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Modulatory effect of diphenyl diselenide in Carioca High- and Low-conditioned Freezing rats

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ABSTRACT

Diphenyl diselenide ([PhSe]$_2$)is an organoselenium compound that has interesting pharmacological properties, including antioxidant, glutathione peroxidase-mimetic, and neuroprotective effects. The objective of the present study was to investigate the possible modulatory effect of (PhSe)$_2$ in 17th-generation Carioca high-and low-conditioned freezing (CHF and CLF) rats, an animal model of generalized anxiety disorders. (PhSe)$_2$ was administered at three doses (10, 50, and 100 mg/kg) in CHF and CLF rats, and their anxiety-like profiles (conditioned freezing patterns) were measured before and 30 min after treatment. A significant difference was found in freezing scores between CHF and CLF animals before treatment ($t_{70}=12.50$, $p<0.001$). Treatment with (PhSe)$_2$ at 10 and 50 mg/kg decreased freezing in CHF rats but significantly increased freezing at 100 mg/kg. (PhSe)$_2$ increased freezing in CLF animals at 50 and 100 mg/kg ($p<0.01$). These results indicate that (PhSe)$_2$ exerts both anxiolytic- and anxiogenic-like effects in bi-directional rat lines. Distinct genetic profiles of the CHF and CLF lines may influence biochemical functions and lead to differential responses to aversive situations and various drugs like (PhSe)$_2$.

1. Introduction

We recently demonstrated an association between oxidative stress and the genesis of anxiety using a novel rat breeding line known as Carioca High-and Low-Conditioned Freezing (CHF and CLF; Hassan et al., 2013). The breeding protocol for these animals is based on defensive freezing responses to contextual cues that are associated with electric footshock (Dias et al., 2009; Castro-Gomes and Landeira-Fernandez, 2008). The anxiety-like profile of these animals was confirmed with several behavioral test protocols (Hassan et al., 2013; Dias et al., 2009; Castro-Gomes and Landeira-Fernandez, 2008). The levels of reactive species (RS) and rate of lipid peroxidation (LPO) were higher in CHF rats than in CLF rats. Consequently, low antioxidant enzymatic status was confirmed in CHF animals, reflected by glutathione peroxidase (GPx) and catalase (CAT) activity in various brain structures, including the cortex, hippocampus, and cerebellum (Hassan et al., 2013). These results are consistent with other studies that reported the direct involvement of oxidative stress in anxiety-related disorders (Salim, 2011). Oxidative stress has been linked to the pathological manifestations of other psychological and neurological disorders (Andersen, 2004).

The use of antioxidants may be beneficial for ameliorating the adverse effects of oxidative bursts and may help reduce anxiety. Various dietary and synthetic antioxidants have been reported to protect against anxiety-related disorders (Salim, 2011; Bouayed and Kalinin, 2011; Augustyniak et al., 2010). Vitamin C, rutin, caffeic acid, and rosmarinic acid have been reported to have antidepressant and anxiolytic effects at lower doses (Atmaca et al., 2004). Masood et al. (2008) recently showed that supplementation with tempol (an antoxidant) reduced buthionine-[*S*,*R*]-sulfoximine (BSO)-induced anxiety-like behavior in rats. Importantly, BSO produces oxidative stress by inhibiting glutathione (GSH) synthesis.

Data regarding synthetic antioxidants are very diverse, and a range of different classes have been reported in the literature (Augustyniak et al., 2010). Organoselenium compounds have gained increasing attention because of their broad applications in organic synthesis and pharmacological efficacy. Various classes of selenium compounds have been shown to have interesting

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biological effects, including glutathione peroxidase-mimetic, lipid peroxidation, radical-scavenging, antiinflammatory, antinociceptive, cardioprotective, and thioredoxin reductase activity (Nogueira et al., 2004). Diphenyl diselenide ($[PhSe]_2$), the simplest diaryl diselenide, has shown remarkable potential in various animal models of pathology (Nogueira and Rocha, 2010). Interestingly, selenium deficiency has been related to depression, mood disorders, and anxiety (Sher, 2000; 2007; Rayman, 2000).Selenium supplementation has been shown to improve both anxious and depressive symptoms (Benton, 2002; Benton and Cook, 1991). Different mono- and diselenides have been used in various models of anxiety and depression. Depending on the chemical structure, route of administration, and dose, various compounds have shown promising antidepressant and anxiolytic activities (Nogueira and Rocha, 2011).

To our knowledge, no study has focused on the possible modulatory effects of $(PhSe)_2$in bi-directional rat lines. With distinct and specific neurochemical and neurogenetic profiles, bi-directional lines are important for exploring the underlying mechanisms of anxiety-related behavior. In the present study, 17th-generation CHF and CLF rats were treated with different doses of $(PhSe)_2$ to gain a better understanding of the possible psychomodulatory effects of this organoselenium compound.

2. Material and methods

2.1. Animals

The present study used rats that were selectively bred for high (CHF) and low (CLF) contextual fear conditioning according to procedures described in our previous work (Castro-Gomes and Landeira-Fernandez, 2008). Female albino Wistar rats from the 17th generation of selective breeding were 15–20 weeks old and weighed 180–300 g at the beginning of the study. They were bred and maintained in the colony room in the Psychology Department of the Pontifícia Universidade Católica do Rio de Janeiro with controlled room temperature (24 ± 1 °C) and a 12 h/12 h light/dark cycle (lights on 7:00 AM–7:00 PM). The animals were housed in groups of three to five, according to their respective lines, in polycarbonate cages ($18 \times 31 \times 38$ cm^3) with food and water available *ad libitum*. All of the behavioral experiments were conducted during the light phase of the light/dark cycle. The animals were handled once daily for a period of 2 min for 5 days before the fear conditioning experiment. The experimental procedures reported herein were performed in accordance with the guidelines for experimental animal research established by the Brazilian Society of Neuroscience and Behavior (SBNeC) and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animal handling and the methods of sacrifice were reviewed and approved by the Committee for Animal Care and Use of PUC-Rio (protocol no. 20/2009).

2.2. Chemicals

$(PhSe)_2$ was prepared in our laboratory according to a previous report (Paulmier, 1986). Analysis of the ^1H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra showed that $(PhSe)_2$ presented analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of the compound (99.9%) was determined by gas chromatography/high-performance liquid chromatography and was stable under storage (room temperature, humidity, and light) conditions.

2.3. Preparation of $(PhSe)_2$ solution

The compound was dissolved in canola oil, and the desired concentrations of $(PhSe)_2$ were analyzed and prepared prior to use. The solutions were stored at 2–8 °C and allowed to warm to room temperature before use. The adult rats were given a single i.p. injection of 10, 50, or 100 mg/kg $(PhSe)_2$. The dosages of $(PhSe)_2$that were used in thepresent study were within the therapeutic range (Nogueira and Rocha, 2011) because $(PhSe)_2$ causes neurotoxicity at very high doses.

2.4. Apparatus

Contextual fear conditioning occurred in four observation chambers ($25 \times 20 \times 20$ cm^3). Each observation chamber was placed inside a sound-attenuating box. A red light bulb (25 W) was placed inside the box, and a video camera was mounted to the back of the observation chamber so the animal's behavior could be observed on a monitor outside the experimental chamber. The floor of each observation chamber was composed of 15 stainless-steel rods (4 mm diameter) spaced 1.5 cm center-to-center, which were wired to a shock generator and scrambler (AVS, SCR04; São Paulo, Brazil). An interface with eight channels (Insight, Ribeirão Preto, Brazil) connected the shock generator to a computer, which allowed the experimenter to apply an electric footshock. A digital multimeter was used to calibrate the shock intensities before each experiment. An ammonium hydroxide solution (5%) was used to clean the chamber before and after each test.

2.5. Procedure

During the acquisition phase, each animal was placed in the observation chamber for 8 min. At the end of this period, three 0.6 mA unsignaled electric footshocks were delivered (1 s duration, 20 s intershock interval). The animals were then returned to their home cage 3 min after the last shock. The next day, the animals were returned to the same chamber for 8 min with no footshock or other stimulation during this period for the phenotyping test session. A time-sampling procedure was used to assess fear conditioning in response to contextual cues. Every 2 s, the animal was observed, and a well-trained observer recorded episodes of freezing, which were defined as the total absence of movement of the body or vibrissa, with the exception of movement required for respiration. One week after this initial test session of contextual aversive conditioning (phenotyping), CHF and CLF rats were tested again in the same observation chamber. Thirty minutes before the test, four independent groups of CHF rats and four independent groups of CLF rats were intraperitoneally injected with either $(PhSe)_2$ at three different concentrations (10, 50, and 100 mg/kg) or vehicle. After the injections,all of the rats were returned to their home cages where they remained before the behavioral test session. The same time-sampling procedure described above was used to record freezing behavior during the 8 min test session.

3. Results

Fig. 1 shows the mean and standard error of the mean (SEM) percentage of time spent freezing in CHF and CLF animals of the 17th generation during the contextual fear conditioning test session. As expected, CHF animals froze more that CLF animals. This difference was confirmed statistically. Student's *t*-test revealed a significant difference between CHF and CLF animals ($t_{70} = 12.50$, $p < 0.001$).

Fig. 2 shows the mean and SEM percentage of time spent