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Stereospecific interactions are necessary for Alzheimer disease amyloid-β toxicity

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Abstract

Previous studies suggest membrane binding is a key determinant of amyloid β (Aβ) neurotoxicity. However, it is unclear whether this interaction is receptor driven. To address this issue, a D-handed enantiomer of Aβ42 (D-Aβ42) was synthesized and its biophysical and neurotoxic properties were compared to the wild-type Aβ42 (L-Aβ42). The results showed D- and L-Aβ42 are chemically equivalent with respect to copper binding, generation of reactive oxygen species and aggregation profiles. Cell binding studies show both peptides bound to cultured cortical neurons. However, only L-Aβ42 was neurotoxic and inhibited long term potentiation indicating L-Aβ42 requires a stereospecific target to mediate toxicity. We identified the lipid phosphatidylserine, as a potential target. Annexin V, which has very high affinity for externalized phosphatidylserine, significantly inhibited L-Aβ42 but not D-Aβ42 binding to the cultured cortical neurons and

Abbreviations: AD, Alzheimer's diseases; APP, amyloid precursor protein; Aβ, amyloid-β peptide; ACSF, artificial cerebral spinal fluid; BCA, bicinchoninic acid; CF, 5-carboxyfluorescein; DBU, 1,8-diazabicyclo[5,4,0]-undec-7-ene; DCF, dichlorofluorescein diacetate; DIV, days *in vitro*; DMF, dimethylformamide; DPH, diphenyl-1,3,5-hexatriene; EM, electron microscopy; EPR, electron paramagnetic resonance; fEPSP, field excitatory post-synaptic potential; HAE, 4-hydroxyalkenals; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; HFS, high-frequency stimulation; LDH, lactate dehydrogenase; LTP, long term potentiation; LUV, large unilamellar vesicles; MDA, malondialdehyde; MALDI-TOF MS, matrix-assisted laser desorption/ionization–time of flight mass spectrometry; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt; PBS, phosphate buffered saline; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; PICUP, photo-induced crosslinking of unmodified proteins; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; POPS, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-[phospho-L-serine]; PS-NBD, 1-oleoyl-2-[6-[(7-nitro-2-1,3-benzoxadiazol-4-yl)amino]hexanoyl]-*sn*-glycero-3-phospho-L-serine; ROS, reactive oxygen species; Ru(Bpy), ruthenium bis(*bpy*); TFA, trifluoroacetic acid.

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significantly rescued L-Aβ42 neurotoxicity. This suggests that Aβ mediated toxicity in Alzheimer disease is dependent upon Aβ binding to phosphatidylserine on neuronal cells.

Keywords: Cortex; Neurotoxicity; Chiral; Annexin V; Long term potentiation; Membrane phospholipid

1. Introduction

Alzheimer disease (AD) is the major cause of age-related dementia. AD is characterized by the abnormal accumulation of the amyloid-β peptide (Aβ) into insoluble aggregates known as plaques. Aβ is derived from the amyloid precursor protein (APP) (Kang et al., 1987), with Aβ40 and Aβ42 the dominant peptide species (Masters et al., 1985). The precise mechanism of Aβ-induced neuronal toxicity remains unresolved. Since Aβ contains part of the putative transmembrane domain of the APP (Kang et al., 1987) the mechanism of Aβ mediated neurotoxicity may involve interactions with the cell membrane (Verdier and Penke, 2004; Verdier et al., 2004). We previously reported that Aβ neurotoxicity correlated with an increased affinity for the neuronal plasma membrane (Ciccotosto et al., 2004; Smith et al., 2006; Tickler et al., 2005). Potential molecular targets on the cell membrane for Aβ includes lipids, proteoglycans, and proteins (Verdier and Penke, 2004).

The plasma membrane lipid composition is heterogeneous and asymmetrical, a process that is essential for normal cellular function (Bretscher, 1972). The choline-containing lipids, phosphatidylcholine (PC) and sphingomyelin, are primarily enriched in the extracellular outer cell surface of the membrane while the amine-containing glycerophospholipids, phosphatidyl-ethanolamine (PE) and phosphatidylserine (PS) are primarily located on the cytoplasmic surface of the plasma membrane (reviewed in Daleke, 2003; Pomorski et al., 2004). The role of externalized PS in neuronal membranes is typically used as an identification marker for cells that are undergoing apoptosis. Recent evidence highlighted that a specific neuronal cell population with increased membrane exposure of externalized PS correlated best with increased membrane Aβ binding (Simakova and Arispe, 2007). Annexin V peptide is a Ca^{2+} dependent phospholipid-binding protein that has a very high affinity for externalized PS (Mukhopadhyay and Cho, 1996; White et al., 2001). Aβ can induce PS flipping to the extracellular surface since fluorescently tagged annexin V binding was dramatically increased following neuronal Aβ treatment, an effect that was inhibited by co-incubation of Aβ peptides with annexin V antibodies (Lee et al., 2002; White et al., 2001).

Numerous hypotheses have been put forward to explain Aβ toxicity (Cappai and Barnham, 2007b) including the generation of reactive oxygen species (ROS), the self-assembly of aggregates that bind and form holes in membranes or Aβ interacting with receptors, such as RAGE. To test if Aβ toxicity and cell membrane binding was receptor mediated i.e. required a stereospecific interaction, we synthesized D- and L-handed versions of Aβ42 because in the absence of a chiral environment, both the variants would behave identically and as such if a receptor was not required for toxicity then it would be expected that both peptides would be equally toxic. When the biological properties of L- and D-Aβ42 were examined, it was observed that unlike L-Aβ42, D-Aβ42 was not neurotoxic to primary cortical cultures and did not inhibit long term potentiation (LTP) in mouse hippocampal slices. When we examined the membrane binding characteristics of the two peptides, it was observed that while both L- and D-Aβ42 enantiomers bound to neurons in culture to a similar extent, annexin V could only inhibit the binding of L-Aβ42 and not D-Aβ42 variant. Based on these observations, we propose that Aβ membrane binding occurs via PS and therefore the externalized PS is functioning as a specific early molecular target for Aβ interaction ultimately leading to cell death and/or inhibition of long term potentiation.

2. Materials and methods

2.1. Peptide synthesis

Peptide synthesis was performed using solid state Fmoc chemistry, in a continuous flow synthesizer; the synthesis of the L-handed (levorotatory) peptide was carried out using Fmoc-L-Ala-PEG-PS resin (Applied Biosystems, Foster City, CA) whilst for D-handed (dextrorotatory) peptide, TentaGel S Trt D-Ala-Fmoc resin (RAPP Polymer GmbH, Tubingen, Germany) was used. Both the synthesis and deprotection steps were undertaken using 2% 1,8-diazabicyclo[5,4,0]-undec-7-ene (DBU) in dimethylformamide (DMF) from the C-terminus to Ser 8 and then changed to 20% piperidine in DMF until the N-terminus. All the DBU steps were performed for 5 min whist the 20% piperidine reactions were extended to 10 min. All the amino acids were coupled to the resin using a 3-fold excess where 0.5 M 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate and 0.5 M of *N*,*N*-diisopropylethylamine were used as activators. Each coupling was performed for 1 h after which the resin was washed with DMF. Upon synthesis completion, the resin was swelled in DMF and either biotin or 5-carboxyfluorescein (CF) was coupled overnight using the standard coupling methods described previously. The resin was washed with DMF and methanol, lyophilized and then the peptide was cleaved from the resin by stirring in a solution of 1% water,