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Diphenyl diselenide diet intake improves spatial learning and memory deficits in hypothyroid female rats

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ABSTRACT

Cognitive deficits have been observed in different animal models of adult-onset hypothyroidism. Thus, this study was delineated to evaluate whether diphenyl diselenide, an organoselenium compound with neuroprotective and antioxidant properties, could afford protection against the detrimental effects of hypothyroidism on behavioral parameters. Hypothyroidism condition was induced in female rats by continuous exposure to methimazole (MTZ) at 20 mg/100 ml in the drinking water, during 3 months. MTZ-induced hypothyroid rats were fed with either standard or a diet containing 5 ppm of diphenyl diselenide for 3 months. Behavioral assessments were performed monthly, in the following order: elevated plus maze, open field and Morris water maze. The levels of thyroid hormones in the animals exposed to MTZ were lower than control until the end of experimental period. The rats exposed to MTZ had a significant weight loss from the first month, which was not modified by diphenyl diselenide supplementation. In elevated plus maze test, MTZ exposure caused a reduction on the number of entries of animals in closed arms, which was avoided by diphenyl diselenide supplementation. In Morris water maze, the parameters latency to reach the platform and distance performed to find the escape platform in the test session were significantly greater in MTZ group when compared to control. These cognitive deficits observed in MTZ-induced hypothyroid rats were restored by dietary diphenyl diselenide. The group fed with diphenyl diselenide alone exhibited a better spatial learning and memory capability in some parameters of Morris water maze when compared to the control group. In summary, our data provide evidence of the effectiveness of dietary diphenyl diselenide in improving the performance of control and hypothyroid rats in the water maze test.

1. Introduction

Hypothyroidism is one of the most common thyroid disorders in the general population, especially prevalent in women (Morganti et al., 2005). Clinical observations have shown that this thyroid disease is closely related to psychiatric and cognitive disorders, such as impaired memory, anxiety and depression (Demet et al., 2002; Van Boxtel et al., 2004; Guimarães et al., 2009). In fact, the thyroid hormones are essential for maturation and normal brain functions in vertebrates and their deficiency, especially during a critical period of development, affects cognitive functions and learning (Darbra et al., 1995, 2003; Vara et al., 2002; Sala-Roca et al., 2008).

It has been postulated that the thyroid gland requires high concentrations of selenium for selenoproteins expression, which are important in maintaining the physiological levels of active hormones T3 (triiodothyronine) and T4 (thyroxine) (Köhrle, 1999; Köhrle and Gärtner, 2009); and that selenium deficiency is linked with a decrease in the levels of these hormones (Arthur et al., 1992, 1993). Really, this trace element is considered essential to the biosynthesis, activation and metabolism of the thyroid hormones (Köhrle, 1999; Köhrle and Gärtner, 2009).

The importance of selenium to the thyroid hormone biosynthesis came from myxedematous cretinism cases, predominantly observed in Central Africa, which can be caused by combined iodine and selenium deficiency with exposure to nutritional goitrogens (Vanderpas et al., 1990). In this context, experimental evidence

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have demonstrated that the supplementation with iodine alone does not promote the improvement of thyroid function (Schweizer et al., 2004) and that inadequate selenium supply associated with iodine deficiency increases the severity of hypothyroidism (Arthur et al., 1993). Additionally, recent findings in literature reported that selenium supplementation improves cerebrum and cerebellum impairments as well as alleviates oxidative stress and hematological disorders in rats with hypothyroidism induced by the antithyroid drug methimazole (MTZ) (Amara et al., 2009, 2010). Thus, the use of selenium has received a growing interest to the hypothyroidism therapy.

Numerous selenium compounds have been proposed as potential pharmacological agents mainly due their thiol-peroxidase-like and antioxidant activities (Rayman, 2000; Posser et al., 2008; Nogueira and Rocha, 2010). In fact, selenium element acts as a cofactor of the glutathione-peroxidase family that recycles glutathione, reducing lipid peroxidation by catalyzing the reduction of peroxides (Navarro-Alarcon and Cabrera-Vique, 2008). However, selenium compounds also have been found to induce toxic effects in rodents, especially when used at high doses (Nogueira et al., 2001, 2004; Brito et al., 2006; Nogueira and Rocha, 2010). Regarding the toxicity, there are several points of evidence in the literature indicating that the toxic action exhibited by some selenium compounds is highly associated with their pro-oxidant activity, which can be connected with ROS formation and thiol depletion (Nogueira et al., 2004; Nogueira and Rocha, 2010).

Diphenyl diselenide is a simple synthetic organoselenium compound, which exhibits numerous interesting effects when used in pharmacological doses. It has been reported that this compound is a potent anti-inflammatory, antioxidant, antidepressant and anxiolytic-like agent in different *in vivo* experimental models (Nogueira et al., 2004; Ghisleni et al., 2008; Savegnago et al., 2007, 2008). In addition, experimental data support the idea that diphenyl diselenide improves cognitive function and learning of mice (Rosa et al., 2003) and enhances acquisition and retention of spatial memory in rats (Stangherlin et al., 2008). On the order hand, there are also strong evidence that diphenyl diselenide may induce convulsant activity in rodents (Nogueira et al., 2003). The occurrence of seizures episodes caused by compound depends on the routes of administration, dose and animal species (Nogueira et al., 2003; Prigol et al., 2009).

Based on the evidence addressed previously, this study aims to investigate whether the intake of a diet supplemented with diphenyl diselenide could attenuate the behavioral changes related to motor and exploratory activities, anxiety, learning and memory in MTZ-induced hypothyroid female rats.

2. Material and methods

2.1. Animals and reagents

Forty adult female Wistar rats (150–200 g) purchased from our own breeding colony were acclimated for 10 days before the beginning of the experiments. The animals were housed in plastic cages and maintained at 22–24 °C, on a 12 h light/12 h dark cycle, with free access to food (Supra®, Brazil) and water. All experiments were performed in accordance with guidelines of the Committee on Care and Use of Experimental Animal Resources, Federal University of Santa Maria, Santa Maria, RS, Brazil. Methimazole was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Diphenyl diselenide was synthesized according to the literature method (Paulmier, 1986). Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure.

2.2. Experimental protocol

2.2.1. Hypothyroidism induction

Hypothyroidism was induced by continuous exposure to the antithyroid drug MTZ at 20 mg/100 ml in the drinking water, during 3 months. After 1 and 3 months of treatment some animals were euthanized and the whole blood collected by cardiac puncture for measurement of total thyroid hormones (tT3 and tT4) levels. The work was specifically conducted in female rats in order to mimic more appropriately certain features of hypothyroidism that is an endocrine dysfunction whose prevalence and incidence is higher in females (Morganti et al., 2005).

2.2.2. Dietary treatment

The female rats were randomly divided into four experimental groups ($n = 10$): (1) control; (2) methimazole; (3) diphenyl diselenide (Se) and (4) diphenyl diselenide plus methimazole (Se + MTZ). Control and MTZ groups were supplied with a standard diet, whereas the groups Se and Se + MTZ were supplemented with a diet containing 5 ppm of the selenium compound diphenyl diselenide. The choice of this concentration was based on previous studies, which show that chronic diphenyl diselenide diet intake, in the range of 1–10 ppm, did not cause overt signals of toxicity in rats (Barbosa et al., 2008a,b). Oral MTZ exposure and diphenyl diselenide diet intake were concomitantly maintained for a period of 3 months. The food was prepared in an industrial mixer to allow the uniformity of the mixture, and the compound diphenyl diselenide was dissolved in soybean oil. After preparation, the diets were frozen (−20 °C) until they were used. The diets were offered *ad libitum* to the animals and the consumption of feed, water and MTZ solution was evaluated daily.

2.2.3. Behavioral testing

The behavioral assessments were performed monthly, in the following order: elevated plus maze, open field and Morris water maze; and conducted between 8:00 am and 2:00 pm.

2.2.3.1. Elevated plus-maze test. The apparatus consisted of four 10 cm wide, 50 cm long corridors at 90° angles and 50 cm above the floor level. Two opposite corridors were surrounded by 50 cm high wood walls (closed arms), and the other two were not (open arms). Rats were placed in the middle of the four arms facing the open arm and left to explore the apparatus for 5 min. The number of entries and time spent in open and closed arms were evaluated as a measure of anxiety of the animals (Belzung and Griebel, 2001). The apparatus was cleaned between assessments with a 20% ethanol solution.

2.2.3.2. Open-field test. This task was performed in a circular apparatus (56 cm diameter) with the surface divided into 10 areas of equal size. The five areas that had the edges bounded by walls (50 cm height) were termed peripheral, while the five remaining areas and who had no contact with the wall of the apparatus were called central. The rats were gently placed at the center of the apparatus and observed for 5 min. The following parameters were evaluated: number of central and peripheral crossings, number of rearing, number and time of grooming, defecation (number of bolus) and time of freezing. The apparatus was cleaned between assessments with a 20% ethanol solution.

2.2.3.3. Morris water-maze. This task was adapted from paradigm described by Morris (1981) in order to investigate spatial learning and memory in the laboratory rat, extensively revised by D'Hooge and Deyn (2001). The apparatus consisted in a black circular pool (180 cm diameter, 60 cm height) filled with water (depth 30 cm, 24 ± 1 °C) and located in a room that was rich in spatial cues (flags on two walls, a large door and experimenters). The pool was divided into four quadrants of equal size and one of the quadrants contained an escape platform (10 cm diameter) in the middle and submerged 1.5 cm. The escape platform was maintained in all trials sessions. Swimming activity of each animal was monitored using a video tracking system to analyze the latency to reach the escape platform, time spent in platform and opposite quadrant, number of crossings, swimming velocity and distance traveled by experimental groups. Briefly, the rats were monthly submitted to four trials sessions. The trial began by placing the rat in the water facing the wall of the pool at one of the starting points demarcated by dividing the pool into four quadrants. All four start positions were used once in a random sequence in each trial session with an interval of 10 min. If the animal did not find the platform within 60 s of the trial session, it was gently led to the escape platform by the experimenter and remained there for 10 s. After each trial session, the animals were dried and heated by a lamp before returning to their cages. Twenty-four hours after the last training, the rats were submitted to a test session without the escape platform. The test session was performed by placing the rat in water for 1 min. The time spent to find the original position of the escape platform and the swimming velocity were measured. The rats were then dried and heated by a lamp and returned to their cages.

2.2.4. Thyroid hormones determination

Plasma levels of tT4 and tT3 were measured by microparticle enzyme immunoassay (MEIA) using AxSYM® system (Abbott Laboratories, Abbott Park, Illinois, USA), according to suppliers' instructions.

2.3. Statistical analysis

Data of behavior testing were analyzed by nonparametric methods, using Kruskal–Wallis test (kw) followed by Dunn's multiple comparisons test when appropriate. Data of non-parametric analysis are represented as medians and ranges (interquartile interval) and the data of parametric analysis as means and SD. Body weight and hormones levels were analyzed by three-way ANOVA (2 MTZ × 2