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Differential effect of arachidonic acid and docosahexaenoic acid on age-related decreases in hippocampal neurogenesis

Hisanori Tokuda[a], Masanori Kontani[a], Hiroshi Kawashima[a], Yoshinobu Kiso[a], Hiroshi Shibata[a], Noriko Osumi[b,*]

[a] Institute for Health Care Science, Suntory Wellness Ltd., Osaka, Japan
[b] Department of Developmental Neuroscience, Tohoku University Graduate School of Medicine, Sendai, Japan

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ABSTRACT

Hippocampal neurogenesis affects learning and memory. We evaluated in rats effects of ingestion of arachidonic acid (ARA) and/or docosahexaenoic acid (DHA) on age-related decreases in proliferating neural stem/progenitor cells (NSPCs) or newborn neurons (NNs). Rats were fed with ARA- and/or DHA-containing diet from 2 to 18 months old and then sacrificed 1 day or 4 weeks after 5-bromo-2-deoxyuridine (BrdU) injections at 2, 6 and 18 months. The numbers of NSPCs (SOX2+/BrdU+) and NNs (NeuN+/BrdU+) were determined immunohistochemically. The number of BrdU+ cells 1 day after BrdU injections decreased with age, but increased 65% after ARA ingestion compared to the control at 18 months. The SOX2+/BrdU+ cell ratio was unchanged by aging or ingestion of ARA or DHA. The number of NeuN+/BrdU+ cells 4 weeks after BrdU injections decreased with age, but increased 34% (yet not statistically significant) after DHA ingestion compared to the control at 18 months. These results indicate that ARA ingestion can ameliorate the age-related decrease in the number of NSPCs in rats. The functions of ARA and DHA in hippocampal neurogenesis appear to be different in aged rats; ARA may maintain an NSPC pool, whereas DHA may support NN production and/or survival.

1. Introduction

Arachidonic acid (ARA) and docosahexaenoic acid (DHA) are important components of brain phospholipids (PLs) that decrease with age (Söderberg et al., 1991; McGahon et al., 1997, 1999). Age-related deficits in hippocampal functions, including learning and memory, have recently been reported to be ameliorated by ARA or DHA supplementation in aged rats (Okaichi et al., 2005; Kotani et al., 2003; Gamoh et al., 2001); however, the mechanisms involved remain unknown.

Neurogenesis in the dentate gyrus (DG) of the hippocampus occurs throughout the lifetime of humans and rodents (Eriksson et al., 1998; van Praag et al., 2002) and is related to learning and memory in primates and rodents (Aizawa et al., 2009; Imayoshi et al., 2008). Newborn neurons (NNs) are generated from neural stem/progenitor cells (NSPCs) in the subgranular zone (SGZ) of the DG through differentiation and maturation. The numbers of both proliferating NSPCs and NNs in the DG decrease with age (Kuhn et al., 1996; Abrous et al., 2005). These findings suggest that maintaining the number of proliferating NSPCs will ameriorate the age-related decrease in NNs (Rao et al., 2005) and hippocampal function.

Several previous reports have addressed the relationship between neurogenesis and ingestion of ARA and DHA. Maekawa et al. showed that ingestion of ARA during postnatal days 2–31 increases the number of NSPCs in neonatal rats (Maekawa et al., 2009). Administration of DHA for 7 weeks increases the number of NNs in aged n-3 polyunsaturated fatty acid (PUFA)-deficient rats in the third generation of diet-deficient breeding (Kawakita et al., 2006). In addition, DHA promotes neuronal differentiation from embryonic cerebral cortex in a neurosphere assay (Kawakita et al., 2006; Katakura et al., 2009). Recently, Sakayori et al., using a neurosphere assay, showed that ARA and DHA have different effects on maintenance and differentiation of NSPCs (Sakayori et al., 2011). However, the effects of ARA and DHA ingestion on the age-related decline in neurogenesis in normal-aged rats remain unclear, although ARA and DHA may have different roles in neurogenesis.

ARA and DHA are biosynthesized from linoleic acid (LA) and α-linolenic acid (ALA), respectively, in the body (Fig. 1). The conversion reactions from LA and ALA are catalyzed by the same enzymes common to the n-6 and n-3 series, and they compete with each other. The n-6/n-3 ratio is an important factor for brain functions

* Corresponding author. Tel.: +81 22 717 8201.
E-mail address: osumi@med.tohoku.ac.jp (N. Osumi).

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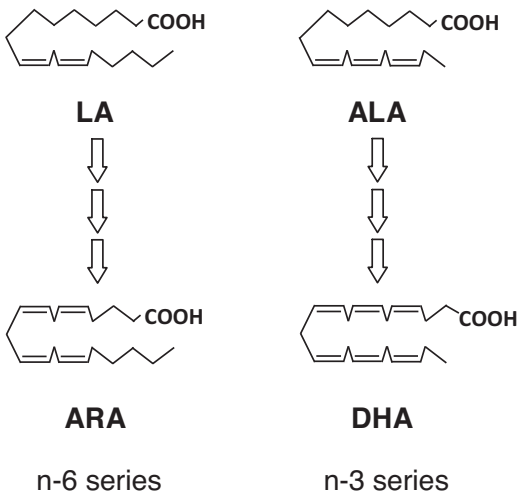

Fig. 1. Biosynthetic pathways from linoleic acid and α-linolenic acid to ARA and DHA, respectively. LA: linoleic acid; ALA: α-linolenic acid; ARA: arachidonic acid; DHA: docosahexaenoic acid.

because this ratio in the diet affects cognitive functions in humans and other vertebrates (Loef and Walach, 2013). Therefore, to properly evaluate the effects of ARA and DHA on hippocampal neurogenesis, the effects of variation in the n-6/n-3 ratio should be excluded from the experimental conditions as much as possible.

The purpose of this study was to evaluate the effects of ingestion of ARA and/or DHA on age-related decreases in NSPCs or NNs in rats. We used diets containing ARA and/or DHA with the same n-6/n-3 ratio. We observed age-related decreases in ARA and DHA in the hippocampus and in the number of hippocampal NSPCs, and these phenomena were ameliorated by ARA and/or DHA ingestion.

2. Materials and methods

2.1. Animals

Male Fisher 344 rats, aged 7 weeks, were obtained from Oriental Bioservice Inc. (Kyoto, Japan). For 1 week prior to experimentation, the animals were fed a commercial diet (CRF-1, Oriental Yeast Co. Ltd., Tokyo, Japan) and tap water *ad libitum* and were housed at 25 ± 1 °C and 60 ± 5% humidity under a 12-h light-dark cycle. Four (at 2 and 6 months) and 4–6 rats (at 18 months) in each group were examined. Experiments were approved by the Animal Care and Use Committee of Suntory Holdings Limited according to the guidelines for animal experiments prescribed by the Science Council of Japan on June 1, 2006.

2.2. Diets

We prepared four different diets by altering the fatty acid composition of AIN-76A: the control, ARA(+), DHA(+), and ARA(+)DHA(+) diets (Table 1). The groups of rats were fed each diet as follows: Control diets (Cont), ARA(+) diets (ARA), DHA(+) diets (DHA), and ARA(+)DHA(+) diets (A + D). The diets were prepared to have almost the same n-6 PUFA: n-3 PUFA and PUFA: monounsaturated fatty acid: saturated fatty acid ratios (2:1 and 1:1:1, respectively). The diets were stored at 4 °C and protected from light to prevent oxidation and denaturation. The rats were fed with these special diets from the age of 8 weeks until the end of the experimental period.

Table 1
Fatty acid composition of the modified AIN-76A diets used in this experiment.

FA	Control	ARA(+)	DHA(+)	ARA(+)DHA(+)
Palmitic acid	26.7	26.0	26.0	26.1
Stearic acid	4.4	4.8	4.1	4.5
Oleic acid	32.1	29.9	31.6	29.6
Linoleic acid	22.3	18.2	22.2	17.6
α-Linolenic acid	11.1	11.6	7.2	7.2
Arachidonic acid	0.0	4.0	0.0	3.9
Eicosapentaenoic acid	0.0	0.0	0.0	0.0
Docosahexaenoic acid	0.0	0.0	3.9	3.9
Others	3.5	5.7	5.1	7.2
Total polyunsaturated FA	33.7	35.0	33.7	34.1
Total monounsaturated FA	32.6	30.4	32.2	30.3
Total saturated FA	31.3	32.1	30.4	31.9
n-6/n-3	2.0	2.0	2.0	2.1

FA: fatty acid. FA composition (%) of the total FA in each diet.

2.3. BrdU labeling assay

Two-, 6-, and 18-month-old rats fed with the control, ARA(+), DHA(+), or ARA(+)DHA(+) diet received intraperitoneal injections of BrdU (Sigma., St. Louis, MO, USA) dissolved in 0.01 M phosphate-buffered saline (PBS) at 50 mg/kg body weight, four times a day, and were sacrificed 1 day or 4 weeks after the injections.

2.4. Immunohistochemistry

To identify markers related to cell proliferation of NSPCs and NNs, rats were deeply anesthetized with sodium pentobarbital and then transcardially perfused with 4% paraformaldehyde and 0.5% picric acid in PBS. The brains were removed and further immersion-fixed in the same fixative at 4 °C for 24 h. Coronal sections of 14 μm thickness were prepared using a cryostat (CM1900, Leica, Wetzlar, Germany) and mounted onto slide glasses. The sections were washed with PBS containing 0.1% Triton X100 (PBS-Tx). For immunostaining of BrdU+ cells on day 1 after BrdU injections, the sections were boiled in 0.01 M citric acid for 2 min, incubated in 2 N HCl for 10 min at 37 °C, and then neutralized with 0.1 M borate buffer (pH = 9.0) for 1 min, washed in PBS-Tx, and blocked in 2% normal goat serum. The sections were incubated at 4 °C overnight with mouse IgG anti-BrdU antibodies (1:100) (Becton Dickinson, Franklin Lakes, NJ, USA). To visualize antigens, the sections were incubated with goat anti-mouse IgG Alexa594-conjugated antibodies (1:250) (Invitrogen, Life Technologies Corp., Carlsbad, CA, USA) at room temperature for 1 h. For nuclear counterstaining, the sections were incubated with 20 μg/mL 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen, Life Technologies Corp., Carlsbad, CA, USA) in PBS-Tx at room temperature for 5 min, and covered by coverslips with PermaFluor™ (Thermo Fisher Scientific Inc., Waltham, MA, USA). For double-immunostaining of SOX2+/BrdU+ cells on day 1 after BrdU injections, the sections were incubated with mouse monoclonal IgG2A anti-SOX2 antibodies (1:200) (R&D Systems, Minneapolis, MN, USA) at room temperature for 2 h. The sections were then incubated with goat anti-mouse IgG Alexa488-conjugated antibodies (1:250) (Invitrogen, Life Technologies Corp., Carlsbad, CA, USA) at room temperature for 1 h. The sequential procedure for detecting BrdU was similar to that described above, except that rat anti-BrdU antibodies (1:125) (Oxford Biotechnology Ltd., Oxford, UK) and goat anti-rat IgG Alexa594-conjugated antibodies (1:250) (Invitrogen, Life Technologies Corp., Carlsbad, CA, USA) were used. For double-immunostaining of NeuN+/BrdU+ cells 4 weeks after BrdU injections, the sections were incubated with mouse monoclonal IgG anti-NeuN antibodies (1:200) (Chemicon, Billerica, MA, USA) at room temperature for 2 h. The sections were then incubated with goat anti-mouse IgG Alexa488-conjugated antibodies (1:250) (Invitrogen, Life Technologies Corp., Carlsbad,