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Original article

Antidepressant and antinociceptive activities in animal models and *in vitro* assessment of the anti-thyroid activity of bis(DL-pyroglutamate) magnesium complex

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ABSTRACT

Background: Magnesium is an essential element related with biochemistry of the brain and different types of depression have been associated with its deficiency.
Methods: The structure of a novel magnesium bis(DL-pyroglutamate) ($Mg(DL\text{-}pGlu)_2$) was elucidated by X-ray crystallography. Wistar rats were used in the *in vivo* experiments. The antidepressant-like effect was assessed by the forced swim test (FST) and the antinociceptive activity was evaluated using hot plate test. In both, non-specific effects were evaluated by the open field test. Anti-thyroid activity was examined using Lang's method. Albumin binding behavior was evaluated by 3D fluorescence spectroscopy.
Results: For the $Mg(DL\text{-}pGlu)_2$ complex (30 mg/kg), the FST test on Wistar rats revealed a decrease of 22% in the immobility time and an increment of 106% in the swimming time. The compound alters neither the locomotor activity nor the body weight after chronic administration. At the same dose, it showed antinociceptive activity, increasing the response latency. It blocks iodination reactions generating a charge transfer complex with iodine hence indicating anti-thyroid activity (K_c = 45366.5 ± 29 M^{-1}). Albumin 3D fluorescence spectroscopy experiments showed intensity increase of peak A and decrease of peak B.
Conclusions: The results showed that the new compound produced a lowering of the immobility time and an increment of the swimming ability of the rats. The compound is able to increase the response latency in 70.0%, to capture iodine (anti-thyroid activity) and to interact with albumin through covalent type of interaction of the free NH groups.
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Introduction

Magnesium is an essential element and one of the main intracellular earth-metal cation with a cytosolic concentration of about 0.5 mM being the second most abundant cation. Mg(II) acts as a cofactor of several enzymatic reactions [1,2] and its insufficiency were described for various pathological conditions in humans. Magnesium compounds began to be prescribed as a supplement and there are many formulations for (i) prophylaxis [3], (ii) electrolyte balance restoration [4], (iii) improving nutrition, cognitive [5], (iv) Alzheimer's disease, attention deficit hyperactivity disorder, Parkinson's disease, anxiety disorder, *etc* [6], (v) pain [7], (vi) stimulating epidermal renewal [8]. In addition, pyroglutamic acid and their metal derivatives (pidolates) have long been used as supplementation drugs [9,10] and cosmetics [11].

Depression is a mental disorder characterized by persistent sadness, loss of interest, loss of energy, appetite change, anxiety, feelings of worthlessness, *etc*. (WHO 2017) [12]. Depression,

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anxiety and apathy have been related with magnesium insufficiency [13]. Preclinical and clinical reports support therapeutic application of magnesium compounds [14]. Magnesium has also been associated with the lowering of pain. Patients with chronic fatigue treated with magnesium improved energy levels, emotional state and suffered less pain [14]. Patients with depression and chronic pain showed magnesium deficiency and those having chronic stress (including pain) increased the requirement of magnesium administration [15].

There seems to be a link between depressive disorder and hyperthyroidism disease. Studies showed that depression aggravates the prognosis of antithyroid drug-treated hyper-thyroidism [16] and restores the levels of circulating thyroxine after treatment [17]. Clinical studies on thyroxicosis linked magnesium deficiency with hyperthyroidism [18]. It is known that some antidepressants may act as anti-thyroid drugs [19] and that there is of pharmacological interest to investigate drugs in tablets and ophthalmic solutions [20]. The above information prompted us to study the anti-thyroid activity of the new compound.

The binding ability of the magnesium complex to albumin was also assessed considering the extraordinary ligand carrier capacity of this protein as main modulator of fluid distribution in the body [21].

Materials and methods

All chemicals including solvents were of analytical grade: $Mg(NO_3)_2.6H_2O$ and $MgCl_2.6H_2O$ (Biopack), DL-pyroglutamic acid (MP Biomedicals), iodine (Merck). Albumin and all the other analytical grade chemicals used were purchased from Sigma. Elemental analyses were performed using a Carlo Erba EA 1108 analyzer. Equipment related to thermo-gravimetric analysis, FTIR, Raman, UV–vis and fluorescence spectroscopies have been described in reference [22].

Synthesis of [Mg(DL-pGlu)$_2$]

Solvothermal reaction of 1 mmol of $Mg(NO_3)_2{\cdot}6H_2O$ and 1 mmol of DL-pyroglutamic acid (DL-pGlu acid) in 3.5 mL of *N, N*-dimethylacetamide solvent at 100 °C for 5 days produced single crystals suitable for structural X-ray diffraction. Crystals were filtered out, washed with *N,N*-dimethylacetamide and air dried. This reaction was also carried out under the same experimental conditions but with higher concentrations of the compounds to obtain a microcrystalline powder. This solid produced an X-ray diffraction pattern which is coincident with the one calculated from the single crystal X-ray structure (see supplementary information for single crystal data, structure solution, refinement, FTIR, UV–vis and conductivity). Elemental analyses for C, H, N confirm the composition: $C_{10}H_{12}MgN_2O_6$: Calculated: 42.8 %C, 4.3% H; 10%N. Found: 42.5% C, 4.1%H, 9.8% N.

In vitro *biological and pharmacological activities*

Anti-thyroid activity

Iodine complexation was assayed based on Lang's method [23] Iodine was bi-sublimated and kept in dark in a desiccator containing P_2O_5. Spectroscopic grade solvent were used. Solutions of iodine, DL-pGlu acid and Mg(DL-pGlu)$_2$ were prepared just before the beginning of experimentation. Iodine concentration was kept constant (ethanol, 2×10^{-4} M), and the concentrations of the ligand and the complex were varied between 1×10^{-4} and 1×10^{-3} M (DMF-H_2O 10:1). The reaction was carried out directly in the spectrophotometric cell by mixing 1.5 mL solutions of the donors and the acceptor (iodine). Spectra were recorded immediately on a double beam UV–vis spectrophotometer. The temperature of the solutions was kept at 25 ± 1 °C during the measurements. Three independent replicates of each solution were measured. The formation constant (K_c) and the molar extinction coefficient were determined. This method has been used to determine the formation constants of 1:1 stoichiometric complexes at the wavelength under analysis using:

$$[A_0][D_0] / d_c = ([A_0] + [D_0] - d_c/\varepsilon_c) / \varepsilon_c + 1 / K_c \varepsilon_c \quad (1)$$

In Eq. (1), d_c is the absorbance, ε_c is the molar extinction coefficient and K_c is the formation constant of the CT-complex. Parameters were adjusted using a program designed by our research group.

Eq. (1) can be re-written in the form:

$$Y = (1/\varepsilon_c) X + 1 / K_c \varepsilon_c \quad (2)$$

where $Y = [A_0][D_0] / d_c$ and $X = [A_0] + [D_0] - d_c/\varepsilon_c$.

and a straight line with slope $1/\varepsilon_c$ and Y-intercept $1 / (K_c \varepsilon_c)$ has been obtained.

Iteration and linear regression methods were used to solve Eq. (2). A suggested initial value of ε_c was dispensed and an X value was calculated. From the slope of the line, a new ε_c was determined and the process was iterated several times until convergence of ε_c and K_c values.

In addition, the free energy change ΔG^0 was calculated from Gibbs free energy of formation according to the equation: $\Delta G^0 = -RT \ln K_c$, where ΔG^0 is the free energy change of the CT-complex, R is the gas constant (8.31 J/Kmol), T is the temperature in Kelvin and K_c is the formation constant of donor-acceptor complex.

Experiments concerning lactoperoxidase from bovine milk (LPO)-inhibition were performed using phosphate buffer (pH 7) at 25 °C according to Roy et al. procedure [24]. In this study, we employed the Fe-containing LPO as a model for TPO, because both of the enzymes have shown similar properties. The LPO enzyme activity was followed by catalysis of ABTS oxidation monitoring the UV absorption at 411 nm. The enzyme activity, upon complex addition, was expressed as a percentage of the activity in the absence of inhibitors (control). In the experiment, solutions of 100 mM ABTS and 30 mM hydrogen peroxide solutions were freshly prepared in deionized water. Lactoperoxidase enzyme (0.15–0.25 unit mL^{-1}) was prepared in cold deionized water and used immediately. In a 1 mL reaction mixture, concentrations of 12.9 nM LPO, 28.7 mM H_2O_2, 1.4 mM ABTS, and 1–500 μM of the inhibitor were fixed in the final solution.

In vivo *pharmacologic screening*

Animals

Experiments were carried out on male Wistar rats weighing 170–290 g. The animals were housed in groups of 4- rats/polyethylene cages ($55 \times 38 \times 30$ cm) at ambient room temperature of 22 ± 2 °C and relative humidity of 50 ± 5% and maintained under a 12:12 h dark light cycle (lights on at 08:00 h). Food and water were available *ad libitum* except during specific experimental protocol [22]. Rats were used only for one type of experiment. Their weights were recorded at the beginning and end of each experiment. Ambient temperature of the room and humidity were maintained consistent in all the tests.

All studies described were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the National Institutes of Health, USA and AVMA Guidelines for the Euthanasia of Animals, 2013 Ed. The experiments were performed after approval of the protocol by the Ethics Committee for the care and use of Laboratory Animals of the Universidad Nacional de La Rioja, Argentina (SNB Res. N^{0}072/15).

Antidepressant activity

Drugs and treatment

Based on previous researches, the doses of 20 and 30 mg (Mg)/kg were chosen as starting doses in the experiments [25]. The doses of the magnesium compound were calculated as the quantity of magnesium element. Doses of DL-pGlu are calculated as amounts corresponding to those present in [Mg(DL-pGlu)$_2$]. Rats were treated with the saline solution (sal, rats control) or DL-pGlu acid, $MgCl_2$ and [Mg(DL-pGlu)$_2$] once a day and all solutions were administered by oral route in a constant volume of 10 ml/kg body weight. The rats were randomly distributed into seven groups which were subjected to the following treatments: Group 1: saline (control group), Group 2: $MgCl_2$ (60 mg, 20 mg(Mg)/kg), Group 3: $MgCl_2$ (87.5 mg, 30 mg (Mg)/kg), Group 4. DL-pGlu acid (73 mg/kg corresponding to 20 mg(Mg)/kg in the complex), Group 5. DL-pGlu acid (110 mg/kg corresponding to 30 mg(Mg)/kg in the complex), Group 6: [Mg(DL-pGlu)$_2$] (80 mg, 20 mg(Mg)/kg) and Group 7: [Mg(DL-pGlu)$_2$] (121 mg, 30 mg(Mg)/kg). Saline solution and compounds were administered orally by gavage (*po*) for 14 days.

Forced swimming test (FST)

FST employs forced swimming stimuli as stressor to generate a behavior characterized by increased immobility time [22]. Swimming sessions were conducted by placing rats in individual Plexiglas cylinders (46 cm tall x 20 cm in diameter), filled with water (23–25 °C) up to 30 cm from bottom. All swimming sessions were carried out between 12:00 and 16:00 h and two sessions were conducted: an initial 15 min pre-test on day 1 followed by a 5 min test on day 15. Drug treatments began on day 1 after the pre-test session and it was administered from day 1 to 14 [22]. At the end of both swimming sessions, rats were removed from the cylinders, dried with towels, placed in heated cages for 15 min, allowed to rest and recover, and then returned to their home cages. The cylinders were emptied and cleaned between rat tests. Each animal was assigned randomly to a treatment, and was only employed for one pre-test/test session. Data were analyzed with two-way ANOVA followed by Tukey's test for multiple comparisons.

Behavioral scoring

For behavioral sampling, rats were rated at 5 s intervals throughout the duration of the forced swimming session. At each 5 s interval, the predominant behavior was assigned to one of three categories: (1) immobility: floating in the water without struggling, and making only those movements necessary to keep the head above the water; (2) swimming: making active swimming motions, more than necessary to merely keep the head above water (*i.e.*, moving around in the cylinder); and (3) climbing: making active movements with forepaws in and out of the water, usually directed against the walls. Scores for each behavior were expressed as total behavioral counts per 5-min session [22].

Thermal stimulus-induced pain (analgesic activity-hot plate test)

The hot plate consisted of an electrically heated surface (Socrel DS-35, Ugo Basile, Comerio, VA, Italy) kept at a constant temperature of 54 ± 0.8 °C. The rats were kept inside a circular transparent plastic cage on the hot plate. The time taken for the rats to respond to the thermal stimulus (licking paws or jumping surface) was recorded as the response latency (in seconds). If the animals did not respond within 45 s (cut-off time), they were removed from the plate to avoid tissue damage [22]. Animals were distributed in 4 groups: Group 1: saline solution (0.4% NaCl) (control), Group 2: DL-pGLu acid, Group 3: $MgCl_2$ and Group 4: [Mg(DL-pGlu)$_2$]. The doses are the same as for the antidepressant effect. Saline solution and compounds were administered orally by gavage (*po*) for 14 days. The antinociceptive activity chronic administration (for 14 days), was evaluated using hot plate method. Statistical analysis was carried out by one-way ANOVA followed by Bonferroni's test.

Open field test (OFT)

The locomotor activity may influence immobility in the FST or in the latency response in the hot plate test [22]. To determine whether different treatments that were effective in both tests had non-specific effects, the OFT was performed.

The apparatus used consisted of a black, square open field (60 cm × 60 cm) with the floor divided in squares (15 × 15 cm) by means of white lines. The open field was placed in a quiet room only illuminated with a 75 W electric bulb hung 75 cm above it. Testing was performed between 14:00 and 17:00 h.

Each animal was placed in the center of the open-field arena and the locomotor activity was expressed as the number of floor units the animal entered with all of its limbs over a period of 5 min (ambulatory counts). After each animal was removed, the open field was carefully cleaned with a damp cloth.

Until day 14, these studies were conducted exactly as the FST or hot plate test studies; but instead of re-testing in the FST or testing in the hot plate test on day 14, animals were subjected to an open field session. The behavior was scored by an observer who was unaware of the experimental procedures previously performed on the animals and the results were expressed as mean ± SEM.

Results

The structure of [Mg(DL-pGlu)$_2$] was characterized by single crystal X-ray analysis. Fig. 1 is an ORTEP [26] drawing of the salt (Tables S1–S7). There is in general agreement with the reported structure of free pyroglutamic acid [27] and the major difference arises as a consequence of deprotonation at the —COOH group. The Mg(II) ion is sited on a crystallographic two-fold axis in an octahedral environment, coordinated to the carboxylic oxygen atoms of six neighboring and symmetry-related ligands (Additional chemical information in supplementary material).

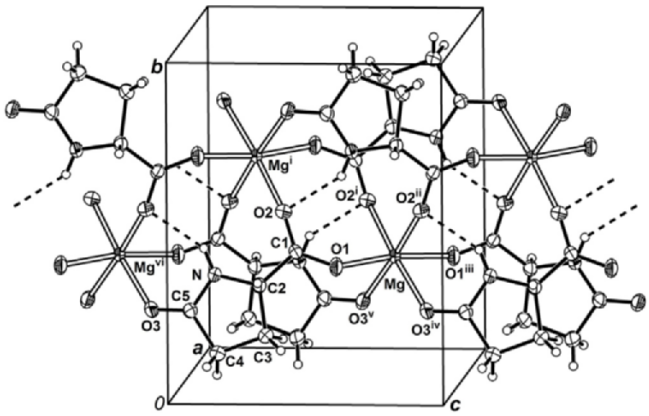

Fig. 1. View of [Mg(DL-pGlu)$_2$] down the crystal *a*-axis showing the labeling of the non-H atoms and their displacement ellipsoids at the 50% probability level. Ligand-metal bonds are indicated by open lines and H-bonds by dashed lines.

In vitro *determination of potential biological-pharmacological activities*

Anti-thyroid activity

Based on the chemical possibilities of the ligand and the complex (presence of NH group), the ability to form charge transfer-iodine complex and to inhibit LPO have been evaluated [19,23]. Neither the ligand nor the [Mg(DL-pGlu)$_2$] complex have absorption bands that masked the CT-complex band.

The ligand did no interact with iodine. A progressive increment of the [Mg(DL-pGlu)$_2$] concentration produced an increment in the absorbance of the band at 363 nm (Fig. 2A) indicating complexation with molecular iodine. Applying the Langs method to the experimental data (Fig. 2B), the formation constant K_c = 45366.5 ± 29 M^{-1} (ΔG^0 = − 26.55 KJ/mol) and the molar extinction coefficient of the CT complex ε = 2079.5 ± 25 M^{-1} cm^{-1} were determined.

Despite the anti-thyroid ability displayed by the complex, unfortunately both the complex and the ligand did not show inhibition of LPO.

In vivo *assessment of potential pharmacological activities*

Antidepressant activity

The antidepressant activity of DL-pyroglutamic acid and [Mg(DL-pGlu)$_2$] was evaluated using the FST [22]. $MgCl_2$ was also tested to evaluate a synergistic potential behavior and to compare with previously reported data [25]. The effects of a chronic treatment with [Mg(DL-pGlu)$_2$] on the total duration of immobility time in rats are shown in Fig. 3A. Contrasting with the control group (sal), $MgCl_2$ (20 and 30 mg(Mg)/kg) and DL-pGlu acid (the equivalent quantity to 20 and 30 mg(Mg)/kg) in the complex) failed to affect the immobility time. Interestingly, at the higher tested dose of [Mg(DL-pGlu)$_2$] (30 mg(Mg)/kg) the immobility time decreased (22%, $p < 0.05$) and a significant increase in swimming time (106%, $p < 0.05$) were observed without affecting considerably the frequency of the climbing behavior in comparison with the control group (Fig. 3A, sal). At a low dose of the complex (20 mg(Mg)/kg), it did not significantly change immobility time compared with the treatment of saline solution and $MgCl_2$ (30 mg(Mg)/kg). Additionally, the effect of [Mg(DL-pGlu)$_2$] (20 and 30 mg(Mg)/kg) on spontaneous locomotor activity was evaluated. Fig. 3B includes the effects of $MgCl_2$ (20 and 30 mg(Mg)/kg) and DL-pGlu acid (the equivalent quantity to 20 and 30 mg(Mg)/kg in the complex) for the sake of comparisons. Compared with the control group, none of these treatments affected the activity levels when rats were tested in an open field chamber instead of the forced swimming cylinders during the re-test (Fig. 3B). Similarly, the body weight of the rats was no affected after the treatments (Fig. S8).

Thermal stimulus-induced pain (hot plate test)

The antinociceptive activity of the compounds was determined. It has to be considered that an increase in reaction time (latency, sec) compared to basal (saline solution) is proportional to analgesic activity of the tested compounds. It can be appreciated (Fig. 4) that $MgCl_2$ (20 and 30 mg(Mg)/kg) and DL-pGlu acid (the equivalent quantity to 20 and 30 mg(Mg)/kg in the complex) treatments failed to alter the reaction time significantly while the treatment with [Mg(DL-pGlu)$_2$] (30 mg(Mg)/kg) had a greater antinociceptive effect (increment of 70%, $p < 0.05$) compared with the control group (Fig. 4, sal).

3D fluorescence spectroscopy

Fig. 5 presents the 3D fluorescence spectra of BSA in the absence and presence of DL-pGlu acid and [Mg(DL-pGlu)$_2$] complex, respectively. It is shown that Peak A, associated with the Trp and Tyr residues (Fig. 5, Table 1) slightly increases its intensity for the magnesium complex and in opposition in the ligand slightly decreases in comparison with BSA. On the contrary, Peak B, associated with the polypeptide backbone decreases its intensity for the metal complex and increases for the ligand.

Discussion

In the present study a crystal of a new coordination complex, [Mg(DL-pGlu)$_2$], was obtained (Fig. 1) in contrast to the Mg(II)-pyroglutamate salt (magnesium pidolate) whose crystalline structure is unknown [25]. This compound has similar octahedral environment than calcium pyroglutamate [28], same structure in the polycrystalline material and in solution in opposition to other magnesium compounds (magnesium gluconate) [29]. For this compound, antithyroid, antidepressant and antinociceptive activities were evaluated.

It is known that the overproduction of L-tri-iodothyronine (T_3) and thyroxine (T_4) leads to hyperthyroidism and that specific inhibitors control them by blocking the thyroid hormone

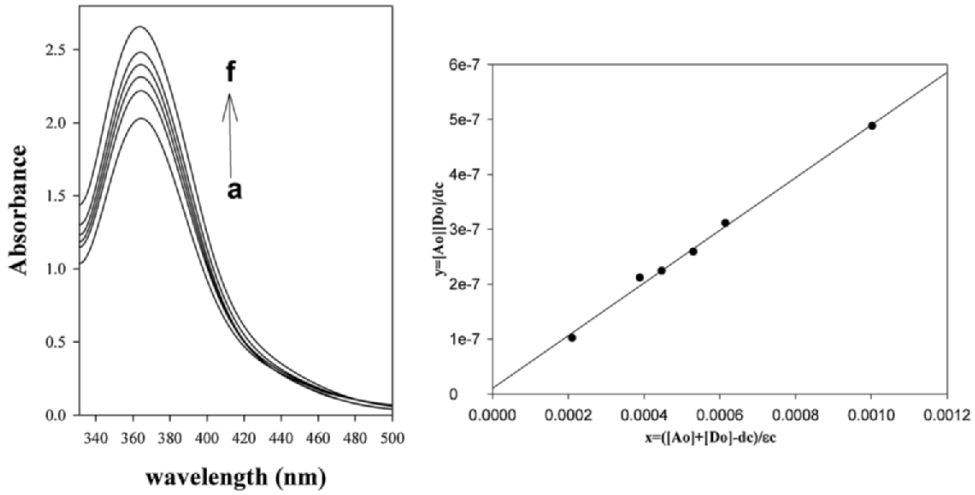

Fig. 2. (A) UV–vis spectra for the reaction of [Mg(DL-pGlu)$_2$] (a = 1 × 10^{-4} M, b = 2 × 10^{-4} M, c = 4 × 10^{-4} M, d = 6 × 10^{-4} M, e = 8 × 10^{-4} M, f = 1 × 10^{-3} M, DMF-water 10:1 solutions) with iodine (2 × 10^{-4} M, ethanol) at 298 K, 1 cm of optical path length. (B) Lang s method.

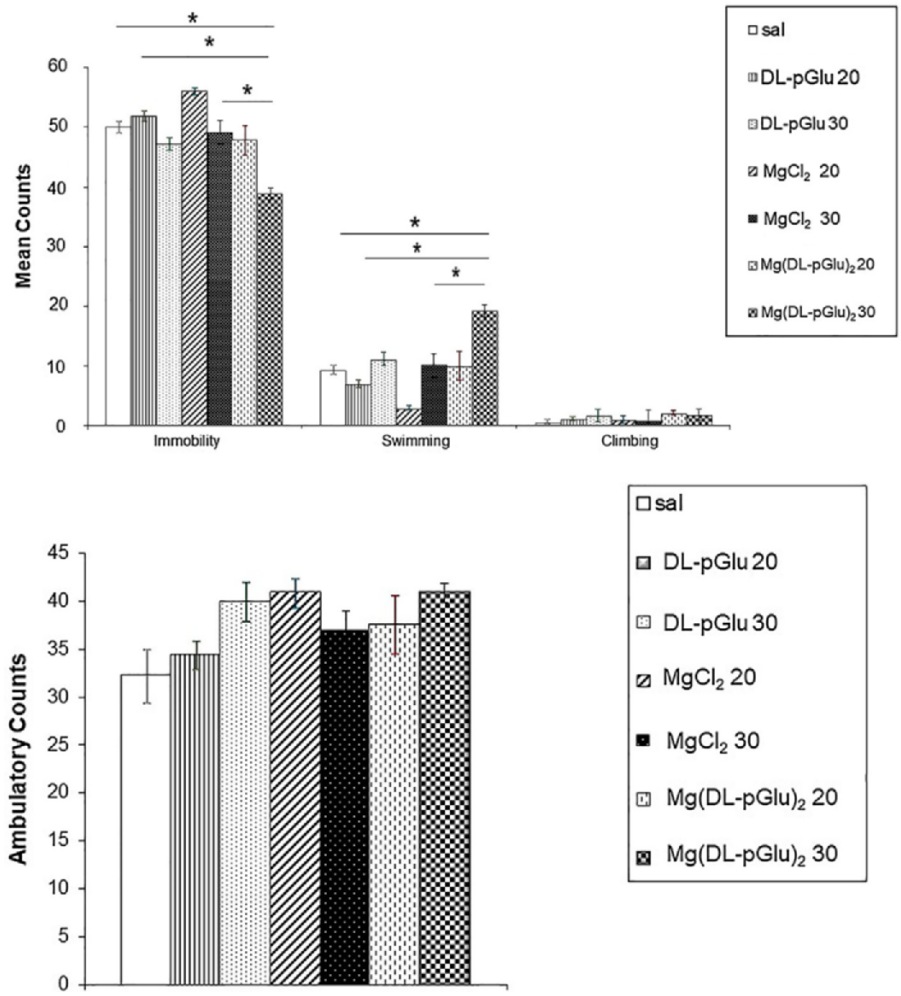

Fig. 3. Effects of [$Mg(DL\text{-}pGlu)_2$], $MgCl_2$ and DL-pGlu acid treatments on (A) FST test. Data were analyzed with two-way ANOVA, followed by Tukey's test for multiple comparisons. $p < 0.05$ compared with the control group, n=6–10 rats per group. (B) Open Field Test. Bars represent the mean number of counts over the 5-min period of the test (± SEM). Results are expressed as mean ± SEM, n = 5–10 rats per group.

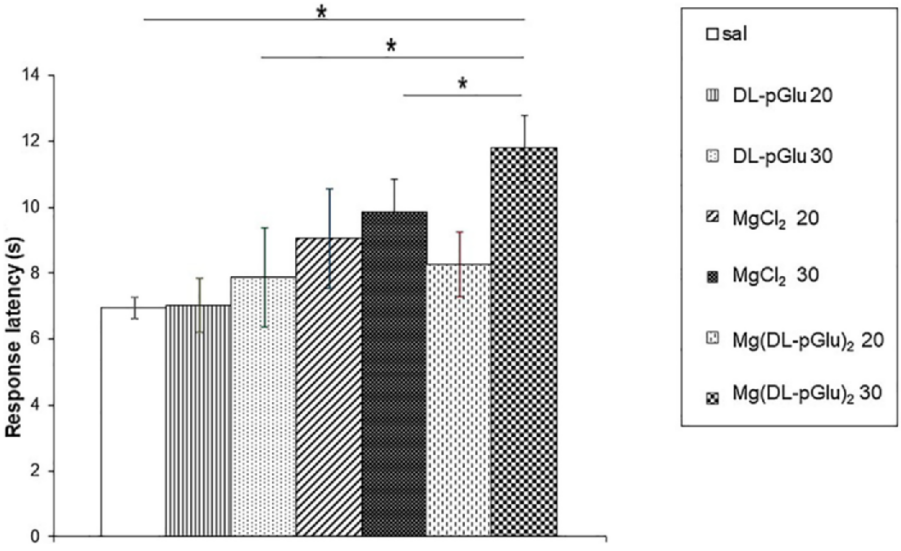

Fig. 4. The antinociceptive action of [$Mg(DL\text{-}pGlu)_2$], $MgCl_2$ and DL-pGlu acid in the hotplate test. Doses: for [$Mg(DL\text{-}pGlu)_2$] and $MgCl_2$ (20 and 30 mg(Mg)/Kg) and for DL-pGlu acid the numbers 20 and 30 refer to the proportional doses that were used for a metal complex considering 20 and 30 mg(Mg)/Kg. Values are expressed as mean ± SEM for 6–10 rats per group. $^*p < 0.05$, compared with the control group. Data were analyzed with one-way ANOVA and followed Bonferroni's test.

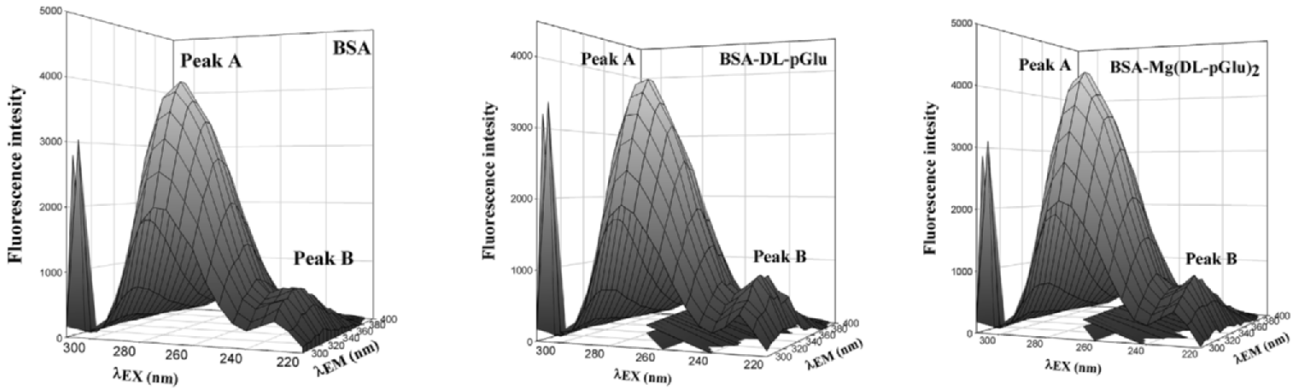

Fig. 5. Three-dimensional fluorescence spectra of 6 μM BSA, 6 μM BSA-500 μM DL-pGlu acid and 6 μM BSA-500 μM [$Mg(DL\text{-}pGlu)_2$].

Table 1
3D Fluorescence Spectral Parameters.

	Peak position	λex/λem (nm/nm)	Δλ	Intensity
BSA	A	280/337.9	57.9	4043.5
	B	230/340.3	110.3	577.6
BSA-DL-pGlu acid	A	280/335.5	55.5	3814.3
	B	230/339.0	109	673.1
BSA-[$Mg(DL\text{-}pGlu)_2$]	A	280/337.3	57.3	4215.3
	B	230/334.4	54.4	559.3

biosynthesis or reducing the conversion of T_4 to T_3 [22]. Antidepressants may act also as antithyroid drugs because of their ability to form charge-transfer complexes with iodine [19] and to block *in vivo* the iodination of the TPO enzyme. The inactivation may occur through the formation of hydrogen bonding between the distal histidine group (NH group) of the molecule and the active center of the enzyme [30]. Using K_c constant as a criterion for evaluation of potential antithyroid activity [19], the compound ($K_c > 100\ M^{-1}$, negative ΔG^0 value) can be considered a potential antithyroid agent in a spontaneous process. The presence of non-coordinated NH groups could be the reason for the revealed activity. Nevertheless a direct comparison with other molecules could not be performed; K_c constant is in the same order of magnitude of methimazole [31]. The ligand was not able to interact with iodine and the enzyme inhibition experiments suggest that neither the ligand nor the complex were able to inhibit LPO.

The antidepressant *in vivo* experiments were performed on Wistar rats (Fig. 3A). In the last years, the antidepressant effects exerted by Mg(II) ions were determined. Poleszak et al. [25] using magnesium chloride, sulfate and hydroaspartate (acute and chronic experiments, 20 and 30 mg(Mg)/Kg, intraperitoneally (*ip*) administration) on albino Swiss mice observed almost a 20–30% reduction on the immobility behavior. Similar results were obtained by Decollogne et al. [32] on OF1 mice (acute treatment, *ip* administration and 30 mg(Mg)/Kg doses). Other tests were performed on the basis of magnesium depletion [33] on mice (C57BL/6 J mice, 4 weeks) and humans [34] or by joint administration of Mg(II) with typical antidepressants [35,36]. Poleszak et al. performed experiments on male Wistar rats and demonstrated the influence of magnesium on the lowering of the immobility time [37]. In our experiments, Wistar rats were used and the compounds were given orally because of their high solubility in water. Yamamoto et al. [38] studied the antidepressant effect of pyroglutamyl peptides in mice and in this context determined the activity of pyroglutamic acid (10 mg/Kg, *ip*) which resulted ineffective. In a previous report [25], the Mg(II)-pyroglutamate salt (Mg(II) content 8%, magnesium pidolate) is mentioned but it was not used for the measurement of the antidepressant effect. Thus, for a first time, a magnesium complex was proved to act as antidepressant.

FST experiments revealed that the effective dose of the complex was the higher tested concentration (30 mg(Mg)/Kg, Fig. 3A) in which the complex produced one of the most important decrease in the immobility time (22%) and increment in swimming behavior (106%). This effect resulted effective contrary to the lower tested dose (20 mg(Mg)/kg). Because of the inactivity of the ligand and $MgCl_2$ in their individual determinations (Fig. 3A), the observed effect can be entirely attributed to the presence of the magnesium complex and therefore a synergistic effect can be discarded. Data concerning to antidepressant activity of magnesium complexes are scarce. Mg(II)-hydroaspartate salt resulted effective at 30 mg (Mg)/kg (*ip*) on albino Swiss mice but a direct comparison with this data cannot be performed.

Locomotor activity test (Fig. 3B) showed that the FST test did not affect the activity levels supporting the fact that the immobility changes can be exclusively attributed to the effect produced by the new compound. Finally, experiments controlling the body weight of the rats showed that the treatments did not induced changes in weight compared to the control. Thus, this compound can be proposed as a potential antidepressant agent without affecting locomotor activity and body weight in contrast to other antidepressants [39].

Several pre-clinical studies revealed antinociceptive properties of some magnesium compounds. Administration of the $MgCl_2$ before arthritis induction prevented carrageenan-induced hyperalgesia [40]. It was also proved in patients suffering osteoarthritis [41] or undergoing arthroscopic knee surgery. Close to the coordination complex [$Mg(DL\text{-}pGlu)_2$], magnesium-pidolate (oral administration) was able to reduce the occurrence of painful days in patients with sickle cell disease [42].

The results of this study show that the [$Mg(DL\text{-}pGlu)_2$] complex (30 mg(Mg)/Kg) had a significant antinociceptive effect (70.0%) after chronic administration when compared with the control. In addition, the increased response to thermal nociception through this treatment can be considered specific since it is not attributable to changes in locomotor activity.

The *in vivo* experiments let us propose a dual action of the magnesium complex as a potential antidepressant and analgesic agent.

The investigation of the binding between drugs and serum albumin is relevant in pharmacology and pharmacodynamic fields.

3D fluorescence spectroscopy is used to study the conformational changes of BSA after the interaction with the compound of interest. Quenching is typically expected but sometimes the enhancement of the fluorescence intensity occurs associated to a possible intercalation process [43]. BSA 3D spectrum presents: Peak A (Trp and Tyr residues, Fig. 5) which reflects the polarity of the microenvironment and Peak B (polypeptide backbone structures) associated to exposition of hydrophobic regions [44]. Different kinds of interactions were produced by the ligand and the complex. Peak A slightly increases its intensity for the magnesium complex and in opposition the interaction with the ligand slightly decreases its intensity in comparison with BSA. On the contrary, Peak B decreases its intensity for the metal complex and increases for the ligand. These differences may arise from the total charge. The ligand, at physiological pH is probably deprotonated ($-COO^-$) and electrostatic interactions were involved. Neutral complex possible preferred covalent type of interaction through the free NH groups in the molecule.

Conclusions

In the last years magnesium has spurred much interest to elucidate its participation in depression and pain. In this work we demonstrated that the presence of the racemic DL-Pyroglutamic acid favors crystallization of $[Mg(DL\text{-}pGlu)_2]$ complex. We obtained promising results (i) antidepressant effects, regarding the lowering of the immobility time (22%) and the increment of the swimming ability (106%) with a 30 mg (Mg)/kg dose; (ii) analgesic activity by increasing the response latency in 70.0% and (iii) anti-thyroid activity assayed in the ability of the complex to capture iodine (K_c>100). By 3D fluorescence spectroscopy, the interaction with the carrier protein albumin was also ascertained.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.pharep.2019.07.009.

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