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Maintenance of membrane organization in the aging mouse brain as the determining factor for preventing receptor dysfunction and for improving response to anti-Alzheimer treatments

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ABSTRACT

Although a major risk factor for Alzheimer's disease (AD), the "aging" parameter is not systematically considered in preclinical validation of anti-AD drugs. To explore how aging affects neuronal reactivity to anti-AD agents, the ciliary neurotrophic factor (CNTF)–associated pathway was chosen as a model. Comparison of the neuroprotective properties of CNTF in 6- and 18-month old mice revealed that CNTF resistance in the older animals is associated with the exclusion of the CNTF-receptor subunits from rafts and their subsequent dispersion to non-raft cortical membrane domains. This age-dependent membrane remodeling prevented both the formation of active CNTF-receptor complexes and the activation of prosurvival STAT3 and ERK1/2 pathways, demonstrating that age-altered membranes impaired the reactivity of potential therapeutic targets. CNTF-receptor distribution and CNTF signaling responses were improved in older mice receiving dietary docosahexaenoic acid, with CNTF-receptor functionality being similar to those of younger mice, pointing toward dietary intervention as a promising adjuvant strategy to maintain functional neuronal membranes, thus allowing the associated receptors to respond appropriately to anti-AD agents.

1. Introduction

Aging of the brain is a physiological process that reflects the long-term exposure of the individual to a number of environmental factors, including diet and lifestyle (smoking, alcohol consumption, etc.). Although age is an established risk factor for Alzheimer's disease (AD), preclinical testing for drug development is often based on pathologic AD animal models without consideration of the aging process, which needs to be clearly understood to develop effective therapeutics for the elderly AD patient.

Numerous studies have provided strong evidence for associations between chronic conditions such as metabolic disorders and dyslipidemia with AD-related cognitive decline (Frisardi et al., 2010), suggesting that disruption of lipid homeostasis is linked to risk of AD. Physiological brain aging has indeed been associated with alterations of lipid metabolism and bioavailability, including that of polyunsaturated fatty acids (PUFAs) such as omega-6 arachidonic (ARA) and omega-3 docosahexaenoic (DHA) acids (Gao et al., 2013; Taha et al., 2012), both of which influence membrane fluidity (Feller et al., 2002; Stillwell and Wassall, 2003), with consequences on brain functions including synaptic plasticity and neurotransmission (Kawakita et al., 2006; Latour et al., 2013). Furthermore, both qualitative and quantitative age-associated alterations in neuronal membrane lipid composition (Martín et al., 2010; Ohno-Iwashita et al., 2010) may affect a variety of cellular functions such as membrane fusion, receptor function, and activation of signaling pathways (Ledesma et al., 2012), all of which have been reported to be altered in AD. Lipid rafts serve as signaling platforms for these membrane-dependent cell activities, providing appropriate microenvironments for specific associations of receptors and signaling proteins, and the means to regulate cell functions including survival/death balance and neuroprotection (Davis et al., 2007; Sui et al., 2006). These highly ordered

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cholesterol- and sphingolipid-rich membrane microdomains are distinct from nonraft regions, and their specific architecture can significantly influence biophysical membrane properties, receptor activation, signal transduction and related pathways, as well as amyloid-β peptide (Aβ) release and cerebral vulnerability to neuronal stress conditions (Colin et al., 2016; Díaz et al., 2015). We, therefore, initiated this study to evaluate age-dependent changes in these signaling platforms, as well as the functional consequences with regard to AD susceptibility.

To do this, we selected as a model the ciliary neurotrophic factor (CNTF), whose neuroprotective properties against Aβ-induced neuronal damage and behavioral impairment have previously been demonstrated in our in vivo AD model (Garcia et al., 2010). CNTF is of particular interest for this study since its effects result from its interaction with a tripartite CNTF-R receptor (CNTFRα/LIFRβ/gp130) and the associated JAK-STAT transduction pathway, which depends on lipid raft architecture and composition (Sehgal et al., 2002). Furthermore, CNTF-R activation depends on its 2 subunits gp130 and LIFR that are located in possibly distinct microdomaines (Buk et al., 2004), but that must cluster together with CNTFRα in lipid rafts for transduction of CNTF signaling to occur (Port et al., 2007).

We show here that aging modulated the structural organization of neuronal membranes, resulting in altered functional properties of CNTF-R and significantly attenuated the neuroprotective effect of CNTF. In contrast, the CNTF neuroprotective activity was maintained in older mice on dietary DHA supplementation, suggesting that dietary lipid-based preventive management could improve the efficiency of therapeutic agents by regulating the direct microenvironment of their targets in neuronal membranes.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals and reagents were obtained from Sigma, unless otherwise specified. Aβ was obtained from Bachem, and soluble oligomers of Aβ were prepared at the concentration of 0.5 mM in sterile 0.1-M phosphate-buffered saline (PBS), pH 7.4 as previously described (Kriem et al., 2005), and aliquots were stored at −20 °C until use. Analysis of oligomeric Aβ preparations by electrophoresis in nondenaturing conditions indicated the presence of monomers, trimers, and tetramers (Garcia et al., 2010). Soluble human CNTF was prepared at 1 μg/μL in sterile 0.1-M PBS, pH 7.4, and aliquots were stored at −20 °C until use.

2.2. Animals and diets

Three- or 15-month old male C57BL/6J mice were purchased from Janvier Breeding (Le Genest-St Isle, France). Experiments were conducted on male mice to limit endocrine influences on both neuroprotective action in the brain and lipid metabolism. Animals were housed in pathogen-free, certified animal facility with access to food and water *ad libitum*. They were kept in a controlled environment (22 °C ± 1 °C, 50% ± 20% relative humidity) with a 12-h light/dark cycle. At the beginning of the experiments, body masses of young and old animals were (mean ± SD) 24.1 ± 0.2 and 32.3 ± 0.1 grams, respectively. The group of young mice received a standard diet (StD, 3.08 kcal/g), containing 18% lipids (Harlan Laboratories, Gannat, France), whereas the older mice were randomly divided into 2 groups. One group received the StD diet (Supplementary Table 1), and the other group was placed on a DHA+ diet (3.11 kcal/g), corresponding to the StD diet supplemented with 0.3% (w/w) DHA-ethyl ester (KD Pharma, Bexbach, Germany). Animals received StD or DHA+ diet for 10 weeks. Animal handling and experimental protocols were authorized in accordance with the European Communities Council Directive (86/609/EEC) and the French Directive for animal experimentation (2013/118) for the use and care of laboratory animals and in conformity with PHS policy on Humane Care and Use of Laboratory Animals, incorporated in the Institute for Laboratory Animal Research Guide for Care and Use of Laboratory Animals. The experimental protocol was approved by the institutional ethic committee (CELMEA-2012-0026).

2.3. Surgical procedures

All surgical procedures were performed on mice anesthetized by intraperitoneal injection of 0.8% (w/v) ketamine (Virbac, Carros, France) in physiological serum containing 0.1% (w/v) xylazine (Bayer HealthCare), at the dose of 1-mg ketamine/10 g body weight. One μL of soluble Aβ oligomers (0.5 nmol), CNTF (1 μg), or saline were injected into the right ventricle (stereotaxic coordinates from the bregma in mm: Aβ-0.22, L-1.0, and D 2.5) using a 5-μL Hamilton microsyringe fitted with a 26-gauge needle as previously described (Garcia et al., 2010). After surgery, the mice were returned to their cages until the day of Y-maze testing, which was 4 days after intracerebroventricular (icv) injections. At the end of the experimental period, all animals were anesthetized with isoflurane, followed by decapitation to remove the brain. Cortical tissues were removed and immediately placed on ice and stored at −80 °C.

2.4. Evaluation of short-term memory

Immediate spatial working memory performance was evaluated by recording spontaneous alternation behavior in a Y maze 4 days after icv injection, as described by Garcia et al. (2010).

2.5. Duolink labeling

Mouse brains freshly dissected were immediately placed in precooled (−20 °C) methylbutane for 30 minutes at −20 °C, removed, and then stored at 80 °C. Brain sections (14-μm thickness) were prepared using a cryostat HM550 (Microm-Microtech, Francheville, France) and then mounted on gelatin-coated slides. Brain sections were fixed by treating with 4% (w/v) paraformaldehyde in PBS for 15 minutes at room temperature, after which all steps were performed at room temperature. After washing in PBS, the sections were stained using the Duolink in situ kit (Olink Bioscience, Uppsala, Sweden) according to manufacturer's instructions. This method is a proximity ligation assay which allows the detection of 2 closely located proteins. To evaluate the number of fluorescence spots corresponding to protein interaction, 5 independent fields were counted using a confocal microscope (Fluoview Fv10i, Olympus), for brain sections from 3 mice, and quantification was performed using Image J image-processing software (http://imagej.nih.gov).

2.6. Preparation of protein homogenates

Brain cortex tissues were dissected and immediately placed in RIPA lysis buffer (Millipore) containing 1-mM phenylmethylsulfonyl fluoride (PMSF), 1-mM sodium orthovanadate, and complete protease inhibitor cocktail (Roche). After homogenization with a syringe through a 23G × 1¼″ needle, the samples were lysed using 2 cycles of freezing and thawing, and centrifuged at 4 °C for 30 minutes at 10,000 × *g* to collect supernatants. Protein concentrations in the supernatants were determined by BCA (Thermo Fisher Scientific).