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Short Communication

Threshold to *N*-methyl-D-aspartate-induced seizures in mice undergoing chronic nutritional magnesium deprivation is lowered in a way partly responsive to acute magnesium and antioxidant administrations

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Magnesium deficiency may be induced by a diet impoverished in magnesium. This nutritional deficit promotes chronic inflammatory and oxidative stresses, hyperexcitability and, in mice, susceptibility to audiogenic seizures. Potentiation by low-magnesium concentrations of the opening of *N*-methyl-D-aspartate (NMDA) receptor/calcium channel in *in vitro* and *ex vivo* studies, and responsiveness to magnesium of *in vivo* brain injury states are now well established. By contrast, little or no specific attention has been, however, paid to the *in vivo* NMDA receptor function/excitability in magnesium deficiency. The present work reports for the first time that, in mice undergoing chronic nutritional deprivation in magnesium (35 *v.* 930 parts per million for 27 d in OF1 mice), NMDA-induced seizure threshold is significantly decreased (38 % of normal values). The attenuation in the drop of NMDA seizure threshold (percentage of reversal) was 58 and 20 % upon acute intraperitoneal administrations of magnesium chloride hexahydrate (28 mg magnesium/kg) and the antioxidant ebselen (20 mg/kg), respectively. In nutritionally magnesium-deprived animals, audiogenic seizures are completely prevented by these compound doses. Taken as a whole, our data emphasise that chronic magnesium deprivation in mice is a nutritional *in vivo* model for a lowered NMDA receptor activation threshold. This nutritional model responds remarkably to acute magnesium supply and moderately to acute antioxidant administration.

Nutritional magnesium deprivation: *N*-methyl-D-aspartate receptor: Ebselen: Magnesium chloride hexahydrate: *N*-methyl-D-aspartate-induced seizure: Seizure threshold: Audiogenic seizures

Magnesium and its deficiency present with many facets. The magnesium blockade of the *N*-methyl-D-aspartate (NMDA) receptor–calcium channel was reported more than 20 years ago[1]. Magnesium deficiency, which may be induced by chronic nutritional deprivation, has been shown to be a particular state affecting a great many tissue and cell physiological events. Among others, magnesium deficiency induces inflammatory and oxidative stresses, being associated with central hyperexcitability[2] and susceptibility towards audiogenic seizures. These seizures, which develop into four successive phases (latency, wild running, seizure and recovery), have been shown to respond to both anticonvulsant and neuroprotective compounds in a relatively discriminative way[3–5]. Protection given by compounds in this peculiar nutritional model may result from either intrinsic anti-seizure or intrinsic antioxidant/anti-inflammatory neuroprotective properties, or both.

Magnesium occupancy of the calcium channel lumen of the NMDA receptor results in a physiological block, which needs to be removed to allow the entry of calcium into the cell via this channel. In this respect, Nowak *et al.* [1] in their initial work demonstrated that NMDA receptor–calcium channel opening was greatly potentiated by exposure to low-magnesium concentrations. Despite this finding being corroborated by many *in vitro* and *ex vivo* (tissue slices) experiments, little or no direct information is currently available about this relationship in the

Abbreviation: NMDA, *N*-methyl-D-aspartate.

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whole animal; notably, the susceptibility of magnesium-deprived mice to seizures induced by a compound selectively targeting the NMDA receptor function, for instance NMDA, remains to be explored. So, this work was aimed at determining in chronic nutritional magnesium deprivation (*v.* normal nutritional magnesium intake conditions) the status of the threshold for NMDA-induced seizures. Responses to magnesium chloride hexahydrate and ebselen (a glutathione peroxidase mimic antioxidant chosen here to depress oxidative stress taking place in magnesium deficiency) were determined. Susceptibility to audiogenic seizures was also studied.

Materials and methods

Compounds

Magnesium chloride hexahydrate, magnesium sulphate heptahydrate, ebselen, NMDA, dimethylsulfoxide (DMSO) and polyethylene glycol 300 (PEG 300) were from Sigma-Aldrich Fine Chemicals (St Quentin Fallavier, France).

Experimental protocols

The present experimental protocols and procedures complied with the European Communities Council Directives of 24 November 1996 (86/609/EEC).

Chronic nutritional magnesium deprivation protocol

Nutritional magnesium deprivation was performed as described in the work of Bac *et al.* [3], except that a 35 ppm instead of a 50 parts per million (ppm) magnesium content of the diet was used here in order to induce in adult OF1 mice (Janvier, Le Genest St Isle, France) susceptibility to audiogenic seizures in 100 % animals over a period of 27 d instead of 42 d. The control and magnesium-deficient semi-synthetic diets were essentially prepared as described by Rayssiguier's group[6,7], and in this study they contained (g/kg): 200 casein; 700 sucrose; 10 sunflower oil; 40 corn oil; 3 D,L-methionine; 2 phosphorylcholine; 35 modified AIN-76 mineral mix; 10 AIN-76A vitamin mix (ICN Biomedicals, Orsay, France). MgO was omitted from the mineral mix in the magnesium-deficient diet. The diets were stored at −20°C before being lyophilised and compacted until consumption by the mice as described elsewhere[7]. Magnesium contents of the diets were determined as described in a previous work[3]. The diets were high in the oxidant stressor fructose[8] and low in anti-inflammatory *n*-3 fatty acids. OF1 mice were chosen because their use as animals undergoing magnesium deficiency has been largely described in the literature. A major reason could be that, in contrast to some other mouse strains, deaths in OF1 mice submitted to severe nutritional magnesium deprivation remain occasional only. For instance, this strain may survive successfully to long periods (6 months) of severe nutritional magnesium deprivation[9].

Susceptibility to audiogenic seizures

Essentially, the audiogenic seizure test was started by exposing tested animals to a 15 s auditory signal of 10 ± 0·1 kHz frequency and 100 ± 1 db intensity. The intensity of 100 db characterising the 10 kHz acoustic stimulus is the intensity previously used to validate and standardise the audiogenic test; it usually induces audiogenic seizures in 90–100 % of OF1 mice fed on the present magnesium-deprived diet. The 10 kHz frequency is the frequency at which magnesium-deficient, and not control, OF1 mice develop audiosensitive seizures.

Development of these seizures was characterised by the succession of four phases (latency, wild running, seizure and recovery) as previously described[3–5].

Susceptibility to N-*methyl-*D*-aspartate-induced seizures*

NMDA was dissolved in a 0·9 % saline solution and was administered by the intraperitoneal route, and the lowest dose of NMDA inducing lethal seizures in 100 % animals was determined. This dosage was referred to as the threshold for NMDA-induced seizures.

Reversion of susceptibility to seizures by acute compound administration

Acute administration of compounds (magnesium chloride hexahydrate (see the range of given doses in the next paragraph) and 20 mg/kg ebselen) was performed intraperitoneally 30 min before the administration of NMDA or exposure to the acoustic stimulus. Solubilisation of compounds prior to the administration was performed as follows: magnesium chloride hexahydrate was dissolved in a 0·9 % saline water solution, and ebselen in a 10 µl DMSO/10 µl PEG 300 mixture solution. At these dosages, the vehicles (saline, DMSO/PEG 300) were without significant effects on the parameters investigated throughout this study.

Acute administration of magnesium

The amount of magnesium ingested by mice given the deficient diet corresponded to *grosso modo* a daily supply of 5·6 mg magnesium/kg body weight. A half to several folds of this daily dose was given to mice acutely by the intraperitoneal route in the form of magnesium chloride hexahydrate, 46·8 mg of which contained 5·6 mg magnesium.

Statistical analysis

Quantitative data were expressed as the means with their standard errors for each treatment group. Means were compared using ANOVA with Fischer's, Scheffe's or Dunnett's multiple comparison of means test (StatView™ 512+, Brain Power, Inc., Calabasas, CA).

Results

Susceptibility of magnesium-deprived animals to N-*methyl-*D*-aspartate-induced seizures*

In magnesium-deprived mice, threshold to NMDA-induced seizures was lowered to 38 % of normal values, normal and deficient animals having thresholds equal to 137 and 52 mg NMDA/kg, respectively (Table 1).

British Journal of Nutrition

Table 1. Comparative seizure susceptibility of mice given a standard animal chow and a magnesium-deprived diet, and ability of compounds to reverse changes related to magnesium deprivation

	Control mice	Magnesium-deprived mice		
	No compounds	No compounds	Magnesium (28 mg/kg)	Ebselen (20 mg/kg)
Sensitivity to audiogenic seizures (%)	0	100	0	0
Sensitivity to NMDA-induced seizures (threshold expressed as mg NMDA/kg)	137 (SEM 1)	52 (SEM 1)[a]	101 (SEM 4)[b,c]	69 (SEM 2)[a,d]

The results are mean values expressed as percentages of unprotected animals for mice submitted to the audiogenic seizure test, and are means with their standard errors for mice given *N*-methyl-D-aspartate (NMDA) (*n* 6).
[a] $P<0{\cdot}001$ (comparison of magnesium-deprived mice with controls).
[b] $P<0{\cdot}01$ (comparison of magnesium-deprived mice with controls).
[c] $P<0{\cdot}001$ (comparison of magnesium-deprived mice given a compound with deficient mice that received no compounds).
[d] $P<0{\cdot}01$ (comparison of magnesium-deprived mice given a compound with deficient mice that received no compounds).

Susceptibility of magnesium-deficient mice to audiogenic seizures

The nutritional protocol was characterised by the magnesium content of the diet and duration of diet administration such that the resulting magnesium deprivation caused a susceptibility to audiogenic seizures in 100 % of tested magnesium-deprived animals (Table 1). By contrast, mice given the control diet and exposed to the acoustic stimulus were all refractory to audiogenic seizure development (Table 1).

Reversion of susceptibilities to seizures

The drop induced by magnesium deficiency in the threshold to NMDA-induced seizures was partly reversed by acute administrations of 28 mg/kg magnesium (58 % of reversal) and 20 mg/kg antioxidant ebselen (20 % of reversal; Table 1). Increasing the doses of each element or compound did not lead to substantial gain in reversing the threshold shift, magnesium doses superior to 30 mg/kg body weight (from 30 to 40 mg/kg) became progressively toxic and finally lethal for the magnesium-deficient animals (data not shown).

Susceptibility of magnesium-deprived mice to audiogenic seizures was fully reversed by either 28 mg/kg magnesium or 20 mg/kg ebselen (Table 1).

A similar dose of magnesium given in the form of magnesium sulphate heptahydrate instead of magnesium chloride hexahydrate was also successful in enhancing by a comparable percentage the threshold to NMDA-induced seizures and to protect completely magnesium-deficient animals against audiogenic seizures (data not shown).

Other aspects of susceptibilities and reversals

The extent of magnesium to restore, in magnesium-deprived mice, threshold to NMDA-induced seizures and protection against audiogenic seizure was found to be dose dependent (Fig. 1). When 2·8, 5·6 or 11·2 mg/kg magnesium was given in a single dose, no substantial increase of threshold to NMDA-induced seizures was observed in magnesium-deficient mice. Acute administrations of 16·8 and 22·4 mg/kg magnesium enhanced partially and progressively the threshold to NMDA-induced seizures in deficient animals. The latter two magnesium doses provided mice with full protection against audiogenic seizures. The 11·2 mg/kg magnesium corresponded to a dose protecting 50 % of animals against audiogenic seizures. The acute administration of 5·6 mg/kg magnesium was sufficient to protect a weak percentage of animals against audiogenic seizures, while halving this acute administration of magnesium was devoid of protective effects towards the development of these seizures.

Discussion

This work shows that, in the whole animal, chronic nutritional magnesium deprivation lowers seizure threshold to NMDA. Partial but substantial reversion of this effect by acute magnesium chloride (notably 28 mg magnesium/kg) might result from increasing magnesium availability and hence magnesium block within the calcium channel of NMDA receptor. Reversion by ebselen was lower. It might be linked to redox sensitivity of NMDA receptor, ebselen having been previously

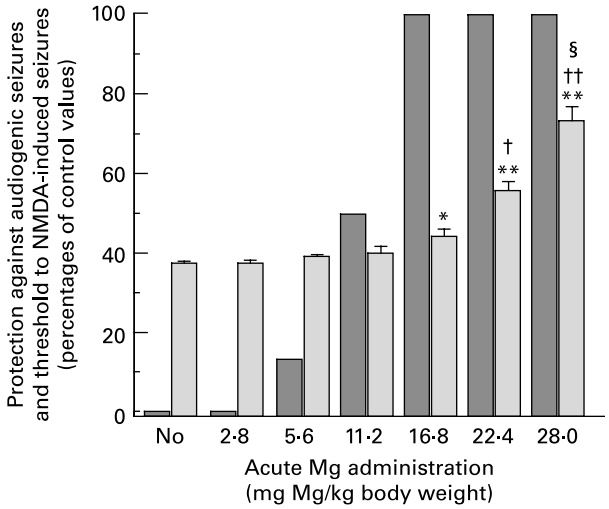

Fig. 1. Differential effect of acute magnesium administrations on audiogenic seizure susceptibility (■) and *N*-methyl-D-aspartate (NMDA) seizure threshold lowering (□) encountered in the mice fed a diet impoverished in magnesium. Each group of mice (*n* 6) was given chronically the magnesium-deficient diet before being (2·8, 5·6, 11·2, 16·8, 22·5 and 28 mg magnesium/kg body weight) or not (No) submitted to acute magnesium administration. Note that the effects illustrated in this figure and those shown in Table 1 are induced by the tested compounds (here, magnesium chloride hexahydrate) 30 min after the intraperitoneal administration. * and **: $P<0{\cdot}005$ and $P<0{\cdot}001$, respectively (comparison of mice given acute magnesium *v.* the 'no' group). † and ‡: $P<0{\cdot}01$ and $P<0{\cdot}001$, respectively (comparison is made between 22·4 and 28 mg/kg groups with the group of mice given 16·8 mg/kg magnesium). §: $P<0{\cdot}01$ when comparing the groups of mice receiving 28 mg/kg with the group given 22·4 mg/kg magnesium.

British Journal of Nutrition

shown to interact with the redox modulatory site of NMDA receptor[10]. The weak impact of ebselen on NMDA receptor excitability shown here in magnesium-deprived animals might be in agreement with its limited neuroprotective activity in preclinical and clinical studies[11]. By contrast, the rapidity and extent to which acute administration of magnesium chloride hexahydrate restored NMDA-induced seizure threshold suggest major, though not strict, magnesium dependency of threshold reduction occurring during chronic nutritional magnesium deprivation. Magnesium chloride effects reported here were on the other hand mimicked by magnesium sulphate. This potent action of magnesium salts on the NMDA receptor might explain the success of magnesium-based therapies in neurological stress or insult[12–14]. In contrast to NMDA-induced seizures, audiogenic seizures were fully responsive to acute administrations of magnesium chloride hexahydrate and ebselen. In understanding the mechanisms by which an antioxidant compound such as ebselen (this work) or a synthetic ovothiol analogue[5] prevents audiogenic seizures, we should take into account that, importantly, these and other antioxidants/anti-inflammatory compounds do not control acutely the seizures in classic animal models and hence in human epileptic patients. Their activity in audiogenic seizures might hold in the audiogenic nature of seizures, which develops in conditions, for instance exposure to kanamycin[15], cerebral ischaemia[16,17] or magnesium deprivation[3], associated with free radical generation[18–23], ebselen being capable of preventing noise-induced toxicity[24].

Enrichment of the magnesium-deficient diet with *n*-3 fatty acids (i.e. full replacement of sunflower and corn oils by rapeseed (colza) oil in the semi-synthetic diet) had little to no effect in the decrease of NMDA-induced seizure threshold (the authors, unpublished results), while it reduced the number of mice becoming sensitive to audiogenic seizures[25], further validating the view that audiogenic seizures are more sensitive than NMDA-induced seizures to antioxidant manipulations during magnesium deficiency.

In this respect, the better sensitivity to magnesium of audiogenic seizures (*v.* NMDA-induced seizures) might be accounted for by anti-inflammatory[26] and/or antioxidant properties of magnesium. Magnesium is a cofactor for the enzymes in glutathione biosynthesis (γ-glutamylcysteine synthetase and glutathione synthetase)[27,28] and NADPH-producing pentose phosphate pathway (6-phosphogluconate dehydrogenase and transketolase)[29,30], explaining why magnesium may affect glutathione biosynthesis and recycling. In normal diet conditions, the physiological cellular loss of reduced glutathione is balanced by substantial intracellular biosynthesis rates. In magnesium deficiency, cellular loss is maintained, whereas biosynthesis decreases, resulting in a net depletion in cellular levels in reduced glutathione (and hence in antioxidant defences)[20]. Reversing magnesium levels (as performed here by acute magnesium administrations) can restore biosynthesis enzyme activities and hence cell antioxidant status[20]. The latter antioxidant properties of magnesium are consistent with its protective properties towards audiogenic seizures.

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NS British Journal of Nutrition

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British Journal of Nutrition