Archives of Gerontology and Geriatrics xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Archives of Gerontology and Geriatrics

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

Long-term food restriction prevents aging-associated sphingolipid turnover dysregulation in the brain

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ARTICLE INFO

Article history:
Received 19 December 2012
Received in revised form 10 December 2013
Accepted 18 December 2013
Available online xxx

Keywords:
Aging
Brain
Sphingomyelin turnover
Calorie restricted diet
N-acetylcysteine
α-Tocopherol acetate

ABSTRACT

Abnormalities of sphingolipid turnover in the brain during normal aging and age-related neurological disorders were associated with the neurons loss and cognitive malfunction. Calorie restriction (CR) prevented age-related deficits in hippocampal long-term potentiation and improved cognitive function at old age. In the paper we investigated the ceramide and sphingomyelin (SM) levels in the brain regions, which are critical for learning and memory of 3- and 24-month-old rats, as well as the correction of sphingolipid turnover in the brain of old rats, by means of the CR diet and modulators of SM turnover. Using the [methyl-^{14}C-choline]SM, the neutral, but not the acid SMase activity has been observed to increase in both the hippocampus and brain cortex of 24-month-old rats with respect to 3-month-old animals. Age-dependent changes of neutral SMase activities were associated with ceramide accumulation and SM level drop in the brain structures studied. Treatment of the rats with the CR diet or N-acetylcysteine (NAC) or α-tocopherol acetate, but not an inhibitor of acid SMase imipramine, reduced the ceramide content and neutral SMase activity in the hippocampus of 24-month-old animals with respect to control rats of the same age. These results suggest that redox-sensitive neutral SMase plays important role in SM turnover dysregulation in both the hippocampus and neocortex at old age and that the CR diet can prevent the age-dependent accumulation of ceramide mainly via neutral SMase targeting.

1. Introduction

Calorie restricted diet beneficial effects on mental health have been reported in human studies and aged animals (Shetty, Galeffi, & Turner, 2011). Calorie restriction prevented age-related deficits in hippocampal long-term potentiation and improved cognitive function at old age and in animal models of Parkinson's and Alzheimer's disease (AD) (Gomez-Pinilla, 2008; Maalouf, Rho, & Mattson, 2009). Improved cognitive function correlated with reduced neuronal loss in neocortex and hippocampus. Diminished energy uptake was followed by decreased levels of amyloid beta-peptide (Aβ) and phosphorylated protein *tau* in the hippocampus of the mouse model of AD as compared with the control diet group (Halagappa et al., 2007).

A diffuse loss of neurons and synapses in the neocortex, hippocampus and other subcortical regions of the brain at AD (Puglielli, Ellis, Saunders, & Kovacs, 2003) is associated with a ceramide accumulation (Costantini, Weindruch, Della Valle, & Puglielli, 2005; Cutler et al., 2004). Ceramide can regulate both the amyloid precursor protein processing and Aβ generation by affecting the beta-secretase (BACE1) stability (Puglielli, Tanzi, & Kovacs, 2003).

Ceramide is a core constituent of sphingolipids and an important signaling molecule that regulates diverse cellular processes including cell growth, differentiation, and apoptosis. Different enzymes involved in the regulation of ceramide level in the cells and sphingomyelinases (SMases) are among them. In the hippocampal neurons the neutral SMase as well as acid SMase activity was stimulated during Aβ treatment (He, Huang, Li, Gong, & Schuchman, 2010). However, by using the primary human neurons and astrocytes, it has been found in transwell experiments that the fibrillar Aβ-activated astroglia kills neurons via neutral SMase but not acid SMase (Jana & Pahan, 2010). Knockdown of neutral SMase, but not acid SMase, by either antisense oligonucleotides or specific inhibitors of SMases (GW4869 and imipramine) prevented the induction of proinflammatory molecules, activation of nuclear factor-κB in the activated astroglia and protected neurons from fibrillar Aβ-induced death.

Abnormalities of sphingolipid turnover and ceramide accumulation have been determined in the brain during normal aging

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http://dx.doi.org/10.1016/j.archger.2013.12.005

(Babenko & Semenova, 2010; Costantini et al., 2005; Cutler et al., 2004). Moreover, significant increase of ceramide levels in the hippocampus and brain cortex have been defined in aged rats, chronically maintained on calorie high diet (Babenko, Semenova, & Kharchenko, 2009). Ceramide accumulation in brain structures was associated with farther decline in cognitive function of old animals as compared with the age-matched normal control. Elevated ceramide content in the glial cells induced AD-like changes in neurons. Glial ceramide increased amyloidogenesis and hyperphosphorylation of *tau* and induced abnormal cerebral glucose metabolism (Patil, Melrose, & Chan, 2007). The inhibition of ceramide production in astroglia by the inhibitor of key enzyme of sphingolipid synthesis (serine palmitoyltransferase)–L-cycloserine reduced significantly ceramide content in the cells. Moreover, L-cycloserine nullified the ceramide-induced AD-like features in cortical neurons. Recent studies have shown that normal aging increases Aβ generation in the cerebral cortex by acting through the p75 neurotrophin receptor ($p75^{NTR}$)-mediated ceramide accumulation (Costantini et al., 2005). Aging-induced ceramide production and Aβ accumulation in mice brain can be blocked by both the calorie restricted diet and inhibitor of neutral SMase–manumycin A. The above results indicated that activation of different metabolic sphingolipid pathways could lead to ceramide accumulation and ceramide-induced AD-like changes in brain structures.

In the present study, we showed that in the hippocampus and brain cortex the neutral SMase, but not the acid SMase activation, led to ceramide accumulation at old age. Calorie restricted diet reducing the neutral SMase normalized ceramide and SM contents in the brain regions of old rats and had no effect on the SM turnover of young animals. NAC, as well as α-tocopherol acetate (inhibitors of neutral SMase), but not an inhibitor of acid SMase imipramine, imitated the calorie restricted diet effect on ceramide content in the hippocampus and neocortex of old rats. The age-dependent and calorie restriction-initiated changes of sphingolipid turnover appeared to be more pronounced in the hippocampus as compared with the neocortex. The results obtained have demonstrated that the calorie restriction and NAC/α-tocopherol acetate targeting of neutral SMase can be used for modulation of the SM and ceramide levels in brain structures at old age.

2. Materials and methods

2.1. Materials

[Methyl-^{14}C-choline]sphingomyelin (52 mCi/mmol) was from PerkinElmer (USA). Silica gel plates for thin-layer chromatography were from Sorbfil (Russia). Lipid standards (ceramide, SM) and N-acetylcysteine were obtained from Sigma–Aldrich. α-Tocopherol acetate and melipramin (imipramine) were from ZAT Texnolog (Ukraine) and Egis (Hungary), respectively. The other chemicals used were of chemically pure grade.

2.2. Animals

The 1-, 3- (young) and 24-month-old (old) male Wistar rats were used in the experiments. They were kept at 24 °C on a cycle of 12 h light/12 h darkness and had free access to a standard chow diet and drinking water ad libitum. Experimental procedures were approved by the Institutional Animal Care and Use Committees at the Kharkov Karazin National University. 1-Month-old rats were divided into 2 groups, singly housed, and either kept on the ad libitum diet or on a calorie restricted diet (McCay, Crowell, & Maynard, 1935; Nikitin, 1984). The final calorie restricted diet consisted of a 60% reduction in total calories without reduction in essential nutrients. Caloric restriction without a reduction in essential nutrients extends the median and maximum lifespan of rats. The median lifespan of control and calorie restricted rats was 630 ± 12 and 765 ± 15 days, respectively. The maximum lifespan of control and calorie restricted rats was 1080 and 1395 days, respectively. Animals were weighed twice a week and the amount of food was adjusted individually in order to suppress the growth of rats down to 10 g per 100 days. Caloric restricted animals were used at the age of 3 (16 rats) and 24 months (16 rats), respectively. 3- (16 animals) and 24-month old (16 animals) rats, singly housed, and kept on the ad libitum diet, were used as control rats. The body weights of the rats used in the experiments were as follows: 214.3 ± 6.96 and 483.4 ± 14.79 for the 3- and 24-month-old animals, respectively, and 73.3 ± 0.67 and 122.7 ± 5.33 for the 3- and 24-month-old calorie restricted rats, respectively. The 24-month-old rats which had free access to a standard chow diet and drinking water ad libitum were divided into 6 groups (12 animals in each group): NAC-, α-tocopherol acetate- and imipramine-treated and controls. Rats of the NAC-fed group were fed with the NAC (3 mM/kg body weight) intragastrically daily for 14 days. Control 24-month-old animals were fed with 0.9% NaCl intragastrically daily for 14 days. Rats of vitamin E-treated group were fed with the α-tocopherol acetate (100 μg/100 g body weight) intragastrically daily for 14 days. Control 24-month-old animals were fed with corn oil. Rats of imipramine-treated group were intra-muscularly injected with a drug (10 mg/kg body weight) daily for 14 days. Control 24-month-old animals were intra-muscularly injected with 0.9% NaCl for 14 days. Prior to experiment the animals were starved overnight. Their hippocampuses, neocortexes, kidney cortexes, skeletal muscles (soleus) and blood serum were obtained 24 h after the last drug treatment or 0.9% NaCl treatment. Tissues were homogenated and used for lipid analysis as described below.

2.3. Experiments with hippocampus and neocortex

Brains were quickly removed, dissected on ice. Hippocampuses and neocortexes were isolated and washed in Krebs-Ringer bicarbonate buffer, pH 7.4. For SM and ceramide separation, the hippocampus and neocortex homogenates prepared in the Krebs-Ringer bicarbonate buffer, pH 7.4, were used. The lipids were extracted and analyzed as described below. The hippocampus and neocortex homogenates (10%) prepared in 50 mM Tris–HCl, pH 7.4, 1 mM EDTA, 10 mM magnesium chloride, 0.65% Triton X-100, were used to determine the neutral SMase activity as described below. Homogenates prepared in 50 mM sodium acetate, pH 5.0, were used to determine the acid SMase activity as described below.

2.4. Determination of sphingolipids turnover

Activities of the neutral and acid SMases were determined using the hippocampus and neocortex homogenates and [methyl-^{14}C-phosphorylcholine]sphingomyelin (52 mCi/mmol). The reaction mixture which contained 50 mM Tris–HCl, pH 7.4, 1 mM EDTA, 10 mM magnesium chloride, 0.65% Triton X-100, 1.5 mg protein and 38,000 dpm [methyl-^{14}C]sphingomyelin in a final volume of 200 μl was used to determine the neutral SMase activity. To study the acid SMase activity, the reaction mixture containing 50 mM sodium acetate, pH 5.0, 1 mM EDTA and 0.65% Triton X-100 was used. The reaction proceeded up to 1 h at 37 °C and then was terminated by the addition of 1.5 ml of chloroform/methanol (1:2, v/v) followed by 1 ml of chloroform and 1 ml of H_2O. The mixture was centrifuged for 5 min at 3000 rpm. After the phase separation, a portion of the upper aqueous phase, containing [^{14}C]phosphorylcholine, was removed and the radioactivity was determined by liquid scintillation counting. To determine the remaining [methyl-^{14}C]sphingomyelin, the lower phase was analyzed as described below.

Please cite this article in press as: Babenko, N.A., Shakhova, E.G., Long-term food restriction prevents aging-associated sphingolipid turnover dysregulation in the brain. Arch. Gerontol. Geriatr. (2014), http://dx.doi.org/10.1016/j.archger.2013.12.005