Rapeseed oil and magnesium manipulations affect the seizure threshold to kainate in mice*

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Abstract: *We have previously shown that the drop in N-methyl-D-aspartate (NMDA)-induced seizure threshold caused by nutritional magnesium deprivation responded well to the w-3 polyunsaturated fatty acid (PUFA) alpha-linolenate (ALA) (5% rapeseed oil) diet when compared to w-6 PUFA diet. In the present work, kainate-induced seizures are shown to be also exacerbated by magnesium deprivation. ALA diet better attenuates this seizure exacerbation when compared to the non-ALA diet. The reversion of the drop in kainate seizure threshold induced in these conditions by magnesium administration was, however, better under the non-ALA diet in comparison with the ALA diet. Taken as a whole, present data indicate that kainate like NMDA brain injury is attenuated by ALA diet. On the other hand, the relative failure of ALA diet to potentiate reversion induced by magnesium might suggest that magnesium and ALA protections are not additive.*

Key words: *rapeseed oil, corn: sunflower oil, omega-3, alphalinolenic acid, magnesium deficiency, kainate receptor, magnesium chloride hexahydrate, kainate-induced seizure, seizure threshold*

Magnesium deprivation is long known to cause brain vulnerability to inflammatory, oxidative and convulsant injuries. In mice, this particular nutritional animal model may be exploited for its susceptibility to audiogenic seizures to evaluate *in vivo* brain activity of anticonvulsant, neuroprotective, and anti-inflammatory/antioxidant compounds (Bac *et al.*, 1998; Vamecq *et al.*, 2003; Maurois *et al.*, 2008; Maurois *et al.*, 2009; Pagès *et al.*, 2010; Vamecq *et al.*, 2010; Pagès *et al.*, 2011). Chronic magnesium deprivation based on a vegetable oil diet devoided of ϖ-3 polyunsaturated fatty acids (ϖ3PUFA) (diet containing 5% corn/sunflower oil) in mice was also recently shown to represent an interesting nutritional model for *in vivo* exacerbated NMDA receptor function (reduction of NMDA seizures threshold) which responds remarkably to acute magnesium supply, adding experimental evidence that magnesium administration is a promising approach of glutamate-mediated brain disorders (Maurois *et al.*, 2009).

PUFAs are essential components of the central nervous system (CNS) and are brought exclusively by food (Rapoport *et al.*, 2007). Many recent studies have documented the beneficial effects of ϖ-3 PUFA on cardiovascular diseases (Heurteaux *et al.*, 2006) and neurological disorders (Vreugdenhil *et al.*, 1996; Xiao and Li, 1999; Lauritzen *et al.*, 2000; Kim *et al.*, 2001; Blondeau *et al.*, 2009; Delattre *et al.*, 2010) including epilepsy (Heurteaux *et al.*, 2006; Pagès *et al.*, 2011). These studies mainly focused on beneficial effects of docosahexaenoic acid (DHA) and eicosapentaenoic (EPA) acids, and to a less extent on the effects of alpha-linolenic acid (ALA) (18:3 n-3). ALA is supplied with diet *via* different vegetal origins: it represents 9% of the rapeseed oil composition, which also contains 60% monounsaturated fatty acids (MUFA) (18:1 fatty acid) and 20% ϖ6PUFA. Its ϖ6/ϖ3 ratio is low (close to 3) contrasting with the high ratio (more than 80) characterizing corn:sunflower oils which contain 28% MUFA, 56% ϖ-6 PUFA and 0.6% ϖ-3 PUFA (Pagès *et al.*, 2011). Diet containing 5% rapeseed oil (rich in ϖ-3 alpha-linolenate (ALA) improves protection against experimental seizures including NMDA induced seizures to a higher extent than diet containing 5% corn/sunflower oil devoided of ϖ-3 PUFA (Pagès *et al.*, 2011), supporting modulation of glutamate neurotransmission by ϖ-3 PUFAs. Glutamate-driven excitatory synaptic neurotransmission in the mammalian central nervous system is also mediated in major part by receptors other than the NMDA-type receptor and

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including those coupled to channels highly permeable to Na^+ and K^+ and less permeable to Ca^{2+} ions (Hatt *et al.*, 1988; Colquhoun et al., 1992). When activated, the non-NMDA glutamate receptor-channels are typically associated with inward Na^+ current, these channels being classified into kainate- and α-amino-3-hydroxy-5-methylisoxazole (AMPA)- preferring types (Collingridge and Ras, 1989; Monaghan *et al.,* 1989; Lodge, 1997).

The aim of the present study was to study whether dietary rapeseed oil could also protect mice against kainate-induced seizures in adult mice fed magnesium deficient (35 ppm) or normal magnesium containing (900 ppm) diets containing 5% lipids brought by either corn: sunflower oil or rapeseed oil.

Materials and methods

The investigation was conforming to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institute of Health (NIH, No 85-23, revised 1996). Female Swiss OF1 mice, were purchased from Janvier (Le Genest-St-Isle, France) and divided into 3 groups (n=20).

The control group was fed a diet containing normal magnesium levels (≥900 ±50 ppm) (control Mg+ diets) under the form of industrial pellets containing soya lipids (UAR, France)

The two magnesium-deficient groups received different diets (magnesium-deficient (Mg-) diets). These diets impoverished in magnesium were designed by restricting severely the magnesium content to 35±5 ppm as described previously (Maurois *et al.*, 1989; Maurois *et al.*, 2009) and differed in fat content: ALA-poor (a mix of corn and sunflower oils (3:1)) or ALA-rich (pure rapeseed oil). Mice were placed eight per cage and maintained on a 12:12h light-dark schedule at 21±1 °C. They had free access to food and to distilled water which avoids additional magnesium input. In current practice, in order to prevent food oxidation, fresh diets were lyophilized and frozen at –20 °C. They were given to mice every day in sufficient amount.

At the end of the magnesium deprivation period (30 days), kainate seizure tests were performed by evaluating the capacity of the various diets to provide protection against threshold seizures through determination of the lethal dose 100 (LD_{100}, minimal dose inducing death of 100% tested animals). The reversion of susceptibility to kainate seizures was studied for intraperitoneal administration of magnesium chloride hexahydrate (dissolved in a 0.9% saline water solution) performed 30 min before kainate administration. The daily amount of magnesium delivered to mice by a deficient diet corresponded to *grosso modo* 5.6 mg magnesium/kg body weight. This daily amount, or several-fold this amount, was given intraperitoneally to mice acutely in the form of magnesium chloride hexahydrate, 46.8 mg of which contained 5.6 mg magnesium).

Statistical analysis: Data were expressed as mean ± SEM and analyzed by Student's t-test.

Results

Threshold to kainate-induced seizures in mice fed a normal magnesium diet was found to be 45 mg/kg. In magnesium-deprived mice, kainate seizure threshold was significantly ($p<0.05$) lowered to 32 and 39% of these values in groups fed diets containing corn: sunflower (ALA poor) and rapeseed (ALA rich) oils, respectively. Thresholds in these two respective groups were significantly ($p<0.05$) different 14.5 and 17.5 mg/kg *(table 1)*.

The drop induced by magnesium deficiency in the threshold to kainate-induced seizures was partly reversed by acute intraperitoneal administrations of 28 mg/kg magnesium which increased by 213 and 154% the kainate seizure threshold of mice given a magnesium-deficient diet supplemented with corn: sunflower (ALA poor) and rapeseed (ALA rich) oils, respectively (*table 1*, $p<0.05$). The threshold was re-heightened significantly ($p<0.05$) to 31 and to 27 mg kainate/kg, under corn: sunflower (ALA poor) and rapeseed (ALA rich) oils, respectively. However, the levels did not reach the initial values observed with normal magnesium diet (only 60% of Mg+ diet).

Increasing the doses of acute magnesium administration did not induce substantial gain in further reversing these thresholds, magnesium doses superior to 30 mg/kg body weight (from 30 to 40 mg/kg) in the form of chloride salt becoming progressively toxic and finally lethal for the magnesium-deficient animals (data not shown).

Discussion

In the wake of previous studies, the present work originally highlights a lowering of threshold to kainate seizures in OF1 mice induced by chronic exposition to nutritional deprivation in magnesium. The shift observed in this threshold was operated from 45 mg/kg (normal magnesium fed animals) to 14.5 and to 17.5 mg/kg in mice given a magnesium-deficient diet based on corn: sunflower (ALA-poor diet) or rapeseed oils (ALA-rich

Table 1. *Effects magnesium-deficient diets on threshold to kainate-induced seizures*

Diets		**Threshold to kainate-induced seizures (mg/kg)**	**Threshold to kainate-induced seizure after acute $MgCl_2$ injection (mg/kg)**
Mg+	Commercial diet	45.0±2.2*[a] (reference threshold)	
Mg-	ALA poor diet (corn/sunflower)	14.5±3.1*[b]	31.0±2.0*[d]
	ALA rich diet (rapeseed)	17.5±1.6*[c]	27.2±1.5*[d]

Evaluations were performed on 10 mice in each experimental group and condition. Mg+, normal magnesium-containing diet; Mg-, magnesium-deficient diets; ALA, alpha-linolenic acid.*, $p<0.05$;
*[a], *[b],*[c], groups significantly different $p<0.05$; *[d] groups acutely injected versus corresponding non injected groups significantly different $p<0.05$

diet), respectively. Partial reversions (213 and 154% under corn: sunflower and rapeseed oils, respectively) in magnesium deficiency-driven drop of kainate seizure threshold were provided by acute intraperitoneal administration of magnesium chloride hexahydrate. In the present series of experiments, previously reported abilities of acute magnesium administration and of rapeseed oil-based magnesium-deficient diet to protect fully and partly, respectively, mice against audiogenic seizures were again observed. The main contribution of this study is the evidence of a better ability of ϖ3PUFA-rich oil (*vs* ϖ3PUFA-poor oil) to protect brain against the drop induced by magnesium deficiency in kainate seizure threshold. The fact that, paradoxically, rapeseed oils (ALA-rich diet) *vs* corn: sunflower (ALA-poor diet) offer to magnesium administration a lower capacity to reverse this drop might further suggest that magnesium and ϖ3PUFA-mediated brain protective mechanisms are not additive. Elucidation of these emerging and intriguing issues is in progress.

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