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Brain protection by rapeseed oil in magnesium-deficient mice

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ABSTRACT

Diets given for 30 days with various mono-(MUFA) and poly-(PUFA) unsaturated fatty acid contents were evaluated for brain protection in magnesium-deficient mice: a commercial and three synthetic diets (n-6PUFA, n-3PUFA and MUFA-based chows enriched with 5% corn/sunflower oils 1:3, with 5% rapeseed oil and with 5% high oleic acid sunflower oil/sunflower oil 7:3, respectively). Unlike magnesium deprivation, they induced significant differences in brain and erythrocyte membrane phospholipid fatty acid compositions. n-3PUFA but not other diets protected magnesium-deficient mice against hyperactivity and moderately towards maximal electroshock- and NMDA-induced seizures. This diet also inhibited audiogenic seizures by 50%, preventing animal deaths. Because, like n-6PUFA diet, matched control MUFA diet failed to induce brain protections, alpha-linolenate (ALA) rather than reduced n-6 PUFA diet content is concluded to cause n-3PUFA neuroprotection. Present *in vivo* data also corroborate literature *in vitro* inhibition of T type calcium channels by n-3 PUFA, adding basis to ALA supplementation in human anti-epileptic/neuroprotective strategies.

1. Introduction

Long-chain polyunsaturated fatty acids (PUFA) are essential lipid components of the central nervous system and are supplied exclusively by the diet [1]. In the last decade, *in vivo* and *in vitro* studies have documented beneficial effect of PUFAs of the n-3 family towards cardio-vascular [2] and neurological [3–8] disorders. Supplementations with n-3 PUFAs and notably with alpha-linolenic acid (ALA (18:3 n-3)) have been proposed in cerebral protection after focal brain ischemia, and against epileptic seizures [2,9]. Inversely, chronic low intake of n-3 PUFA predisposes to brain toxicity through weak anti-inflammatory on strong pro-inflammatory signal imbalance [10]. The abundance of ALA is by far higher (10-fold and more) in vegetable, including rapeseed, soya and walnut oils, than in some other oils such as corn and sunflower oils [11]. ALA represents 9% of rapeseed oil which contains also 60% of monounsaturated fatty acids (18:1 fatty acid) and 20% linoleic acid [LA] (α n-6 PUFA). Its n-6/n-3 ratio is low (close to 3) whereas that of corn:sunflower oils is by far higher (more than 80).

PUFAs generate oxygenated lipid-derived eicosanoids which modulate the inflammatory response [12,13]. n-6 PUFAs and notably arachidonate (ARA; 20:4n-6) are the precursors of various pro-inflammatory eicosanoids (prostaglandins of the 2-series and leukotrienes of the 4-series) which are incriminated in adverse inflammatory processes. However, one of these eicosanoids, PGE2, may restrict the inflammatory response by inhibiting the production of inflammatory leukotrienes and by inducing the inflammation resolving lipoxin A4 [12]. On the other hand, production of ARA-derived-eicosanoids is lowered by n-3 fatty acids, eicopentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3), which are incorporated partly at the expense of ARA and other n-6 PUFAs into cell membrane phospholipids [1,12]. This reduced incorporation of ARA into cell membrane phospholipids lowers the amount of substrate available for release of ARA and hence for synthesis of ARA-derived-eicosanoids.

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In addition, EPA is a substrate for both cyclooxygenase and 5-lipooxygenase, giving rise to an alternative family of eicosanoids with anti-inflammatory properties (prostaglandins of the 3-series and leukotrienes of the 5-series) [12,14]. Finally, a class of EPA and DHA-derived lipid mediators with potent anti-inflammatory properties called resolvins E and D, respectively, has been recently described [12,14]. DHA can generate also a dihydroxy derivative, neuroprotectin D1, a docosanoid with also potent anti-inflammatory properties [12,15,16]. As a whole, derivatives of the n-3 series essentially mediate major anti-inflammatory action, justifying their dietary supplementation to control disorders in which inflammation contributes to the pathogenesis. Fig. 1 illustrates the metabolic pathway (elongation, desaturase) involved in the synthesis of PUFAs from the n-6 and n-3 series.

A particular condition associated with a general inflammatory and/or oxidant state is severe magnesium deficiency [17–19]. It can alter brain function, leading to central nervous hyperexcitability (CNHE) and to decreased protection against experimental seizures [20,21]. In this respect, experimental magnesium deficiency in mice has been shown to lower seizure threshold to N-methyl-D-aspartate (NMDA) [22] and to induce susceptibility to audiogenic seizures [23]. Magnesium deficiency-dependent audiogenic seizures (MDDAS) represent a useful murine experimental *in vivo* tool to identify a large spectrum of brain protective drugs and mechanisms including anti-inflammatory, anti-oxidant, neuroprotective and/or anti-epileptic compound properties [22–28]. The pro-convulsant status induced by magnesium deficiency may be the result of a loss of protective effects normally provided by magnesium. Regarding these protective effects, magnesium is endowed with anti-NMDA, anti-inflammatory and anti-oxidant properties. In a previous work, anti-NMDA properties of magnesium were shown to take place *in vivo* [22]. On the other hand, magnesium is a cofactor for the enzymes in glutathione biosynthesis (γ-glutamylcysteine synthetase and glutathione synthetase) [29,30] and NADPH-producing pentose phosphate pathway (6-phosphogluconate dehydrogenase and transketolase) [31,32], explaining why magnesium deficiency may affect reduced glutathione biosynthesis and recycling. In normal diet conditions, the physiological cellular loss of reduced glutathione is counterbalanced by intracellular glutathione biosynthesis. In magnesium deficiency, cellular loss is maintained, whereas biosynthesis decreases, resulting in a drop of cell reduced glutathione content and anti-oxidant potential [33]. Anti-inflammatory properties of magnesium are not fully elucidated and have been proposed to result from different mechanisms including inhibition of calcium-based processes (priming of phagocytic cells, NMDA receptor activation), prevention of the release of neuropeptides (e.g. substance P) and of the activation of NF-κB signaling [17]. Magnesium deficiency-driven pro-convulsant susceptibilities in mice including MDDAS were here challenged by chronic diets with distinct n-6/n-3 PUFA ratio contents of magnesium-deficient chows. Erythrocyte and brain phospholipid fatty acid compositions under the various diet conditions were also studied. Part of data appearing in this manuscript was the topic of an invited lecture with a subsequent written account [34] at the Journées Chevreul 2007 symposium (AFECG and DGF Joint Meeting, Lipids and Brain: PUFA Metabolism, Function and Protection Against Diseases, Paris (France), 13 and 14th March 2007).

2. Materials and methods

2.1. Chemicals

Pentylenetetrazol (Ptz) and N-methyl-D-aspartate (NMDA) were purchased from Sigma-Aldrich Fine Chemicals (Saint Quentin Fallavier, France). Fresh commercial oils were from Lesieur (Asnières-sur Seine, France).

2.2. Animals

Female Swiss OF1 mice, 3-weeks old, weighing 20–22 g, were purchased from Janvier (Le Genest-St-Isle, France) and were

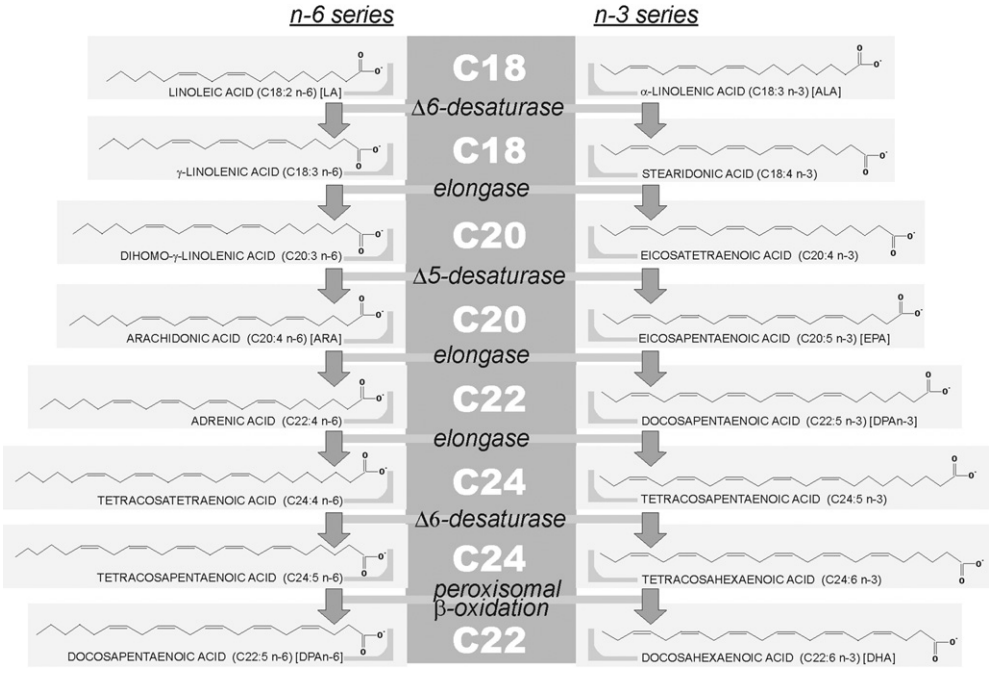

Fig. 1. PUFAs of the n-6 and n-3 series. Linear structure representation of PUFAs is illustrated and their names are given between parentheses. Abbreviations used throughout the text are further given between brackets. C20 PUFAs are the reservoir for synthesis of eicosanoids and C22 PUFAs obtained by peroxisomal β-oxidation-driven retroconversion of C24 precursors are the source for docosanoids. Other comments are the text.