Neurobiology of Disease 91 (2016) 94–104

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

A single amino acid difference between the intracellular domains of amyloid precursor protein and amyloid-like precursor protein 2 enables induction of synaptic depression and block of long-term potentiation

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ARTICLE INFO

Article history:
Received 11 October 2015
Revised 29 January 2016
Accepted 23 February 2016
Available online 24 February 2016

Keywords:
Alzheimer disease
Synaptic plasticity
APP intracellular domain
APLP2 intracellular domain

ABSTRACT

Alzheimer disease (AD) is initially characterized as a disease of the synapse that affects synaptic transmission and synaptic plasticity. While amyloid-beta and tau have been traditionally implicated in causing AD, recent studies suggest that other factors, such as the intracellular domain of the amyloid-precursor protein (APP-ICD), can also play a role in the development of AD. Here, we show that the expression of APP-ICD induces synaptic depression, while the intracellular domain of its homolog amyloid-like precursor protein 2 (APLP2-ICD) does not. We are able to show that this effect by APP-ICD is due to a single alanine vs. proline difference between APP-ICD and APLP2-ICD. The alanine in APP-ICD and the proline in APLP2-ICD lie directly behind a conserved caspase cleavage site. Inhibition of caspase cleavage of APP-ICD prevents the induction of synaptic depression. Finally, we show that the expression of APP-ICD increases and facilitates long-term depression and blocks induction of long-term potentiation. The block in long-term potentiation can be overcome by mutating the aforementioned alanine in APP-ICD to the proline of APLP2. Based on our results, we propose the emergence of a new APP critical domain for the regulation of synaptic plasticity and in consequence for the development of AD.

1. Introduction

Alzheimer disease (AD) is the most common neurodegenerative disease of the elderly, causing memory impairment and, in its final stages, the loss of higher cognitive function (Selkoe and Schenk, 2003). Post-mortem brains of AD patients show neuronal loss, neurofibrillary tangles composed of tau protein and senile plaques containing amyloid-beta (Aβ). Studies of AD patients revealed, that the degree of dementia is poorly predicted by the burden of amyloid plaques but is highly correlated with synaptic marker loss (Terry et al., 1991). This result has led to the notion that, at least during its early stages, AD appears to be primarily a disease of the synapse (Selkoe, 2002).

Aβ is the proteolytic product of the amyloid-precursor protein (APP). Besides Aβ, APP cleavage produces the extracellular soluble APP fragment and the APP intracellular domain (APP-ICD). While Aβ remains the most studied peptide in the etiology of AD, recent years have given evidence that the production of APP-ICD may also play a significant role in AD (Ghosal et al., 2009; Vogt et al., 2011).

In addition to APP, neurons express in equal amounts two homologs of APP: the amyloid precursor like proteins 1 and 2 (APLP1 and APLP2) (Slunt et al., 1994). APP and APLP2 have a certain degree of redundancy during development since APLP2 and APP double knock-out mice are lethal while mice with a single deficiency for APP or APLP2 are viable (Heber et al., 2000; von Koch et al., 1997). The intracellular domains of APP and APLP2 are highly homologous (Fig. 2A) and have all known key regulatory sites and domains in common: the Thr-668 phosphorylation site in APP has an equivalent site in APLP2, the caspase cleavage site and the critical YENPTY motif in APP are perfectly conserved in APLP2. As a consequence of this high degree of homology, APP and APLP2 share common interaction partners, such as Fe65 (Walsh et al., 2003), Mint (X11) (Orcholski et al., 2011) and Dab1 (Howell et al., 1999). While APP-ICD transgenic mice show a deficit in learning and memory, the effects of APP-ICD or APLP2-ICD on synaptic plasticity remain largely unknown.

Our study aims to analyze whether the APP-ICD affects synaptic transmission and plasticity and whether the homologous APLP2-ICD can mimic APP-ICD dependent effects. We find that APP-ICD causes synaptic depression, enhanced LTD and a block in LTP induction, while, unexpectedly, APLP2-ICD has no effect on synaptic transmission or synaptic plasticity. The different effects of APP-ICD vs. APLP2-ICD on synaptic function are caused by a single amino-acid difference between APP-ICD and APLP2-ICD. Exchanging this single amino acid between APP-ICD and APLP2-ICD, we find that APP-ICD loses its effects on synaptic transmission and synaptic plasticity, while the APLP2-ICD gains the ability to induce synaptic depression. Taken together, we show that a

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Available online on ScienceDirect (www.sciencedirect.com).

http://dx.doi.org/10.1016/j.nbd.2016.02.016
0969-9961/

single amino-acid difference between APP-ICD and APLP2 is necessary and sufficient for the induction of synaptic depression and the block of long-term potentiation.

2. Materials and methods

2.1. cDNA constructs and virus production

APP(PML)-ICD$_{(20-22)}$, APP(AAV)-ICD$_{(20-22)}$, APP(PAV)-ICD$_{(20)}$, APP(AML)-ICD$_{(20)}$ and APP(VEVA)-ICD point mutations were made by site directed mutagenesis, using iProof High-fidelity DNA polymerase (Bio-Rad), directly in the pIRES vector (Clontech) containing either APP-ICD or APLP2-ICD followed by GFP as an IRES expression system. Cloning of chimeric APLP2-ICD and APP-ICD constructs was achieved by nested PCR with overlapping primers and the end product was inserted into the pIRES vector.

Subsequently, all constructs were cut from pIRES and inserted into the viral pSinRep5 expression vector (Invitrogen). In vitro mRNA synthesis followed the protocol of Invitrogen for Sindbis virus production. BHK cells were electroporated with the RNA transcripts using the MammoZapper Cloning Gun electroporation system (Tritech Research). 48 h later, viral particles in the cell medium were collected by ultracentrifugation and stored at −80 °C.

2.2. Hippocampal slice cultures

Organotypic hippocampal slice cultures were prepared from p7 rat pups, and maintained in culture for 10 to 12 days as described before (Tamburri et al., 2013). At this time, CA1 neurons in organotypic cultures display NMDA-receptor 2A subunit expression levels indicative of mature neurons (Gambrill and Barria, 2011). CA1 pyramidal neurons were infected with viral solution 16 h prior to recording, using a thin glass electrode in combination with a pico-spritzer (see Fig. 1A). The animal protocol (Permit Number: 14-170) followed the guidelines of the "Comité de déontologie de l'expérimentation sur les animaux" (CDEA) of the Université de Montréal.

2.3. Imaging

Overview images of the whole infected hippocampal slice were obtained with an Olympus BX51 fluorescent microscope using Neurolucida software (MBF Bioscience). Close-up imaging of infected neurons was performed with an Olympus Fluoview-1000 laser scanning confocal microscope. Image analysis was performed with Fluoviewer 4.1 and ImageJ softwares on Z-stack images.

2.4. Drugs

NMDA-receptor antagonist D,L-APV (50 μM, Tocris) for Fig. 2C and Caspase-3 inhibitor Z-DEVD-FMK (50 μM, MBL) for Fig. 5B were added to the slice culture media 1 h after viral infection.

2.5. Electrophysiological recordings

Dual (control and infected neuron, identified under fluorescent guidance) and single whole cell patch clamp recordings of CA1 neurons

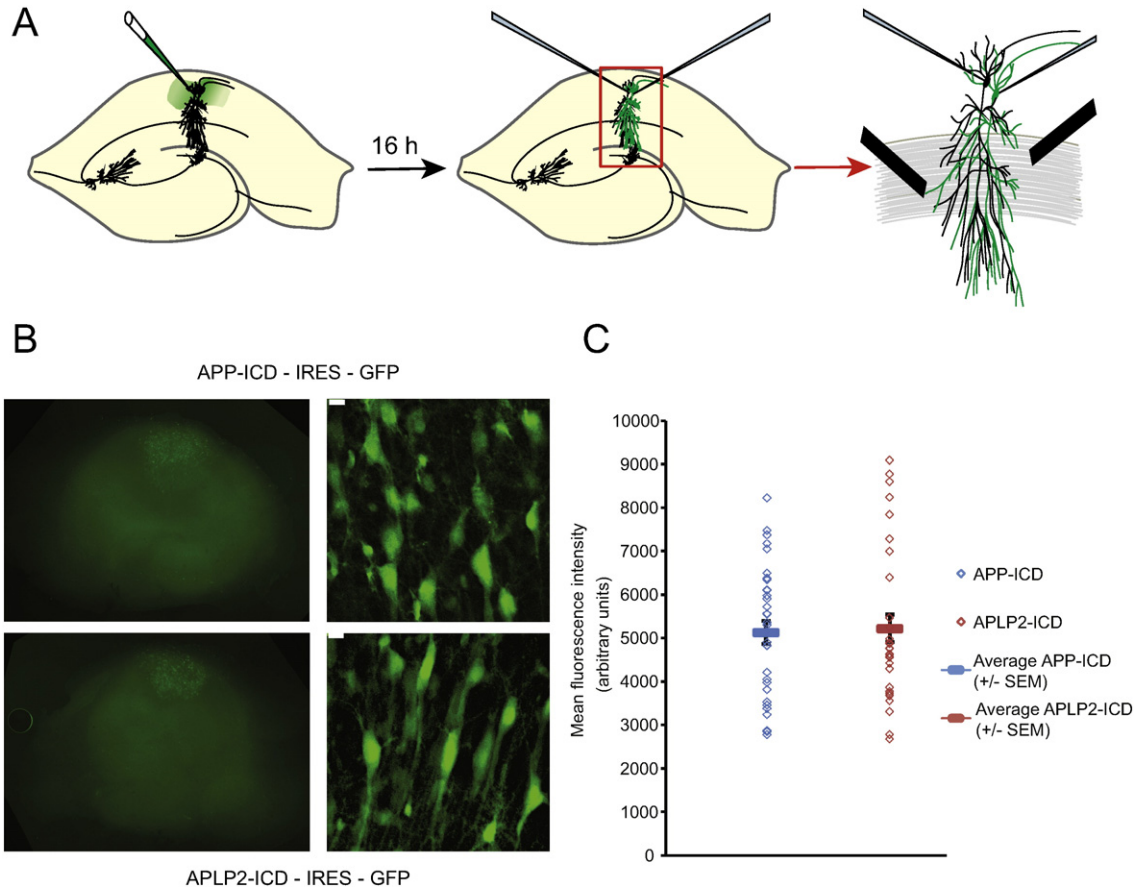

Fig. 1. Illustration of the experimental approach. (A) Local infection of CA1 pyramidal neurons in organotypic hippocampal slice cultures with virus and, after 16 h, recording of evoked synaptic responses in whole cell patch clamp configuration of infected (co-expressing GFP together with the construct of interest) and non-infected CA1 neurons by stimulating Schaffer collaterals. (B) Left: Overview of APP-ICD–IRES-GFP and APLP2-ICD–IRES-GFP infected hippocampal slices, showing that the viral expression of constructs (indicated by GFP expression) is restricted to the injection site in the CA1 area. Right: close-up of the infected CA1 area (bar: 10 μm). (C) Comparison of fluorescent intensity in APP-ICD–IRES-GFP and APLP2-ICD–IRES-GFP infected CA1 neurons, showing that the mean expressions of both constructs do not differ (n = 32 for each construct). Error bar = SEM.