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The impact of a diphenyl diselenide-supplemented diet and aerobic exercise on memory of middle-aged rats

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HIGHLIGHTS

- $(PhSe)_2$-supplemented diet and swimming exercise improved memory in middle-aged rats.
- $(PhSe)_2$-supplemented diet and swimming exercise did not alter hippocampal p-CREB levels.
- Middle-aged rats in the swimming exercise group had the best performance in memory.

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ABSTRACT

Selenium is an essential trace element for human health and has received attention for its role as a nutrient. The combination of exercise and nutrients has been proposed to promote health. The aim of this study was to determine the effects of a diet supplemented with diphenyl diselenide $(PhSe)_2$ and swimming exercise on memory of middle-aged rats. Male Wistar rats (12 months) received standard diet chow supplemented with 1 ppm of $(PhSe)_2$ for 4 weeks. Rats were submitted to swimming training (20 min per day for 4 weeks). After 4 weeks, memory was evaluated in the object recognition test (ORT) and in the object location test (OLT). The hippocampal levels of phosphorylated cAMP-response element-binding protein (CREB) were determined. The results of the present study demonstrated that the association of $(PhSe)_2$-supplemented diet and swimming exercise improved short-term memory, long-term memory and spatial learning, and this effect was not related to the increase in hippocampal p-CREB levels in middle-age rats. This study also revealed that middle-aged rats in the swimming exercise group had the best performance in short- and long-term memory. In conclusion, we demonstrated that swimming exercise, $(PhSe)_2$-supplemented diet or the association of these factors improved learning and memory functioning. The hippocampal levels of CREB were not directly related to the benefits of swimming exercise and $(PhSe)_2$-supplemented diet association in memory of middle-aged rats.

1. Introduction

Selenium has been used as a component of a multi-nutrient combination which improves memory function and preserves functional brain network organization in mild Alzheimer's disease and in experimental models of memory performance [1,2]. Selenium bioavailability varies according to the selenium source and nutritional status of the subject, being significantly higher for organic forms of selenium [3].

Selenium in its organic speciation has been investigated in different experimental models of learning and memory [4,5]. In this way, the organoselenium diphenyl diselenide improves cognitive function and learning of mice [6] and enhances acquisition and retention of spatial memory in rats [7]. The addition of diphenyl diselenide in diet has proven to be effective in improving the performance of hypothyroid rats in the water maze test [8].

Exercise increases the overall health [9] and promotes hippocampal neurogenesis [10]. Moreover exercise reduces oxidative stress [11], increases the levels of brain-derived neurotrophic factor (BDNF) [12] and cAMP-response element-binding protein (CREB) [13,14] and causes a variety of morphological changes [15]. Regarding memory, exercise improves memory in rats [11,16].

In view of the above considerations and the fact that the progressive decline of neurological functions such as learning and memory is an unavoidable consequence of aging [17,18], the aim of this study was to determine the effects of a diet supplemented with diphenyl diselenide and swimming exercise on memory of middle-aged rats.

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2. Materials and methods

2.1. Drugs

Diphenyl diselenide $(PhSe)_2$ was prepared and characterized in our laboratory by the method previously described [19]. Analysis of the ^1H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of $(PhSe)_2$ (99.9%) was determined by GC/MS.

All other chemicals were obtained from analytical grade and standard commercial suppliers.

2.2. Animals

Experiments were conducted using adult (3 months) and middle-aged (12 months) male Wistar rats. The animals were obtained from a local breeding colony and were kept in a separate air-conditioned (22 ± 2 °C) room, on a 12-h light/12-h dark cycle with lights on at 7:00 a.m. The animals were housed in separate plastic cages with free access to food and water. All manipulations were carried out between 08.00 a.m. and 04.00 p.m. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil.

2.3. Experimental design

The experimental design of this study (Fig. 1) consisted of separating animals into five groups as follows: group I (adult control group) — adult animals that did not swim, group II (control group) — middle-aged sedentary animals that did not swim, group III (exercise group) — swimming-trained animals, group IV ($(PhSe)_2$ group) — animals that did not swim and were supplemented with 1 ppm of $(PhSe)_2$ diet and group V — (exercise + $(PhSe)_2$) group — swimming-trained animals and supplemented with 1 ppm of $(PhSe)_2$ diet.

2.3.1. Dietary supplementation

The supplementation began after the period of adaptation to swimming training. The animals were fed daily with standard diet chow or standard chow supplemented with 1 ppm of $(PhSe)_2$ during 4 weeks. The preparation and concentration of supplemented standard chow was based on a previous study [20]. The standard diet was pulverized with ethyl alcohol, whereas the supplemented diet was pulverized with $(PhSe)_2$ [1 mg of $(PhSe)_2$/100 g standard chow] dissolved in ethyl alcohol (1 mg/100 ml). The standard and supplemented diets were stored at room temperature for 3 h to evaporate the alcohol and then kept at 4 °C for no more than 1 week.

2.3.2. Exercise training protocol

The exercise and exercise + $(PhSe)_2$ groups were submitted to the pre-training session with duration of 20 min/day for 1 week. After adaptation, rats were submitted to a swimming training session with a workload (3% of body weight, 20 min per day for 4 weeks) [21]. The swimming training was performed between 01:00 and 03:00 p.m. in water at a temperature of 32 °C ± 1. Rats from controls and $(PhSe)_2$ groups were placed in the bottom of a separate tank with shallow water (5 cm) at 32 °C ± 1, without the workload (adaptation to the water).

2.4. Behavioral tests

The behavioral tests were performed 24 h after the last swimming training day (Fig. 1).

2.4.1. Object recognition test (ORT)

All animals were submitted to a habituation session where they were allowed to freely explore the open field for 5 min. No objects were placed in the box during the habituation trial [22]. Twenty-four hours after arena exploration, training was conducted by placing individual rats for 5 min in the field, in which two identical objects (objects 1A and 2A; duple Lego toys) were positioned in two adjacent corners, 9 cm from the walls. In a short-term memory (STM) test given 1.5 h after training, the rats explored the open field for 5 min in the presence of one familiar (A) and one novel (B) object. All objects presented similar textures, colors and sizes, but distinctive shapes. The results were expressed as exploratory preference, a recognition index was calculated for each animal by the ratio TB / (TA + TB) × 100 [TA = time spent exploring the familiar object A; TB = time spent exploring the novel object B]. Between trials, the objects were washed with 10% ethanol solution. In a long-term memory (LTM) test given 24 h after training, the same rats explored the field for 5 min in the presence of familiar object A and a novel object C [23].

2.4.2. Object location test (OLT)

The apparatus used for this test was the same field used in the ORT as the LTM objects (object A and object C). The OLT, a hippocampal-dependent spatial memory task, was performed to evaluate potential cognitive deficits resulting from aging. The period of acclimation was performed as in the ORT.

In the sample trial, objects A and C were placed in the apparatus as described in the ORT. After 5 min of the object exploration, the rats were returned to their home cages for a 4 h interval. Subsequently, in the test trial, object C was moved to a location that was diagonally opposite to object A, and the rat was left in the field for 5 min exploration [24]. The time spent exploring novel and familiar objects location was recorded. The exploration criterion and the results were expressed as in ORT.

2.4.3. Locomotor activity

The animals were pre-exposed to the chamber before testing, and activity was monitored under light and sound-attenuated conditions and the spontaneous locomotor activity was tested with the purpose of excluding some motor abnormality. Each animal initially was placed in the center of the testing chamber and allowed to freely move while being tracked by an automated tracking system. The data (distance traveled and velocity) were collected and recorded during 5 min. Testing took place in a clear acrylic chamber (500 × 480 × 500 mm) equipped with 16 infrared sensors for the automatic recording of horizontal activity (Model EP149, Insight Instruments Ltda, São Paulo, BR).

2.5. Western blot assay

After behavioral tests (Fig. 1), all animals were killed by decapitation, brains were collected and samples of hippocampus were separated.

Samples of hippocampus were homogenized in 10 mM Tris–HCl, 1 mM EDTA, pH 7.4, and centrifuged (9800 ×g at 4 °C for 5 min) to concentrate the proteins. The pellet was reconstituted in a buffer solution

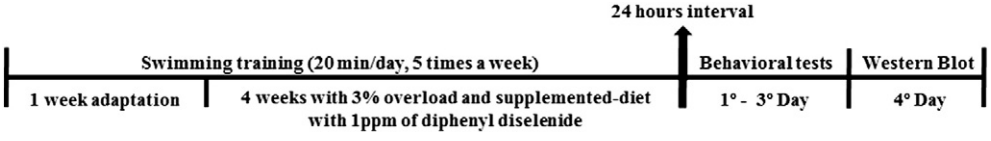

Fig. 1. Illustration of experimental design of this study.