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Amyloid β peptide adversely affects spine number and motility in hippocampal neurons

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Elevated levels of amyloid-beta peptide (Aβ) are found in Down's syndrome patients and alter synaptic function during the early stages of Alzheimer's disease. Dendritic spines, sites of most excitatory synaptic contacts, are considered to be an important locus for encoding synaptic plasticity. We used time-lapse two-photon imaging of hippocampal pyramidal neurons in organotypic slices to study the effects of Aβ on the development of dendritic spines. We report that exposure of hippocampal neurons to sub-lethal levels of Aβ decreased spine density, increased spine length and subdued spine motility. The effect of Aβ on spine density was reversible. Moreover, Aβ's effect on dendritic spine density was blocked by rolipram, a phosphodiesterase type IV inhibitor, suggesting the involvement of a cAMP dependent pathway. These findings raise the possibility that Aβ-induced spine alterations could underlie the cognitive defects in Alzheimer's disease and Down syndrome.

Introduction

The majority of excitatory synaptic connections in the central nervous system are located on small dendritic protrusions called dendritic spines (Gray, 1959). Spines are enriched in signaling molecules and serve to compartmentalize individual postsynaptic structures (Nimchinsky et al., 2002). Dendritic spines vary greatly in their morphology, and spine size correlates with the strength of the synapses they form (Kasai et al., 2003; Matsuzaki et al., 2004; Wallace and Bear, 2004). Therefore, alterations in spines can influence synaptic function, and may play a role in disorders associated with cognitive defects, such as Down's syndrome (DS) and Alzheimer's disease (AD). Significantly, aberrations in dendritic spine development are common in patients with mental retardation (Marin-Padilla, 1972; Purpura, 1974; reviewed in Fiala et al., 2002).

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The well-known pathological hallmarks of AD are associated with increased levels of amyloid β peptide 1–42 (Aβ 42). Individuals affected by Down syndrome (DS), the most common type of mental retardation, have an extra copy of chromosome 21, and overexpress amyloid precursor protein (APP) (Rumble et al., 1989) and BACE, one of the enzymes that cleaves APP (Barbiero et al., 2003). Elevated levels of soluble Aβ can be detected in DS in early childhood (Teller et al., 1996; Head and Lott, 2004) and these patients almost invariably develop AD at midlife (Wisniewski et al., 1985; Isacson et al., 2002). Impairments in memory and synaptic function are well correlated and precede the formation of amyloid plaques or neuronal death in mouse models of AD (Chapman et al., 1999; Hsia et al., 1999; Selkoe, 2002; Rowan et al., 2003; Cleary et al., 2005; Lesne et al., 2006). Synaptic transmission and plasticity were shown to be altered in neurons acutely overexpressing APP (Kamenetz et al., 2003) or exposed to elevated levels of Aβ (Hartley et al., 1999; Freir et al., 2001; Vitolo et al., 2002), perhaps due to altered trafficking of glutamatergic receptors (Almeida et al., 2005; Roselli et al., 2005; Snyder et al., 2005). Despite studies on the effects of Aβ on mature synapses, the effects of elevated levels of soluble Aβ on the development of synaptic connections are not known. Here we demonstrate that sub-lethal levels of Aβ alter dendritic spine number, morphology and dynamics in developing hippocampal neurons.

Results

Sub-lethal levels of Aβ reduce spine density on pyramidal hippocampal neurons in culture

To examine the effects of sub-lethal levels of Aβ on dendritic spine morphogenesis, we exposed hippocampal slices to 200 nM Aβ for 7 days. Western blot analysis with an antibody against Aβ (6E10) determined that the sample contains mainly monomers, dimers, trimers and tetramers as has been previously described (Dahlgren et al., 2002, data not shown). Chronic treatment of slices with 200 nM Aβ did not increase cell death. Uptake of calcein-AM and ethidium

homodimer by live and dead cells, respectively, was not different in the control ($n=8$) and Aβ-treated ($n=11$) slices (Fig. 1). In contrast, ethanol-fixed slices had a much decreased calcein-AM uptake and increased ethidium homodimer uptake, validating this viability assay in brain slice cultures.

To visualize hippocampal neurons in slices, we imaged green fluorescent protein (GFP)-transfected neurons with two-photon laser scanning microscopy. Pyramidal neurons had typical morphologies and the dendritic trees of pyramidal neurons in slices treated with Aβ for 7–8 days showed no gross differences compared to controls (Figs. 2A, B), leading us to focus on changes in dendritic spines. After 7 DIV, spine density in neurons treated with Aβ was lower compared to controls (Figs. 2C, D). We quantified spine density as the number of spines per micron of dendritic segment. Because there was no significant difference in spine density between the basal and apical regions of pyramidal neurons at this age (basal: 0.356±0.035, $n=17$ dendrites, apical: 0.369±0.028, $n=18$ dendrites, $p=0.77$), we pooled the measurements of the two regions for further analysis. The mean spine density on neurons in the Aβ-treated slices was 45% lower than that in the control slices (Fig. 2F and Supplementary Fig. 1, Control: 0.363±0.022, $n=35$ dendrites, 11 cells and Aβ: 0.199±0.021, $n=31$ dendrites, 10 cells, independent samples t test, $p<0.001$). We conclude that exposure of developing neurons to sub-lethal levels of Aβ leads to reduced density of dendritic spines. Immunostaining against the presynaptic protein synaptophysin as well as electron microscopy analysis of control and Aβ-treated slices (Fig. 3), indicates that numerous differentiated presynaptic terminals are present in the Aβ-treated slices and are contacting the remaining dendritic spines.

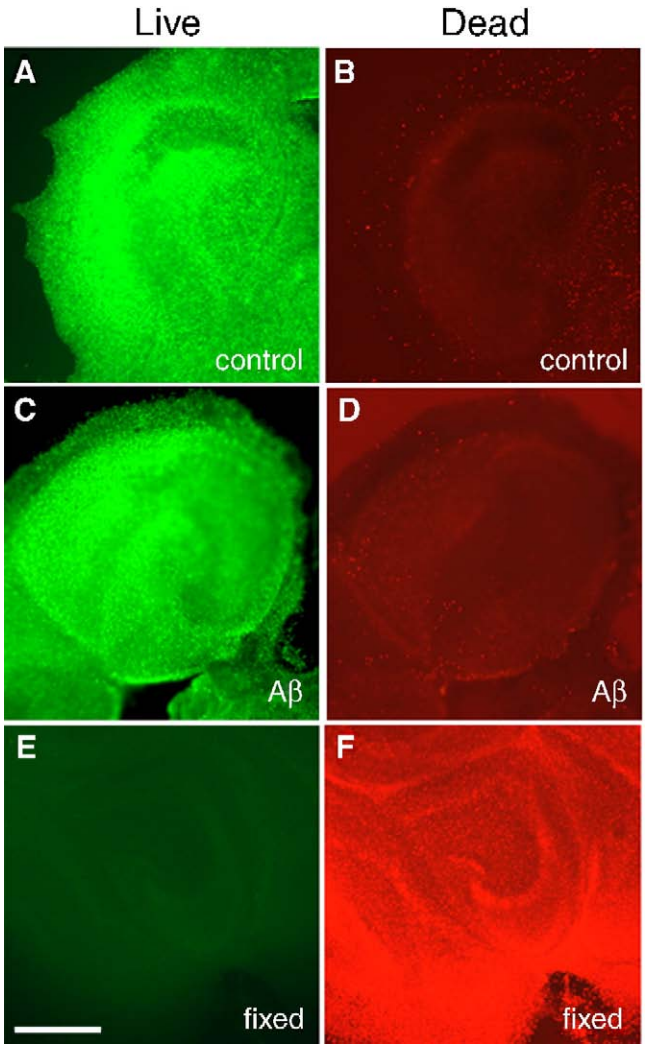

Fig. 1. Low dose of Aβ oligomers does not lead to cell death in organotypic hippocampal slice cultures. A viability assay was used on control (A, B), Aβ-treated (C, D) and ethanol fixed (E, F) hippocampal slice cultures. Live cells were labeled with 4 μM calcein-AM (A, C and E), while dead cells were labeled with 2 μM ethidium homodimer (B, D and F). Scale bar: 0.5 mm.

Aβ perturbs spine morphology and motility

Since spine dimensions are known to correlate with synaptic strength (Kasai et al., 2003), we analyzed spine morphology. No significant difference was detected in mean spine head diameter (Figs. 4C, D; Control: 0.69±0.01, $n=639$ spines; Aβ: 0.70±0.01 μm, $n=279$ spines; Mann–Whitney U test: one-sided $p=0.222$). However, spines on the Aβ-treated neurons were longer (7.3%) than those on the control cells (Figs. 4A, B; Control: 1.37±0.03 μm, $n=639$ spines; Aβ: 1.47±0.04 μm, $n=279$ spines; Mann–Whitney U test: one-sided $p=0.03$). Thus, sub-lethal level of Aβ leads to aberrant spine morphology.

Dendritic spines are highly motile structures exhibiting changes in morphology on a time scale of minutes (Fischer et al., 1998). Spines have been observed to elongate, retract, "morph" and emit short filopodia from the spine head (Fig. 5A, Dunaevsky et al., 1999). This motility is actin dependent and developmentally regulated (Dailey and Smith, 1996; Dunaevsky et al., 1999; Deng and Dunaevsky, 2005). To test if exposure to Aβ changes spine dynamics, we performed two-photon time-lapse imaging of GFP-labeled hippocampal neurons. We first scored the number of spines emerged or disappeared during the imaging period and did not detect a difference between the Aβ-treated and control slices (data not shown). We next calculated the Motility Index of spines (Dunaevsky et al., 1999, 2001; Deng and Dunaevsky, 2005). Spine motility in the Aβ group was 23.5% lower than in the control group (Figs. 5B, C; Control: 1.15±0.08, $n=75$ spines; Aβ: 0.88±0.04, $n=70$ spines, Mann–Whitney U test: one-sided $p=0.003$, $n=145$ spines, see movies in Supplementary material). Thus, sub-lethal levels of Aβ oligomers reduce spine number as well as render the remaining spines less dynamic.

Aβ effect on dendritic spine density is mediated by a cAMP dependent pathway

Rolipram, a phosphodiesterase type 4 inhibitor that increases cAMP levels and stimulates the cAMP/PKA/CREB pathway, enhances synaptic plasticity in slices (Bach et al., 1999; Navakkode et al., 2004) and improves memory in mice (Barad et al., 1998). Rolipram blocks the Aβ-induced inhibition of PKA and CREB and LTP reduction in slices (Vitolo et al., 2002). We tested if the reduction of spine density by Aβ is blocked by inhibition of the cAMP pathway with rolipram. Slices were incubated in hippocampal medium containing 200 nM Aβ and 1 μM rolipram or with 1 μM rolipram alone for 7–8 days. Spine density in the Aβ+rolipram group was comparable to that in the control group, and was significantly higher than that in the Aβ group (Figs. 2E, F and Supplementary Fig. 1; Rolipram+Aβ: 0.34±0.03 spines/