Experimental Gerontology 53 (2014) 67–73

Contents lists available at ScienceDirect

Experimental Gerontology

journal homepage: www.elsevier.com/locate/expgero

Caffeine and diphenyl diselenide improve long-term memory impaired in middle-aged rats

Marlon R. Leite [a], Marcel Henrique Marcondes Sari [a], Mayara L. de Freitas [b], Lia P. Oliveira [b], Laíza Dalmolin [b], Ricardo Brandão [b], Gilson Zeni [a,*]

[a] Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil
[b] Departamento de Análises Clínicas e Toxicológicas, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, RS, Brazil

ARTICLE INFO

Article history:
Received 21 October 2013
Received in revised form 3 March 2014
Accepted 6 March 2014
Available online 15 March 2014

Section Editor: Christian Humpel

Keywords:
Caffeine
Organoselenium
Memory
Object recognition
Middle-aged

ABSTRACT

The aim of the present study was to evaluate the effects of diphenyl diselenide $(PhSe)_2$ supplemented diet (10 ppm) associated to the administration of caffeine (15 mg/kg; i.g.) for 30 days on the novel object recognition memory in middle-aged rats. The present findings showed that $(PhSe)_2$-supplemented diet enhanced short-term memory, but not long-term memory, of middle-aged rats in the novel object recognition task. The $(PhSe)_2$ supplemented diet associated with caffeine administration improved long-term memory, but did not alter short-term memory, impaired in middle-aged rats. Daily caffeine administration to middle-aged rats had no effect on the memory tasks. Diet supplemented with $(PhSe)_2$ plus caffeine administration increased the number of crossings and rearings reduced in middle-aged rats. Caffeine administration plus $(PhSe)_2$ diets were effective in increasing the number of rearings and crossings, respectively, in middle-aged rats, $[^3H]$ glutamate uptake was reduced in hippocampal slices of rats from $(PhSe)_2$ and caffeine plus $(PhSe)_2$ groups. In addition, animals supplemented with $(PhSe)_2$ showed an increase in the pCREB/CREB ratio whereas pAkt/Akt ratio was not modified. These results suggest that the effects of $(PhSe)_2$ on the short-term memory may be related to its ability to decrease the uptake of glutamate, influencing the increase of CREB phosphorylation. $(PhSe)_2$-supplemented diet associated to the administration of caffeine improved long-term memory impaired in middle-aged rats, an effect independent of CREB and Akt phosphorylation.

1. Introduction

The global phenomenon of increase life expectancy in recent decades has reflected directly in incidence of age-related diseases. Particularly, it is observed a deficiency on cognitive functions in elderly subjects, causing a decrease in a variety of brain functions including processing speed, inductive reasoning, spatial learning and memory (Hedden and Gabrieli, 2004). Thus, the age-dependent loss of cognitive functions has stimulated the development of strategies to contain this decline.

Caffeine is certainly the most widely consumed psychoactive substance in the world. It is estimated that more than 50% of the world's adult population consume caffeine daily (Fredholm et al., 1999). The wide consumption of caffeine associated with common beverages, such as teas and coffee, together with the impact of xanthines on biomedical research, prompted many studies that have focused on specific caffeine effects (Daly, 2007; Ferre, 2008). Caffeine crosses the blood–brain barrier and triggers its effects in the central nervous system (CNS) by antagonizing primarily A_1 and A_{2A} adenosine receptors (AR) (Fredholm et al., 2005). It has been reported that caffeine induces several cellular and pharmacological responses, such as the CNS and motor activity stimulation (Fredholm et al., 1999), anxiety and sleep disturbance (Nardi et al., 2009; Paterson et al., 2009), antioxidant activity (Noschang et al., 2009; Shi et al., 1991) among others.

In addition, animal studies have reported the effectiveness of caffeine in enhancing cognition (Duarte et al., 2012; Leite et al., 2011; Vila-Luna et al., 2012). The beneficial effects of caffeine on cognition also have been shown in humans. In fact, epidemiological studies in elderly population demonstrated that habitual caffeine consumption lower cognitive decline in men (van Gelder et al., 2007) and women (Santos et al., 2010).

Abbreviations: Akt, also called protein kinase B (PKB); CaMKII and CaMKIV, Ca^{2+}/calmodulin-dependent protein kinase II and IV; CNS, central nervous system; CREB, cAMP response element-binding protein; $(PhSe)_2$, diphenyldiselenide; LTM, long-term memory; MAPK, mitogen-activated protein kinase; NMDA, N-methyl-D-aspartate; OFT, open field test; ORT, object recognition task; PI3K, phosphatidylinositol-3 kinase; PKA, protein kinase A; STM, short-term memory; KAc, acetyl-lysine.

* Corresponding author at: Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, RS 97105-900, Brazil. Tel.: +55 55 32209611; fax: +55 55 32208978.
E-mail address: gzeni@quimica.ufsm.br (G. Zeni).

http://dx.doi.org/10.1016/j.exger.2014.03.008
0531-5565/

Selenium has an important biological role in mammalian species. In fact, it is present in the form of a selenocysteine residue in the active sites of enzymes (Behne et al., 1990; Forstrom et al., 1978; Holmgren, 1985). Persuasive evidence has been found to indicate that organoselenium compounds are promising pharmacological agents (Nogueira and Rocha, 2011). Particularly, the compound diphenyl diselenide $(PhSe)_2$ has been proven to be neuroprotector (Posser et al., 2008). Regarding memory, $(PhSe)_2$ enhances cognition of mice in a model of cognitive impairment induced by scopolamine (Souza et al., 2010) and in the novel object recognition test (Rosa et al., 2003) and improves the performance of rats in the water-maze test (Stangherlin et al., 2008).

Several signaling pathways are involved in the process of memory formation, among them phosphatidylinositol-3 kinase (PI3K)/Akt (also known as protein kinase B) pathway (Cassilhas et al., 2012). The phosphorylated Akt can lead to upregulation of cAMP response element-binding protein (CREB) phosphorylation, which in turn, regulates gene expression necessary for the formation of long-term memory. In addition, the increased Ca^{2+} influx via N-methyl-D-aspartate (NMDA) receptor can also lead to increased CREB phosphorylation by activation Ca^{2+}/calmodulin-dependent protein kinase (CaMKII) and CaMK-IV (Hu et al., 2013).

Based on the above considerations the aim of the present study was to evaluate the effects of $(PhSe)_2$-supplemented diet associated to the administration of caffeine on the novel object recognition memory in middle-aged rats.

2. Materials and methods

2.1. Animals

Male adult (3 month-old, 9–12% lifespan) and middle-aged (18 month-old, 57–61% lifespan) Wistar rats were obtained from a local breeding colony. Animals were kept in an air conditioned room (22 ± 2 °C) with free access to water and commercial diet (Guaiba, RS, Brazil, under a 12 h light/dark cycle). 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 (040/2012), the Federal University of Santa Maria, Brazil.

2.2. Drugs

Diphenyl diselenide $(PhSe)_2$ was prepared in our laboratory according to the method described by Paulmier (Paulmier, 1986) and the chemical purity (99.9%) was determined by gas chromatography–mass spectrometry (GC/MS). Analysis of ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra showed analytical and spectroscopic data in full agreement with its assigned structure. Caffeine was purchased from Sigma-Aldrich (Dorset, UK). L-[^{3}H]glutamate (specific activity 50 Ci/mmol) was purchased from Amersham International, UK. Choline chloride was purchased from Sigma Chemical CO (St. Louis, MO, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

2.3. Dietary supplementation

Animals were fed daily with ~150 g/animal standard diet chow or standard chow supplemented with $(PhSe)_2$ (3–4 animals per cage). The standard diet was pulverized with ethyl alcohol, whereas the supplemented diet was pulverized with $(PhSe)_2$ dissolved in ethanol (1 mg/10 ml). The standard and supplemented diets were kept at room temperature for 3 h to evaporate the alcohol and then kept at 4 °C by not more than 1 week. The selenium content in the $(PhSe)_2$-supplemented chow was previously determined (Barbosa et al., 2008).

2.4. Experimental procedure

The rats were divided into five groups of seven animals each, as following.

Adult: rats received standard diet chow and saline;
Middle-aged:
Control: rats received standard diet chow and saline;
$(PhSe)_2$: rats received 10 ppm of $(PhSe)_2$-supplemented diet for 30 days and saline;
Caffeine: rats received standard diet chow and 15 mg/kg of caffeine (dissolved in saline 0.9%;i.g., by gavage) for 30 days;
$(PhSe)_2$ + Caffeine: rats received both $(PhSe)_2$ plus caffeine.

The dosage and regimen of caffeine and $(PhSe)_2$ were chosen based on previous studies (Barbosa et al., 2008; Fredholm et al., 1999).

2.5. Behavioral tests

2.5.1. General methods

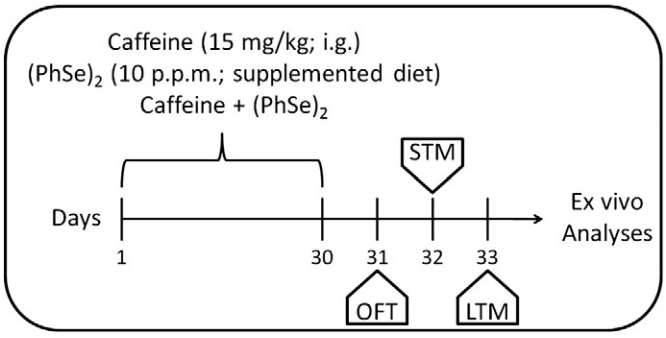
All behavioral tests lasted 5 min. Besides assessing locomotor and exploratory activities, the open field test (OFT) was also used as a familiarization period of animals to the object recognition task (ORT). Regarding the ORT, all objects presented similar textures, colors and sizes, but distinctive shapes. The exploration of objects by the animal is traditionally defined as approaching the object headfirst within a short distance. In this study, exploration was defined as sniffing or touching the object with the nose and/or forepaws. Sitting on the object was not considered exploration. A longer exploration of the new object represents that the animal remembers. Between trials the arena and objects were washed with 10% ethanol solution.

2.5.2. OFT

The open field test was performed 24 h after the last treatment day (Scheme 1) in order to discard possible sensorimotor effects induced by caffeine and $(PhSe)_2$. The open field was a 40 × 45 cm arena surrounded by 50 cm high walls, made of plywood with a frontal glass wall. The floor of the arena was divided into 9 (3 × 3) equal squares by black lines. Line crossings and rearings were counted and were used as indicative of locomotor and exploratory activities, respectively (Walsh and Cummim, 1976).

2.5.3. ORT

Twenty-four hours after the OFT (Scheme 1), the animals were trained and tested in a novel object recognition task as previously described (de Lima et al., 2005). The ORT required that the rats recalled which of two plastic objects they had been previously familiarized with the environment where the test was performed. Training was conducted by placing individual rats into the field, in which two identical objects (objects A1 and A2; duple Lego toys) were positioned in two adjacent corners, 9 cm from the walls. Animals were left to explore the objects



Scheme 1. Experimental design.