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Amyloid-β precursor protein mediates neuronal toxicity of amyloid β through Go protein activation

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Abstract

Amyloid beta (Aβ) is a metabolic product of amyloid-β precursor protein (APP). Deposition of Aβ in the brain and neuronal degeneration are characteristic hallmarks of Alzheimer's disease (AD). Aβ induces neuronal degeneration, but the mechanism of neurotoxicity remains elusive. Here we show that overexpression of APP renders hippocampal neurons vulnerable to Aβ toxicity. Deletion of the extracellular Aβ sequence of APP prevents binding of APP to Aβ, and abolishes toxicity. Aβ toxicity is also abrogated by deletion of the cytoplasmic domain of APP, or by deletions comprising the Go protein-binding sequence of APP. Treatment with Pertussis toxin (PTX) abrogates APP-dependent toxicity of Aβ. Overexpression of PTX-insensitive Gα-o subunit, but not Gα-i subunit, of G protein restores Aβ toxicity in the presence of PTX, and this requires the integrity of APP-binding site for Go protein. Altogether, these experiments indicate that interaction of APP with toxic Aβ-species promotes toxicity in hippocampal neurons by a mechanism that involves APP-mediated Go protein activation, revealing an Aβ-receptor-like function of APP directly implicated in neuronal degeneration in AD.

Keywords: Amyloid beta (Aβ); Amyloid-β precursor protein (APP); Alzheimer's disease (AD); Neuronal degeneration; Pertussis toxin (PTX); Go protein

1. Introduction

Alzheimer's disease (AD) is the most frequent cause of memory loss and dementia in the elderly human population. The abundance of senile plaques and neuronal degeneration are characteristic features of AD histopathology. Senile plaques are complex lesions mainly composed of aggregated amyloid-β (Aβ) protein, and typically surrounded by dystrophic neurites. Aggregation confers toxicity to Aβ (Pike et al., 1991; Busciglio et al., 1992), suggesting its direct involvement in AD neurodegeneration. The mechanism of Aβ toxicity is not completely understood. Recent evidence suggests that different Aβ-aggregated species, such as Aβ-fibrils or Aβ-oligomers induce toxicity through distinct mechanisms (Deshpande et al., 2006). Toxicity of Aβ fibrils (fAβ), the most conspicuous species of Aβ in AD brain, appears to require the interaction of the fibrils with cell-surface receptors, resulting in altered modulation of signal transduction pathways. Consistent with this possibility it was shown that fAβ abnormally modulates the activity of focal adhesion proteins (Grace and Busciglio, 2003), actin-binding proteins (Heredia et al., 2006; Mendoza-Naranjo et al., 2007) and tau (Busciglio et al., 1995), indicating that fAβ may alter signaling-cascades resulting in neuronal degeneration around senile plaques.

Aβ is a metabolic product of the amyloid-β precursor protein (APP), and familial forms of AD can arise from mutations in APP or the Presenilins (Selkoe, 2001). These mutations alter APP-processing and the generation of Aβ predisposing to Aβ aggregation, which implicates an altered APP processing in the pathogenesis of familial AD (Hardy and Selkoe, 2002). More recently, it was found that increased gene dosage of wild-type APP is sufficient

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to cause early onset AD (Rovelet-Lecrux et al., 2006), indicating that a slight elevation in APP-expression predisposes to AD-neurodegeneration. The protein structure and cellular functions of APP are consistent with its potential role as a cell-surface receptor implicated in cell adhesion, although a natural ligand for APP remains to be established (for a review see Zheng and Koo, 2006). Importantly, APP binds fAβ and modulates their toxicity (Lorenzo et al., 2000; Van Nostrand et al., 2002), suggesting that fAβ may be a pathological ligand for APP. Moreover, deposition of fAβ promotes cell-surface accumulation of APP (Heredia et al., 2004), in a way that resembles clustering of cell-adhesion receptors upon binding to their extracellular ligands. However, it is unclear whether APP may directly mediate signaling events required for fAβ toxicity. In this work, we show that interaction of fAβ with the ectodomain of APP promotes APP-dependent activation of heterotrimeric Go protein, resulting in degeneration of hippocampal neurons. Taken together these results reveal that, in addition of being the source of Aβ, APP might function as a receptor for fAβ that may be directly implicated in neuronal degeneration in AD.

2. Material and methods

2.1. Neuronal cultures

Rat hippocampal cultures were established from embryonic days 18–19 fetuses as described previously (Heredia et al., 2004). Briefly, neurons were plated at a density of 80,000 cells/well with DMEM (Invitrogen, Gaithersburg, MD) plus 10% horse serum (Hyclone, Logan, UT) on poly-L-lysine (0.25 mg/ml)-coated 96 multiwell dishes; after 2 h, the medium was replaced with DMEM plus N2 and B27 supplements (Invitrogen, Gaithersburg, MD). The cultures were maintained at 37 °C in a 5% CO2 humidified atmosphere. Neuroblastoma cell line B103 was kindly provided by Dr David Schubert (The Salk Institute for Biological Studies, San Diego, CA), and cultured under standard conditions (Schubert and Behl, 1993).

2.2. Expression plasmids and transfections

Full-length wild-type human APP695 and mutant forms of APP were inserted in the expression plasmids pcDNA3.1 (Invitrogen, Gaithersburg, MD). Mutant forms of APP were generated on human APP695. The entire Aβ-juxtamembrane domain (aa 597-624) was deleted in APPΔβ. APP-ΔT carries a deletion of the entire intracytoplamic domain (amino acids 649-695). APP-Δ1 lacks 14 amino acids (KKKQYTSIHHGVVE) of the intracellular-juxtamembrane region of APP. APP-Δ2 lacks 18 amino acids (VDAAVTPEERHLSKMQQN) on the middle region of APP-cytoplasmic domain. APP-Δ3 lacks the last 15 amino acids (GYENPTYKFFEQMQN) of the c-terminus of APP. Full-length wild-type human APLP1 inserted in the expression plasmids pCEP (Invitrogen, Gaithersburg, MD) was kindly provided by Dr Stefan Kins (University of Heidelberg, Germany). Pertussis toxin-insensitive forms of Gαi/o proteins (i-Gαo, i-Gαi2, and i-Gαi3) inserted in pcDNA3.1 expression plasmid were generously provided by Dr J. Silvio Gutkind (National Institutes of Health, Bethesda, MD). Transient transfections were performed in 2 DIV rat hippocampal cultures grown in 96 multiwell culture dishes. Cultures were co-transfected with 0.04 μg/well of pEGFP-N1 plasmid encoding for the green fluorescent protein (GFP), and 0.015–0.12 μg/well of pcDNA3.1 plasmid encoding for the wild-type APP, or deletion-mutant forms of APP, by using Lipofectamine 2000 (0.4 μl/well; Invitrogen, Gaithersburg, MD) in 60 μl/well OptiMEM transfection medium (Invitrogen, Gaithersburg, MD). For some experiments, cultures were triple-transfected with pEGFP-N1 (0.04 μg/well) encoding the GFP, 0.06 to 0.12 μg/well of pcDNA3.1 plasmid encoding wild-type or deletion-mutant forms of APP and 0.06 μg/well of pcDNA3.1 plasmid encoding Pertussis toxin-insensitive forms Gαi/o proteins. Control cultures were transfected with identical amounts of the corresponding empty vectors. After 2 h, the transfection medium was replaced with DMEM plus N2 and B27 supplement (Invitrogen, Gaithersburg, MD). Treatment with Aβ was initiated immediately after transfection. After the indicated time, cultures were fixed and analyzed by fluorescence microscopy.

2.3. Treatments

Synthetic Aβ1-40 and Aβ1-42 were from Biopeptide Inc. (San Diego, CA). To prepare Aβ1-40 fibrils (fAβ), the peptide was dissolved in sterile double-distilled water to a concentration of 1 mM, incubated for 3 days at 37 °C, then diluted in PBS to 0.5 mM, and further incubated at 37 °C for 3–5 days to allow fibril formation. To prepare Aβ1-42 fibrils (fAβ42), the peptide was dissolved in double-distilled water to a concentration of 1 mM, incubated for 5–7 days at 37 °C. These Aβ preparations (fAβ) were added to the culture medium at final concentrations of 20 μM. For some experiments, the fAβ preparation was centrifuged at 14,000 rpm for 1 h in order to separate the supernatant containing aggregated/soluble Aβ (fAβs) from the pellet containing insoluble-fibrillar Aβ (fAβp), which was resuspended in a volume of PBS equal to the supernatant. These fractions were added directly to the neurons at final Aβ concentrations calculated using the initial concentration of the monomeric form of the peptide. Monomeric Aβ (mAβ) was prepared by dissolving the peptide in water immediately before use. Pertussis toxin (Sigma, St. Louis, MO) was used at a final concentration of 200–400 ng/ml.

2.4. Western blot

Cultures were harvested at 4 °C in RIPA buffer with protease inhibitors (Complete mini, Roche, Indianapolis, IN). The cell homogenates were then diluted in Laemmli sam-