Biological Psychiatry

Archival Report

The Amyloid Precursor Protein Intracellular Domain Is an Effector Molecule of Metaplasticity

Emilie Trillaud-Doppia and Jannic Boehm

ABSTRACT

BACKGROUND: Human studies and mouse models of Alzheimer's disease suggest that the amyloid precursor protein (APP) can cause changes in synaptic plasticity and is contributing to the memory deficits seen in Alzheimer's disease. While most of these studies attribute these changes to the APP cleavage product Aβ, in recent years it became apparent that the APP intracellular domain (APP-ICD) might play a role in regulating synaptic plasticity.

METHODS: To separate the effects of APP-ICD on synaptic plasticity from Aβ-dependent effects, we created a chimeric APP in which the Aβ domain is exchanged for its homologous domain from the amyloid precursor-like protein 2.

RESULTS: We show that the expression of this chimeric APP has no effect on basal synaptic transmission or synaptic plasticity. However, a synaptic priming protocol, which in control cells has no effect on synaptic plasticity, leads to a complete block of subsequent long-term potentiation induction and a facilitation of long-term depression induction in neurons expressing chimeric APP. We show that the underlying mechanism for this effect on metaplasticity is caused by caspase cleavage of the APP-ICD and involves activation of ryanodine receptors. Our results shed light on the controversially discussed role of APP-ICD in regulating transcription. Because of the short timespan between synaptic priming and the effect on synaptic plasticity, it is unlikely that APP-ICD–dependent transcription is an underlying mechanism for the regulation of metaplasticity during this time period.

CONCLUSIONS: Our finding that the APP-ICD affects metaplasticity provides new insights into the altered regulation of synaptic plasticity during Alzheimer's disease.

Keywords: Alzheimer's disease, Amyloid precursor protein, Amyloid precursor protein intracellular domain, Caspase cleavage, Metaplasticity, Synaptic plasticity

http://dx.doi.org/10.1016/j.biopsych.2016.12.015

Alzheimer's disease (AD), a progressive neurogenerative disorder, is initially characterized by deficits in learning and memory that will eventually lead to loss of higher cognitive function (1). The cellular mechanism for memory formation is synaptic plasticity, with its two most prominent forms: long-term potentiation (LTP) and long-term depression (LTD) (2). However, the underlying mechanisms for changes in synaptic plasticity in AD remain largely unknown. Genetic studies suggest that the amyloid precursor protein (APP) is critically involved in the development of AD (3). The proteolytic cleavage of APP leads to the production of amyloid-β (Aβ) and the APP intracellular domain (APP-ICD) (4). Although much of AD research still focuses on the role of Aβ, recent results from others and our group have shown that the APP-ICD might play a prominent role of synaptic transmission (5,6).

Along with expressing APP, neurons also express the APP paralogues amyloid precursor-like proteins 1 and 2 (APLP1 and APLP2) in equal amounts (7). APP and APLP2 appear to have overlapping functions during development; APP and APLP2 double knockout mice are not viable, whereas single knockout mice are viable (8,9). The intracellular domains of APP and APLP2 are highly homologous and have most of the known key regulatory sites conserved. However, in a recent publication, we found that the expression of the APP-ICD fragment leads to synaptic depression, while the APLP2-ICD had no effect on synaptic transmission. Interestingly, this effect is caused by a single amino acid difference between the two ICDs (5). APP-ICD is, however, normally expressed not as an isolated fragment, but as part of the full-length APP molecule, which needs to be trafficked to dendritic compartments before its proteolytic processing. Hence, in the present study we aimed at analyzing the effects of APP-ICD on synaptic plasticity, with APP-ICD being part of full-length APP. We created a chimeric APP, in which we exchanged the Aβ domain of APP for its homologous domain from APLP2 to avoid potential interference on synaptic plasticity by Aβ. We find that the expression of chimeric APP has no effect on synaptic transmission or synaptic plasticity. However, chimeric APP affects metaplasticity (i.e., it changes the ability of the neuron to induce synaptic plasticity after previous

 ISSN: 0006-3223

stimulation). We observe that the transient activation of the synapse leads to a complete block of subsequent LTP induction and a facilitation of LTD induction. We can attribute these changes in metaplasticity to the caspase cleavage of APP-ICD and propose changes in ryanodine receptor activation as the underlying mechanism. Taken together, we propose that the APP-ICD is an effector molecule of metaplasticity.

METHODS AND MATERIALS

Complementary DNA Constructs and Virus Production

Cloning of chimeric APP (APP_{Ext}-$APLP2_{TM}$-APP_{ICD} construct) was achieved using APP_{695} for its extracellular domain APP_{Ext} and APPδB-100 internal ribosomal entry site green fluorescent protein, which contains $APLP2_{TM}$-APP_{ICD} (10). APP_{Ext} and $APLP2_{TM}$-APP_{ICD} were linked by nested polymerase chain reaction with overlapping primers in order to form the APP_{Ext}-$APLP2_{TM}$-APP_{ICD} construct. The APP_{Ext}-$APLP2_{TM}$-APP_{ICD} construct was cloned into the viral pSinRep5 expression vector (Invitrogen, Carlsbad, CA). $APLP2_{Full}$ was also cloned as a C-terminal internal ribosomal entry site green fluorescent protein expression system and inserted in the viral pSinRep5 expression vector (Invitrogen). APP_{Ext}-$APLP2_{TM}$-$APP(PAV)_{ICD}$, APP_{Ext}-$APLP2_{TM}$-$APP(T668A)_{ICD}$ and $APLP2(adeno\text{-}associated\ virus\ [AAV])_{Full}$ point mutations were made by site-directed mutagenesis using iProof high-fidelity DNA polymerase (Bio-Rad, Raleigh, NC), directly in the pSinRep5 vector containing either APP_{Ext}-$APLP2_{TM}$-APP_{ICD} or $APLP2_{Full}$. All constructs were in vitro transcribed, following the protocol of Invitrogen for pSindbis virus production. Baby hamster kidney cells were electroporated with the RNA transcripts using the MammoZapper Cloning Gun electroporation system (Tritech Research, Los Angeles, CA). Forty-eight hours later, viral particles in the cell medium were collected by ultracentrifugation and stored at −80°C.

Hippocampal Slice Cultures

Organotypic hippocampal slice cultures were prepared from p7 rat pups and maintained in culture for 10 to 12 days as described by Tamburri *et al.* (11). At this time, CA1 neurons in organotypic cultures display *N*-methyl-D-aspartate receptor (NMDAR) 2A subunit expression levels indicative of mature neurons (12). CA1 pyramidal neurons were infected with viral solution 16 hours before recording, using a thin glass electrode in combination with a picospritzer. Restricted local CA1 expression was confirmed by analyzing green fluorescent protein expression. The animal protocol (permit number 14-170) followed the guidelines of the "Comité de déontologie de l'expérimentation sur les animaux" of the Université de Montréal.

Drugs

Caspase-3 inhibitor Z-DEVD-FMK (50 μM; Medical and Biological Laboratories, Nagoya, Japan) was added to the slice culture media 1 hour after viral infection. Intracellular diffusion of ryanodine receptor antagonist dantrolene was achieved by dissolving the drug in the internal solution of the patch pipette (10 μM), followed by drug diffusion for 10 minutes after obtaining whole-cell patch clamp configuration.

Electrophysiological Recordings

Dual (control and infected neuron, identified under fluorescent guidance) and single whole-cell patch clamp recordings of CA1 neurons were done by transferring hippocampal slices to a recording chamber filled with artificial cerebrospinal fluid containing 2.5 mM KCl, 118 mM NaCl, 1 mM NaH_2PO_4, 26 mM $NaHCO_3$, 10 mM glucose, 100 μM picrotoxin, and 4 μM 2-chloroadenosine and continuously bubbled with carbogen (5% CO_2 and 95% O_2). Before recording, 4 mM $CaCl_2$ and 4 mM $MgCl_2$ were added to the artificial cerebrospinal fluid. All recordings were done at 25°C, except for the induction of LTP, for which the temperature was increased to 30°C. Patch pipettes (3–5 MΩ) were filled with internal solution: 115 mM cesium methanesulfonate, 20 mM CsCl, 10 mM HEPES, 2.5 mM $MgCl_2$, 0.6 mM EGTA, 4 mM Na_2ATP, 0.4 mM Na_3GTP, and 10 mM sodium phosphocreatine at pH 7.2 and 290 mOsm. Two tungsten electrodes for stimulation were placed above the Schaffer collaterals, 150 to 250 μm apart. Alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor and NMDAR currents were recorded while neurons were maintained at −60 mV and 40 mV, respectively. A stimulation of 0.3 Hz was applied.

For the induction of LTD, neurons were depolarized at −30 mV and stimulated with one electrode at 1 Hz for 800 pulses (LTD pathway) while the second electrode remained off (control pathway). Facilitation of LTD induction was tested by using the same LTD protocol but delivering 180 pulses instead of 800 pulses. For the induction of LTP, neurons were depolarized at 0 mV and stimulated with one electrode at 3 Hz for 3 minutes (LTP pathway) while the second electrode remained off (control pathway). The synaptic priming protocol was administered 3 minutes before induction of synaptic plasticity (either facilitated LTD or LTP). During the priming protocol, neurons were depolarized at –30 mV and stimulated twice at 100 Hz for 1 second with a 1-second pause in-between.

Statistics

A paired *t* test was used to compare effects on alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor and NMDAR currents between control and infected neurons. Effects on synaptic plasticity were analyzed by paired *t* test, comparing the normalized size of synaptic currents before (indicated in the figures as B [baseline]) and after induction of synaptic plasticity (see figures for time intervals).

RESULTS

Aβ-deficient APP Affects Metaplasticity

To analyze the isolated effect of the intracellular domain of APP on synaptic plasticity and to separate these effects from those potentially caused by Aβ, we created a chimeric APP in which we exchanged the Aβ domain for the homologous domain from the APLP2, the most conserved APP paralogue