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

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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 anti-convulsant, 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., 2011). Chronic magnesium deprivation based on a vegetable oil diet devoided of ω-3 polyunsaturated fatty acids (ω3PUFA) (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 ω3 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% ω3PUFA. Its ω6/ω3 ratio is low (close to 3) contrasting with the high ratio (more than 80) characterizing corn:sunflower oils which contain 28% MUFA, 56% ω-6 PUFA and 0.6% ω3 PUFA (Pagès et al., 2011). Diet containing 5% rapeseed oil (rich in ω-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 ω-3 PUFA (Pagès et al., 2011), supporting modulation of glutamate neurotransmission by ω-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

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

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including those coupled to channels
drastically permeable to Na⁺ and K⁺ and less
permeable to Ca²⁺ 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 magne-
sium containing (900 ppm) diets con-
taining 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 con-
taining 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 impov-
ished in magnesium were designed by
restricting severely the magnesium con-
tent to 35:±5 ppm as described pre-
viously (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 sched-
ule 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 oxida-
tion, 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
during determination of the lethal dose
100 (LD100, 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 admin-
istration. The daily amount of magne-
sium delivered to mice by a deficient diet
corresponded to gesso 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 hex-
hydroxylate, 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 defi-
cency 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 signifi-
cantly (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 thresh-
holds, 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 ani-
mals (data not shown).

**Discussion**

In the wake of previous studies, the
present work originally highlights a low-
ering 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 mag-
nesium 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

| Diets            | Threshold to kainate-induced seizures (mg/kg) | Threshold to kainate-induced seizure after acute MgCl₂ injection (mg/kg) |
|------------------|-----------------------------------------------|-----------------------------------------------------------------------|
| Mg+              | Commercial diet                                | 45.0±2.2**a (reference threshold)                                       |
|                  | ALA poor diet (corn/sunflower)                 | 14.5±3.1b                                                            |
|                  | ALA rich diet (rapeseed)                       | 17.5±1.6c                                                            |
| Mg-              | ALA poor diet (corn/sunflower)                 | 31.0±2.0*d                                                           |
|                  | ALA rich diet (rapeseed)                       | 27.2±1.5*e                                                           |

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; ** groups significantly different p<0.05; * 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 ∏3PUFA-rich oil (vs ∏3PUFA-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 ∏3PUFA-mediated brain protective mechanisms are not additive. Elucidation of these emerging and intriguing issues is in progress.

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