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Long-term soluble $A\beta_{1-40}$ activates CaM kinase II in organotypic hippocampal cultures

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Abstract

Recent findings suggested a role for soluble amyloid-β (Aβ) peptides in Alzheimer's disease associated cognitive decline. We investigated the action of soluble, monomeric $A\beta_{1-40}$ on CaM kinase II, a kinase involved in neuroplasticity and cognition. We treated organotypic hippocampal cultures short-term (up to 4 h) and long-term (5 days) with $A\beta_{1-40}$ (1 nM–5 μM). Aβ did not induce cell damage, apoptosis or synaptic loss. Short-term treatment down-regulated enzymatic activity of the kinase, by reducing its Thr^{286} phosphorylation. In contrast, long-term treatment (1 nM–5 μM) markedly and significantly up-regulated enzymatic activity, with peak stimulation at 10 nM (three-fold). Up-regulation of activity was associated with increased expression of the α-isoform of CaM kinase II, increased phosphorylation at Thr^{286} (activator residue) and decreased phosphorylation at $Thr^{305-306}$ (inhibitory residues). We investigated the effect of glutamate on CaM kinase II following exposure to 1 or 10 nM $A\beta_{1-40}$. As previously reported, glutamate increased CaM kinase II activity. However, the glutamate effect was not altered by pretreatment of slices with Aβ.

Short- and long-term Aβ treatment showed opposite effects on CaM kinase II, suggesting that long-term changes are an adaptation to the kinase early down-regulation. The marked effect of $A\beta_{1-40}$ on the kinase suggests that semi-physiological and slowly raising peptide concentrations may have a significant impact on synaptic plasticity in the absence of synaptic loss or neuronal cell death.

Keywords: Alzheimer; Amyloid; CaM kinase II; Synaptic plasticity; Protein phosphorylation; Glutamate

1. Introduction

The amyloid hypothesis has attributed a central neuropathological role to amyloid β peptides (Aβ), typical constituents of the hallmark amyloid plaques of Alzheimer's disease (AD). However, several independent findings recently led to a reformulation of the hypothesis, leading to the proposal that early cognitive dysfunction is linked to the action of soluble, low molecular weight, non-deposited Aβ [10,19]. These findings include: (1) the onset of cognitive deficits in transgenic (tg) mice carrying mutations of amyloid precursor protein (APP) is found prior to formation of Aβ plaques [18]; (2) the synaptic loss in patients is inversely correlated with levels of soluble Aβ in brain [15,17]; (3) soluble Aβ and/or soluble oligomeric forms of Aβ acutely induce marked changes in excitability, neurotransmitter release and synaptic plasticity, as well as in related signaling [3,12,24,25,27]. It has been proposed that soluble, oligomeric Aβ peptides induce changes in synaptic physiology and

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related signaling, impairing synaptic plasticity (e.g., long-term potentiation, LTP). These changes could be associated with early cognitive symptoms of AD and in turn lead to synaptic loss preceding degeneration of neuronal pathways.

AD primarily affects memory and cognitive functions. Ca^{2+}/calmodulin-dependent protein kinase II (CaM kinase II) is an enzyme particularly enriched in brain, where it was shown to be essentially required for the formation of new memories and also the induction of synaptic correlates of memory (e.g., LTP) [7,14]. Therefore, it is interesting to assess whether Aβ affects CaM kinase II, particularly with regard to synaptic dysfunction induced by Aβ peptides in early AD. Previous studies suggested an involvement of this kinase in the cellular effects of Aβ. It was shown that $Aβ_{1–40}$-induced translocation of low density lipoprotein receptor-related protein, a proposed protective event, was mediated by activation of CaM kinase II in C6 glioma cells [23]. Recently, it was found that acute in vitro application of $Aβ_{1–42}$ peptide inhibited LTP and related autophosphorylation of CaM kinase II at Thr^{286} [27]; furthermore, we showed that enzymatic activity and regulation of this kinase are deranged in cultured fibroblasts from AD patients [2]. However, although CaM kinase II represents a major effector in memory processes, the long-term (LT) action of Aβ on this kinase was never investigated. In the present study we analyzed the effect of both short- and long-term incubation of organotypic hippocampal cultures with low concentrations (low nanomolar to low micromolar) of soluble, non-oligomeric $Aβ_{1–40}$ peptide on enzymatic activity, expression and phosphorylation of CaM kinase II. We found, in the absence of synaptic loss, apoptosis or cellular toxicity, distinct time- and concentration-dependent effects of Aβ on CaM II kinase expression and activity. These findings indicate that soluble, non-oligomeric forms of Aβ may directly affect synaptic signaling and plasticity.

2. Materials and methods

2.1. Organotypic cultures and treatment with amyloid peptide

All experiments complied with guidelines for use of experimental animals of European Community Council Directive 86/609/EEC. Hippocampal slice cultures were prepared from P7–P9 Sprague–Dawley rat pups and maintained in culture according to Tartaglia et al. [21]. Serum deprivation started from 7 days in vitro (DIV): the medium was changed from 25% horse serum to 10% (7 DIV), 5% (8 DIV), then serum free medium (from 9 to 14 DIV) composed by Neurobasal medium, 2% B27 supplement, 1 mM glutamine (Invitrogen, Carlsbad, CA). Treatment with amyloid started at 9 DIV (LT) or at 14 DIV (short-term, ST). Glutamate stimulation was carried out at 14 DIV in serum-free medium with 10 μM glutamate/1 μM glycine for 15 min. At 14 DIV cell viability was assessed with propidium iodide (PI) staining. PI was added to the medium at final concentration of 2 mM for 3 h. Staurosporine treatment was carried out by adding 100 nM staurosporine for 24 h. Digital images of the slices were acquired by a Zeiss Axiovert 100 fluorescence microscopy fitted with a Nikon Cool Pix990.

The slices were collected on dry ice and homogenized with a Teflon/glass homogenizer in lysis buffer containing 200 mM NaCl, 20 mM HEPES, 0.1 mM DTT, 0.1 mM EGTA, 5 mM $Na_4P_2O_7$, 20 mM NaF, 1 mM Na_3VO_4, 0.1 mM calyculine, 2 μl/ml protease inhibitor cocktail (Sigma, St. Louis, MO). Assays were performed on at least four slices per each single sample in triplicate or greater for each culture preparation. Between four and eight culture preparations were used for each study.

2.2. Soluble Aβ preparation

Disaggregated $Aβ_{1–40}$ (California Peptide Research, Napa, CA) or $Aβ_{40–1}$ (reverse peptide) were prepared as previously described [26]. Briefly, Aβ peptides were prepared by dissolving lyophilized powder to 7.5 mM in dimethyl sulfoxide (DMSO) and sonicating for 30 min in a bath sonicator. The supernatant was snap frozen in small aliquots and stored frozen at −20 °C. Based on previous findings that DMSO dissolution of Aβ produces monomeric solutions containing variable amounts of Aβ dimers or other small oligomers [5,9,22], DMSO-disaggregated Aβ is referred to as soluble Aβ in this paper. The formation of Aβ fibrils was monitored by a thioflavin T (ThT) fluorescence assay as previously described [13].

2.3. Assay of CaM kinase II enzymatic activity

Ca^{2+}-dependent and Ca^{2+}-independent activity of CaM kinase II was assayed by measuring initial rate of phosphate incorporation in the selective peptide substrate autocamtide-3 (Biosource, Camarillo, CA), as previously described [2].

2.4. Western blot

Western blot analysis was carried out as previously described [1]. Monoclonal antibodies used were: αCaM kinase II 1:1000 (Chemicon International, Temecula, CA). Polyclonal antibodies were: phospho-Thr^{286} αCaM kinase II 1:500 (Promega, Milan, Italy), phospho-Thr305-306 αCaM kinase II 1:4000 (courtesy of A. Silva, Department of Neurobiology, UCLA), human $Aβ_{1–17}$, 6E10 1:500 (Sigma) and caspase-3 (Cell Signaling Technology, Massachusetts).

Aβ oligomers were obtained from medium of 7PA2-CHO cells (courtesy of D. Walsh, Department of Neurology, Harvard) [25], cultured to 90% confluence and left in serum-free medium for 18 h. Conditioned medium was concentrated 10× with a stirred ultrafiltration cell 8050 fitted with 44.5 mm, NM WL 1000 cutoff cellulose membrane (Millipore, Billerica, MA). Aβ monomer and oligomers were separated on Tris-tricine, 16.5% polyacrylamide gels; total