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Intraperitoneal administration of docosahexaenoic acid for 14 days increases serum unesterified DHA and seizure latency in the maximal pentylenetetrazol model

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ABSTRACT

Docosahexaenoic acid (DHA) is an omega-3 polyunsaturated fatty acid (n−3 PUFA) which has been shown to raise seizure thresholds following acute administration in rats. The aims of the present experiment were the following: 1) to test whether subchronic DHA administration raises seizure threshold in the maximal pentylenetetrazol (PTZ) model 24 h following the last injection and 2) to determine whether the increase in seizure threshold is correlated with an increase in serum and/or brain DHA.

Animals received daily intraperitoneal (i.p.) injections of 50 mg/kg of DHA, DHA ethyl ester (DHA EE), or volume-matched vehicle (albumin/saline) for 14 days. On day 15, one subset of animals was seizure tested in the maximal PTZ model (Experiment 1). In a separate (non-seizure tested) subset of animals, blood was collected, and brains were excised following high-energy, head-focused microwave fixation. Lipid analysis was performed on serum and brain (Experiment 2). For data analysis, the DHA and DHA EE groups were combined since they did not differ significantly from each other.

In the maximal PTZ model, DHA significantly increased seizure latency by approximately 3-fold as compared to vehicle-injected animals. This increase in seizure latency was associated with an increase in serum unesterified DHA. Total brain DHA and brain unesterified DHA concentrations, however, did not differ significantly in the treatment and control groups. An increase in serum unesterified DHA concentration reflecting increased flux of DHA to the brain appears to explain changes in seizure threshold, independent of changes in brain DHA concentrations.

1. Introduction

Epilepsy is a neurological disorder involving recurrent, spontaneous seizures. Approximately 1% of the population suffers from epilepsy at any given time, with approximately 3–4% of the population being affected at some point during their lives [1,2].

Antiepileptic drugs (AEDs) are the therapy of choice for epilepsy. However, there are approximately 30–40% of patients whose seizures fail to respond to AEDs and continue to display seizures while on medication [3]. Moreover, many side effects are associated with AEDs, including nausea, drowsiness, and – in a rare subset of patients – hypersensitivity reactions [4,5]. Therefore, there is still a need for new therapies for epilepsy.

One suggested therapy for epilepsy is dietary supplementation with omega-3 polyunsaturated fatty acids (n−3 PUFAs) [6]. n−3 PUFAs can be derived from dietary sources such as seafood and are not known to have any serious toxicity [7]. Docosahexaenoic acid (DHA) in particular, is an n−3 PUFA which is highly concentrated in the brain and plays a role in a number of brain functions including the regulation of gene expression [8], the maintenance of membrane fluidity, signaling [9], and the production of anti-inflammatory metabolites [10].

The n−3 PUFAs are known to have antiarrhythmic effects, which appear to be mediated by their actions on voltage-dependent sodium channels (VDSCs) [11,12]. In vitro work on hippocampal slices, for instance, has demonstrated that the n−3 PUFAs reduce sodium currents and increase VDSC inactivation time [13]. Other studies have shown that the n−3 PUFAs increase the action potential firing threshold [14], reduce repetitive action potential firing [14,15], and reduce hippocampal sharp waves [16]. These effects on action potential firing are thought to be

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mediated by the unesterified form of the fatty acids, as ethyl esters are ineffective [14]. It was recently shown, however, that both the free and methyl ester forms of DHA were effective at reducing hippocampal sharp wave firing [16].

It has been suggested that the n−3 PUFAs might have anticonvulsant as well as antiarrhythmic effects. Several studies have been published on the anticonvulsant properties of n−3 PUFAs in vivo. The first of these reported that intraperitoneal (i.p.) administration of 40 mg/kg of a mixture of linoleic acid (LA) and alpha-linolenic acid (ALA) in a 4:1 ratio for a period of 21 days resulted in seizure protection in 4 different seizure models [17]. Our own group was not able to reproduce this effect at 40 mg/kg, but we did find an increase in seizure threshold at a higher dose of 200 mg/kg [18,19].

Alpha-linolenic acid is the precursor to longer chain n−3 PUFAs, including DHA. Studies investigating the specific effect of DHA have shown DHA to increase seizure threshold in several in vivo studies when administered intravenously (i.v.) [20,21], subcutaneously (s.c.) [22,23], and intraperitoneally (i.p.) [21]. It has been previously demonstrated that acute administration of DHA results in increased seizure latency 1 h following injection, but this effect is lost 2 h postinjection [22]. However, it has yet to be determined if multiple injections could increase the duration of efficacy of DHA on seizure latency.

The present study was designed to investigate if subchronic injection can result in an elevation in seizure latency 24 h following the last injection and whether this effect of DHA could be linked to elevations in unesterified DHA in serum and brain. Moreover, the efficacy of injecting the ethyl ester form of DHA was also tested as it has yet to be tested in vivo. Docosahexaenoic acid was administered i.p. for a period of 14 days. Animals were then seizure tested in the maximal PTZ model (Experiment 1) or sacrificed for measurement of DHA in serum and brain (Experiment 2). The maximal PTZ test models tonic–clonic seizures in humans. Docosahexaenoic acid was administered both in an unesterified form (DHA) and in the form of an ethyl ester (DHA EE). It was hypothesized that the esterified form would be ineffective. It was found, however, that DHA EE was just as effective as DHA.

2. Materials and methods

2.1. Subjects

The present experiments were conducted in accordance with the standards of the Canadian Council on Animal Care and were approved by the Animal Care Committee of the Faculty of Medicine of the University of Toronto. Sixty-day-old male Wistar rats were obtained from Charles River (La Prairie, Qc) and were individually housed in a vivarium maintained on a 12 h light–dark cycle (lights on at 7 am) and at a temperature of 21 °C. Rat chow (2018 Teklad Global 18% Protein Rodent Diet; Teklad Global, Madison, WI) and water were available ad libitum. The rat chow contained (in g/kg diet) protein (189), fat (60), carbohydrate (554), fiber (38), ash (59), and moisture (100). The diet fat composition (in percent of total fatty acids) was palmitate (18.5%), stearate (2.8%), oleate (18.5%), linoleate (54.8%), and alpha-linolenate (5.6%) [23].

2.2. Drug preparation

Docosahexaenoic acid and docosahexaenoic acid ethyl ester were obtained from Nu-Check Prep (Elysian, MN). Stock solutions were prepared by mixing 140 μl of DHA or DHA EE with 90 mg of bovine serum albumin (BSA) per ml of physiological saline. The final concentrations of the stock solutions were 128.8 mg/ml and 127.4 mg/ml for DHA and DHA EE, respectively (being equimolar in concentration). A vehicle solution containing 90 mg of BSA dissolved in 1 ml of physiological saline was given to control subjects. Stock solutions were sonicated for 5 min. Stock solutions were made on day 1 of injections and were stored at −80 °C between injections. Stock solutions were thawed prior to injections and were kept on ice during the injections.

Pentylenetetrazol was obtained from Sigma-Aldrich (Oakville, ON). The PTZ solution was prepared by dissolving 50 mg of PTZ per ml of 0.9% saline. Pentylenetetrazol stock solution was made on day 15, prior to seizure testing, and was kept on ice between PTZ injections.

2.3. Experiment 1 — seizure testing

Two groups of 10 animals each received a dose of 50 mg/kg i.p. of either DHA or DHA EE for 14 consecutive days. Vehicle solution, volume-matched to the DHA group, was administered to a third group of control animals (n = 10).

On day 15, each animal received 105 mg/kg i.p. of PTZ. In a pilot study involving a separate group of subjects (n = 10), this dose of PTZ had been shown to reliably induce tonic–clonic convulsions. Following the PTZ injection, the subjects were placed in the open field and observed for 15 min. The latencies to the first myoclonic jerk and the first tonic–clonic seizure were scored by two independent observers. Following seizure testing, all subjects were immediately euthanized with a lethal intracardiac (i.c.) injection of 100 mg/kg of T-61 (Intervet, Kirkland, QC). As per animal care guidelines, if animals did not seize within 15 min, animals were euthanized by CO_2 asphyxiation. Four animals (saline = 2, DHA = 1, and DHA EE = 1) did not have seizures within 15 min and were removed from analysis. No fatty acid analysis was done in the seizure-tested subjects of Experiment 1.

2.4. Experiment 2 — blood and brain analysis

Two other groups of 19 non-seizure tested animals received a dose of 50 mg/kg i.p. of either DHA (n = 9) or DHA EE (n = 10) for 14 consecutive days. Vehicle solution, volume-matched to the DHA group, was administered to a third group of control animals (n = 10).

On day 15, animals were placed under a heat lamp to generate vasodilation. Animals were then cannulated in the right tail vein with 24-gauge (g) angiocath for blood sampling (Becton Dickson, Mississauga, ON). One milliliter of blood was drawn. Blood was gently transferred into a centrifuge tube and placed on ice. Blood was later spun for 5 min at 4 °C, and serum was collected following centrifugation and stored at −80 °C.

Ten minutes following blood collection, animals were euthanized with high-energy, head-focused microwave fixation (13.5 kW for 1.6 s; Cober Electronics Inc., Norwalk, CT) to stop brain lipid metabolism and to prevent postmortem-related ischemic increases in unesterified fatty acid concentrations [24]. Heads were placed on dry ice to cool them. Brains were then quickly removed from the skull and stored at −80 °C.

2.4.1. Folch extraction

Total lipids were extracted from serum and brain by Folch extraction [25]. Total lipids were extracted from 200 μl serum in 2 ml of methanol, 4 ml of chloroform, and 1.6 ml of 0.88% KCl, followed by a second 4 ml chloroform wash. Unesterified (Nu-Chek Prep, Elysian, MN), triacylglyceride (TAG) (Nu-Chek Prep, Elysian, MN), and phosphatidylethanolamine (Avanti Polar Lipids, Inc., Alabaster, AL) heptadecanoic acid were added as an internal standard. Total lipids were then dried down by nitrogen gas and reconstituted in 100 μl chloroform.

Brain samples were weighed prior to Folch extraction. Total lipids were extracted from brain samples in 30 ml of chloroform and methanol (2:1 v/v) and 8 ml of 0.88% KCl using a glass homogenizer. A second 20 ml chloroform wash was performed. Heptadecanoic acid standard (10 nmol) was added to the total lipid extract. Total lipids were reconstituted into 1 ml chloroform.