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SCIENCE DIRECT
Neurochemistry International 46 (2005) 575–583
NEUROCHEMISTRY International
www.elsevier.com/locate/neuint

Aβ(31–35) peptide induce apoptosis in PC 12 cells: Contrast with Aβ(25–35) peptide and examination of underlying mechanisms

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Received 4 November 2004; received in revised form 8 January 2005; accepted 17 January 2005
Available online 24 February 2005

Abstract

The toxic behaviour of the two shorter sequences of the native Aβ amyloid peptide required for cytotoxicity i.e., Aβ(31–35) and Aβ(25–35) peptides, was studied. We have shown that Aβ(31–35) peptide induces neurotoxicity in undifferentiated PC 12 cell via an apoptotic cell death pathway, including caspase activation and DNA fragmentation. Aβ(25–35) peptide, like the shorter amyloid peptide has the ability to induce neurotoxicity, as evaluated by the MTS reduction assay and by adherent cell count, but the Aβ(25–35) peptide-induced neurotoxicity is not associated with any biochemical features of apoptosis. The differences observed between the neurotoxic properties of Aβ(31–35) and Aβ(25–35) peptides might result on their different ability to be internalised within the neuronal cells. Furthermore, this study reveals that the redox state of methionine residue, C-terminal in Aβ(31–35) and Aβ(25–35) peptides affect in a different way the toxic behaviour of these two short amyloid fragments. Taken together our results suggest that Aβ(31–35) peptide induces cell death by apoptosis, unlike the Aβ(25–35) peptide and that role played by methionine-35 in Aβ induced neurotoxicity might be related to the Aβ aggregation state.

Keywords: Amyloid β-peptide; AβP(31–35) fragment; AβP(25–35) fragment; Methionine oxidation; Mitochondria; Apoptosis; Neurotoxicity

1. Introduction

Alzheimer's disease (AD) is a neuronal pathology characterised by the presence of senile plaques in several regions of the brain, particularly concentrated in those zones where neurodegeneration occurs. The major protein component of the plaques is the amyloid β-peptide (AβP), which is a 39–43 amino acid peptide that is formed by a much larger transmembrane protein, the amyloid precursor protein (APP) (Selkoe, 1994; Hardy, 1997). It is widely accepted that AD syndrome starts with various gene defects, leading to altered APP expression or proteolytic processing, or to changes in Aβ stability or aggregation. These in turn result in a chronic imbalance between Aβ production and clearance. Aβ is released extra and intracellularly and can also be accumulated extra and intracellularly (Dickson, 2004). It has been reported that toxic conformation of Aβ is a quaternary structure such as an aggregated fiber (Pike et al., 1991) and its gradual accumulation may represent a starting point of a complex, multistep cascade that include inflammatory changes, gliosis and transmitter loss (Selkoe, 2002). However, other reports have indicated that non-aggregated Aβ may intercalate into plasma membrane and directly alter membrane activities (Arispe et al., 1993; Etcheberrigaray et al., 1994; Muller et al., 1998). Recently, we have reported that Aβ(31–35) peptide, although it does not exhibit aggregation phenomena (Yan et al., 1999), induce several toxic and pro-apoptotic effects in isolated brain mitochondria (Misiti et al., 2004).

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0197-0186/$ – see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuint.2005.01.001

This finding supports the hypothesis that Aβ peptide in non-aggregated form might play a key role in neurotoxicity (Pillot et al., 1996, 1999; Schubert et al., 1995; Lambert et al., 1998; Favit et al., 1998; Arispe et al., 1993; Etcheberrigaray et al., 1994). In addition, we have demonstrated that Aβ(31–35) peptide, with the sulfur of methionine oxidised, shows a toxic behaviour different from that displayed by unoxidised Aβ(31–35) (Misiti et al., 2004; Clementi et al., 2004). These and other reports suggest that the active centre of neurotoxicity of AβP may be the 31–35 core sequence and methionine 35 might play a key role in the Aβ dependent mechanism of toxicity (Butterfield and Bush, 2004; Hou et al., 2002; Varadarajan et al., 2001). Because the relation existing between the oxidation of Met-35 and its influence on β-aggregation, neurotoxicity and fibril formation of AβP (Iversen et al., 1995; Varadarajan et al., 1999; Seilheimer et al., 1997), still remain controversial, in the current study, we further investigate the role of methionine 35, comparing the neurotoxic features of Aβ(25–35) peptide, that exhibit significant levels of molecular aggregation, retaining the toxicity of the full-length peptide (Terzi et al., 1995; Shearman et al., 1994) with those evidenced by the soluble AβP(31–35) peptide and by a series of peptides where the methionine at residue 35 is substituted by norleucine or it is oxidised to a sulfoxide.

Our results show that in neuronal PC 12 cells, the soluble Aβ(31–35) peptide induces cell death by apoptosis, unlike the aggregated Aβ(25–35) peptide does. Furthermore, methionine residue, C-terminal in Aβ(25–35) and Aβ(31–35) peptides mediates the degree of neurotoxicity induced by soluble Aβ(31–35) peptide, but not that by the aggregated form of Aβ(25–35) peptide, suggesting that the differential role played by methionine-35 in Aβ induced neurotoxicity is related to the Aβ aggregation form.

2. Materials and methods

2.1. Preparation of peptides

Aβ(25–35), Aβ(25–35)Met-35^{ox}, Aβ(25–35)Met-35^{Nle}, Aβ(31–35), Aβ(31–35)Met-35^{ox} and Aβ(31–35)Met-35^{Nle} were obtained by Peptide Speciality Laboratories GmbH (Heidelberg, Germany). Analysis of the peptide by reverse phase high-performance chromatography (HPLC) and mass spectrometry revealed a purity >98%. Stock solutions of the soluble Aβ peptides were prepared at 8 mM concentration in DMSO, according to Boland et al. (1996) and kept frozen at −20 °C. Stock solutions of the aggregated form of Aβ(25–35) were prepared according to the manufacturer's instructions by leaving a 4 mM Aβ(25–35) solution in ddH_2O at room temperature for 2 h. Thawing and dilutions to the final concentration in the proper medium was performed immediately before use.

2.2. FITC labelling of peptides

Fluorescein isothiocyanate (FITC) was purchased from Sigma. Labelling was conducted essentially according to the manufacturer's recommendations. In brief, FITC was freshly dissolved in Me_2SO to 1 mg/ml, and added to 2 mg/ml of each peptide in 50 mM potassium phosphate buffer (final pH 7.6) to a final concentration of 25 μg/ml. The calculated molar ratio of FITC to peptides was about 1:10. After incubation for 16 h in the dark at 4 °C, 50 mM NH_4Cl was added to inactivate the residual FITC. The solutions were left in the dark for an additional 2 h at 4 °C, and stored in aliquots at 2.5 mM at −20 °C. At the moment of experiments fluorescent compounds, at a final concentration of 40 μM was added to PC 12 cell suspensions kept in ice. Incubation has been performed for 2 h at +4 and +37 °C. Thereafter cells were washed three times with saline solution (NaCl 0.7% v/v) and observed at a fluorescent phase contrast inverted microscope (Olympus IX-70 System). Images were taken by using a 40× objective. Processing images was done on a PC using the software package Photoshop (Adobe Systems Inc., Mountain View, CA, USA).

2.3. Cell culture and Aβ treatment

The PC 12 cell line maintained in 5% CO_2 atmosphere at 37 °C in RPMI with HEPES 10 mM, glucose 1.0 g/l, $NaHCO_3$ 3.7 g/l, penicillin 100 units/ml, streptomycin 100 μg/ml, 10% fetal calf serum and 15% horse serum. The medium was changed every other day and cells were plated at an appropriate density according to each experiment scale. For determination of cytotoxicity of Aβ peptides, PC 12 (rat pheocromocytoma) were initially plated in 96-well plates at a density of 10,000 cells/well and maintained 16 h in complete medium cells were then incubated for 24, 36 and 48 h in the absence (controls) and presence of 40 μM Aβ(31–35) and Aβ(25–35) peptides with methionine in the oxidised and unoxidised form and with Met → Nle substituted Aβ peptides. Staurosporine 10 μM was used as positive control of 100% of cellular death (data do not shown).

2.4. Direct toxicity study

Cell survival was evaluated by two different methods: the 3-[(4,5-dimethylthiazol-2-yl)-5,3-carboxymethoxyphenyl]-2-(4-sulfophenyl)-2H tetrazolium, inner salt (MTS) reduction assay (CellTiter 96 Aqueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA) and the number of adherent cells on collagen type 1 coated plates (SIGMA, St. Louis, USA). The MTS assay is a sensitive measurement of the normal metabolic status of cells, particularly that of mitochondria, which reflects early cellular redox changes. After incubation, cells were treated with the MTS solution (2 mg/ml) and after incubation for 4 h