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N-truncated amyloid-β oligomers induce learning impairment and neuronal apoptosis

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Abstract

N-terminal-truncated forms of amyloid-β (Aβ) peptide have been recently suggested to play a pivotal role early in Alzheimer's disease (AD). Among them, Aβ3(pE)-42 peptide, starting with pyroglutamyl at residue Glu-3, is considered as the predominant Aβ species in AD plaques and pre-amyloid lesions. Its abundance is reported to be directly proportional to the severity of the clinical phenotype. The present study investigates the effects of soluble oligomeric Aβ3(pE)-42 after intracerebroventricular injection on mice learning ability and the molecular mechanisms of its in vitro neurotoxicity. Mice injected with soluble Aβ3(pE)-42 or Aβ(l-42) displayed impaired spatial working memory and delayed memory acquisition in Y-maze and Morris water maze tests, while those injected with soluble Aβ(42-1) showed no effect. These cognitive alterations were associated with free radical overproduction in the hippocampus and olfactory bulbs, but not in the cerebral cortex or cerebellum. In vitro, Aβ3(pE)-42 oligomers induced a redox-sensitive neuronal apoptosis involving caspase activation and an arachidonic acid-dependent pro-inflammatory pathway. These data suggest that Aβ3(pE)-42 could mediate the neurodegenerative process and subsequent cognitive alteration occurring in preclinical AD stages.

Keywords: Alzheimer's disease; Soluble amyloid-β oligomers; N-truncated amyloid-β; Neuronal apoptosis; Learning and memory

1. Introduction

Alzheimer's disease (AD) is the most common form of senile dementia characterized by irreversible memory impairment, continuous cognitive decline, and behavioral disturbances. Early dysfunction of synaptic integrity associated with apoptotic neuronal death is indeed a typical event in AD pathogenesis (Selkoe, 2002, 2004). It is associated with the 40 to 42-residue β-amyloid (Aβ) peptide that results from the processing of the amyloid precursor protein (APP) and that is known to aggregate in senile plaques, one of the neuropathological hallmarks of this neurodegenerative disease (Drouet et al., 2000; Selkoe, 2004).

Increasing evidence highlights the critical role played by N-terminal-truncated forms of Aβ in AD development (Geddes et al., 1999; Piccini et al., 2005; Tekirian, 2001). N-terminal-truncated Aβ peptides are known to accumulate early in the brain of sporadic AD patients, in early onset familial AD patients, most particularly in PS1 mutation carriers, and in Down syndrome brain (Russo et al., 1997; Saido et al., 1996; Tekirian et al., 1998). Among them, Aβ peptides starting with pyroglutamyl at residues Glu-3 or Glu-11, particularly Aβ3(pE)-42, have been suggested to be early aggregating species in AD (Tekirian, 2001) and are

Abbreviations: Aβ, amyloid-β peptide; AD, Alzheimer's disease; $cPLA_2$, cytosolic calcium-dependent phospholipase A_2; DAPI, 4,6-diamidino-2-phenylindole; DMEM, Dulbecco's modified eagle's medium; icv, intracerebroventricular; MAFP, methyl arachidonyl fluorophosphonate; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline

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considered as an important component of Aβ species in AD plaques (Harigaya et al., 2000; Kuo et al., 1997; Miravalle et al., 2005; Russo et al., 2000). They are also present in pre-amyloid lesions (Lalowski et al., 1996). Most importantly, the increase of the N-terminal-truncated species is proportional to the severity of the disease in terms of course duration and early onset (Russo et al., 1997, 2000). These results indicate that the intensity of neuronal degeneration and the severity of the clinical phenotype are directly proportional to the predominance of the Aβ3(pE)-42 peptide. The origin of these N-terminal-truncated forms remains unknown. They may be derived from the amyloid precursor protein through an alternative β-secretase cleavage (Russo et al., 2000), despite the fact that the formation of Aβ3(pE)-40 and Aβ3(pE)-42 species as products of constitutive APP processing have not been detected (Shirotani et al., 2002). Alternatively, Aβ3(pE)-42 may be produced from the full-length (1-42) form by extracellular peptidases and then modified by glutaminyl cyclase to generate a pyroglutamylated form that might be more resistant to proteolytic degradation (Saido et al., 1995; Shirotani et al., 2002). Interestingly, the proteolysis of N-terminal cyclized parts of Aβ requires neprilysin, a metalloprotease that is reduced in AD brains (Yasojima et al., 2001).

The original amyloid cascade hypothesis causally links AD clinico-pathological process and neuronal cell death to the aggregation and deposition of Aβ (Anderson et al., 1996; Estus et al., 1997; Hardy and Higgins, 1992). However, this hypothesis has been challenged by several clinical observations, including the poor correlation between dementia and amyloid plaque burden (Katzman et al., 1988). Furthermore, the amyloid cascade hypothesis has been recently challenged by our studies and others, strongly suggesting a close association between neuronal loss and a proapoptotic effect of the soluble oligomers of the Aβ peptide (Kriem et al., 2005; Pillot et al., 1999; Sponne et al., 2003; Walsh et al., 2002; Wang et al., 2002). Indeed, it has been recently demonstrated using mouse cerebral slices that soluble oligomers of Aβ are responsible for regiospecific toxicity to hippocampal CA1 neurons, a region involved in cognitive functions (Kim et al., 2003). Moreover, the synaptic loss in AD brain has been correlated with the pool of soluble Aβ peptide rather than with that of fibrillar Aβ (Lue et al., 1999; McLean et al., 1999; Wang et al., 1999). Both neurodegeneration and specific spatial learning deficits associated with early Aβ oligomer accumulation occur without amyloid plaque formation in AD transgenic models (Chang et al., 2003; Hsia et al., 1999; Koistinaho et al., 2001, 2002). These observations have been further corroborated by a recent elegant study showing the targeting of synaptic terminals by Aβ oligomers in AD brain (Lacor et al., 2004). In addition, increasing evidence suggests that oxidative damage is associated with the development of AD (Cardoso-Pelaez et al., 1999; Driver et al., 2000), including oxidative damage to protein, lipids, and nuclear and mitochondrial DNA (Aksenov and Markesbery, 2001; Montine and Morrow, 2005; Perry et al., 2002; Smith et al., 1996). In addition, it has been clearly demonstrated that the generation of reactive oxygen species is involved in fibrillar as well as soluble Aβ peptide-induced neurotoxicity (Butterfield, 2003; Butterfield and Boyd-Kimball, 2005; Florent et al., 2006; Sponne et al., 2003). However, the molecular mechanisms triggering soluble Aβ-mediated cell death remain unclear.

Despite the amount of information describing pyroglutamate-modified N-terminal-truncated Aβ as the molecules most likely related to the amyloidogenic process and to the severity of the disease (Larner, 1999), little and contrasting data are available concerning the neurotoxicity of fibrillar aggregates of Aβ3(pE)-40 and Aβ3(pE)-42 peptides (He and Barrow, 1999; Tekirian et al., 1999). To date, the neurotoxicity of soluble oligomers of N-terminal-truncated Aβ has not been addressed, and no data are available concerning their in vivo effects. We therefore addressed the question of whether cognitive deficits associated with Aβ peptides may be directly caused by soluble Aβ3(pE)-42 oligomers. Furthermore, we performed experiments aimed to determine the molecular mechanisms of neuronal cell death induced by soluble Aβ3(pE)-42 oligomers.

2. Experimental procedures

2.1. Materials

The caspase substrates (Ac-DEVD-AMC and Ac-LEHD-AMC) and inhibitor peptides (ZVAD-fmk, Ac-DEVD-fmk) were purchased from Bachem. Aβ(l-40), Aβ(40-l), Aβ(l-42) and Aβ3(pE)-42 were also obtained from Bachem, and soluble oligomers of Aβ were prepared as previously described (Kriem et al., 2005; Pillot et al., 1999; Sponne et al., 2003). Briefly, to overcome problems of peptide solubility at high concentrations, fresh peptide stock solutions were prepared at 5 mg/ml in hexafluoro-2-propanol (Sigma). For preparation of Aβ oligomers, aliquots of peptide stock solution were quickly dried under nitrogen, directly solubilized into culture medium and incubated for 1 h at room temperature before use. The presence of oligomers of Aβ was detected by SDS-PAGE and immunoblot analysis. Oligomeric preparations of Ap resolve to monomers, trimers and tetramers (15–20 kDa) after SDS-PAGE, as previously described (Dahlgren et al., 2002; Malaplate-Armand et al., 2006; Pillot et al., 1999). After this solubilization protocol, no major differences were observed in the preparation and structural characterization of Aβ(l-42) and Aβ3(pE)-42 (see supplementary data, Fig. 1). Peptide solutions were then applied onto the cells or used for in vivo experiments. The specific $cPLA_2$ inhibitor, methyl arachidonyl fluorophosphonate (MAFP), was obtained from Calbiochem. All other chemicals were purchased from Sigma. Unless otherwise indicated, materials used for cell culture were obtained from Life Technologies (Invitrogen).