Effect of diet with omega-3 in basal brain electrical activity and during status epilepticus in rats

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ARTICLE INFO

Keywords:
Omega-3
ECoG
Brain electrical activity
Status epilepticus
Power spectrum

ABSTRACT

Western diets are high in saturated fat and low in omega-3. Certain animals cannot produce omega-3 from their own lipids, making it necessary for it to be acquired from the diet. However, omega-3s are important components of the plasma membrane, and altering their proportions can promote physical and chemical alterations in the membranes, which may modify neuronal excitability. These alterations occur in healthy individuals, as well as in patients with epilepsy who are more sensitive to changes in brain electrical activity. This study evaluated the effect of a diet supplemented with omega-3 on the basal brain electrical activity both before and during status epilepticus in rats. To evaluate the brain electrical activity, we recorded electrocorticograms (ECoG) of animals both with and without omega-3 supplementation before and during status epilepticus induced by pilocarpine. Calculation of the average brain wave power by a power spectrum revealed that omega-3 supplementation reduced the average power of the delta wave by 20% and increased the average power of the beta wave by 45%. These effects were exacerbated when status epilepticus was induced in the animals supplemented with omega-3. The animals with and without omega-3 supplementation exhibited increases in basal brain electrical activities during status epilepticus. The two groups showed hyperactivity, but no significant difference between them was noted. Even though the brain activity levels observed during status epilepticus were similar between the two groups, neuron damage to the animals supplemented with omega-3 was more slight, revealing the neuroprotective effect of the omega-3.

1. Introduction

Fatty acids consumed in the diet have two primary functions: the formation of triglycerides and the incorporation of phospholipids into the plasma membrane. Phospholipids are formed by two hydrophobic fatty acids and hydrophilic phosphate group joined together by a glycerol molecule. Binding to different types of fatty acids generates a variety of phospholipids. When the fatty acid chain is less saturated, the cellular membrane is more fluid, while chains that are more saturated result in membrane less fluid. Thus, the balance between saturated and unsaturated fatty acids is important for maintaining optimum fluidity in the membrane (Nelson and Cox, 2004; Ouettel et al., 2009).

The presence of omega-3 and omega-6 fatty acids in the human diet is important because we do not have the Delta-12 and Delta-15 desaturase enzymes. These enzymes are responsible for creating double bonds at the carbon prior to the carbon 9 of the methyl group; thus, humans are unable to produce omega-3 and omega-6 fatty acids from fatty acids produced by the body (Gurr et al., 2002). For this reason, the fatty acids in these families are called essential fatty acids. Because Western diets are low in omega-3s and high in saturated fatty acids, it is necessary to include omega-3 fatty acids in the diet.

Furthermore, it is important to maintain a suitable ratio between omega-6 and omega-3 in the diet because they compete for the same enzyme complex. The former originates in response to pro-inflammatory eicosanoids, while the latter promotes an increase in inflammatory and antioxidant eicosanoids to anti-inflammatory responses, primarily the polyunsaturated fatty acids (PUFA), omega-3 acid eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) (Tapiero et al., 2002; Gunstone et al., 2007; Schmitz and Ecker, 2008; Falinska et al., 2012). Diets low in omega-3 have been linked to the development of depression, bipolar disorder and schizophrenia. The relationship between omega-3 and the development of mood disorders is not entirely clear, but it may be related to the anti-inflammatory and antioxidant effects of the fatty acids (Chiappetti and Bejor, 2010; Chiappetti et al., 2012). Based on these
findings, research has been conducted to investigate the neuroprotective effect of omega-3 (Hong et al., 2003).

Studies have shown that compared to triglycerides, PUFAs in the diet are preferentially incorporated by phospholipids (Balogun et al., 2013). DHA and EPA can easily pass through the blood brain barrier and be incorporated into neuron phospholipid membranes (Ouellet et al., 2009). As stated previously, the incorporation of these PUFAs, which also compose the neuron’s myelin sheath, tends to increase membrane fluidity in the neurons (Horrocks and Farooqui, 2004). Thus, it is reasonable to propose that increased fluidity in the membranes of the nervous system cells can facilitate the generation and propagation of action potentials, thereby increasing brain electrical activity.

Factors that alter brain electrical activity should be investigated on epilepsy patients, since epilepsy is one of the most common neurological diseases and is directly linked to pathological brain hyperexcitability. While some studies indicate that omega-3 supplementation reduces the duration and frequency of seizures (Schlanger et al., 2002), others indicate that the supplementation presented no antiepileptic effect (Bromfield et al., 2008). This work investigates the influence of omega-3 supplementation on basal brain electrical activity in healthy subjects and in subjects during status epilepticus.

2. Materials and methods

We used 38 male Wistar rats (Rattus norvegicus albinus) from the biotery of the Department of Animal Morphology and Physiology, Federal Rural University of Pernambuco. The animals were housed at a controlled temperature (23 ± 2 °C), controlled humidity (50%), and a 12-h light–dark cycle with food and water ad libitum.

All animals were carried out in accordance with procedures approved by the Local Committee for the Care and Ethical Use of Animals in Research (CEUA/UFRPE, Recife, PE, Brazil), outlined in protocol number 103/2014 – CEUA/UFRPE.

2.1. Experimental design

Animals were divided into two groups. Group 1 (n = 20) animals were fed a standard balanced diet (Presence, Betel/Paulínia, São Paulo, Brazil) and received 1 ml/day of saline solution (0.9% NaCl) per gavage until the ECoG record and induction of status epilepticus. Group 2 (n = 18) animals were fed a standard balanced diet supplemented with fish oil (Naturalis, São Paulo, São Paulo, Brazil) containing 180 mg of EPA and 120 mg of DHA per gram at a dose of 1 ml/day administered by gavage. The dietary supplementation was performed from 60 to 120 days postnatal. At 120 days of age, 10 animals from each group were chosen for histological analysis, and the others were used to record the brain electrical activity.

2.2. Histological analysis

The animals were divided into 4 groups for the histological analysis:

Group 1: control animals; Group 2: animals submitted to status epilepticus for a period of 4 h; Group 3: animals supplemented with omega-3; Group 4: animals supplemented with omega-3 and submitted to status epilepticus for a period of 4 h.

After being anesthetized, all animals were perfused with saline solution (0.9% NaCl) and with 10% formaldehyde in phosphate-buffered saline (pH 7.4) thereafter. The brains of the animals were collected and fixed in 10% buffered formaldehyde solution. Coronal brain fragments were dehydrated through increasing concentrations of ethanol (70% to 100% by P.A.), diaphanized in xylene, and infiltrated with Paraplast Plus (Sigma-Aldrich, St. Louis, Missouri, USA).

Using a Leica RM 2125 rotary microtome (Leitz-Park, Wetzlar, Germany), coronal hippocampal sections were obtained at a thickness of 4 μm. The sections were taken at the height of the third ventricle, equivalent to section 21 (Bregma: −1.70 mm and level 74), serially.

After this procedure, the sections were stained with hematoxylin and eosin (H/E). Photomicrographs were obtained with a Moticam camera (Motic, Kowloon, Hong Kong, China) attached to a light microscope (Leica, Leitz-Park, Wetzlar, Germany) at 40X to 400X magnification using ImageJ software (Wayne Rasband, National Institutes of Health in Bethesda, Maryland, USA) for histopathological evaluation of hippocampus regions: CA1, CA2, CA3, the dentate gyrus and the hilus of the hippocampal formation.

To quantify the brain tissue lesions caused by status epilepticus, the vacuoles caused by cell death were quantified using Gimp 2.0 software. These histological images were quantified by an RGB histogram (Red-Green-Blue), which is based on the intensity of the luminosity, with image pixel tones varying from 0 to 255. A tone of 0 represents absolute dark, while a tone of 255 represents absolute white (Oberholzer et al., 1996).

2.3. Surgical procedure for electrode implantation

At 115 days of age, the animals were anesthetized with a combination of ketamine (50 mg) and xylazine (20 mg) at a dose of 0.1 ml/100 g body weight, administered intramuscularly. Body temperature was maintained at approximately 37.5 ± 1 °C by an electric heater positioned under the animal. A trichotomy and antisepsis were performed on the animals’ heads. After the head was fixed to the base of a stereotactic apparatus (Insight, Ribeirão Preto, São Paulo, Brazil), a longitudinal skin incision was made on the midline of the skull. Using a small drilling machine, two orifices were made, and two surgical screws were placed on the left hemisphere. A screw was placed in the parietal area of the sensorimotor cortex at 1.5–2.5 mm anterior and 1–2 mm lateral to the bregma. Another screw was placed on the frontal bone (reference electrode). The screws were reinforced with acrylic resin (VipiFlash, Vipi, Pirassununga, São Paulo, Brazil). After the resin was dry, the skin was sutured, leaving only the screws exposed as electrodes.

2.4. Record of the in basal brain electrical activity before and during status epilepticus

After a period of five days to allow for complete healing from the surgery, the animals were placed in a Faraday cage. The ECoG recording was performed by a 410C EMG device (EMG System, São José dos Campos, São Paulo, Brazil). The ECoG was recorded for 60 min, 30 min before (baseline) and 30 min during status epilepticus with a sampling rate of 6000 S/s (Pessoa et al., 2016). After the first 30 min of recording, 350 mg/kg of pilocarpine hydrochloride (Sigma-Aldrich, St. Louis, Missouri, USA) were administered to induce status epilepticus, and the recording was continued for another 30 min. After the recording, the animals were euthanized by anesthesia. The filtering of the signal and the ECoG analysis were analyzed using MATLAB 7.8 software (MathWorks, Natick, Massachusetts, USA).

2.5. Analysis of the ECoG

The ECoG was recorded for 30 min, segmented into 2 min windows, for each experimental condition. In all, 15 segments were obtained for each experimental condition, totaling 30 segments per animal. The segments were imported to the MATLAB 7.8 software, and an algorithm for obtaining the power spectrum of the brainwave was implemented (Pessoa et al., 2016).

2.6. Statistical analyses

The Mann-Whitney test was used to compare the pixel count of the lesion images promoted by status epilepticus in the brain tissue with and without omega 3. The Wilcoxon test was used to compare the paired data of the average power values of the ECoG signals. The results are
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