Neurobiology of Aging 36 (2015) 344–351

Contents lists available at ScienceDirect

Neurobiology of Aging

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

A specific multi-nutrient enriched diet enhances hippocampal cholinergic transmission in aged rats☆

Mehmet Cansev [a,*], Nick van Wijk [b], Mesut Turkyilmaz [a], Fulya Orhan [a], John W.C. Sijben [b], Laus M. Broersen [b]

[a] Department of Pharmacology, Uludag University Medical School, Bursa, Turkey
[b] Nutricia Research, Nutricia Advanced Medical Nutrition, Utrecht, the Netherlands

ARTICLE INFO

Article history:
Received 6 February 2014
Received in revised form 23 June 2014
Accepted 18 July 2014
Available online 24 July 2014

Keywords:
Alzheimer's disease
Fortasyn connect
Souvenaid
Acetylcholine release
Cholinergic neurotransmission
Phospholipid
Synaptic membrane
Choline acetyltransferase
Aged rat

ABSTRACT

Fortasyn Connect (FC) is a specific nutrient combination designed to target synaptic dysfunction in Alzheimer's disease by providing neuronal membrane precursors and other supportive nutrients. The aim of the present study was to investigate the effects of FC on hippocampal cholinergic neurotransmission in association with its effects on synaptic membrane formation in aged rats. Eighteen-month-old male Wistar rats were randomized to receive a control diet for 4 weeks or an FC-enriched diet for 4 or 6 weeks. At the end of the dietary treatments, acetylcholine (ACh) release was investigated by in vivo microdialysis in the right hippocampi. On completion of microdialysis studies, the rats were sacrificed, and the left hippocampi were obtained to determine the levels of choline, ACh, membrane phospholipids, synaptic proteins, and choline acetyltransferase. Our results revealed that supplementation with FC diet for 4 or 6 weeks, significantly enhanced basal and stimulated hippocampal ACh release and ACh tissue levels, along with levels of phospholipids. Feeding rats the FC diet for 6 weeks significantly increased the levels of choline acetyltransferase, the presynaptic marker Synapsin-1, and the postsynaptic marker PSD-95, but decreased levels of Nogo-A, a neurite outgrowth inhibitor. These data show that the FC diet enhances hippocampal cholinergic neurotransmission in aged rats and suggest that this effect is mediated by enhanced synaptic membrane formation. These data provide further insight into cellular and molecular mechanisms by which FC may support memory processes in Alzheimer's disease.

1. Introduction

Synapse loss and membrane-related pathology play a central role in the pathogenesis of Alzheimer's disease (AD) (Arendt, 2009; Selkoe, 2002) and consequently provide viable intervention targets. In early AD, the increased synapse loss is associated with memory decline (Selkoe, 2002; Sperling et al., 2011; Terry et al., 1991). Reducing synapse loss and membrane-related pathology may preserve or improve neurotransmission and, thereby, positively affect memory and other cognitive functions.

Fortasyn Connect (FC) is a specific nutrient combination designed to ameliorate synapse loss and synaptic dysfunction in AD (Van Wijk et al., 2014) by addressing nutritional needs believed to exist in these patients (Lopes da Silva et al., 2013; Mi et al., 2013). FC comprises precursors and cofactors required for the formation and maintenance of neuronal membranes, that is, uridine (as uridine monophosphate, UMP), the omega-3 PUFAs docosahexaenoic acid (DHA) and eicosapentaenoic acid, choline, phospholipids, folic acid, vitamins B12, B6, C, and E, and selenium.

FC is present in Souvenaid, a medical food intended for early AD patients. This medical food significantly improved memory performance in drug-naïve patients with mild AD in 2 recent clinical trials (Scheltens et al., 2010, 2012). In addition, electroencephalography measures suggested that the medical food preserved functional brain network organization, counteracting the pattern usually seen in AD patients (de Waal et al., 2014; Scheltens et al., 2012).

The aim of the present study was to further investigate the physiological mechanism underlying the cognitive enhancing effect of FC by supplementing aged rats with a diet enriched with this specific nutrient combination. It is been well established that improved learning and memory in various tasks is associated with augmented cholinergic neurotransmission in the hippocampus (Fadda et al., 2000; Nail-Boucherie et al., 2000). Therefore, we assessed the effect of an FC-enriched diet on acetylcholine (ACh) release, indicative of hippocampal cholinergic neurotransmission, by in vivo microdialysis

* Corresponding author at: Department of Pharmacology, Uludag University Medical School, Gorukle Campus, 16059, Bursa, Turkey. Tel.: +90 224 295 3568; fax: +90 224 442 8102.
E-mail address: mcansev@uludag.edu.tr (M. Cansev).

0197-4580/$ – see front matter © 2015 The Authors. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neurobiolaging.2014.07.021

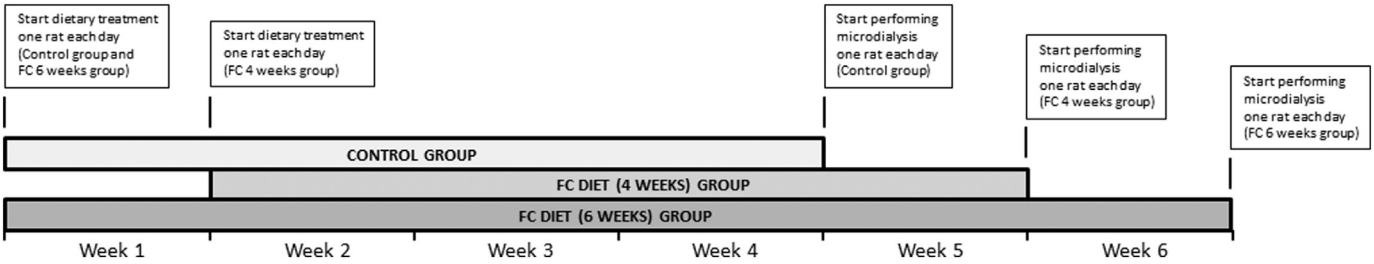

Fig. 1. Timeline of the experimental protocol. To perform microdialysis on each rat on separate days, termination of the experimental procedures for each group was arranged to 3 consecutive weeks by arranging the onset of dietary treatments accordingly: dietary treatment for control and FC diet 6 weeks groups was initiated on the same day whereas dietary treatment for FC diet 4 weeks group was initiated 1 week later. Abbreviation: FC, Fortasyn connect.

in the right hippocampi of aged rats. This was followed by an investigation of the effects of FC on synaptic membrane formation, to further investigate its mode of action. This is the first study to determine the effect of chronic treatment with this specific nutritional combination on hippocampal ACh release in association with its effects on synaptic membranes in aged rats.

We found that dietary supplementation with FC diet for 4 or 6 weeks significantly enhanced hippocampal ACh release, both under basal conditions and after atropine stimulation. This observation was accompanied by enhanced levels of tissue ACh and membrane phospholipids. In addition, FC diet treatment for 6 weeks significantly increased the levels of choline acetyltransferase (ChAT) and the presynaptic and postsynaptic markers Synapsin-1 and PSD-95, respectively, while decreasing levels of Nogo-A, a neurite outgrowth inhibitor. Hence, the present data show that consumption of the FC-enriched diet enhances hippocampal cholinergic neurotransmission, which probably can be ascribed to the concurrently observed enhanced synaptic membrane formation.

2. Methods

2.1. Animals

Eighteen-month-old male Wistar rats (Experimental Animals Breeding and Research Center, Uludag University Medical School, Bursa, Turkey) were group housed in a temperature controlled room with free access to standard rat chow and water under a-12/12 hour light-dark cycle The experimental protocol was approved by the Animal Care and Use Committee of Uludag University, Bursa, Turkey (Approval ID: 2012-03/03), and all experiments conformed to the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23) revised 1996. All efforts were made to minimize the number of animals used and the individual level of discomfort.

2.2. Dietary supplementation

Rats were randomized to control and FC diet groups. Rats in the control group (n = 4) received a regular rat chow for 4 weeks, whereas rats in the FC group were fed the FC diet for either 4 (n = 5) or 6 (n = 5) weeks.

Dietary treatment for control and FC diet 6 weeks groups was initiated on the same day, whereas dietary treatment for FC diet 4 weeks group was initiated 1 week later. With this set-up, we were able to perform microdialysis on each rat on separate days. Timeline of the experimental protocol is provided in Fig. 1.

Both the control diet and the FC-enriched diet were AIN-93 M based (Reeves et al., 1993), isoenergetic, and fulfilled all dietary requirements. Both diets contained the standard vitamin mix (AIN-93-VX) and mineral mix (AIN-93M-MX). The diets differed in composition with regard to the fat blends used, as well as a number of supplemented nutrients, including choline, B-vitamins, antioxidants, UMP, and lecithin. A detailed overview of the contents of diets is presented in Table 1. The diets were formulated by Nutricia Research, Nutricia Advanced Medical Nutrition (Utrecht, the Netherlands), manufactured by Ssniff Spezialdiäten (Soest, Germany) and presented to the animals as pellets. All diets were stored at −20 °C until use, to prevent lipid oxidation. Reanalysis of the diets at the end of the study confirmed that all fatty acids were still present in the original amounts.

No significant difference was observed between treatment groups in terms of mean daily amount of food consumed and weight gain (data not shown) during the treatment.

2.3. Surgical procedures

One day before the completion of dietary treatment, a probe was inserted in the right hippocampus of rats in each group to perform in vivo microdialysis. Rats were anesthetized with ketamine and xylazine (80 mg/kg and 10 mg/kg, respectively) and then placed in a stereotaxic frame. Subsequently, skulls were exposed, and a small hole was drilled over the right hippocampus. A hand-made probe

Table 1
Detailed compositions of the 2 experimental diets

Ingredients (g/100 g diet)	Diets	
	Control	Fortasyn
Cornstarch, pregelatinized	35.6	32.5
Caseine	14.0	14.0
Maltodextrin, 10 DE	15.5	15.5
Sucrose	10.0	10.0
Dextrose	10.0	10.0
Soy oil	1.900	
Coconut oil	0.900	0.100
Corn oil	2.200	1.700
Fish oil		3.200
Cellulose powder	5.0	5.0
Mineral mix (AIN-93M-MX)	3.5	3.5
Vitamin mix (AIN-93-VX)	1.0	1.0
L-cystine	0.180	0.180
Choline chloride (50%)	0.230	0.922
Tert-butylhydroquinone	0.0008	0.0008
UMP (UMP disodium salt)		1.0
Soy lecithin		0.755
Vitamin B6 (pyridoxin hydrochloride, 100%)		0.00328
Folic acid (100%)		0.00060
Vitamin B12 (cyanocobalamin, 0.1%)		0.00350
Ascorbic acid (100%)		0.160
Vitamin E (tocopherol acetate, 50%)		0.4650
Selenium (sodium selenite pentahydrate, 100%)		0.00034
Total	100.0	100.0

All amounts of nutrients are indicated in g/100 g of diet.
Key: UMP, uridine monophosphate.