Effect of oral administration of Magnesium N-Acetyltaurinate on synaptic plasticity in rodents

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Abstract. While magnesium deficiency is common and its effects well known on the nervous system, very few studies has been dedicated to the efficiency of magnesium replacement treatments on the central nervous system. In this study, the effects of oral administration of magnesium salts of acetyl-taurinate on the central manifestations of magnesium deficiency is described in rats submitted to low-magnesium diet and in a murine model of Alzheimer's disease. We tested the effect of ATA Mg®, a salt combining magnesium and taurine, on the hippocampus, a critical component of cognition.

7-10-month-old rats were submitted to dietary magnesium deprivation for 64 days. The effect of magnesium deficiency was studied in *ex vivo* hippocampal slices. We showed that long-term potentiation of synaptic transmission in the hippocampus was significantly improved by the oral administration of ATA Mg® at a dose of 50 mg/kg bw/day, which is comparable to the recommended dose in humans.

7-10-months-old transgenic APP/PS1 mice, a model of Alzheimer's disease, received ATA Mg® during 24 days at a dose of 700 mg/kg bw/day which is the dose used in previous studies demonstrating the positive effect of magnesium supplementation. We showed that long-term potentiation was significantly improved in the treated mice. Moreover, the expression of NR2B subunit of NMDA receptors, known to be involved in synaptic plasticity, was significantly increased in the hippocampus.

These results demonstrate the ability of ATA Mg® to improve the symptoms related to chronic magnesium deficiency at the level of the hippocampus suggesting its bioavailability and effectiveness in reaching the central nervous system.

Key words: electrophysiology, hippocampus, glutamate, Alzheimer's disease, magnesium

Introduction

Magnesium is an essential trace element, cofactor of more than 300 metabolic reactions involved in glucose metabolism, ATP production, protein synthesis, etc. It is also essential for the stability of neuromuscular and cardiovascular membranes and the regulation of hormonal and immunological function. The normal magnesium extracellular concentration is 2.4 mg/dL, 60% being free and biologically active and the recommended daily intake is of 300 to 400 mg/day for an adult [1]. Magnesium deficiency can occur due to the reduction of Mg content in processed and industrial food. This deficiency increases the risk of type II diabetes, neuromuscular disorder, cardiac failure and neurological disorders [2, 3].

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Brain magnesium content is relatively stable in case of hypomagnesemia [4]. Magnesium concentration is higher in cerebrospinal fluid (CSF) than in plasma and is tightly regulated. Nevertheless, decrease in magnesium concentration can be observed in the brain during ageing leading to alteration of neuronal excitability, decrease in synaptic transmission and impairment in cognition [5]. Magnesium is essential for presynaptic transmitter release [6] and postsynaptic activity of glutamatergic NMDA receptors [7]. Magnesium regulates the activity of racemase and D-serine availability and long-term reduction of magnesium concentration decreases synaptic NMDA receptors inducing synaptic plasticity impairments [8, 9]. In rodents, hippocampus-dependent learning impairments have been demonstrated in mice and aged rats [10, 11] submitted to magnesium depletion.

On the contrary, an increase in magnesium concentration improves memory in young rats, reverse memory deficit in aged rats and prevent memory impairment in Alzheimer's disease (AD) mice [12] by increasing NR2B-containing NMDA receptors [13].

Brain magnesium is decreased in the brain of Alzheimer's patients and the hippocampus is one of the first brain region affected in AD [14]. A lot of different rodent models of AD have been developed to study the mechanism of AD and potential therapeutic strategies. APP/PS1 transgenic mice, presenting a double mutation on APP and PS1 genes, overproduce amyloid peptides (Aβ42) which have a direct impact on synaptic NMDA receptors and synaptic plasticity. In these mice, Li et al. (2014) showed that magnesium supplementation increased NR2B subunit expression and NMDA receptor activity through inhibition of calcineurin [12]. This protection of NMDA receptors was sufficient to ameliorate AD-related memory deficits.

In animals, magnesium deficiency can induce audiogenic seizures that can be reversed by oral administration of magnesium. In this model, compared to $MgCl_2$ and PCMH (magnesium pyrrolidone 2 carboxylate), ATA Mg was showed to offer a long-term protection (72 hours) against audiogenic seizure [15]. The positive effect of ATA Mg was also demonstrated in a rat model combining Mg-deficiency and Kainic acid (KA) toxicity [16]. In this model, the behavioral deficits induced by KA were prevented by ATA Mg administration either in a chronic (10 days) or acute (single dose) preventive way or in curative treatment while other magnesium salts (pidolate, aspartate, lactate, gluconate) were showed to be ineffective. In the same way, the neuroprotective effect of magnesium acetyltaurate was demonstrated in a model of NMDA-induced retinal ganglion cell (RGC) loss [17, 18]. In this model, NMDA was injected in eyes inducing RGC apoptosis and poor visual function. Intravitreal injection of magnesium acetyltaurate prevented cell death and preserved color recognition.

In this study, we focused our attention on the potential beneficial effect of ATA Mg® on cognition in two different neuropathological models, rats submitted to low-magnesium diet and a murine model of Alzheimer's disease. The hippocampal formation, embedded in the temporal lobe, is a crucial element of the neuronal networks involved in higher cognitive functions, such as learning and memory. It is generally accepted that long-lasting modification of synaptic transmission in the hippocampus is correlated to information storage and that NMDA-dependent long-term potentiation (LTP) is the more reliable cellular model of this process [19, 20]. NR2B subunit of NMDA receptors are essential for synaptic plasticity [21] and learning and memory [22]. They composed NMDA receptors both at the synapse and in extrasynaptic sites and are abundant in the hippocampus where they have been showed to be involved in long-term potentiation [23].

By using these models, we showed that oral administration of ATA Mg® improved synaptic plasticity in the hippocampus and increased the level of expression of the NR2B subunit of NMDA glutamatergic receptors.

Methods

Animals

All experiments were performed in accordance with European and regional directives on animal experimentation and were supervised by an local ethical committee (Protocol RI/08/01). 7-10-month-old male Sprague-Dawley rats (Charles River, France), weighting between 450 and 550 g, were group-housed (2 to 3 animals per cage) in 12/12 light/dark cycles with food and water ad libitum. Low-magnesium diet was applied for 64 days with food pellets containing

50 mg of magnesium per kg of food (Altromin C1035 Carfil-Pavan, Belgium). Aged-matched rats receiving regular food (700 mg of magnesium per kg of food, Carfil-Pavan, Belgium) were used as controls.

7-10-month-old male APP/PS1 mice (APPswe, PSEN1dE9, Jackson laboratories, USA), weighting between 28 and 35 g, were group-housed (3 to 5 animals/cage) in 12/12 light/dark cycles with food and water ad libitum. Aged-matched male WT mice were used as controls. In the animal facility, temperature was regulated between 18 °C and 20 °C and humidity was maintained between 40 and 60 %.

Drugs

For rats submitted to low-magnesium diet: after 40 days of low-magnesium diet, three types of treatment were applied: one group received water without supplementation (Low-Mg Diet), one group received 50 mg/kg bw/day ATA Mg® supplementation (3.7 mg/kg bw/day elemental Mg) in water and one group received 700 mg/kg bw/day ATA Mg® supplementation (51 mg/kg bw/day elemental Mg) in water during the last 24 days of low-magnesium diet. AD mice were divided in two groups: one non-treated group (APP/PS1) and one group receiving 700 mg/kg bw/day ATA Mg® supplementation in water for 24 days (APP/PS1 + ATA Mg®).

Electrophysiology

After 24 days of ATA Mg® supplementation, animals were anesthetized and killed to extract the brain. Hippocampus was quickly dissected and cut in 400 µm slices with a McIlwain tissue chopper [24]. Slices were maintained in interface in warm and oxygenated aCSF during 1h30 before recording (FST, Canada). Artificial CSF was composed of 124 mM NaCl, 26 mM $NaHCO_3$, 10 mM D-glucose, 4.4 mM KCl, 1 mM NaH_2PO_4, 2.5 mM $CaCl_2$ and 1.3 mM $MgSO_4$. fEPSP was induced by stimulating Schaeffer collaterals by twisted bipolar Ni/Cr electrodes and recorded in the CA1 stratum radiatum with a glass microelectrode (2-5 MOhm). The intensity of stimulation was adjusted to obtain 40% of the maximal fEPSP and a 30-minute baseline was recorded (one stimulation/minute). Four consecutives traces were averaged to measure the slope of fEPSP.

In rat slices, long-term potentiation (LTP) was induced by 4 trains of HFS (100 Hz, 1 sec) separated by 5 minutes. Then potentiation of fEPSP was evaluated for 4 hours. In mouse slices, LTP was induced by 1 train of HFS (100 Hz, 1 sec) and the potentiation was evaluated for two hours.

The fEPSPs slopes after HFS were normalised against the baseline to obtain a relative percentage of potentiation. Stimulation protocol, data acquisition and analysis were performed using the WinLTP program (http://www.winltp.com, RRID: SCR_008590). Data were presented as mean ± SEM. Repeated two-way ANOVA, followed by Holm-Sidak post hoc test, was used to compare groups.

Western blotting

After 24 days of ATA Mg® supplementation, animals were anesthetized and killed to extract the brain. Hippocampus was quickly dissected and snap frozen in liquid nitrogen. Then, samples were homogenised in ice-cold lysis buffer (pH 6.8) containing 50 mM Tris-HCl, 0.1% SDS, 0.1% Triton X-100, 2 mM EDTA, and mQH2O with protease inhibitor cocktail (cOmplete Mini Tabs, Roche). The tissues were disintegrated mechanically using micropipettes and were then sonicated and centrifuged at 12000 rpm to obtain protein lysates. Protein quantification was performed with a PierceTM BCA Protein Assay Kit (Thermo Fisher Scientific). The samples were denatured in 2x Laemmli buffer (0.25 M Tris-HCl, 8 % SDS, 40 % Glycerol, bromophenol blue, mQH2O, pH 6.8) containing 5% 2-mercaptoethanol (Bio-Rad) at 95 °C for 5 min. Proteins with equal amounts per sample (20 µg/lane) and Page RulerTM Plus Prestained Protein Ladder (5 µL, Thermo Fisher Scientific) were loaded onto 4-20 % polyacrylamide gels (Mini-Protean® Precast TGXTM gels, Bio-Rad) for standard SDS-PAGE electrophoresis (45 min, 30 mA, in 1x Tris-Glycine-SDS buffer) and transferred at a 4 °C environment (1h45, 260 mA, in 20 % Methanol - 1x Tris-Glycine buffer) on nitrocellulose blotting membranes (AmershamTM ProtranTM, GE healthcare). The membranes were then blocked in 5 % milk - 0.1% Tween-20 - TBS and incubated overnight at 4 °C with the primary antibody diluted in blocking solution. After washes with 0.1 % Tween-20 - TBS, the membranes were incubated

for 1 h with secondary antibody conjugated to horseradish peroxidase 1:5000 diluted in 0.1 % Tween-20 - TBS. After washing, bands were revealed using a chemiluminescent system (AmershamTM ECLTM Western Blotting Detection Reagents and Hyperfilm, GE Healthcare), and quantified by densitometry with ImageJ software. A mouse monoclonal anti-β-actin antibody 1:5000 diluted in 0.1 % Tween-20 - TBS (Sigma, AB_476692) was used to normalise the protein amount.

The expression of glutamatergic receptors was analyzed with antibodies targeting GluR2/3 subunits of AMPA receptors (Sigma-Aldrich, G-5665), NR2A (Millipore, 07-632) and NR2B (Millipore, 06-600) subunits of NMDA receptors and GIRK1 subunit of kainate receptors (Abcam, AB-67404). The expression of gabaergic receptors was analyzed with antibody directed against GABA-A1 subunit (Millipore, 06-868).

Data were presented as the relative percentage of protein expression by normalising the sample values from the same blot with the corresponding non-treated sample. T-test were used to compare treated animals to non-treated ones.

Results

Magnesium N-Acetyltaurinate improves hippocampal synaptic plasticity in adult rats submitted to low-magnesium diet

Seven-month-old rats were fed with low-magnesium diet for 64 days to provoke a relative magnesium deficiency. Long-term potentiation (LTP) was induced in acute hippocampal slices by High Frequency Stimulation (HFS) of Schaeffer collaterals. Field potential was measured in CA1 pyramidal neurons.

In this condition, potentiation induced by four train of stimulation was impaired in comparison to non-deprived controls. The mean level of potentiation between 160 and 240 minutes after the trains was 419 ± 61 % in control rats (n = 6) and 249 ± 47 % in Mg-deprived rats (n = 6) (Two-way Anova for Repeated measures, from T = 160 to T = 240 minutes, Holm-Sidak post-hoc test, $p < 0.05$).

In order to assess the potential benefit of ATA Mg®, 6 rats were submitted to 64 days-low-magnesium diet and received ATA Mg® supplementation in the water (50 mg/kg bw/day) during the last 24 days This low dose was comparable to the dose recommended in humans.

In rats submitted to low-magnesium diet, LTP was slowly decaying over time (*figure 1*A) and did not show the stabilization known to be associated with long-term memory. While in rats supplemented with ATA Mg®, LTP was maintained at the same level during the four-hour recording session. The increase in slope was significantly higher in ATA Mg® treated group compared to non-treated rats. The mean level of potentiation between 160 and 240 minutes after the trains was 451 ± 70 % in ATA Mg® treated group compared to 249 ± 47 % in Mg-deprived non-treated rats (Two-way Anova for Repeated measures, from T = 160 to T = 240 minutes, Holm-Sidak post-hoc test, $p < 0.05$).

Magnesium N-Acetyltaurinate improves hippocampal synaptic plasticity in APP/PS1 mice, a murine model of Alzheimer's disease

Long-term potentiation induced in 7-10-month-old APP/PS1 mice (n = 5) was lower than in aged-matched WT littermates (n = 6). The mean level of potentiation between the train and 140 minutes after the train was 223 ± 25 % in WT mice compared to 140 ± 25 % in APP/PS1 mice (Two-way Anova for Repeated measures, From T = 16 to T = 140 minutes, Holm-Sidak post -hoc test, $p < 0.05$).

To evaluate the potential effect of ATA Mg® on LTP in 7-10-month-old APP/PS1 mice, 5 mice received ATA Mg® supplementation in water (700 mg/kg bw/day) during 24 days. This high dose was chosen in comparison to the dose used for other magnesium organic salts in the same model [25].

LTP induced in APP/PS1 mice was small with an induction peak at 226 ± 32 % (Mean ± SEM) and a potentiation reaching only 128 ± 13 % after two hours. While the induction of LTP in ATA Mg® treated group reached 313 ± 29 % and the potentiation was maintained stable at 176 ± 14 % after two hours (*figure 1*C). The increase in fEPSP slope was significantly higher in ATA Mg® treated group compared to non-treated mice with a mean potentiation of 220 ± 13 % between the train and 140 minutes after the

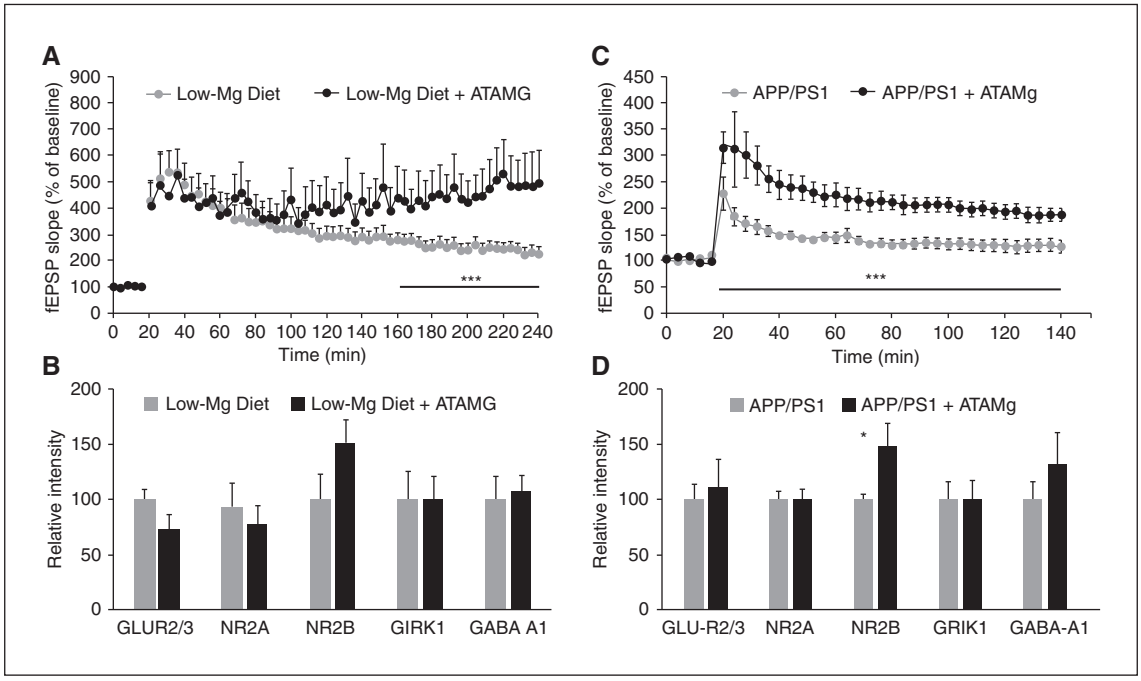

Figure 1. ATA Mg® administration improves long-term synaptic plasticity and increases NMDA receptor subunit NR2B expression in two rodent pathological models. **A-B**. Adult rats submitted to low-magnesium diet. **C-D**. Adult APP/PS1 mice. **A and C**. In rats submitted to low-Mg Diet (**A**), the increase in fEPSP slope was significantly higher in ATA Mg® treated group (n = 6, 50 mg/kg bw/day ATA Mg®) compared to non-treated rats (n = 6) (Two-way Anova for Repeated measures, from T = 160 to T = 240 minutes, Holm-Sidak post hoc test, $p < 0.05$). In APP/PS1 mice (**C**), the increase in fEPSP slope was significantly higher in ATA Mg® treated group (n = 5, 700 mg/kg bw/day ATAMg®) compared to non-treated mice (n = 5) (Two-way Anova for Repeated measures, from T = 16 to T = 140 minutes, Holm-Sidak Post hoc test, $p < 0.05$). **B and D**. Different subunits of synaptic receptors were analyzed by western blotting. Loading was controlled by actin immunolabelling. The intensity of the signal obtained in ATA Mg® treated animals (n = 6 rats and n = 5 mice) was normalized against the intensity of the signal of non-treated animals (n = 6 rats and n = 6 mice). The increase in the expression of NR2B was significant in mice (t-test, $p = 0.017$) but not in rats (t-test, $p = 0.12$).

train in ATA Mg® treated mice compared to 140 ± 25 % in APP/PS1 non-treated mice (Two-way Anova for Repeated measures, From T = 16 to T = 140 minutes, Holm-Sidak post-hoc test, $p < 0.05$).

Magnesium N-Acetyltaurinate increases NR2B subunit expression in the hippocampus

The level of expression of glutamatergic receptors in the hippocampus was evaluated by western blotting in the two models. In rats submitted to low-Mg Diet, ATA Mg® treated group (700 mg/kg bw/day) (n = 6) was compared to non-treated animals (n = 6). In mice, non-treated APP/PS1 mice (n = 6) were compared to APP/PS1 mice treated with ATA Mg® (700 mg/kg bw/day) (n = 5).

The level of AMPA receptor subunits GluR2/3 or kainate receptor subunit GIRK1 was not modified by treatment (*figure 1B and 1D*) both in rats and mice. For NMDA receptors, the level of NR2A subunit was not modified by the treatment while an increase in NR2B subunit was observed in both rats and mice. The increase in the expression of NR2B was significant in mice (t-test, $p = 0.017$) but not in rats (t-test, $p = 0.12$).

For GABA-A receptors, the level of expression of GABA-A1 subunit did not show any difference between treated and non-treated groups.

Discussion

In this study, we showed that oral administration of ATA Mg® was able to restore LTP impairment observed in magnesium-deficient rats and in a mouse model of Alzheimer's disease. This result showing a beneficial effect of ATA Mg® on synaptic plasticity in the hippocampus suggests that ATA Mg® could have a positive effect on cognition and memory.

If it is clear from the literature that an increase in magnesium concentration in the brain can be beneficial for neuronal activity and synaptic plasticity, boosting brain magnesium via chronic oral administration is challenging because magnesium concentration is tightly regulated in the CSF and is not directly impacted by plasma concentration due to the presence of blood brain barrier (BBB).

Previous studies demonstrated that inorganic magnesium salts and most of organic salts were inefficient to increase brain magnesium having a poor bioavailability and presenting little absorption through the BBB. Sun et al. [26] demonstrated that magnesium L-threonate was able to increase magnesium concentration in the brain and inside neurons in a much more efficient way than other organic salt such as citrate, gluconate or malate.

The aim of this study was to analyze the potential efficacy of another organic magnesium salt, ATA Mg®, a N-acetyl-based organic magnesium whose bioavailability has already been demonstrated. Bagatela et al. (2018) measured the concentration of Mg in the blood and the brain 8 hours after oral administration of three magnesium compounds, Mg Sulfate, Mg Oxide and ATA Mg in rats [27]. They showed a significant increase in magnesium concentration in the brain of rats treated with ATA Mg (221.8 $\pm$ 1.8 mg/g compared to 145.4 $\pm$ 3.7 mg/g in control rats). ATA Mg was also showed to increase magnesium concentration in the brain in a more efficient way than inorganic (oxide or sulfate) and organic compounds (citrate or malate): Uysal et al. (2018) demonstrated that a single dose of Mg acetyltaurate (400 mg/70 kg bw elemental Mg) administrated orally in rats was able to significantly increase Mg concentration in the brain measured by atomic spectrophotometry (216.9 $\pm$ 2.9 mg/g compared to 189.3 $\pm$ 1.6 mg/g in control rats) which was not the case of the other compounds [28].

More recently, Ates et al. (2019) studied the effect of oral administration of different organic salts in mice. By using three different doses (45, 135 and 405 mg/70 kg bw elemental magnesium), they demonstrated that if different organic salts are able to increase blood magnesium concentration, Mg acetyltaurate was more efficient to increase Mg concentration in the brain than Mg glycinate, Mg citrate or Mg malate. Indeed, no increase in brain Mg concentration was observed after Mg malate administration while an increase was measured 24 hours after administration of high doses of glycinate and malate and after all doses of Mg acetyltaurate [29]

Interestingly, we were able to reproduce the positive effect of magnesium L-threonate in AD mice at the same dose as the one used in previous studies [25], but we also demonstrated a positive effect on LTP in rats at a much more lower dose, comparable to the one recommended in humans as already demonstrated in the amygdala by Hosgorler et al (2019). They demonstrated that twelve days of oral administration of ATA Mg (50 mg/kg bw/day), in rats having undergone a mild traumatic brain injury, were able to significantly reduce neuronal apoptosis and to restore oxytocin and vasopressin levels in the amygdala confirming the effectiveness of ATA Mg to reach the brain [30].

In both models, improvement of long-term potentiation was associated to an increase in the expression of the NR2B subunit of glutamatergic NMDA receptor. The mechanism probably involves the decrease in calcium flux induced by higher extracellular magnesium concentration: the decrease in intracellular calcium leading to the upregulation of NR2B subunit of NMDA receptors [31]. Indeed, the effect of magnesium concentration in the brain is paradoxical. A short-term increase in magnesium concentration induces a decrease in NMDA currents because Mg is a non-permeant ion of NMDA receptor channel. But a long-term increase in magnesium concentration (from 0.8 mM to 1.2 mM) induces a decrease in calcium flux leading to an increase in NMDA currents and in the number of NMDA receptors.

This result was not significant in rats due to higher variability of the level of expression observed in control animals. This variability could be related to the age of rats as NMDA receptors subunits have been repeatedly showed to be downregulated in ageing rats in many different ways [32].

This result confirms the long-term impact of ATA Mg® on the central nervous system and its potential beneficial effect on cognition.

In conclusion, our results demonstrate the capability of ATA Mg® to improve the symptoms related to chronic magnesium deficiency in the hippocampus suggesting its bioavailability and its effectiveness in reaching the central nervous system.

Disclosure

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Competing interests: Philippe Danhier reports his patent application on ATA Mg®. All other authors report no financial interests or potential conflicts of interest related to the current study.

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