15–16 CD1 wild type or Tg2576 mice overexpressing 695 amino acids isoform of human APP containing K670N and M671L Swedish mutations, as previously described (Berezovska et al., 1999; Wu et al., 2010). Briefly, the cells re-suspended in chemically defined Neurobasal Media (Gibco, Gaithersburg, MD) containing 10% fetal bovine serum were plated on poly-D-lysine (Sigma-Aldrich, St. Louis, MO) coated dishes. Twenty-four hours after the plating, the cell culture media were exchanged to Neurobasal Media containing 2% B27 supplement (Gibco, Gaithersburg, MD), and neurons were maintained for 11–14 days in vitro (DIV) prior to treatment with inhibitory or excitatory agents, or with Aβ (see sections below).

Pharmacological treatment

To modulate neuronal activity in 11–14 DIV primary neurons we adopted the protocol described by Kamenetz et al (2003) using 10 mM $MgCl_2$ (N-methyl-D-aspartate (NMDA) receptor blocker), 1 μM tetrodotoxin (sodium channel blocker), 100 μM picrotoxin (non competitive antagonist for $GABA_A$ receptor chloride channel) (all from Sigma-Aldrich, St. Louis, MO) or H_2O (vehicle control). The cells were treated for various periods of time (see Results), ranging from 6 to 24 h. Neuronal viability and potential toxicity due to the treatment were assessed by visual inspection of neuronal morphology, by CellTiter-Glo luminescent cell viability assay (Promega, Madison, WI), or by monitoring the release of adenylate kinase in the culture medium using the ToxiLight Bio Assay kit (Cambrex, Rockland, ME). After 6 to 24 h of treatment, conditioned media were collected for Aβ-ELISA, and cells were immunostained prior to FLIM experiments.

Aβ treatment

Synthetic $A\beta_{42}$, $A\beta_{40}$ and reversed $A\beta_{40\text{-}1}$ peptides (BioSource International, Camarillo, CA) were prepared as 10 μM stock solution, stored at −20 °C and reconstituted in neuronal culture medium to 1 nM concentration immediately prior to neuronal treatment. Low oligomeric $A\beta_{42}$ was prepared by applying 100 μl of 1 mg/ml synthetic $A\beta_{42}$ peptide to size exclusion chromatography (SEC) on Superdex75 10/300 GL column (GE healthcare), and eluting in 10 mM Tris buffer (pH 7.4) with AKTA purifier 10 (GE healthcare) at a flow rate of 0.5 ml/min. The presence of Aβ oligomers in the selected eluted fractions was confirmed by western blotting. Protein concentration was measured by BCA assay (Thermo Scientific, Rockford, IL), and 1 nM low order oligomeric $A\beta_{42}$ fraction B2 was used for pulse-chase experiments (Supplementary Fig. 4). Alternatively, neurons were treated with naturally generated Aβ extracted from the brain of an AD patient or an age matched control individual. Briefly, cortical gray matter of temporal lobe from AD or non-demented control brain was homogenized in 4 volumes of Tris-buffer saline solution (TBS) with protease inhibitor cocktail (Roche) with 25 strokes on a mechanical Dounce homogenizer and centrifuged at 260,000 × g for 20 min at 4 °C. 75 μl of TBS soluble fractions of AD brain or control brain was applied to SEC Superdex75 10/300 GL column in 50 mM ammonium acetate (pH 8.5) with AKTA purifier 10, and eluted at a flow rate of 0.5 ml/min (Townsend et al., 2006). The presence of Aβ in the eluted fractions was confirmed by western blotting (Supplementary Fig. 4). Aβ-containing fractions (11–17 kD) were dialyzed against PBS overnight at 4 °C, and mixed with culture medium at a 1:1 ratio to treat primary neurons for 1 h.

Immunocytochemistry

After pharmacological or Aβ treatment, cells were washed briefly in PBS and fixed with 4% paraformaldehyde in PBS for 20 min. After three washing steps with PBS, cells were permeabilized with 0.1% Triton-X 100 for 20 min and incubated in 1.5% normal donkey serum (NDS) blocking solution for 45 min. Primary antibodies mAb PS1 loop and rabbit anti-APP CT diluted in 1.5% NDS were applied overnight at 4 °C, whereas corresponding Alexa 488- and Cy3-conjugated secondary antibodies were applied at room temperature for 1 h. Before and after antibody application, cells were washed three times in PBS for 5 min each to minimize nonspecific staining. After immunostaining, cells were covered with glass coverslips using GVA mounting solution (Zymed, South San Francisco, CA) and were used for the FLIM assay to evaluate endogenous PS1 and APP interactions. Alternatively, to distinguish between the PS1 interaction with either full length APP or APP C-terminal fragments, the primary neurons were transfected with C99-GFP (FRET donor) for 24 h prior to the treatment (see above). Treated cells were fixed and immunostained with mAb PS1 loop followed by Cy3-conjugated secondary antibody (FRET acceptor). BACE-CT or ADAM-10 CT antibodies (both from Calbiochem, Darmstadt, Germany) were used to detect β- and α-secretases, respectively. The primary neuronal cultures were immunostained with GAD65 or GluR2 antibodies to determine the percentage of GABAergic or glutamatergic neurons, respectively. MAP2 was used as a neuronal marker (total number of neurons); GFAP and Iba-1 antibodies were used to label astrocytes and microglia, respectively.

Fluorescence lifetime imaging microscopy (FLIM)

The proximity between fluorophore labeled endogenous PS1 and APP was assessed by previously validated FLIM assay as described (Berezovska et al., 2003; Lleo et al., 2004). Briefly, the baseline lifetime ($t1$) of the Alexa 488 fluorophore (negative control, FRET absent) was measured in the absence of an acceptor fluorophore. Upon excitation of the donor fluorophore in the presence of Cy3