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RESEARCH ARTICLE

p-Chloro-diphenyl diselenide, an organoselenium compound, with antidepressant-like and memory enhancer actions in aging male rats

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Abstract The aim of this study was to evaluate the protective effects of *p*-chloro-diphenyl diselenide $(p\text{-ClPhSe})_2$ on depressant-like action and cognitive impairment caused by aging in male rats. For this purpose, old rats were orally treated with $(p\text{-ClPhSe})_2$ (10 or 25 mg/kg) for seven days. Then, rats were tested in experimental models of ambulation, memory and depression. In addition, Na^+ K^+ ATPase activity and reactive species (RS) levels were measured in rat cortex and hippocampus. Our findings demonstrated that treatment of old rats with $(p\text{-ClPhSe})_2$ (10 and 25 mg/kg) reversed spatial memory deficit in the object location test and depressant-like action in the forced swimming test (FST) caused by aging. Reduction in exploratory behavior (rearings) in the open-field test caused by aging was not altered by $(p\text{-ClPhSe})_2$ administration. Moreover, the increase of RS levels and inhibition of Na^+ K^+ ATPase activity in cortex and hippocampus resulting from aging were restored by the highest dose of $(p\text{-ClPhSe})_2$. To assess the mechanisms involved in the antidepressant-like effect of $(p\text{-ClPhSe})_2$, old rats received WAY100635 (0.1 mg/kg, subcutaneous, a selective 5-HT_{1A}R antagonist), ritanserin (1 mg/kg, intraperitoneal, a 5-$HT_{2A/2C}$R antagonist) or ondansetron (1 mg/kg, intraperitoneal, a 5-HT_3R antagonist) 15 min before $(p\text{-ClPhSe})_2$ (25 mg/kg) treatment. After 30 min, the FST was performed. Results showed that in addition to the antioxidant action, the modulation of 5-HT_{1A} and 5-HT_3 receptors may be at least partly involved in the antidepressant-like action elicited by $(p\text{-ClPhSe})_2$ in old rats. These findings highlight the beneficial potential of $(p\text{-ClPhSe})_2$ in aged male rats.

Keywords Memory · Mood · Depression · Aging · Selenium · Male rats

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Introduction

Similar to other differentiated tissues, neurons in the central nervous system are affected by aging as indicated by a decline of several physiological abilities including sensory, motor and cognitive functions (Hofer et al. 2003). Moreover, aging has been associated with an increased risk of developing depression (Burgut et al. 2006) and neurodegenerative diseases such as Alzheimer's disease (Burgut et al. 2006; Granholm et al. 2008). Due to the fact that depressive symptoms are frequently part of early stage of dementia and that dementia is a significant risk factor for depression, an interrelationship between both diseases has emerged. It is becoming clear that

the comorbidity of depression and dementia does not occur by chance, but rather is an inevitable consequence of pathologic relationships between these conditions (Sun and Alkon 2002).

Aging is also characterized by an increased susceptibility to nutritional deficiencies (Palaniyappan and Alphonse 2011) as well as accumulation of oxidative damage mainly in the brain due to its high energy metabolism and the relative low activity of antioxidative defense mechanisms (Müller et al. 2010). In this regard, dietary or pharmacological supplementation with antioxidants could be a newsworthy alternative in order to mitigating oxidative damage associated to age (Palaniyappan and Alphonse 2011). Among the antioxidants, selenium plays an important role. A growing interest exists in understanding the biological role of selenium, in particular its physiological role in human health, in the prevention of diseases, and its potential use as a therapeutic agent (Papp et al. 2007). Mood and cognition are examples of physiological aspects modified by selenium intake (Berr et al. 2000; Benton 2002). It becomes more apparent that selenium plays a critical role in the maintenance of proper functioning of the nervous system. Selenium is a potent protective agent for neurons through the expression of selenoproteins, which are mostly involved in regulation of redox status under physiological conditions and in antioxidant defense (Schweizer et al. 2004). In line with this, organic forms of selenium generally are more bioavailable and less toxic than the inorganic forms (Doucha et al. 2009).

Organoselenium compounds exhibit a variety of interesting pharmacological actions, namely antioxidant property, which can account for their in vitro and in vivo beneficial effects in a wide range of human pathology models (PATAI 2012). These compounds, capable of propagating the redox cycle of selenium, with the property of imitating the redox physiological chemistry of selenol/selenolate groups, might supplement natural cellular defenses against the oxidizing agents (Arteel and Sies 2001). In addition to the antioxidant action, some neuroprotective properties have been assigned to diselenides, such as memory improvement (Souza et al. 2010), antidepressant-like (Savegnago et al. 2007; Brüning et al. 2011) as well as anticonvulsant (Prigol et al. 2009) in rodents. Thus, the brain appears to be one of the target organs of diselenides, and this proposal is reinforced by the fact that diselenides have highly lipophilic nature (Nogueira and Rocha 2010).

Based on the neuroprotective properties presented by synthetic organoselenium compounds, the purpose of the present study was to investigate the possible protective effect of subacute treatment with *p*-chlorodiphenyl diselenide $(p\text{-ClPhSe})_2$, a disubstituted diaryl diselenide, on depressant-like action and cognitive impairment caused by aging in male rats.

Materials and methods

Animals

Male young (3 months-old, 10% lifespan completed, weighing 170–250 g) and aged (23 months-old, 76.6% lifespan completed, weighing 400–550 g) Wistar rats were obtained from a local breeding colony. Only male rats were used in this experimental design to avoid the interference of ovarian hormones in $(p\text{-ClPhSe})_2$ effects. Taking into account that the ovarian steroid hormones regulate a wide variety of functions in the central nervous system by interacting with molecular and cellular processes (Foy 2011), male rats were selected based on the following reasons: (1) young female rats are not adequate control for the present protocol since they would suffer hormonal fluctuations due to cyclical reproductive hormones (estrous cycle, 4 to 5 days) and behaviors are conducted on different days and, (2) during reproductive senescence, aging female rats undergo a loss of cyclicity but continue to maintain constant, moderate levels of ovarian hormones (Markham and Juraska 2002). Male animals were housed in cages (2 rats per cage) with free access to food and water. They 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. Commercial diet (Guaiba, RS, Brazil) and tap water were supplied ad libitum. Animal care and all experimental procedures were conducted in compliance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH publications No. 80-23, revised in 1996) and in accordance with the guidelines of the Committee on Care and Use of Experimental Animal Resources, from the Federal University of Santa Maria, Brazil. All efforts were made to minimize the number of animals used and their suffering.

Drugs

(p-ClPhSe)$_2$ (Fig. 1) was prepared and characterized by the method previously described (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. The chemical purity of (p-ClPhSe)$_2$ (99.9%) was determined by GC-MS. 2′,7′-dichlorofluorescein diacetate (DCHF-DA), ouabain, adenosine triphosphate (ATP), bovine serum albumin, WAY100635, ritanserin and ondansetron were obtained from Sigma (St. Louis, MO, USA). All other chemicals were obtained from analytical grade and standard commercial suppliers.

(p-ClPhSe)$_2$ was dissolved in canola oil. WAY100635 and ondansetron were dissolved in saline. Ritanserin was sonicated in 2% Tween 80 and diluted with saline.

Exposure

Old male rats were orally (p.o.) treated with (p-ClPhSe)$_2$ (10 or 25 mg/kg, daily per 7 days) or vehicle (canola oil, 1 ml/kg). Young male rats received only vehicle. The chosen doses of (p-ClPhSe)$_2$ and the treatment time were based on a pilot study conducted in our research group. The animals ($n = 10$ rats per group) were divided into four groups as following:

- Group I: young male rats
- Group II: old male rats
- Group III: old male rats + (p-ClPhSe)$_2$ 10 mg/kg
- Group IV: old male rats + (p-ClPhSe)$_2$ 25 mg/kg

After 7 days of treatment, animals were tested in experimental models of ambulation, memory and depression. On the 7th day, the animals were subjected to the open field test (OFT). On the 8th day, the same animals were submitted to the object recognition test (ORT). On the 9th day, the animals were submitted to the object location test (OLT). Forced swimming training was conducted on the 10th day. After 24 h, the forced swimming test (FST) was performed. Animals were then allowed to rest until 14th day in order to avoid behavioral stress, when they were killed by decapitation for ex vivo experiments (Scheme 1).

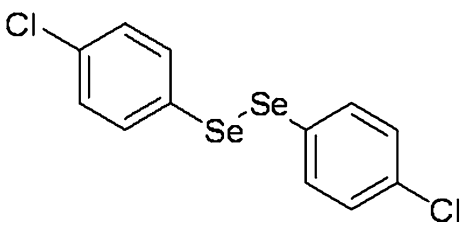


Fig. 1 Chemical structure of (p-ClPhSe)$_2$

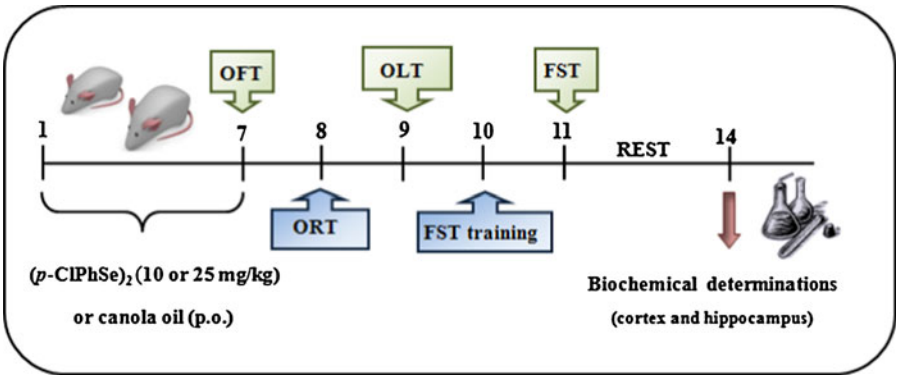
Behavioral tests

OFT

The ambulatory behavior of rats was assessed in the OFT 3 h after the last injection. 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. Each animal was placed individually at the center of the apparatus and the number of squares crossed with all paws (crossings) and rearings were counted in a 4-min session (Walsh and Cummins 1976). The arena floor was cleaned between experiments and the test was carried out in a temperature and light controlled room. The 4-min session of OFT was also useful to familiarize the rats with the arena as a context habituation trial for the recognition memory task.

ORT

Twenty-four hours after OFT, animals were trained in the same arena in order to perform a novel object recognition test as previously described (de Lima et al. 2005). The object recognition test, a non-spatial memory task, required that the rats recalled which of two plastic objects they had been previously familiarized with the environment where the test was performed. For this purpose, after arena exploration, training was conducted by placing individual rats into the field, in which two identical objects (objects A and A'; duple Lego toys) were positioned in two adjacent corners, a little away from the walls. Animals were allowed to explore the objects for 5 min. After 4 h, the novel object recognition task was performed to assess the short-term memory. In the test, 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 time spent exploring the familiar object A



Scheme 1 Experimental design

and the novel object B was recorded. Exploration was defined as sniffing or touching the object with the nose and/or forepaws. Periods in which the rat moved around, climbed over or sat on the objects were not recorded. To prevent olfactory cues, the objects and the field were cleaned with ethanol/water solution after each trial. The results were expressed as exploratory preference according to the following formula: [time spent in the novel object B/(time spent in the familiar object A + time spent in the novel object B)] × 100.

OLT

The apparatus used for this test was the same field used in the ORT. The OLT, a hippocampal-dependent spatial memory task, was performed to evaluate potential cognitive deficits resulting from aging. The discriminated objects, D and E, were two identically sized (5 cm diameter and 10 cm height). The period of acclimation was performed as in the ORT. In the sample trial, objects D and E were placed in the apparatus as described in the ORT. After 5 min object exploration, the rats were returned to their home cage for a 4-hour interval. Subsequently, in the test trial, object E was moved to a location that was diagonally opposite to object D, and the rat was left in the field for 5 min exploration (De Rosa et al. 2005). The time spent exploring novel and familiar object location was recorded. The exploration criterion used was similar to ORT, i.e., the results were expressed as exploratory preference according to the following formula: [time spent in the novel location/(time spent in the familiar location + time spent in the novel location)] × 100.

FST

The FST has been employed as a successful screening tool for assessing antidepressant-like action of drugs in rodents. The perseverance of the animal struggling to escape from an inescapable aversive scenario (a cylinder full of water) has been proved to be inversely correlated with a depressive-like profile. The FST was performed in a cylinder filled with water (23–24°C) to a depth of 40 cm, such that animals could not lay their rear paws on the bottom without being totally submersed, and with a height of 20 cm above the water level, thus preventing the escape of animals. A first trial in the FST (training) was done approximately 1 h after the EPMT and lasted 15 min. Twenty-four hours later, rats underwent the test session. In the test session, rats were again placed in cylinders filled with water, and the duration of immobility was recorded for 6 min. Each rat was recorded as immobile when floating motionless or making only those movements necessary to keep its head above water (Porsolt et al. 1977).

Evaluation of mechanisms involved in the antidepressant-like action of (p-ClPhSe)$_2$ in old male rats

The possible contribution of the serotonergic system to the effect of (*p*-ClPhSe)$_2$ in reducing the immobility time of old rats in the FST was assessed using a subset of animals ($n = 6$ per group). The FST was carried out over 2 days, i.e., a day for the training session and a day for the test session, as already described. To evaluate the involvement of serotonin (5-HT) receptor subtypes in the antidepressant-like effect caused by

(p-ClPhSe)$_2$ in the FST, old rats were pretreated with WAY100635 (0.1 mg/kg, subcutaneous, s.c., a selective 5-HT$_{1A}$ receptor antagonist), ritanserin (1 mg/kg, intraperitoneal, i.p., a 5-HT$_{2A/2C}$ receptor antagonist) or ondansetron (1 mg/kg, i.p., a 5-HT$_3$ receptor antagonist) (Savegnago et al. 2007) and after 15 min they received (p-ClPhSe)$_2$ (25 mg/kg, p.o.) or vehicle. Thirty min later, old rats were tested in the OFT (4 min session) and FST (6 min session) to record the crossing number and immobility time, respectively.

Soon after behavioural tests, animals were killed by decapitation, the brains were removed and the hippocampi were separated for monoamine oxidase (MAO) activity assay.

MAO activity

Preparation of cerebral mitochondria A preparation of cerebral mitochondria was carried out as described by Soto-Otero et al. (2001). Rat whole brain was removed; hippocampus was separated and washed in ice-cold isolation medium (pH 7.4, Na_2PO_4/KH_2PO_4 isotonized with sucrose). Mitochondria from hippocampus were then obtained by differential centrifugation. Briefly, hippocampus was 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 centrifuged again 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.

Enzymatic assay MAO activity was determined as described by Krajl (1965) with some modifications of Matsumoto et al. (1984). An aliquot of 100 µl of samples (100 µg of protein) was 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), 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 were 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 cooling and centrifugation at 3,000×g for 15 min, an aliquot of 1 ml of the supernatant was added to 1 ml of 1 M NaOH. The fluorescence intensity was detected spectrofluorimetrically with excitation at 315 nm and emission at 380 nm. The concentration of 4-hydroxyquinoline was estimated from a corresponding standard fluorescence curve of 4-hydroxyquinoline. MAO A and B activities were expressed as nmol 4-OH quinoline/mg protein/min.

Biochemical determinations

On the 14th day, rats were killed by decapitation, brains were removed and hippocampus and cortex were separated. For determination of biochemical parameters the cerebral structures were homogenated in 50 mM Tris–HCl, pH 7.4 (1:4, w/v), and centrifuged at 2,400×g for 10 min to obtain the low-speed supernatant (S_1).

Reactive species (RS) measurement

The RS levels were determined by a spectrofluorometric method, using 2′,7′-dichlorofluorescein diacetate (DCHF-DA) assay (Loetchutinat et al. 2005). The oxidation of DCHF-DA to fluorescent dichlorofluorescein (DCHF) is measured for the detection of intracellular RS. To estimate the level of RS, 10 µl of S_1 were added to 2.98 ml of 50 mM Tris–HCl (pH 7.4) and incubated with 10 µl of 1 mM DCHF-DA. The DCHF fluorescence intensity emission was recorded at 520 nm (with 480 nm excitation) 15 min after the addition of DCHF-DA to the medium. The RS levels were expressed as arbitrary units.

Na^+ K^+ ATPase activity

Since an age-related decline in Na^+ K^+ ATPase activity has been reported in the parietal cortex and hippocampus of rats (Kaur et al. 1998), the activity of this enzyme was determined in the present study. The reaction mixture for Na^+ K^+ ATPase activity assay contained 3 mM MgCl, 125 mM NaCl, 20 mM KCl and 50 mM Tris–HCl, pH 7.4, in a final volume of 500 µl. The reaction was started by the addition of ATP to a final concentration of 3.0 mM. For obtaining the ouabain-sensitive activity, samples were carried out under the same conditions with the addition of

0.1 mM ouabain. The samples were incubated at 37°C for 30 min, the incubation was stopped by adding trichloroacetic acid solution (10%) with 10 mM $HgCl_2$. Na^+ K^+ ATPase activity was calculated by subtracting the ouabain-sensitive activity from the overall activity (in the absence of ouabain). Released inorganic phosphate (Pi) was spectrophotometrically measured at 650 nm as described by Fiske and Subbarow (1925) and enzymatic activity was expressed as nmol Pi/mg protein/min.

Protein quantification

Protein concentration was determined by the method of Bradford (1976), using bovine serum albumin as the standard.

Statistic analysis

Data are expressed as means ± SEM statistical analysis was performed using a One-way Analysis of Variance (ANOVA) or Two-way ANOVA (interaction of (p-ClPhSe)$_2$ with the pharmacological agents) followed by Newman–Keuls test when appropriate. Values of $P < 0.05$ were considered statistically significant. Main effects are presented only when interaction was not significant.

Results

OFT

Statistical analysis showed that the number of crossings in the OFT was not significantly altered by age or (p-ClPhSe)$_2$ treatment ($F_{(3,\ 36)} = 2.28$; $P < 0.0958$) (Fig. 2a).

The one-way ANOVA revealed a reduction in the number of rearings ($F_{(3,\ 36)} = 7.46$; $P < 0.0005$) for old male rats. Treatment with (p-ClPhSe)$_2$, at doses of 10 and 25 mg/kg for seven days, was not effective in reversing this alteration (Fig. 2b).

ORT

Statistical analysis performed using the one-way ANOVA yielded that the exploratory preference of male rats in the ORT was not significantly modified by age or (p-ClPhSe)$_2$ treatment ($F_{(3,\ 36)} = 2.07$; $P < 0.1209$) (Fig. 3a).

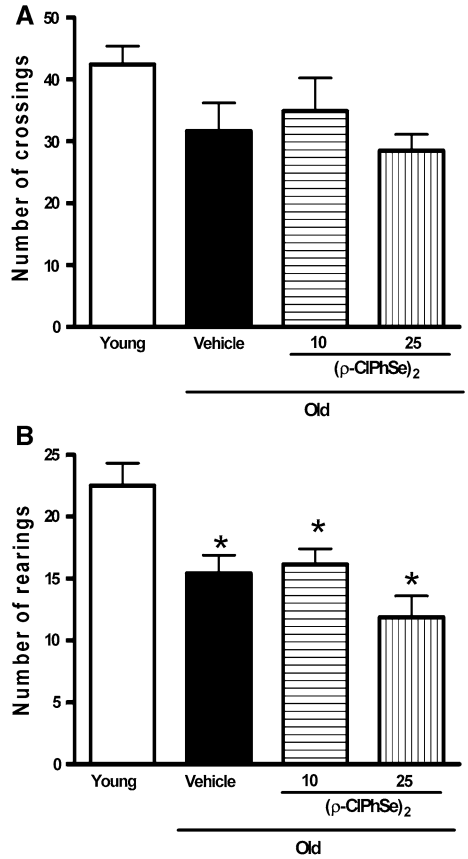


Fig. 2 Effect of treatment with (p-ClPhSe)$_2$ (10 or 25 mg/kg, p.o.; for 7 days) on **a** locomotor and **b** exploratory behavior of old male rats in the OFT. OFT was conducted on the 7th day, 3 h after the last (p-ClPhSe)$_2$ administration. Values are expressed as mean ± SEM ($n = 10$ rats per group). Data were analyzed by One-way ANOVA followed by Newman–Keuls test. *Significance*: $^*P < 0.05$ as compared to young rats

OLT

The one-way analysis revealed significant effects on the exploratory preference by novel object location in the OLT ($F_{(3,\ 36)} = 7.21$; $P < 0.0007$). Old male rats had a decrease in the preference exploratory by the novel object location. The results demonstrated that the treatment of old rats with (p-ClPhSe)$_2$, at doses of 10 and 25 mg/kg, reversed the impairment in spatial memory (Fig. 3b).

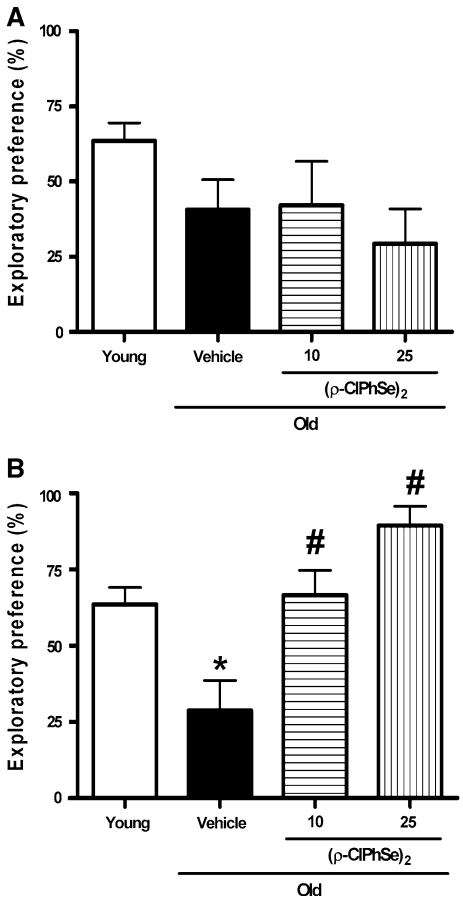


Fig. 3 Effect of treatment with $(p\text{-ClPhSe})_2$ (10 or 25 mg/kg, p.o.; for 7 days) on short-term memory of old male rats in the **a** ORT and **b** OLT. ORT and OLT were carried out on 8th and 9th days, respectively. Recognition memory task was performed after 4 h of training for both tests. Values are expressed as mean ± SEM ($n = 10$ rats per group). Data were analyzed by One-way ANOVA followed by Newman–Keuls test. *Significance*: $^{*}P < 0.05$ as compared to young rats; $^{\#}P < 0.05$ as compared to old rats treated with vehicle

FST

As revealed by the one-way statistical analysis, significant effects on immobility time were observed in the FST ($F_{(3,\ 36)} = 4.78$; $P < 0.0066$). Figure 4 shows that aging caused a depressant-like action in male rats, evidenced by an increase in immobility time of old rats. The oral administration of $(p\text{-ClPhSe})_2$ for seven days, at doses of 10 and 25 mg/kg, was effective in reversing the increase in immobility time in old male rats.

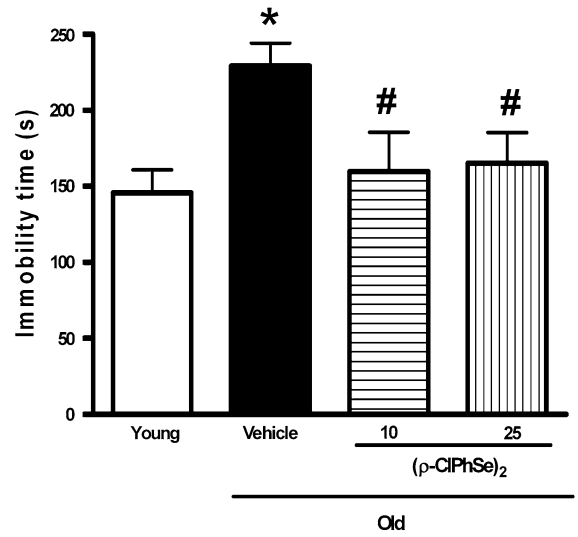


Fig. 4 Effect of treatment with $(p\text{-ClPhSe})_2$ (10 or 25 mg/kg, p.o.; for 7 days) on immobility time of old male rats in the FST. Rats were submitted to FST on the 11th day. Values are expressed as mean ± SEM ($n = 10$ rats per group). Data were analyzed by One-way ANOVA followed by Newman–Keuls test. *Significance*: $^{*}P < 0.05$ as compared to young rats; $^{\#}P < 0.05$ as compared to old rats treated with vehicle

Mechanisms of (p-ClPhSe)$_2$ antidepressant-like action

Figure 5a shows that pretreatment of male rats with ritanserin (a 5-$HT_{2A/2C}$ receptor antagonist) was not effective in preventing the antidepressant-like action of $(p\text{-ClPhSe})_2$ in the FST. The two-way ANOVA revealed a significant main effect of $(p\text{-ClPhSe})_2$ ($F_{(1,\ 20)} = 60.44$; $P < 0.0001$). Post hoc comparisons revealed that acute administration of $(p\text{-ClPhSe})_2$ (25 mg/kg, p.o.) to old male rats resulted in a decrease of immobility time in the FST.

As shown in Fig. 5b, pretreatment of male rats with WAY100635 (a 5-HT_{1A} receptor antagonist) was effective in preventing the antidepressant-like action of $(p\text{-ClPhSe})_2$ in the FST. The two-way ANOVA revealed a significant main effect of $(p\text{-ClPhSe})_2$ ($F_{(1,\ 20)} = 26.48$; $P < 0.0001$). Post hoc comparisons revealed that acute administration of $(p\text{-ClPhSe})_2$ (25 mg/kg, p.o.) to old rats resulted in a decrease of immobility time in the FST.

Figure 5c shows that pretreatment of male rats with ondansetron (a 5-HT_3 receptor antagonist) was effective in preventing the antidepressant-like action of

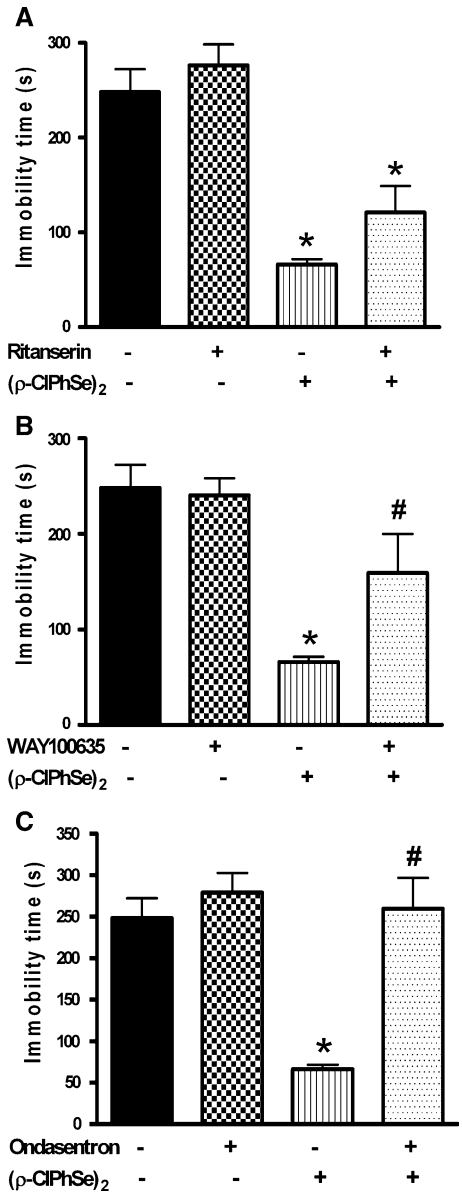


Fig. 5 Effect of pretreatment with **a** WAY100635 (0.1 mg/kg, s.c., a selective 5-HT_{1A} receptor antagonist), **b** ritanserin (1 mg/kg, i.p., a 5-$HT_{2A/2C}$ receptor antagonist) or **c** ondansetron (1 mg/kg, i.p., a 5-HT_3 receptor antagonist) on the antidepressant-like action resulting from acute $(p\text{-ClPhSe})_2$ treatment (25 mg/kg; p.o.) in the FST. 5-HT antagonists were administered 15 min before $(p\text{-ClPhSe})_2$. FST was conducted 30 min after $(p\text{-ClPhSe})_2$ administration and immobility duration (s) was recorded. Values are expressed as mean ± SEM ($n = 6$ rats per group). Data were analyzed by Two-way ANOVA followed by Newman–Keuls test. *Significance*: $^{*}P < 0.05$ as compared to old rats treated with vehicle or 5-HT antagonists; $^{\#}P < 0.05$ as compared to old rats treated with $(p\text{-ClPhSe})_2$

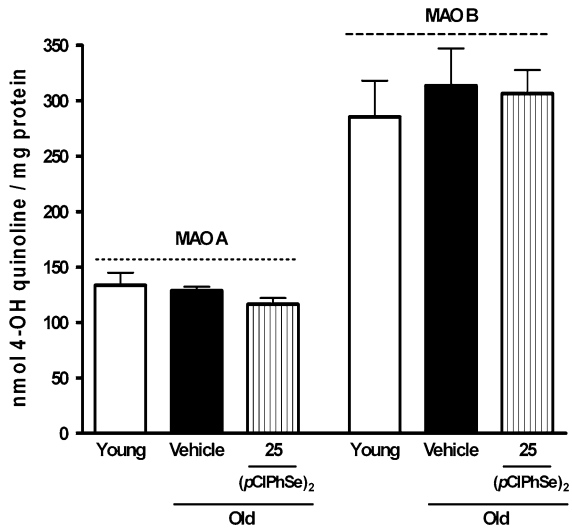


Fig. 6 Effect of acute treatment with $(p\text{-ClPhSe})_2$ (25 mg/kg, v.o) on MAO A and MAO B activities in hippocampus of old male rats. Rats were killed 30 min after $(p\text{-ClPhSe})_2$ administration. MAO activity was expressed as nmol 4-OH quinoline/mg protein/min. Values are expressed as mean ± SEM ($n = 6$ rats per group). Data were analyzed by One-way ANOVA followed by Newman–Keuls test

$(p\text{-ClPhSe})_2$ in the FST. The two-way ANOVA of immobility duration demonstrated significant differences for $(p\text{-ClPhSe})_2$ pretreatment ($F_{(1,\ 20)} = 15.08$; $P < 0.0007$), ondansetron treatment ($F_{(1,\ 20)} = 19.62$; $P < 0.0003$) and $(p\text{-ClPhSe})_2 \times$ ondansetron interaction ($F_{(1,\ 20)} = 10.28$; $P < 0.0044$).

There were no significant differences in the crossing number of animals from all experimental groups evaluated in the OFT (data not shown).

Hippocampal MAO activity

The one-way ANOVA showed that there was no alteration in MAO A ($F_{(2,\ 15)} = 1.35$; $P < 0.2888$) and MAO B ($F_{(2,\ 15)} = 0.25$; $P < 0.7848$) activities in hippocampi of old rats (treated ot not) in comparison to young rats (Fig. 6).

Ex vivo experiments

RS measurement

The one-way ANOVA demonstrated significant differences in RS levels in rat hippocampus ($F_{(3,\ 36)} = 8.99$; $P < 0.001$) and cortex ($F_{(3,\ 36)} = 6.51$; $P < 0.0012$).

Table 1 Effect of treatment with $(p\text{-ClPhSe})_2$ (10 or 25 mg/kg) by 7 days on RS levels and Na^+ K^+ ATPase activity in hippocampus and cortex of old male rats

Groups	Hippocampus		Cortex	
	RS	Na^+ K^+ ATPase	RS	Na^+ K^+ ATPase
Young + vehicle	59.85 ± 2.68	28.51 ± 2.44	54.76 ± 4.80	21.30 ± 2.29
Old + vehicle	90.09 ± 5.35*	14.70 ± 1.44*	89.04 ± 8.99*	10.34 ± 1.37*
Old + $(p\text{-ClPhSe})_2$ 10	80.51 ± 8.00*	20.13 ± 3.00	55.02 ± 3.83$^{\#}$	18.63 ± 3.27
Old + $(p\text{-ClPhSe})_2$ 25	65.85 ± 1.78$^{\#}$	38.01 ± 7.23$^{\#}$	67.87 ± 5.33$^{\#}$	25.94 ± 5.08$^{\#}$

The results are expressed as mean ± SEM, $n = 10$ rats/group. The RS levels were expressed as arbitrary units; Na^+ K^+ ATPase activity as nmol Pi/mg protein/min. One-way ANOVA followed Newman–Keuls test. *Significance*: * $P < 0.05$ as compared to young rats; $^{\#}$ $P < 0.05$ as compared to old rats treated with vehicle (canola oil)

Old male rats had an increase in hippocampal and cortical RS levels in comparison to young rats. Treatment of old rats with $(p\text{-ClPhSe})_2$ at the dose of 25 mg/kg, but not at 10 mg/kg, reversed the increase in hippocampal RS levels. Both doses of $(p\text{-ClPhSe})_2$ were effective in normalizing RS levels in cortex of old rats (Table 1).

Na^+ K^+ ATPase activity

Statistical analysis demonstrated significant changes in Na^+ K^+ ATPase activity in cortex ($F_{(3,\ 36)} = 5.35$; $P < 0.0038$) and hippocampus ($F_{(3,\ 36)} = 7.90$; $P < 0.0004$) of rats. In fact, the enzymatic activity was reduced in both brain structures of old male rats. At the dose of 25 mg/kg $(p\text{-ClPhSe})_2$ treatment was effective in protecting against inhibition of Na^+ K^+ ATPase activity in cortex and hippocampus of old rats (Table 1).

Discussion

Results of the present study provide evidence for the protective potential of $(p\text{-ClPhSe})_2$ on depressant-like action and cognitive impairment caused by aging in male rats. Our findings demonstrated that treatment of old male rats with $(p\text{-ClPhSe})_2$ for seven days was effective in reversing spatial memory deficit, depressant-like action as well as the increase of RS levels and the inhibition of Na^+ K^+ ATPase activity in cortex and hippocampus. Moreover, the modulation of 5-HT_{1A} and 5-HT_3 receptors may be at least partly involved in the antidepressant-like action elicited by $(p\text{-ClPhSe})_2$ in old male rats.

Researchers have started to investigate the possibility of a biological and/or clinical connection between the triad of symptoms: movement, cognition and emotion since these functions are all affected by the physiological aging process (Granholm et al. 2008). Here, we showed that the rearing behavior performed by old male rats in the OFT was reduced in comparison to young rats. In addition, old rats crossed less squares in the OFT, although not statistically significant. In accordance with a study carried out by Pardon et al. (2000), our results revealed that the advanced age can cause a reduction of exploratory behavior of rodents. In this context, treatment with $(p\text{-ClPhSe})_2$ for seven days did not protect against the impairment of exploratory behavior caused by aging. With agreement with Viggiano et al. (2006) that have reported the effects of Annurca apple-rich diet (characterized for a very high antioxidant power) on exploratory behavior of old rats, our data demonstrated that the antioxidant $(p\text{-ClPhSe})_2$ did not reverse the impairment in exploratory behavior in aged rats.

It is well-known that the aging involves impairment in cognitive function (Hofer et al. 2003). Regarding non-spatial memory, evaluated in the ORT, no significant memory impairment was found in old male rats treated or not with $(p\text{-ClPhSe})_2$. These results are consistent with those shown by Cavoy and Delacour (1993), who have found no aging-related deficits in object recognition task in rats. By contrast, old male rats had an age-related deficit in spatial memory as evidenced in the OLT, a hippocampal-dependent spatial memory task. Here, $(p\text{-ClPhSe})_2$ exerted an ameliorative action on spatial cognition since it caused an increase of the exploratory preference by new object location in aging rats. The cognitive modulation by $(p\text{-ClPhSe})_2$ is also supported by the fact that its administration did not alter the locomotor and exploratory activity of old rats in the OFT. $(p\text{-ClPhSe})_2$, an

enhancer of spatial memory, requires a relatively short time (subacute treatment) to modulate its molecular targets. In line with our results, Leite et al. (2011) also reported that beneficial effects of caffeine or SCH58261 on memory were obtained after 10 days of administration and interestingly this effect is linked to the reduction of RS levels and restoration of Na^+ K^+ ATPase activity in brain of age rats. Moreover, Shi et al. (2006) demonstrated that treatment for 7 days with *Alpinia* protocatechuic acid, a phenolic compound with antioxidant property, improves the cognition of aged rats in Y-maze and reduces the content of lipid peroxide, supporting the idea that 7 days of treatment are enough to modulate redox status and cognitive functions depending on the chemical compound.

The gradual development and extended time course of dementia and depression suggest some common underlying neuroplastic processes: (1) both diseases are described as dysfunctions of similar brain regions/net-work; (2) the same types of morphological changes in neural structures are observed; (3) neural structures that are involved in memory and mood regulation are vulnerable to stress; (4) major depression and dementia are known to be associated with dramatic brain structure shrinkage (e.g. hippocampus atrophy) and (5) mood and memory disorders share the same phenomena of an involvement of multiple transmitter systems (Sun and Alkon 2002). Since there is consistent evidence of an association between impaired memory performance and depression (Austin et al. 2001), we investigated the link between advanced age and development of experimental mood disorder. In this sense, an important aspect revealed by the present data is the role played by age in rat behavioral despair, since an increase of immobility duration (depressant-like action) in the FST was recorded in old male rats. Of particular importance, $(p\text{-ClPhSe})_2$ caused an improvement of the depressive-like status induced by age, since both doses of this organoselenium compound were effective in reversing the increased despair behavior in old rats. Likewise, other diselenides have been reported as antidepressant-like drugs in rodents (Savegnago et al. 2007; Brüning et al. 2011). Taken together these findings encourage additional studies to investigate the organic forms of selenium as food supplements, since supplements currently commercially available use inorganic selenium forms.

It is interesting to note that the brain is very sensitive to cumulative oxidative damage of proteins, lipids, and DNA that occurs during normal aging (Müller et al. 2010). In fact, antioxidant enzymes are decreased in aging brain (Kumar et al. 2011). So, a general decline in biochemical and physiological functions is noted with the age. In the present study, we demonstrated that aging led to an increase in RS levels as well as an inhibition of Na^+ K^+ ATPase activity in cortex and hippocampus of male rats. Na^+ K^+ ATPase is responsible for maintenance of the neuronal membrane integrity and is vital for a wide variety of neuronal functions, such as regulation of cell volume, restoration of Na^+ and K^+ gradients after neuronal excitation and energy supply (via Na^+ gradient) for other transport mechanisms (Na^+/Ca_2^+ exchange and uptake of neurotransmitters) (Lees 1991). A decline in Na^+ K^+ ATPase activity has been found in the brains of aging animals (Bagh et al. 2008; Leite et al. 2011). Thiol (–SH) groups of this enzyme, necessary for its catalytic action, are highly susceptible to oxidizing agents (Siems et al. 1996). Thus, the inhibition of Na^+ K^+ ATPase activity in cortex and hippocampus of old rats could be tentatively related to increased RS levels. The data reported by Bagh et al. (2008) showing that an inhibition of Na^+ K^+ ATPase activity is associated with an accumulation of peroxidative damage products in aged brain further reinforce this idea.

Furthermore, some authors have introduced the hypothesis that an inhibition of Na^+ K^+ ATPase activity in brain is associated with mood disorders (EI-Mallakh and Wyatt 1995; Gamaro et al. 2003) and cognitive impairments (Leite et al. 2011), which provide us plausible explanation to central impairment found in this study. Interestingly, we found that $(p\text{-ClPhSe})_2$ treatment for seven days, at the highest dose, restored RS levels and Na^+ K^+ ATPase activity modified by aging. A number of studies emphasize that organoselenium compounds can effectively scavenge and eliminate RS. In fact, diselenides can be good mimetics of glutathione peroxidase, glutathione *S*-transferase and dehydroascorbate reductase activities in vitro (Bhabak and Mugesh 2010; Luchese and Nogueira 2010). It is important to note that $(p\text{-ClPhSe})_2$ is effective in reducing lipoperoxidation in brain of rodents in vitro and that antioxidant activity is related to its thiol peroxidase like-properties (Meotti et al. 2004). Thus, we postulate that the antioxidant

action of $(p\text{-ClPhSe})_2$ could be in part responsible for its pharmacological effects. The antioxidant $(p\text{-ClPhSe})_2$ could lead to the reduction of RS levels and thus to maintenance of Na^+ K^+ ATPase activity, a sulfhydryl enzyme. These biochemical alterations could be related to reversing spatial memory impairment and depressant-like action induced by aging. However, we do not rule out the possibility of a direct interaction between the enzyme and $(p\text{-ClPhSe})_2$, since Nogueira and Rocha (2010) have suggested that redox modulation of specific high molecular weight thiol-containing molecules (e.g. redox sensitive enzymes and receptors) can contribute to the pharmacological effects of organochalcogens.

Taken these results together one can hypothesize that $(p\text{-ClPhSe})_2$ crosses the blood–brain barrier and then ameliorates spatial cognition and despair behavior in old rats. In support of this possibility Prigol et al. (2010) have reported that the parent compound of $(p\text{-ClPhSe})_2$, $(PhSe)_2$, has been detected in brains of rodents. We believe that pharmacological actions demonstrated in this study, after discontinuation of treatment, are assigned to administration of $(p\text{-ClPhSe})_2$. This assumption is closely related to the fact that the same treatment discontinuation was given to control animals, that instead of drug received vehicle. This idea is further supported by the fact that a chlorine substituent in organic molecules produces simultaneously an increase in lipophilicity and a metabolic obstruction (Bazzini and Wermuth 2008), which results in prolonged permanence in the body. Also, we do not rule out that $(p\text{-ClPhSe})_2$ can be biotransformed to active metabolite, however this hypothesis remains to be elucidated.

Another important point to be addressed is that $(p\text{-ClPhSe})_2$, at doses tested, did not elicit dose dependent effects on behavioral profile of aged rats, i.e., the dose was not directly related to the effectiveness of the drug since the doses used had similar effects. We suppose that $(p\text{-ClPhSe})_2$ needs low doses to exert its beneficial effects and that the dose of 10 mg/kg is a sufficient amount to reach a plateau of pharmacological efficacy. We demonstrated that although $(p\text{-ClPhSe})_2$ reversed the depressant-like action and spatial memory deficit without altering locomotor activity, it was not effective to recovery exploratory behavior impaired by age in rats. This fact indicates that although movement, cognition and emotion are all affected by the physiological aging process, $(p\text{-ClPhSe})_2$ at doses tested possibly modulates only pathways common to mood and memory.

In the brain, the proper monoaminergic signaling, i.e., regulation of neurotransmitter level such as serotonin, nor-epinephrine and dopamine, is regarded as one key mechanism for the modulation of mood and emotion (Mahesh et al. 2011). It is also important to know that molecular mechanisms and cascades that underlie memory may be shared by mood regulation. In fact, many antidepressants are reported to have positive impact on learning and memory. On the other hand, agents that appear to have memory-enhancing or antidementia value are frequently found to exhibit antidepressant activity in patients and animal depression models (Sun and Alkon 2002). For these reasons, we investigated the involvement of serotonin receptors and MAO activity in the antidepressant-like action elicited by acute administration of $(p\text{-ClPhSe})_2$ in old male rats. Moreover, this investigation was also performed in order to explain additional mechanisms involved in the pharmacological actions of $(p\text{-ClPhSe})_2$, since its administration at the dose of 10 mg/kg reversed memory impairment and depressant-like effect induced by aging, but did not recover significantly hippocampal and cortical Na^+ K^+ ATPase activity.

Therefore, we demonstrated that the pretreatment of old male rats with WAY100635 (5-HT_{1A} receptor antagonist) or ondansetron (5-HT_3 receptor antagonist) was effective in blocking the antidepressant-like action elicited by $(p\text{-ClPhSe})_2$, without influencing the baseline locomotion. Based on these results, we suggest that the modulation of 5-HT_{1A} and 5-HT_3 receptors is involved in the antidepressant-like action of $(p\text{-ClPhSe})_2$ in old rats. It is worth mentioning that an important drug used for the clinical treatment of anxiety and depression, buspirone, is a full agonist of presynaptic and a partial agonist of postsynaptic 5-HT_{1A} receptors (Butkevich et al. 2011). In addition, clinical and preclinical studies have suggested that 5-HT_3 receptors may be a relevant target in the treatment of affective disorders (Bétry et al. 2011). Lastly, hippocampal MAO A and/or B activity as well as $5\text{-HT}_{2A/2C}$ receptors seem not be involved in the antidepressant-like action of $(p\text{-ClPhSe})_2$. Based on the considerations above, we propose that $(p\text{-ClPhSe})_2$ by some common underlying mechanisms improved behavioral despair and enhanced spatial memory deficit in old rats. In the present study, experiments

were performed only in male rats, so the results obtained can not be extrapolated to female rats since ovarian hormones can exert a wide range of effects on central nervous system and thus on behavior (Foy 2011). However, future studies evaluating the gender influence on pharmacological action of $(p\text{-ClPhSe})_2$ in aged rats are quite relevant.

In summary, our findings demonstrate that $(p\text{-ClPhSe})_2$ protected against depressant-like action and cognitive impairment caused by aging in male rats. In addition to the antioxidant action and restoration of $Na^+ K^+$ ATPase activity, the modulation of 5-HT_{1A} and 5-HT_3 receptors may be at least partly involved in the antidepressant-like action elicited by $(p\text{-ClPhSe})_2$ in old male rats. Thus, these results strongly contribute to the research of novel therapeutic interventions for geriatric comorbidity of depression and memory deficit.

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