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2-ARACHIDONOYLGLYCEROL METABOLISM IS DIFFERENTLY MODULATED BY OLIGOMERIC AND FIBRILLAR CONFORMATIONS OF AMYLOID BETA IN SYNAPTIC TERMINALS

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Abstract—Alzheimer's disease (AD) is the most prevalent disorder of senile dementia mainly characterized by amyloid-beta peptide (Aβ) deposits in the brain. Cannabinoids are relevant to AD as they exert several beneficial effects in many models of this disease. Still, whether the endocannabinoid system is either up- or down-regulated in AD has not yet been fully elucidated. Thus, the aim of the present paper was to analyze endocannabinoid 2-arachidonoylglycerol (2-AG) metabolism in cerebral cortex synaptosomes incubated with Aβ oligomers or fibrils. These Aβ conformations were obtained by ''aging'' the 1-40 fragment of the peptide under different agitation and time conditions. A diminished availability of 2-AG resulting from a significant decrease in diacylglycerol lipase (DAGL) activity was observed in the presence of large $A\beta_{1\text{-}40}$ oligomers along with synaptosomal membrane damage, as judged by transmission electron microscopy and LDH release. Conversely, a high availability of 2-AG resulting from an increase in DAGL and lysophosphatidic acid phosphohydrolase activities occurred in the presence of $A\beta_{1\text{-}40}$ fibrils although synaptosomal membrane disruption was also observed. Interestingly, neither synaptosomal mitochondrial viability assayed by MTT reduction nor membrane lipid peroxidation assayed by TBARS formation measurements were altered by $A\beta_{1\text{-}40}$ oligomers or fibrils. These results show a differential effect of $A\beta_{1\text{-}40}$ peptide on 2-AG metabolism depending on its conformation.

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Abbreviations: 2-AG, 2-arachidonoylglycerol; AD, Alzheimer's disease; AEA, anandamide; APP, amyloid precursor protein; Aβ, amyloid-beta peptide; CBR, cannabinoid receptors; CC, cerebral cortex; DAG, 1,2-diacyl-sn-glycerol; DAGL, diacylglycerol lipase; ECS, endocannabinoid system; HRP, horseradish peroxidase; LDH, lactate dehydrogenase; PET, positron emission tomography; TBA, thiobarbituric acid; TCA, trichloroacetic acid; TEM, transmission electron microscopy; TLC, thin layer chromatography.

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Key words: Alzheimer's disease, amyloid-beta peptide, 2-arachidonoylglycerol, synaptosomes.

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative process that depends on aging but differs from physiological aging. The first event in the pathogenesis of AD is the deposition of amyloid-beta peptide (Aβ) which precedes the aggregation of hyperphosphorylated tau protein generating neurofibrillary tangles (LaFerla, 2010). AD progression leads to synaptic loss, reduced dendritic arbors and neuronal loss in several brain regions, thus affecting multiple neurotransmitter systems (Duyckaerts and Dickson, 2011). The progressive accumulation of Aβ is due to an imbalance between its production and clearance (Crews and Masliah, 2010; Querfurth and LaFerla, 2010). Aβ derives from the amyloid precursor protein (APP) by a proteolytic process (Haass and De Strooper, 1999; Walter et al., 2001) that generates peptides of 40 and 42 amino acid residues. The assembly of monomeric Aβ into multimeric structures leads first to the formation of oligomers which finally, by an elongation phase, form larger polymers called fibrils (Kumar and Walter, 2011). Although deposits of Aβ fibrils into plaques have been proposed as the neurotoxic agents of AD pathology, current evidence relates oligomers to the initial state of this disease. In this context, it has been suggested that synaptic plasticity is inhibited by oligomers (Cleary et al., 2005). This leads to a different hypothesis on the neuropathology of AD whose first event could be a consequence of oligomer-induced synaptic dysfunction which causes memory loss (Hardy and Selkoe, 2002; Lacor et al., 2004). On the other hand, oligomer aggregations into fibrils and their deposition into plaques could represent an inactive reservoir of neurotoxic oligomers (Lacor et al., 2004).

Endocannabinoid-triggered signaling may modulate several processes that occur prior to the onset of dementia in neurodegenerative pathologies including AD (Stella et al., 1997; Marsicano et al., 2003; Aso and Ferrer, 2014). The endocannabinoid system (ECS) is a cell communication mechanism which comprises endogenous ligands, mainly anandamide (AEA) and 2-arachidonoylglycerol (2-AG) (Devane et al., 1992; Stella et al., 1997), cannabinoid receptors (CBR) (Matsuda et al., 1990; Munro et al., 1993), and enzymes which

are involved in the biosynthesis and inactivation of endocannabinoids (Jonsson et al., 2006; Piscitelli and Di Marzo, 2012) Endocannabinoids have been shown to be involved in a large number of important pathophysiological processes (Cravatt and Lichtman, 2004; Di Marzo et al., 2005; Viveros et al., 2005). In line with this, it has been observed that the ECS plays an important role in AD (Ramirez et al., 2005). It has been reported that two key brain regions implicated in learning and memory, such as cortex and hippocampus, which are targets of this pathology, express high levels of some ECS components (Hopper and Vogel, 1976; Mackie, 2005). Interestingly, many components of the ECS undergo different changes depending on the stage of AD (Basavarajappa et al., 2017), which are detailed in the Discussion section. It has also been suggested that the activation of CBR by synthetic and plant cannabinoids has beneficial effects on AD by reducing the detrimental Aβ action and tau phosphorylation as well as by activating repair mechanisms in the brain (Aso and Ferrer, 2014). As stated above, AD is an age-dependent neurodegenerative process. In this respect, we have previously demonstrated that aging modifies 2-AG metabolism decreasing its availability (Pascual et al., 2013) and also decreases CB1 and CB2 protein expression (Pascual et al., 2014a), in rat cerebral cortex (CC) synaptosomes. Furthermore, we have shown that 2-AG metabolism is modulated by CB1 and/or CB2 receptor antagonists in adult and aged rat CC synaptosomes (Pascual et al., 2014a). We have also observed that fatty acid amidohydrolase (FAAH) activity, an enzyme involved not only in AEA but also in 2-AG hydrolysis, decreases in the frontal cortex from AD patients and that this effect is mimicked by Aβ1-40 (Pascual et al., 2014b). Taking into account that certain cannabinoid compounds exert neuroprotection against Aβ, (Ruiz-Valdepenas et al., 2010) and that 2-AG metabolism in neurodegenerative processes has been only partially explored, the main purpose of the present study was to analyze if the activities of the enzymes involved in 2-AG synthesis (lysophosphatidic acid phosphohydrolase-LPAase- and diacylglycerol lipase -DAGL- activities) and hydrolysis (mainly monoacylglycerol lipase -MAGL- activity) are modified by the presence of $A\beta_{1\text{-}40}$ peptide in an oligomeric or fibrillar conformation. To this end, we analyzed 2-AG metabolism in CC synaptic terminals, which are highly vulnerable neuronal structures in AD pathology.

EXPERIMENTAL PROCEDURE

Materials

[2-^{3}H]Glycerol (2 Ci/mmol) was obtained from PerkinElmer (Boston, MA, USA). Preblended Dry Fluor (98% PPO and 2% bis-MSD) was obtained from Research Products International Corp. (Mt. Prospect, IL, USA). Lysophosphatidic acid, 1-oleoyl [oleoyl-9,10-^{3}H (N)]-(54 Ci/mmol) was obtained from American Radiolabeled Chemicals, Inc. (Saint Louis, MO, USA). Oleoyl-L-α lysophosphatidic acid, *N*-ethylmaleimide, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Triton X-100, thiobarbituric acid (TBA) and bovine serum albumin (BSA) were obtained from Sigma–Aldrich (St. Louis, MO, USA). $A\beta_{1\text{-}40}$ was obtained from PolyPeptide (Strasbourg, France) and $A\beta_{1\text{-}42}$ from was obtained from Sigma–Aldrich (St. Louis, MO, USA). The kit (LDH-P UV AA) for measuring lactate dehydrogenase (LDH) activity was generously supplied by Wiener Laboratory (Rosario, Santa Fe, Argentina). Monoclonal antibody against Aβ peptide (sc-28365) and the horseradish peroxidase (HRP)-conjugated mouse IgGκ light-chain binding protein (m-IgGκ BP-HRP, sc-516102) were generously supplied by Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). All other chemicals were of the highest purity available.

Preparation of synaptosomes

Wistar-strain adult rats (4-month-old) were kept and killed, and CC was dissected, as previously described (Pascual et al., 2013). All procedures were carried out following the guidelines issued by the Animal Research Committee of the *Universidad Nacional del Sur* (Argentina) in accordance with the Guide of the Care and Use of Laboratory Animals of the Institute for Laboratory Animal Research (ILAR) of the National Academy of Science (Bethesda, MD). Synaptosomal isolation protocol was followed as described elsewhere (Pascual et al., 2013).

Preparation of human amyloid $\beta_{1\text{-}40}$ and amyloid $\beta_{1\text{-}42}$

Oligomers and fibrils were obtained from monomers, based on the protocols described by Uranga and co-workers (Uranga et al., 2010) and by Martin-Moreno and co-workers (Martin-Moreno et al., 2011), respectively, introducing minor modifications. Aβ was resuspended in DMSO at a concentration of 10 mg/ml. From this solution, dilutions in PBS were prepared at a concentration of 80 μM and were ‘‘aged” at 37 °C for 2 h with constant shaking (300 rpm) to obtain oligomers. Incubation was continued for 22 h (24 h in total) at 37 °C with constant shaking (150 rpm) to obtain fibrils. At each time as well as prior to peptide incubation, aliquots of the different conformations were taken to be analyzed by transmission electron microscopy (TEM) and to be incubated with synaptosomes.

Characterization of amyloid $\beta_{1\text{-}40}$ sizes by Western Blot analysis

$A\beta_{1\text{-}40}$ peptide preparations (0.1–1.5 μg) were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) in 8% and/or 16% acrylamide–bisacrylamide gels under nonreducing conditions. After transferring to Immobilon P membranes using a Mini Trans-Blot cell electro blotter (BIO-RAD Life Science Group, CA), membranes were blocked for 5 h with 5% BSA in Tris-buffered saline (20 mM Tris–HCl, 150mMNaCl) pH 7.5 containing 0.1% Tween 20 (TTBS). Membranes were incubated with anti-Aβ 1/500 for at 4 °C overnight and, after washing with TTBS, incubation with m-IgGκ BP-HRP 1/500 for 2 h at room temperature was performed. Proteins were visualized by chemiluminescence with Pirce ECL Western Blotting substrate (Thermos Scientific) using standard X-ray film