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Acute administration of docosahexaenoic acid increases resistance to pentylenetetrazol-induced seizures in rats

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ABSTRACT

Objective: Docosahexaenoic acid (DHA), an omega-3 fatty acid, has been reported to raise seizure thresholds. The purpose of the present study was to test the acute anticonvulsant effects of unesterified DHA in rats, using the maximal pentylenetetrazol (PTZ) seizure model, and also to examine DHA incorporation and distribution into blood serum total lipids and brain phospholipids and unesterified fatty acids. Sedation was measured to monitor for the potential toxicity of DHA.

Methods: Male Wistar rats received subcutaneous injections of saline, oleic acid (OA), or DHA. An initial pilot study (Experiment 1) established 400 mg/kg as an effective dose of DHA in the maximal PTZ seizure test. A subsequent time–response study, using 400 mg/kg (Experiment 2), established 1 hour as an effective postinjection interval for administering DHA subcutaneously. A final study (Experiment 3) comprised two different groups. The first group ("seizure-tested rats") received saline, OA, or DHA (400 mg/kg) subcutaneously, and were seizure tested in the maximal PTZ test 1 hour later to confirm the seizure latency measurements at that time. The second group ("assay rats") received identical subcutaneous injections of saline, OA, or DHA (400 mg/kg). One hour postinjection, however, they were sacrificed for assay rather than being seizure tested. Assays involved the analysis of serum and brain DHA. Sedation was measured in both Experiment 3 groups during the 1-hour period prior to seizure testing or sacrifice.

Results: As noted above, 400 mg/kg proved to be an effective subcutaneous dose of DHA (Experiment 1), and 1 hour proved to be the most effective injection–test interval (Experiment 2). In Experiment 3, in the seizure-tested animals, subcutaneous administration of 400 mg/kg of DHA significantly increased latency to PTZ seizure onset 1 hour postinjection relative to the saline- and OA-injected controls, which did not differ significantly from each other ($P > 0.05$). In the assay animals, no significant effects of treatment on blood serum total lipids or on brain phospholipid or unesterified fatty acid profiles ($P > 0.05$) were observed. There were also no differences in sedation among the three groups ($P > 0.05$).

Conclusion: DHA increases resistance to PTZ-induced seizures without altering measures of sedation and, apparently, without changing DHA concentrations in serum or brain.

1. Introduction

Seizures are self-sustained, usually time-limited, episodes of neuronal hyperexcitability, involving synchronous discharges in large neuronal populations [1]. In people with epilepsy, spontaneous seizures occur because of a chronically low seizure threshold in some part of the brain [1–3]. Epilepsy is usually treated with anticonvulsant medications, which control seizures in approximately 60–70% of patients [4]. About 30–40% of patients, however, have seizures that are medication resistant or "intractable." Better therapies are needed for these patients.

It has been known for many years that the high-fat ketogenic diet can control seizures, including seizures that resist control by anticonvulsant drugs (for reviews, see Prasad et al. [5] and Swink et al. [6]). The ketogenic diet is usually used only in children, however, and is so nutritionally unbalanced that patients are seldom kept on it longer than 3 years [7]. Thus, the ketogenic diet offers no long-term solution for intractable seizures, although it does indicate that the dietary control of seizures is possible.

Omega-3 polyunsaturated fatty acids (*n*-3 PUFA) are dietary lipids that have been reported to raise seizure thresholds in rodents and that might provide the basis for a nutritionally sound, long-term anticonvulsant diet. Yehuda and colleagues, for instance, have reported that chronic administration of the *n*-3 PUFA α-linolenic

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acid (ALA) with linoleic acid (LA) in a 1:4 ratio (i.e., the "SR-3 mixture") increases resistance to pentylenetetrazol (PTZ)-induced seizures in rats [8], possibly by raising brain levels of the *n*-3 PUFA docosahexaenoic acid (DHA) [9]. LA was provided in order to potentiate the synthesis of DHA from ALA [9]. DHA is the final compound in the *n*-3 PUFA synthetic pathway and the most abundant *n*-3 PUFA found in the brain [10].

A subsequent study that used the same SR-3 dose (40 mg/kg) and seizure model failed to replicate Yehuda and co-workers' findings [11]. Recently, however, two independent studies have shown that chronic administration of *higher* doses of the SR-3 PUFA mixture (200 mg/kg) or of a PUFA mixture consisting of 30% ALA and 70% LA (6000 mg/kg), do raise seizure threshold in the PTZ seizure model [12,13]. These findings suggest that co-administration of ALA with LA can raise seizure thresholds in rats when higher doses are given.

Although the effects of ALA, when administered with LA, are thought to be mediated by DHA [9], animal studies that have used diets containing DHA have yielded conflicting results [14,15]. Confusion about the anticonvulsant effects of DHA might be resolved by studies of injected DHA. To date, however, only one study has examined the acute effects of DHA *injection* on seizure occurrence. Voskuyl and colleagues [16] reported that the acute intravenous infusion of DHA increases seizure threshold in a cortical stimulation model. Although suggestive, this study did not use a widely used pharmacological seizure model.

Clinical studies in humans have also yielded conflicting results. In an open-labeled trial, Schlanger et al. reported a significant reduction in seizure frequency in patients with drug-resistant epilepsy following daily dietary supplementation with an *n*-3 PUFA spread containing 3.3 g of eicosapenaenoic acid (EPA, 20:5*n*-3) and DHA for 6 months [17]. These findings, however, were not confirmed in subsequent double-blinded, randomized control trials, which used lower doses of the *n*-3 PUFA for shorter periods (3 months only) [18–20]. The discrepancies in both the clinical and animal literature may relate to differences in study design, dose, and duration of treatment [21].

The goal of the present study was to examine the direct anticonvulsant effects of unesterified, albumin-bound DHA, injected subcutaneously, in the maximal PTZ seizure model and also to investigate levels of DHA in serum and brain following acute injection. Unesterified DHA is the form of DHA available to the brain. We therefore hypothesized that direct administration of a bolus dose of unesterified DHA would raise seizure threshold after subcutaneous injection and would increase DHA levels in serum and brain.

The maximal PTZ test was chosen because it has been used in past studies to model generalized tonic–clonic attacks in humans, and as a screening tool for new anticonvulsant drugs [22,23]. DHA levels in serum and brain were measured by gas chromatography after acute subcutaneous administration. Possible sedation was measured using a noninvasive rating scale.

Three different experiments were conducted to determine the anticonvulsant effects of DHA in rats. Experiment 1 was a pilot dose–response study designed to determine the dose of DHA that would most increase seizure latency in the maximal PTZ test. Experiment 2 was a time–response study that measured the effects of that DHA dose on seizure latency at postinjection intervals from 15 to 480 minutes. Experiment 3 used two different groups of rats. The first group was used to confirm the effects of DHA on seizure latency at the dose and time indicated by Experiments 1 and 2 ("seizure-tested rats"). The second group was sacrificed at the same time and dose to measure the distribution of DHA in serum and brain lipids following acute subcutaneous injection ("assay rats"). Sedation was measured in both Experiment 3 groups during the 1-hour period prior to seizure testing or sacrifice.

2. Materials and methods

2.1. Drug preparation

Saline-containing albumin, oleic acid (OA), and DHA stock solutions were prepared on the day of the experiment. Saline–albumin was prepared by dissolving 90 mg of albumin per milliliter of 0.9% saline. Unesterified OA and DHA (Sigma–Aldrich, St. Louis, MO, USA) were each dissolved in 0.9% saline containing 90 mg of albumin per milliliter, at a concentration of 140 μL/mL. All stock solutions were sonicated for 5 minutes and kept on ice throughout the experiment to minimize oxidation of the fatty acids. The final pH of the fatty acid mixtures was approximately 5.65.

PTZ (Sigma–Aldrich) was prepared by dissolving 50 mg of PTZ per milliliter of 0.9% saline. The PTZ solution was also kept on ice throughout the experiment.

2.2. Subjects

All experimental protocols were approved by the Animal Care Committee of the Faculty of Medicine of the University of Toronto, and were conducted in accordance with the guidelines of the Canadian Council on Animal Care.

Male Wistar rats (Charles River, La Prairie, QC, Canada), initially aged 53 days, served as subjects for all experiments. Subjects were housed individually in plastic cages with corn-cob bedding in a vivarium maintained on a 12-hour-light, 12-hour-dark cycle (lights on at 7 AM) and a temperature of 21 °C. Rats were allowed access to water and regular rat chow ad libitum (Teklad Global, 2018 18% Protein Rodent Diet). Before testing, each subject was handled daily for 6 consecutive days, starting on the second day after arrival from the breeding farm and continuing until the day prior to the experiments.

2.3. Experiment 1

Experiment 1 was a pilot study involving a small number of subjects that was designed to determine the best dose of DHA to be used in Experiment 2. After 7 days in the facility, the subjects were weighed to calculate the injection doses of PTZ and DHA. The subjects were then randomly allocated to the following treatment groups: saline ($n = 3$), DHA 200 mg/kg ($n = 3$), DHA 400 mg/kg ($n = 3$), and DHA 800 mg/kg ($n = 2$). At the time of testing, subjects were injected subcutaneously with an appropriate dose of DHA or saline. Ten minutes following the saline or DHA injection, subjects received an intraperitoneal injection of 80 mg/kg PTZ. The rats were then placed in an open field for a 30-minute observation period, and latency to the first myclonic jerk was scored by two independent observers. Following testing, all subjects were euthanized with a lethal intraperitoneal injection of sodium pentobarbital (100 mg/kg).

2.4. Experiment 2

Experiment 1 suggested that a subcutaneous dose of 400 mg/kg DHA was most effective at increasing latency to seizure onset. Experiment 2, therefore, was a time–response study using a subcutaneous dose of 400 mg/kg. It was carried out in a separate group of rats and was designed to establish the best postinjection interval for testing DHA. Subjects were obtained and housed as described above. After 7 days in the vivarium, they received subcutaneous injections of OA (isocaloric control) or DHA at a dose of 400 mg/kg (volume of injection ~1 ml). They were then injected intraperitoneally with 105 mg/kg PTZ at the following post-DHA time points: 15, 30, 60, 120, 240, and 480 minutes ($n = 8$ rats per group