Neurobiology of Aging 53 (2017) 112–121

Contents lists available at ScienceDirect

Neurobiology of Aging

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

Subacute ibuprofen treatment rescues the synaptic and cognitive deficits in advanced-aged mice

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ARTICLE INFO

Article history:
Received 21 September 2016
Received in revised form 1 February 2017
Accepted 1 February 2017
Available online 9 February 2017

Keywords:
Aging
Ibuprofen
Anti-inflammatory responses
Cognitive function
Synaptic plasticity

ABSTRACT

Aging is accompanied by increased neuroinflammation, synaptic dysfunction, and cognitive deficits both in rodents and humans, yet the onset and progression of these deficits throughout the life span remain unknown. These aging-related deficits affect the quality of life and present challenges to our aging society. Here, we defined age-dependent and progressive impairments of synaptic and cognitive functions and showed that reducing astrocyte-related neuroinflammation through anti-inflammatory drug treatment in aged mice reverses these events. By comparing young (3 months), middle-aged (18 months), aged (24 months), and advanced-aged wild-type mice (30 months), we found that the levels of an astrocytic marker, glial fibrillary acidic protein, progressively increased after 18 months of age, which preceded the decreases of the synaptic marker PSD-95. Hippocampal long-term potentiation was also suppressed in an age-dependent manner, where significant deficits were observed after 24 months of age. Fear conditioning tests demonstrated that associative memory in the context and cued conditions was decreased starting at the ages of 18 and 30 months, respectively. When the mice were tested on hidden platform water maze, spatial learning memory was significantly impaired after 24 months of age. Importantly, subacute treatment with the anti-inflammatory drug ibuprofen suppressed astrocyte activation and restored synaptic plasticity and memory function in advanced-aged mice. These results support the critical contribution of aging-related inflammatory responses to hippocampal-dependent cognitive function and synaptic plasticity, in particular during advanced aging. Our findings provide strong evidence that suppression of neuroinflammation could be a promising treatment strategy to preserve cognition during aging.

1. Introduction

By 2050, the aged population who are older than 85 years are expected to reach 21 million in the United States (Vincent and Velkof, 2010). Increasing evidence demonstrates that aging accelerates the risk for neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (Mattson and Magnus, 2006). More importantly, aging drives the vulnerability to cognitive impairments even in the absence of neurodegenerative diseases (Hedden and Gabrieli, 2004; Mattson and Magnus, 2006; Morrison and Baxter, 2012). Thus, it is critical to understand how aging affects neuronal systems and cognitive function in both normal and pathological aging. In humans, cognitive aging usually starts at middle age with an increase in forgetting, which may be an early sign of impaired synaptic transmission and plasticity (Christensen et al., 1999; Colsher and Wallace, 1991; Schonknecht et al., 2005). The aging-related cognitive decline is progressive, where spatial memory, working memory, executive function, and processing speed are gradually impaired (Kukolja et al., 2009; Plancher et al., 2010; Uttl and Graf, 1993).

Memory deficits are related to impaired hippocampal function; progressive decline of memory is often associated with a decrease in hippocampal volume (Kramer et al., 2007; Mueller et al., 2007; Mungas et al., 2005; Reuter-Lorenz and Park, 2010). Hippocampal-mediated cognitive processes are most vulnerable to aging. Studies in humans and animal models suggest that aging-related cognitive decline is caused by disturbances of synaptic integrity in the

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http://dx.doi.org/10.1016/j.neurobiolaging.2017.02.001

hippocampus (Morrison and Baxter, 2012). These synaptic alternations result in increased slow afterhyperpolarizations (Disterhoft et al., 1996; Landfield and Pitler, 1984; Moyer et al., 1992), deficits in long-term potentiation (LTP) (Barnes, 1994; Barnes and Kidd, 1979; Shankar et al., 1998), and long-term depression (Norris et al., 1996, 1998). The processes that underlie aging-related cognitive decline are vastly complex. Thus, studies that investigate brain aging, the molecular mechanisms involved, neuronal plasticity, and possible therapeutic strategies to combat aging-related cognitive deficits are urgent needs for our aging society.

Aging is associated with increased neuroinflammation, diminished motor function, and cognitive decline in both humans and mice. In fact, nonsteroidal anti-inflammatory drugs, such as ibuprofen, have been associated with a reduction in the incidence of AD (in t' Veld et al., 2001) and increased longevity in simple organisms (He et al., 2014). Therefore, in this study, we used young (3-month old), middle-aged (18-month old), aged (24-month old), and advanced-aged (30-month old) mice to address the age-dependent effects on synaptic plasticity, cognitive function, and neuroinflammation throughout the life span of mice. The ages of 3, 18, 24, and 30 months in mice are estimated to correspond to 20, 56, 69, and 81 years of age in humans, respectively (Fox, 2007). Furthermore, the 30-month-old mice were subacutely treated with ibuprofen to determine if synaptic plasticity and cognitive deficits are reversible at an advanced age. We report here the aging-dependent synaptic deficits and cognition decline and show that anti-inflammatory drug ibuprofen is beneficial in rescuing these detrimental effects.

2. Methods

2.1. Animals

Male mice with different ages (3, 18, 24, and 30 months) were obtained from the National Institute on Aging. All mice were housed in standard 12 hours light-dark cycle and fed normal chow ad libitum. In some experiments, mice (30-month-old) were administrated with 0.14 mg/mL ibuprofen in drinking water according to previous methods (Kofidis et al., 2006) and allowed to drink ad libitum. All animal procedures were approved by the Animal Study Committee at Mayo Clinic and in accordance with the regulations of the American Association for the Accreditation of Laboratory Animal Care.

2.2. Western blot and enzyme-linked immunosorbent assay

Samples were homogenized and incubated in phosphate-buffered saline containing 1% Triton X-100, supplemented with protease inhibitor mix and PhosSTOP (Roche). Equal amounts of protein (by Bradford assay) were resolved by SDS-PAGE and transferred to polyvinylidene difluoride membranes. After the membranes were blocked, proteins were detected with primary antibody for overnight at 4 °C. Membrane was probed with LI-COR IRDye secondary antibodies and detected using the Odyssey infrared imaging system. The following antibodies were used in this study: anti-PSD-95 (Cell Signaling), anti-synaptophysin (Millipore), anti-glial fibrillary acidic protein (GFAP) (Millipore), and anti-β-actin (Sigma) antibodies. Levels of PSD-95 and GFAP were also determined by ELISA as previously described (Shinohara et al., 2013).

2.3. Extracellular recordings

After at least 1 week following behavioral testing, mice were sacrificed, and hippocampi were dissected out for electrophysiological experimental paradigms in ice-cold cutting solution containing 110-mM sucrose, 60-mM NaCl, 3-mM KCl, 1.25-mM NaH_2PO_4, 28-mM $NaHCO_3$, 0.6-mM sodium ascorbate, 5-mM glucose, 7-mM $MgCl_2$, and 0.5-mM $CaCl_2$ as previously described (Rogers et al., 2011, 2013). Field excitatory postsynaptic potentials (fEPSPs) were obtained from area CA1 stratum radiatum with the use of a glass microelectrode (2–4 mΩ) filled with artificial cerebrospinal fluid containing 125-mM NaCl, 2.5-mM KCl, 1.25-mM NaH_2PO_4, 25-mM $NaHCO_3$, 25-mM glucose, 1-mM $MgCl_2$, and 2-mM $CaCl_2$. fEPSPs were evoked through stimulation of the Schaffer collaterals using a 0.1 msec biphasic pulse delivered every 20 seconds. After a consistent response to a voltage stimulus was established, threshold voltage for evoking fEPSPs was determined, and the voltage was increased incrementally every 0.5 mV until the maximum amplitude of the fEPSP was reached (I/O curve). All other stimulation paradigms were induced at the same voltage, defined as 50% of the stimulus voltage used to produce the maximum fEPSP amplitude, for each individual slice. Paired-pulse facilitation (PPF) was induced with 2 paired pulses given with an initial delay of 20 msec, and the time to the second pulse incrementally increased 20 msec until a final delay of 300 msec was reached. A fEPSP baseline response was then recorded for 20 minutes. The tetanus used to evoke LTP was a theta-burst stimulation (tbs) protocol consisting of 5 trains of 4 pulse bursts at 200 Hz separated by 200 msec, repeated 6 times with an intertrain interval of 10 seconds. Following tbs, fEPSPs were recorded for 60 minutes. Potentiation was measured as the increase of the mean fEPSP descending slope following tbs normalized to the mean fEPSP descending slope of baseline recordings.

2.4. Fear conditioning test

This test was conducted in a sound attenuated chamber with a grid floor capable of delivering an electric shock, and freezing was measured with an overhead camera and FreezeFrame software (Actimetrics, Wilmette, IL, USA). Mice were initially placed into the chamber and undisturbed for 2 minutes, during which time baseline freezing behavior was recorded. An 80-dB white noise served as the conditioned stimulus (CS) and was presented for 30 seconds. During the final 2 seconds of this noise, mice received a mild foot shock (0.5 mA), which served as the unconditioned stimulus (US). After 1 minute, another CS-US pair was presented. The mouse was removed 30 seconds after the second CS-US pair and returned to its home cage. Twenty-four hours later, each mouse was returned to the test chamber, and freezing behavior was recorded for 5 minutes (context test). Mice were returned to their home cage and placed in a different room than previously tested in reduced lighting conditions for a period of no less than 1 hour. For the auditory CS test, environmental and contextual cues were changed by wiping testing boxes with 30% isopropyl alcohol instead of 30% ethanol; replacing white house lights with red house lights; placing a colored plastic triangular insert in the chamber to alter its shape and spatial cues; covering the wire grid floor with opaque plastic; and altering the smell in the chamber with vanilla extract. The animals were placed in the apparatus for 3 minutes, and then, the auditory CS was presented, and freezing was recorded for another 3 minutes (cued test). Baseline freezing behavior obtained during training was subtracted from the context or cued tests to control for animal variability.

2.5. Hidden platform water maze (HPWM)

Mice were trained to locate an escape platform hidden just beneath the water surface. The training trials lasted for 4 days, 4 trials per day with an inter-trail interval of 1 minute. Goal latencies were obtained using automated video tracking software (ANY-maze). On day 5, the platform was removed, and mice were