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Research report

Involvement of the serotonergic system in the anxiolytic-like effect caused by *m*-trifluoromethyl-diphenyl diselenide in mice

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ABSTRACT

The organoselenium compound diphenyl diselenide $(PhSe)_2$ has shown interesting antioxidant and neuroprotective activities. On the other hand, this compound has also presented some toxic effects. *m*-Trifluoromethyl-diphenyl diselenide $(m\text{–}CF_3\text{–}C_6H_4Se)_2$, a structural analog of $(PhSe)_2$, has proven to be antipsychotic and antioxidant in mice. The present study was designed to investigate the anxiolytic-like effect of $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ in female mice, employing light/dark box and elevated plus-maze (EPM) tests. The involvement of 5-hydroxytryptamine (5-HT) receptors and monoamine oxidase (MAO) activity in the anxiolytic-like effect was also evaluated. $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ (0.1, 10 and 100 mg/kg, p.o.) did not affect locomotor activity as evaluated in the open-field test (OFT). $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ at the dose of 100 mg/kg produced an anxiolytic-like action, both in light–dark box and the EPM tests. To evaluate the role of 5-HT receptors in the anxiolytic-like effect of $(m\text{–}CF_3\text{–}C_6H_4Se)_2$, a selective 5-$HT_{1A}$ receptor antagonist, WAY100635 (0.1 mg/kg, s.c.), a non-selective 5-$HT_{2A/2C}$ receptor antagonist, ritanserin (2 mg/kg, i.p.) and a selective 5-HT_3 receptor antagonist, ondansetron (0.1 mg/kg, i.p.) were used. All the antagonists used were able to abolish the anxiolytic-like effect of $(m\text{–}CF_3\text{–}C_6H_4Se)_2$. $(m\text{–}CF_3\text{–}C_6H_4Se)_2$, at the dose of 100 mg/kg, inhibited the MAO-A activity in mice brain. Taken together these data demonstrated that the anxiolytic-like effect caused by $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ seems to be mediated by the involvement of the serotonergic system.

1. Introduction

Anxiety, which may be understood as the pathological counterpart of normal fear, is manifested by disturbances of mood, as well as of thinking, behavior, and physiological activity. Additionally, data from the National Comorbidity Survey (NCS) of the United States, demonstrated that women were much more likely to have anxiety disorders than men. Prevalence estimates based on the NCS data indicate that one of every three women meet criteria for at least one anxiety disorder during their lifetime [16]. The etiology of most anxiety disorders, although not fully understood, has come into sharper focus in the recent past. Studies have shown that abnormalities in serotonin [5-hydroxytryptamine (5-HT)] neurotransmission have been implicated in the etiology of several psychiatric and neurological disorders [15,47]. Monoamine neurotransmitter, 5-HT is believed to be involved in pathogenesis of anxiety, and plays important role in mediating behavioral effects of anxiolytic drugs [1,50]. Monoamine oxidase (MAO that occurs as two subtypes, MAO-A and MAO-B) is the key enzyme that is associated with metabolism of this monoamine thus, regulating its intracellular concentration in the brain. Therefore, the abnormal function of this enzyme has been implicated in the etiology and treatment of depression and anxiety disorders [26,49].

Selenium is an essential trace element nutritionally important to mammals, with physiological roles as a structural component of several antioxidant enzymes involved in the peroxide decomposition [35,44]. Studies have reported that insufficient selenium intake may affect some psychological parameters and that selenium supplementation was found to be associated with an improvement in mood and depression status [3,4].

Under this point of view, our group of research and others have widely studied the pharmacological properties of organoselenium compounds [19,30,31,37]. Of particular importance, diphenyl diselenide [$(PhSe)_2$], a simple diaryl diselenide, displays pharmacological properties, such as anti-inflammatory [37] and antioxidant [31]. Moreover, $(PhSe)_2$ produces an anxiolytic-like effect [38] which has been related to the interaction with $GABA_A$ and 5-HT receptors [13].

On the other hand, $(PhSe)_2$ causes toxic effects. Chronic exposure to high $(PhSe)_2$ doses causes central effects in mice brain,

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and there are evidences of teratogenic effects in mice [25,36,46]. In this way, toxicological and pharmacological studies have been performed in our research group to verify whether the introduction of functional groups into the aromatic ring of $(PhSe)_2$ alters its effects [24,39]. Nogueira et al. [24] reported that $(PhSe)_2$ when administered by i.p. route induced seizure and death in mice and *m*-trifluoromethyl-diphenyl diselenide [$(m\text{–}CF_3\text{–}C_6H_4Se)_2$], an analog of $(PhSe)_2$ with CF_3 functional group, does not display the proconvulsant activity at the same doses that $(PhSe)_2$ caused seizures in this species, suggesting that $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ might be less toxic. Recently it was demonstrated some pharmacological properties of $(m\text{–}CF_3\text{–}C_6H_4Se)_2$, such as the ability to attenuate apomorphine-induced stereotypy [21], antimutagenic and antigenotoxic activities [22] and antioxidant action [32].

Considering $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ properties and the lack of data on the potential pharmacology and toxicology of this diselenide, the aim of this study was to investigate the anxiolytic-like effect of $(m\text{–}CF_3\text{–}C_6H_4Se)_2$, employing the light–dark box and elevated plus-maze (EPM) test in female mice. Moreover, we investigated whether the effect of $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ on the EPM is dependent on its interaction with the serotonergic system, employing 5-HT antagonists and assessing the MAO activity.

2. Materials and methods

2.1. Experimental animals

The behavioral experiments were conducted using Swiss female mice (25–35 g). Female mice were randomly selected without monitoring the estrous cycle [14]. The animals were maintained at 22–25 °C with free access to water and food, under a 12:12 h light/dark cycle. All manipulations were carried out between 08.00 a.m. and 04.00 p.m. All experiments were performed on separate groups of animals and each animal was used only once in each test. 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. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

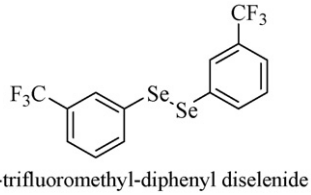
2.2. Drugs

$(m\text{–}CF_3\text{–}C_6H_4Se)_2$ (Fig. 1) was prepared and characterized in our laboratory by the method previously described [28]. 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 $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ (99.9%) was determined by GC/HPLC. All other chemicals were of analytical grade and obtained from standard commercial suppliers. All drugs were dissolved in saline except $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ that was dissolved in canola oil. The mice received all drugs in a constant volume of 10 ml/kg body weight. Appropriate vehicle-treated groups were also assessed simultaneously. The pre-treatment time of 30 min for administration of $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ was based on previously published report [33]

2.3. Behavioral tests

2.3.1. Light–dark box

The light–dark box is a sensitive model to detect activity in disorders related to generalized anxiety [8]. The test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior in response to novel environment and light [10]. The apparatus consisted of two compartments: an open topped rectangular box (46 cm × 27 cm × 30 cm high), is divided into a small (18 cm × 27 cm) area and a large (27 cm × 27 cm) area with an opening door (7.5 cm × 7.5 cm) located in the center of the partition at floor level. The smaller compartment was painted black and covered with a roof. The other compartment had no roof and was brightly illuminated by a 60 W bulb located 25 cm above the box. Each animal was placed at the center of the illuminated compartment, facing one of the dark areas, and total number of transitions between the two compartments, latency to enter the dark and the time spent in the light compartment was recorded during 5 min after 30 min of $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ (0.1, 10 and 100 mg/kg, p.o.) or canola oil (vehicle, p.o) administration. Anxiolytic activity was evaluated by time spent in the illuminated compartment, the latency for the first transition and the number of transitions.



m-trifluoromethyl-diphenyl diselenide

Fig. 1. Chemical structure of *m*-trifluoromethyl-diphenyl diselenide $(m\text{–}CF_3\text{–}C_6H_4Se)_2$.

2.3.2. Elevated plus-maze (EPM)

This test has been widely validated to measure anxiety in rodents [29]. The apparatus consists of two elevated (26 cm high) and open arms (16 cm × 5 cm) positioned opposite to one another and separated by a central platform (5 cm × 5 cm) and two arms of the same dimension, but enclosed by walls (16 cm × 5 cm × 10 cm) forming a cross. The maze is lit by a dim light placed above the central platform. 30 min after the p.o. administration of $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ (0.1, 10 and 100 mg/kg) or canola oil (vehicle), each mice was placed at the center of the maze, facing one of the open arms. During a 5 min test period, the number of entries either the open or enclosed arms, plus the time spent in the open arms were recorded. An entry was defined as placing all four paws within the boundaries of the arm. The following measures were obtained from the test: (a) time spent in the open arms relative to the total time spent in the plus-maze (300 s), expressed as percentage; (b) number of entries into the open arms relative to the total number of entries into both open and closed arms, expressed as percentage. The anxiolytic effectiveness of a drug is illustrated by a significant statistical augmentation of parameters in open arms (time and/or entries) [9].

2.3.2.1. The role of the serotonergic system in the anxiolytic-like effect of $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ on the EPM. To address the role of the serotonergic system in the anxiolytic-like effect of $(m\text{–}CF_3\text{–}C_6H_4Se)$ we chooses the EPM test. Distinct groups of animals were treated with different classes of drugs. For this purpose, mice were pre-treated with WAY100635, a selective 5-HT_{1A} receptor antagonist (0.1 mg/kg, s.c., a dose that produces no effect in the EPM), ritanserin, a non-selective 5-$HT_{2A/2C}$ (2 mg/kg, i.p., a dose that produces no effect in the EPM) or ondansetron, a selective 5-HT_3 receptor antagonist (0.1 mg/kg, i.p., a dose that produces no effect in the EPM). 15 min after WAY100635, ritanserin or ondansetron, $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ (100 mg/kg, p.o., a dose effective in all parameters of the EPM) or canola oil were administered, and 30 min later the EPM was carried out.

2.3.3. Open-field test (OFT)

The locomotor and exploratory behavior was assessed in an OFT. The open-field was made of plywood and surrounded by walls 30 cm in height. The floor of the open-field, 45 cm in length and 45 cm in width, was divided by masking tape markers into 09 squares (3 rows of 3). Animals were evaluated 30 min after a single oral dose of canola oil (vehicle) or $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ (0.1, 10 and 100 mg/kg). Each animal was placed individually at the center of the apparatus and observed for 4 min to record the locomotor (number of segments crossed with the four paws) and exploratory activities (expressed by the number of time rearing on the hind limbs) [48].

To verify whether the administration of $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ with WAY100635, ritanserin or ondansetron impairs motor abilities mice were pre-treated with WAY100635 (0.1 mg/kg, s.c.), ritanserin (2 mg/kg, i.p.) or ondansetron (0.1 mg/kg, i.p.) and 15 min after $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ (100 mg/kg, p.o.) or canola oil was administered. Thirty min later, the OFT was carried out.

2.4. Monoamine oxidase (MAO) assay

2.4.1. Preparation of cortex mitochondria

A preparation of cortex mitochondria was used in MAO assay as described by Soto-Otero et al. [42]. Swiss female mice ($n = 5$) were pre-treated with $(m\text{–}CF_3\text{–}C_6H_4Se)_2$ (100 mg/kg, p.o.) or vehicle (canola oil, p.o.), after 30 min, animals were killed and cerebral cortices were immediately removed and washed in ice-cold isolation medium (pH 7.4, Na_2PO_4/KH_2PO_4 isotonized with sucrose). Cortex mitochondria were then obtained by differential centrifugation. Briefly, after removing blood vessels and pial membranes, cerebral cortices were manually homogenized with four volumes (w/v) of the isolation medium. Then, the homogenate was centrifuged at 900 × *g* at 4 °C for 5 min. The supernatant was centrifuged at 12,500 × *g* for 15 min. The mitochondria pellet was then washed once with isolation medium and recentrifuged under the same conditions. Finally, the mitochondrial pellet was reconstituted in a buffer solution (Na_2PO_4/KH_2PO_4 isotonized with KCl, pH 7.4). MAO activity was performed immediately after mitochondria isolation.

2.4.2. MAO activity

MAO activity was determined as described by Krajl [18] with some modifications of Matsumoto et al. [23]. An aliquot of 100 μl of samples were incubated at 37 °C for 5 min in a medium containing buffer solution (Na_2PO_4/KH_2PO_4 isotonized with KCl, pH 7.4) and specific inhibitors, selegiline (a MAO-B inhibitor, 250 nM) or clorgiline (a MAO-A inhibitor, 250 nM), at a final volume of 600 μl. Then 20 μl of kynuramine dihydrobromide was added to the reaction mixture (final concentration, 90 μM (MAO-A) and 60 μM (MAO-B)) as substrate. Samples were then incubated at 37 °C for 30 min. After incubation, the reaction was terminated by adding 10% of TCA. After