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Amyloid precursor protein (APP) regulates synaptic structure and function

Sheue-Houy Tyan [a,*], Ann Yu-Jung Shih [a,1], Jessica J. Walsh [b], Hiroko Maruyama [a], Floyd Sarsoza [a], Lawrence Ku [a], Simone Eggert [a,2], Patrick R. Hof [b], Edward H. Koo [a], Dara L. Dickstein [b,**]

[a] Department of Neurosciences, University of California San Diego, La Jolla, CA, USA
[b] Fishberg Department of Neuroscience and Friedman Brain Institute, Mount Sinai School of Medicine, New York, NY, USA

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ABSTRACT

The amyloid precursor protein (APP) plays a critical role in Alzheimer's disease (AD) pathogenesis. APP is proteolytically cleaved by β- and γ-secretases to generate the amyloid β-protein (Aβ), the core protein component of senile plaques in AD. It is also cleaved by α-secretase to release the large soluble APP (sAPP) luminal domain that has been shown to exhibit trophic properties. Increasing evidence points to the development of synaptic deficits and dendritic spine loss prior to deposition of amyloid in transgenic mouse models that overexpress APP and Aβ peptides. The consequence of loss of APP, however, is unsettled. In this study, we investigated whether APP itself plays a role in regulating synaptic structure and function using an APP knock-out (APP−/−) mouse model. We examined dendritic spines in primary cultures of hippocampal neurons and CA1 neurons of hippocampus from APP−/− mice. In the cultured neurons, there was a significant decrease (~35%) in spine density in neurons derived from APP−/− mice compared to littermate control neurons that were partially restored with sAPPα-conditioned medium. In APP−/− mice in vivo, spine numbers were also significantly reduced but by a smaller magnitude (~15%). Furthermore, apical dendritic length and dendritic arborization were markedly diminished in hippocampal neurons. These abnormalities in neuronal morphology were accompanied by reduction in long-term potentiation. Strikingly, all these changes in vivo were only seen in mice that were 12–15 months in age but not in younger animals. We propose that APP, specifically sAPP, is necessary for the maintenance of dendritic integrity in the hippocampus in an age-associated manner. Finally, these age-related changes may contribute to AD pathology independent of Aβ-mediated synaptic toxicity.

Introduction

The amyloid precursor protein (APP) is considered to play a central role in the pathogenesis of Alzheimer's disease (AD). APP is the precursor of amyloid β-protein (Aβ), the main component of amyloid plaques, and as such has given rise to the amyloid cascade hypothesis for AD, which posits that the misprocessing and abnormal regulation of APP lead to the gradual overproduction or accumulation of Aβ into oligomers and extracellular plaques, initiating a sequence of events that results in AD (Hardy and Selkoe, 2002). APP is a type I transmembrane protein with a large N-terminal extracellular domain and a short cytoplasmic domain. APP undergoes multiple proteolytic steps: cleavage by either α- or β-secretase releases the large APP ectodomain, sAPPα and sAPPβ respectively; leaving the membrane-anchored C-terminal fragments (CTFs) (Sisodia et al., 1993; Turner et al., 2003). The CTFs can be further cleaved by γ-secretase to generate Aβ peptides, while releasing the APP intracellular domain (AICD), which is thought to contribute to cell signaling or cell death (Konietzko, 2011).

In addition to being the precursor to Aβ, a number of physiological functions have been attributed to APP, including, but not limited to, synapse formation, neuronal survival, and neuritic outgrowth (De Strooper and Annaert, 2000; Mattson, 1995, 1997; Nunan et al., 2001; Russo et al., 1998; Turner et al., 2003). APP belongs to a highly conserved gene family including the amyloid precursor-like proteins, APLP1 and APLP2 (Anliker and Muller, 2006). It is encoded on chromosome 21 and is alternatively spliced into 3 main isoforms, APP695, APP751, and APP770. Individuals with Down syndrome (trisomy 21) or inherited cases of familial AD with point mutations in the APP gene invariably develop AD pathology (Epstein, 1990; Potter, 1991). This link of APP to AD pathology and the many putative functions of APP in neurons led to the establishment of many invertebrate and transgenic

Abbreviations: AD, Alzheimer's disease; APP, amyloid precursor protein; eGFP, enhanced green fluorescent protein; LTP, long-term potentiation; PPF, paired-pulse facilitation; CM, conditioned medium; PBS, phosphate-buffered saline; fEPSP, excitatory postsynaptic potentials; Aβ, amyloid β-protein; CTFs, C-terminal fragments; AICD, APP intracellular domain; KO, knock-out; DIV, days in vitro.

* Correspondence to: S.-H. Tyan, Department of Neurosciences, University of California San Diego, 9500 Gilman Drive, La Jolla CA 92093. USA. Fax: 858 8221021.
** Correspondence to: D.L. Dicktein, Department of Neurosciences Mount Sinai School of Medicine, 1425 Madison Avenue, New York NY, 10029, USA. Fax: 212 8492510.
E-mail addresses: s1tyan@ucsd.edu (S.-H. Tyan), dara.dickstein@mssm.edu (D.L. Dickstein).
[1] Current Address: ActivX Biosciences, La Jolla, CA, USA.
[2] Current Address: Department of Human Biology and Human Genetics, Technical University of Kaiserslautern, Kaiserslautern, Germany.

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mouse models, both knock-in (4 with truncated alleles and 2 with C-terminal point mutation alleles) and knock-out (2 KO and 2 conditional), that suffer from various defects and abnormalities (reviewed in Guo et al., 2011). One APP KO mouse generated by Zheng et al. (1995) shows many deficits in locomotor activity and forelimb grip strength, as well as impairments in learning and memory associated with deficits in long-term potentiation (LTP) (Ring et al., 2007). In fact alterations in synaptic structure and function are some of the most consistent findings of in vitro studies using neurons from either APP-deficient mice or from RNAi-mediated knockdown approaches (Herard et al., 2006; Hoe et al., 2009; Lee et al., 2010; Seabrook et al., 1999; Zheng et al., 1995). This APP mouse model also shows changes in dendritic arborization (Seabrook et al., 1999) and synapse numbers (Lee et al., 2010), although the latter finding was not supported by another study (Phinney et al., 1999). In contrast, a study using autaptic hippocampal cultures derived from a different APP KO mouse line showed an increase in synaptophysin-immunoreactive puncta and miniature synaptic currents (Priller et al., 2006). These conflicting results are compounded by APLP1 and APLP2, which likely play redundant functions that compensate for the loss of APP in the intact animal. However, more recent studies in double APP/APLP2-deficient, but not single APP KO, animals have clearly identified developmental defects in neuromuscular junction synapses with corresponding neurophysiological deficits (Wang et al., 2005). Thus, the situation with single APP KO mice remains unresolved.

Until recently, the conventional methods to investigate structure of synaptic and dendritic morphology have been Golgi staining, immunostaining of synaptic proteins, imaging by fluorescent markers such as GFP labeling, or by ultrastructure. These techniques, however, infer, but do not directly assay, number of synapses and underestimate the number of dendritic spines (Friedland et al., 2006; Pilati et al., 2008). To provide convincing evidence that APP is required for the maintenance of dendritic structure, synapse formation, and synaptic function, we re-examined the effect of APP on synaptic plasticity and dendritic and spine morphology in an APP KO mouse line using 3-dimensional reconstruction of individual neurons and spines to analyze accurately alterations in dendritic and spine structure in vivo. We also conducted in vitro studies in neurons from APP KO mice to assess whether spine density differences were accompanied by alterations in synaptic plasticity or basal synaptic transmission.

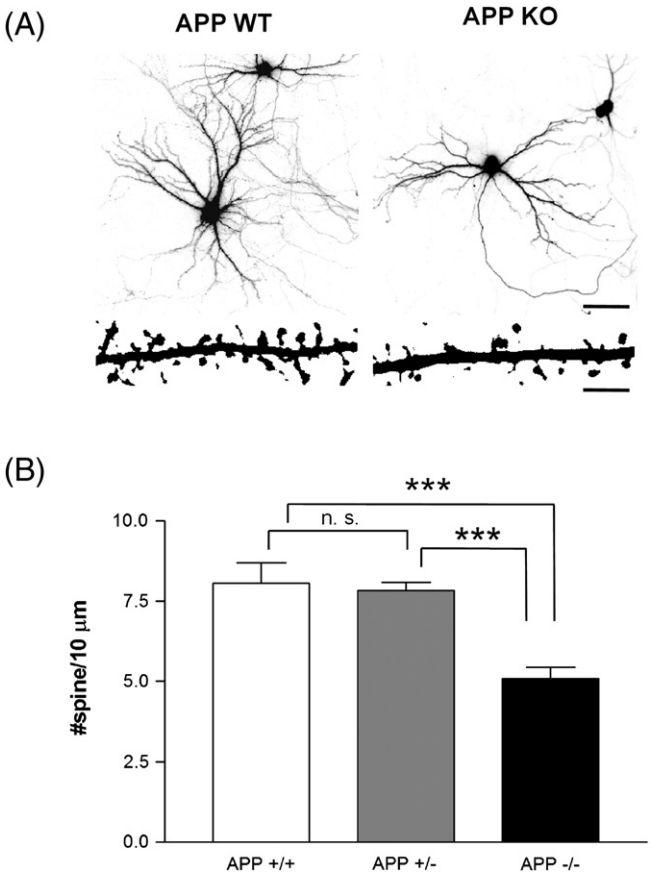

Fig. 1. APP−/− hippocampal neurons show a decrease in spine number. (A) Representative images of primary hippocampal neurons expressing eGFP. Top: APP+/− (left) and APP−/− neurons were from the same parental interbreeding of APP mice. The images were presented in gray and inverted color. Panels at the bottom of each image show high-magnification renderings of dendritic segments. The dendritic images were taken with 0.3-µm step z-section and then stacked with maximum projection. Scale bar: 150 µm (top) and 5 µm (bottom). (B) Quantification of spine density. There was no difference in spine number between APP+/+ and APP+/− ($p>0.05$). APP−/− neurons showed a highly significant decrease in spine density compared to APP+/+ or APP+/− neurons (**, $p<0.01$ and ***, $p<0.001$). Data are expressed as mean ± SEM and tested using a one-way ANOVA with Tukey's post-hoc test.

Results

APP deficiency decreases spine number and dendritic branching in primary hippocampal neurons

We re-examined the potential role of APP in synaptic function by focusing on dendritic spines in APP−/− neurons by both in vitro and in vivo approaches. First, we measured dendritic spine densities from hippocampal neurons cultured from P0-P1 neonatal pups from APP+/+, APP+/−, and APP−/− mice (Fig. 1A, B). The spine density in APP−/− neurons was significantly decreased by almost 40% compared to APP+/+ (36.8%, $p<0.001$, one-way ANOVA with Tukey's post-hoc test) and APP+/− (35%, $p<0.001$). In this analysis, 45 neurons were scored from a total of 11 APP+/+ mice, 64 neurons from 15 APP+/− mice, and 85 neurons from 21 APP−/− mice. Thus, APP−/− primary hippocampal neurons in culture showed a markedly diminished dendritic spine number as a measure of synaptic density. There was, however, no appreciable difference between wild type and hemizygous APP-deficient neurons. Therefore, to simplify subsequent breeding for in vivo analyses, we have chosen to focus our comparison primarily between APP+/− and APP−/− mice. We also noticed a reduction of dendritic complexity in culture hippocampal neurons from APP deficient mice. However, in cultured neurons, it is difficult to precisely define the apical vs. basal dendrites to quantify such changes in neuronal polarity. Therefore, the number of dendritic branch points within a radius of 150 µm from the neuron soma was assayed as a measure of dendritic complexity. We found that the average number of dendritic branch points was significantly reduced in APP−/− neurons compared to APP+/− (Fig. S3), indicating that both dendritic spine numbers and dendritic arborizations were diminished in the absence of APP in cultured hippocampal neurons.

Dendritic and spine morphology in APP knock-out mice

The marked reduction in dendritic spine numbers in cultured hippocampal neurons from APP−/− mice was surprising as this alteration has, to our knowledge, not been reported before. In fact, autaptic neurons from APP−/− mice showed an increase in synaptophysin-immunopositive puncta (Priller et al., 2006). We also observed a decrease in dendritic branching in APP−/− neurons in primary cultures (Fig. 1). Thus, we next evaluated the dendritic and spine morphology in APP−/− mice in vivo in more detail. Brain slices of five to six animals in each group (APP+/− and APP−/−) from two ages were examined (2–4 months-old and 12–15 months-old). We first examined each Lucifer Yellow-injected neuron for various morphometric parameters (i.e. dendritic length and complexity) by performing 3-dimensional tracing of each neuron. Sholl analyses showed that CA1 neurons in old APP−/− mice (~13 months old)