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Aβ PROMOTES VDAC1 CHANNEL DEPHOSPHORYLATION IN NEURONAL LIPID RAFTS. RELEVANCE TO THE MECHANISMS OF NEUROTOXICITY IN ALZHEIMER'S DISEASE

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Abstract—Voltage-dependent anion channel (VDAC) is a mitochondrial protein abundantly found in neuronal lipid rafts. In these membrane domains, VDAC is associated with a complex of signaling proteins that trigger neuroprotective responses. Loss of lipid raft integrity may result in disruption of multicomplex association and alteration of signaling responses that may ultimately promote VDAC activation. Some data have demonstrated that VDAC at the neuronal membrane may be involved in the mechanisms of amyloid beta (Aβ)-induced neurotoxicity, through yet unknown mechanisms. Aβ is generated from amyloid precursor protein (APP), and is released to the extracellular space where it may undergo self-aggregation. Aβ aggregate deposition in the form of senile plaques may lead to Alzheimer's disease (AD) neuropathology, although other pathological hallmarks (such as hyper-phosphorylated Tau deposition) also participate in this neurodegenerative process. The present study demonstrates that VDAC1 associates with APP and Aβ in lipid rafts of neurons. Interaction of VDAC1 with APP was observed in lipid rafts from the frontal and entorhinal cortex of human brains affected by AD at early stages (I–IV/0-B of Braak and Braak). Furthermore, Aβ exposure enhanced the dephosphorylation of VDAC1 that correlated with cell death. Both effects were reverted in the presence of tyrosine phosphatase inhibitors. VDAC1 dephosphorylation was corroborated in lipid rafts of AD brains. These results demonstrate that Aβ is involved in alterations of the phosphorylation state of VDAC in neuronal lipid rafts. Modulation of this channel may contribute to the development and progression of AD pathology.

Key words: voltage-dependent anion channel, amyloid precursor protein, amyloid beta, Alzheimer's disease, lipid rafts, tyrosine phosphatase inhibitor.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative disease mostly related to aged brain, which is clinically characterized by a progressive cognitive decline, eventually leading to dementia. In this disease, there is abundant extracellular aggregation of amyloid-beta (Aβ) peptide that forms senile plaques, and intracellular hyper-phosphorylated tau protein aggregation that forms neurofibrillary tangles (Duyckaerts and Dickson 2003; Lowe et al., 2008). Aβ formation consists of the sequential cleavage of full-length amyloid precursor protein (APP) by β-secretase (BACE1) and γ-secretase complex. The amyloidogenic processing results in the release of Aβ peptides of between 39 and 42 residues in length, depending upon the precise cleavage site. APP is a transmembrane protein abundantly found at the neuronal membrane. There is consensus that part of APP processing takes place in particular membrane microstructures named lipid rafts (Bhattacharyya et al., 2013).

Lipid rafts are plasma membrane microdomains with a distinct structure and lipid composition where numerous signaling proteins are integrated to participate in different intracellular processes (Lingwood and Simons, 2010). In particular in the case of Aβ processing, APP translocates to lipid rafts following palmitoylation (Bhattacharyya et al., 2013), where its intracellular domain interacts with raft-integrated flotillin-1 to initiate amyloigenic processing (Chen et al., 2006; Schneider et al., 2008). Furthermore, a subset of BACE1 and γ-secretase components also partition into lipid rafts, suggesting that Aβ formation occurs in these structures (Ehehalt et al., 2003; Kalvodova et al., 2005). Indeed, recent data have demonstrated that early alterations of membrane microenvironment in lipid rafts from AD brains,

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Abbreviations: Aβ, β-amyloid peptide; AD, Alzheimer's disease; APP, amyloid precursor protein; BACE1, β-secretase; BSA, bovine serum albumin; CI, cell index; DMEM, Dulbecco's Modified Eagle's Medium; DMSO, Dimethyl sulfoxide; EDTA, ethylenediaminetetraacetic acid; ERα, estrogen receptor alpha; FBS, fetal bovine serum; IEF, isoelectrofocusing; IGF-1R, insulin-growth factor-1 receptor; PBS, phosphate-buffered saline; PMSF, phenyl methyl sulfonyl fluoride; p*I*, isoelectric points; PTP, protein tyrosine phosphatase; pTyr, phosphotyrosine; ROI, regions of interest; SDS–PAGE, sodium dodecyl-sulfate–polyacrylamide gel electrophoresis; Tyr, tyrosine; VDAC, voltage-dependent anion channel.

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promotes the accumulation of BACE1 and increases its interaction with APP (Diaz et al., 2014; Fabelo et al., 2014). Overall, these data point to a dynamic system of Aβ production with the participation of different lipid raft modulators.

Another protein highly abundant in neuronal lipid rafts is the voltage-dependent anion channel (VDAC). Although originally characterized as a mitochondrial porin, VDAC present at the plasma membrane appears to play different roles. Thus, this versatile porin has been related to extrinsic apoptotic pathways, cellular ATP release, calcium and metabolite transport, volume control, redox homeostasis and NADH:ferricyanide reductase activity (Akanda et al., 2008; De Pinto et al., 2010; Park et al., 2010). Moreover, in particular in neurons, some studies are starting to reveal the importance of VDAC in the events related to AD pathology. Thus, VDAC1 associates with γ-secretase in lipid rafts (Hur et al., 2012), suggesting the involvement of the porin in Aβ processing. Furthermore, VDAC1 has been related to Aβ-induced toxicity in cultured neurons (Marin et al., 2007). In line with this, immunohistochemical data in, both, APP/PS1 transgenic mouse and human AD brains have shown VDAC intimately related to senile plaques and neurofibrillary tangles (Ferrer, 2009; Ramirez et al., 2009). In AD brains, VDAC expression and modulation appear to be altered, showing an increase in VDAC nitration with the progression of the disease (Sultana et al., 2006).

Activation and opening of VDAC at the plasma membrane may imply the post-translational modification of the channel phosphorylation state (Diaz et al., 2001). At the neuronal membrane, VDAC1 is present in at least three different isoforms phosphorylated at different residues that are conserved in, both, mouse and human brain tissue (Ferrer, 2009; Yoo et al., 2001). Interestingly, alterations in VDAC phosphorylation modulate its activation and gating, as observed by the extracellular application of anti-estrogens for a short period of time (< 15 min). This phenomenon increases cellular toxicity induced by Aβ peptide (Herrera et al., 2011a,b), suggesting that regulation of VDAC phosphorylation may be relevant in the pathogenesis of AD.

To gain insight into the potential participation of VDAC in neurotoxicity as a consequence of membrane disturbance, we have investigated in this work the potential interactions of VDAC with APP and Aβ in neuronal lipid rafts isolated from, both, cultured neurons and human brains. Our results demonstrate the interaction between these molecules, and suggest the presence of mechanisms of Aβ-induced toxicity initiated in lipid rafts that may induce VDAC dephosphorylation.

EXPERIMENTAL PROCEDURES

Materials

Purified recombinant amyloid beta peptide 1–42 ($A\beta_{1-42}$) and Aβ (25–35) and Aβ (1–42) were purchased from Anaspec (Madrid, Spain). The polyclonal antibodies against flotillin 1 and BACE1 were, respectively, from Abcam (Cambridge, UK) and Millipore (Madrid, Spain). The rabbit monoclonal anti-APP antibody, and mouse monoclonal anti-VDAC1 antibody were from Abcam. The monoclonal anti-amyloid beta peptide antibody was purchased from Santa Cruz Biotechnologies (Texas, EEUU). The monoclonal antibody directed against lipid raft scaffolding protein flotillin 1 was from BD Transduction Laboratories (Madrid, Spain).

Mouse monoclonal antibody against the membrane protein Na^+/K^+ ATPase α_1 subunit was from Upstate (Millipore, Madrid, Spain). The monoclonal antibodies against, respectively, anti-phosphotyrosine (anti-pTyr) and 3-nitrotyrosine residues were obtained from Sigma–Aldrich (Tenerife, Spain). Dynabeads sheep anti-rabbit and anti-mouse IgG were from Dynal (Invitrogen, Madrid, Spain). PreCast Criterion sodium dodecyl-sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and immobilized pH 7–11 nonlinear gradient strips for two-dimensional gel electrophoresis were from Bio-Rad Laboratories (Madrid, Spain). The phosphoBLOCKER blocking reagent and the phosphoantibody stripping solution were from Cell Biolabs, Inc. (Madrid, Spain). The phosphatase inhibitor Bipy was from Calbiochem (Barcelona, Spain).

Human brain samples

Post-mortem human brain tissues with AD-related pathology stages I–IV of neurofibrillary tangles and 0-B of β-amyloid plaques according to the nomenclature of (Braak and Braak, 1999; Braak et al., 2006), and from subjects of similar age (> 65 years) without any apparent neurological, psychological or neuropathological disorders were obtained from the Institute of Neuropathology Brain Bank (Bellvitge University Hospital) following legal and ethical guidelines for Biomedical Research involving human subjects and approval of the local Ethics Committee. The postmortem delay was between 3 and 12 h. The frontal cortex (area 8), entorhinal cortex and cerebellum were dissected free of white matter and used for lipid raft isolation. In some cases, lipid raft fractions from the hippocampus of control subjects were also used to run on two-dimensional gel electrophoresis. Five cases were used for each experimental group comprising AD-related pathology stages I–II/0-A; AD-related pathology stage III–IV/A–B, and age-matched controls.

Isolation of lipid rafts and microsomes

Lipid raft isolation was performed following protocols described previously (Mukherjee et al., 2003) with minor modifications. The frontal cortex, entorhinal cortex, hippocampus and cerebellum were homogenized in buffer A (50 mM Tris–HCl pH 8.0, 10 mM $MgCl_2$, 20 mM NaF, 1 mM Na_3VO_4, 5 mM β-mercaptoethanol, 1 mM phenyl methyl sulfonyl fluoride PMSF) in a glass homogenizer grinder, and processed for sucrose gradients differential centrifugation (Ramirez et al., 2009). Six fractions of 2 ml were obtained. Characterization of lipid raft fractions was performed by immunoblotting with lipid raft and non-raft protein markers: flotillin-1 and caveolin-1 for lipid raft fractions, and α1-subunit Na^+/K^+ ATPase and cytosolic Hsp90 for non-raft fractions (data not shown).