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Diphenyl diselenide exerts antidepressant-like and anxiolytic-like effects in mice: Involvement of L-arginine-nitric oxide-soluble guanylate cyclase pathway in its antidepressant-like action

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Abstract

This study investigated the possible antidepressant-like and anxiolytic-like effects of diphenyl diselenide, $(PhSe)_2$ in mice. The involvement of L-arginine-nitric oxide (NO)-cyclic guanosine monophosphate (cGMP) pathway in the antidepressant-like effect was also evaluated. The immobility times in the tail suspension test (TST) and forced swimming test (FST) were reduced by $(PhSe)_2$ (5–100mg/kg; oral route, p.o.). The antiimmobility effect of $(PhSe)_2$ (5mg/kg, p.o.) in the TST was prevented by pretreatment of mice with L-arginine [a substrate for nitric oxide synthase (NOS)], methylene blue [an inhibitor of NO synthase and sGC] and sildenafil [a phosphodiesterase 5 inhibitor]. Furthermore, a sub-effective dose of $(PhSe)_2$ (0.1mg/kg, p.o.) produced a synergistic antidepressant-like effect with N^G-nitro-L-arginine [L-NNA; 0.3mg/kg, i.p. inhibitor of NOS], (1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one [ODQ; 30pmol/site i.c.v., a specific inhibitor of soluble guanylate cyclase (sGC)], fluoxetine and imipramine in the TST. $(PhSe)_2$ (50–100mg/kg, p.o.) induced anxiolytic-like effect in the elevated plus-maze test and light/dark box. Together the results indicate that $(PhSe)_2$ elicited significant antidepressant-like and anxiolytic-like effects. The antidepressant-like action caused by $(PhSe)_2$ seems to involve an interaction with L-arginine-NO-cGMP pathway.

Keywords: Antidepressant-like; Anxiolytic-like; Diphenyl diselenide; Selenium

1. Introduction

A number of novel pharmaceutical agents, which are selenium-based or which target specific aspects of selenium metabolism, are under development due to a variety of organoselenium compounds that possess pharmacological activity (Nogueira et al., 2003a,b, 2004). In fact, organoselenium compounds were found to have antioxidant (Meotti et al., 2004), neuroprotective (Porciúncula et al., 2001; Rossato et al., 2002), antihypertensive, anticancer, antiviral, immunosuppressive, antimicrobial and antiinflammatory properties (Sies, 1993; Nogueira et al., 2003a; Zasso et al., 2005; Savegnago et al., 2007).

Diphenyl diselenide $(PhSe)_2$, a simple diaryl diselenide, is active as a glutathione peroxidase mimic and a safe drug when administered acutely to mice at doses that have antiinflammatory and antinociceptive activities (Nogueira et al., 2003a,b; Meotti et al., 2004). Moreover, recently our research group has reported that the mechanism(s) underlying the antinociceptive action of diphenyl diselenide involves serotoninergic and glutamatergic systems as well as L-arginine-nitric oxide pathway (Zasso et al., 2005; Savegnago et al., 2007).

$(PhSe)_2$ affects a number of neuronal processes e.g. inhibits the cerebral aminolevulinic acid dehydratase from mice brain in vitro (Maciel et al., 2000), increases basal activity of adenylyl cyclase and inhibits [^{3}H]glutamate and [^{3}H]MK-801 binding to

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rat synaptic membranes (Nogueira et al., 2001). On the other hand, $(PhSe)_2$ displays cognitive enhancing properties without inducing behavioral neurotoxicity (Rosa et al., 2003b.). Because of these previous findings, it is important to further evaluate the potential beneficial or toxic effects of this compound using behavioral endpoints of neural function.

Although much research on depression and anxiety has focused on brain noradrenergic, serotoninergic and dopaminergic systems, there are substantial evidences that other systems have important roles in the neurobiology of mood and affective disorders. In this context, several studies have demonstrated that arginine–nitric oxide (NO)-soluble guanylate cyclase (sGC) pathway is involved in the pathophysiology of depression (Dhir and Kulkarni, 2007; Almeida et al., 2006; Kaster et al., 2005; Rosa et al., 2003a; Mantovani et al., 2003; Rodrigues et al., 2002). NO is a signaling molecule in the brain and has been implicated in neurotransmission, synaptic plasticity, learning, perception of pain, aggression (Esplugues, 2002), anxiety and depression (Wiley et al., 1995; da Silva et al., 2000; Harkin et al., 2004; Kaster et al., 2005; Almeida et al., 2006). Moreover, several studies have shown that nitric oxide synthase (NOS) inhibitors have antidepressant- and anxiolytic-like effects in animal models (Faria et al., 1997; Harkin et al., 1999; da Silva et al., 2000; Karolewicz et al., 2001; Volke et al., 2003). In agreement, the administration of NOS inhibitors caused an increase in the effects of 5-HT reuptake inhibitors in the forced swimming test (FST) (Harkin et al., 2004).

Further support for the hypothesis that the inhibition of NO synthesis, with a subsequent decrease in the concentration of cyclic guanosine monophosphate (cGMP) (Snyder, 1992), may cause antidepressant-like effects, at least under certain conditions, comes from the reported reduction in the immobility time in the FST elicited by the administration of the specific inhibitor of sGC activity, ODQ (Heiberg et al., 2002).

Therefore, based on the considerations above, the present study was undertaken to investigate whether diphenyl diselenide causes antidepressant-like effect, employing forced swimming test (FST) and tail suspension test (TST), and anxiolytic-like effect, employing elevated plus-maze test and light/dark box, in mice. Moreover, we investigated whether the effect of diphenyl diselenide on the TST is dependent of its interaction, directly or indirectly, with L-arginine-NO-cGMP pathway.

2. Materials and methods

2.1. Experimental animals

The behavioral experiments were conducted using Swiss mice of either sex (25–35g) maintained at 22–25°C with free access to water and food, under a 12:12hour 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

Diphenyl diselenide $(PhSe)_2$ was prepared and characterized in our laboratory by the method previously described (Paulmier, 1986). 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 diphenyl diselenide (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, diphenyl diselenide that was dissolved in canola oil and (1*H*-[1,2,4] oxadiazolo[4,3-*a*]quinoxalin-1-one) (ODQ) which was dissolved in 1% DMSO The mice received all drugs in a constant volume of 10ml/kg body weight, except ODQ which was administered by intracerebroventricular route (i.c.v.) route (5μL/site). Appropriate vehicle- treated groups were also assessed simultaneously.

2.3. Apparatus

2.3.1. Forced swimming test (FST)

The test was conducted using the method of Porsolt et al. (1977). Briefly, mice were individually forced to swim an open cylinders (25 cm height × 10 cm diameter) containing 19cm of water at 25 ± 1°C. The duration of immobility was scored during the 6min test period as described previously (Zomkowski et al., 2002). Each mouse was recorded as immobile when floating motionless or making only those movements necessary to keep its head above water. Animals were treated with diphenyl diselenide (0.1–100mg/kg, p.o. 30min before) or with canola oil (10mL/kg, p.o.) before being tested.

2.3.2. Tail suspension test (TST)

The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al. (1985). Mice were suspended on the edge of a table 50cm above the floor by the adhesive tape placed approximately 1cm from the tip of the tail. Immobility time defined as the absence of escape oriented behaviours, such as swimming, was scored during 6min, as described previously (Rodrigues et al., 2002; Mantovani et al., 2003). Mice were treated with diphenyl diselenide (0.1–100mg/kg, p.o. 30min before) or with canola oil (10mL/kg, p.o.) before being tested.

To address the role played by the L-arginine-nitric oxide-soluble guanylate cyclase pathway in the antidepressant-like effect caused by diphenyl diselenide in the TST distinct groups of animals were treated with different classes of drugs. For this purpose, mice were pretreated with L-arginine, a precursor of NO (750mg/kg, i.p. a dose that produces no effect in the TST), methylene blue, an inhibitor of NO synthase and sGC (18mg/kg i.p. a dose that produces no effect in the TST) or sildenafil, a specific type 5 phosphodiesterase (PDE5) inhibitor, (5mg/kg, i.p. a dose that produces no effect in the TST). Thirty minutes after L-arginine, methylene blue or sildenafil, diphenyl diselenide (5mg/kg, p.o., a

dose active in the TST) or canola oil was injected, and 30min later the TST was carried out.

In another set of experiments, we investigated the synergistic effect of diphenyl diselenide (0.1mg/kg, p.o., a sub-effective dose) with sub-effective dose of L-NNA (0.3mg/kg, i.p., an inhibitor of NOS) or ODQ (30pmol/site, i.c.v., a specific sGC inhibitor). Diphenyl diselenide or vehicle was administered 30min before the drugs. A further 30min (after i.p. administration of L-NNA) or 15min (after i.c.v. ODQ administration) the TST was carried out.

We also investigated if diphenyl diselenide is capable of potentiating the antidepressant-like effect of fluoxetine and imipramine. To this end, mice were pretreated with a sub-effective dose of diphenyl diselenide (0.1mg/kg, p.o.) or vehicle, and 15min later, the animals were treated with fluoxetine (5mg/kg, i.p.) or imipramine (5mg/kg, i.p.). About 30min later, the TST was carried out.

2.3.3. Elevated plus-maze

This test has been widely validated to measure anxiety in rodents (Pellow et al., 1985). The apparatus consists of two elevated (26cm high) and open arms (16×5cm) positioned opposite to one another and separated by a central platform (5×5cm) and two arms of the same dimension, but enclosed by walls (16×5×10cm) forming a cross. The maze is lit by a dim light placed above the central platform. Thirty minutes after the p.o. injection of diphenyl diselenide (10, 25, 50 and 100mg/kg) or canola oil (vehicle), each mice was placed at the center of the maze, facing one of the open arms. During a 5min test period, the number of entries either the open and enclosed arms, plus the time spent in the open arms was recorded. An entry was defined as placing all four paws within the boundaries of the arm. The following measures were obtained from the test: (1) time spent in the open arms relative to the total time spent in the plus-maze (300s), expressed as percentage; (2) 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) (Clénet et al., 2006).

2.3.4. Light–dark box

The light–dark box is a sensitive model to detect activity in disorders related to generalize anxiety (Costall et al., 1989). 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 (Crawley and Goodwin, 1980). The apparatus consisted of two compartments: an open topped rectangular box (46×27×30 cm high), is divided into a small (18×27 cm) area and a large (27×27 cm) area with an opening door (7.5×7.5 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 60W bulb located 25cm 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 5min after 30min of injection of diphenyl diselenide (10, 25, 50 and 100mg/kg, p.o.) or canola oil (vehicle). Anxiolytic activity could be evaluated by time spent in the illuminated compartment and the number of transitions.

2.3.5. Open-field test

The locomotor and exploratory behavior was assessed in an open-field test. The open-field was made of plywood and surrounded by walls 30cm in height. The floor of the open-field, 45cm in length and 45cm in width, was divided by masking tape markers into 09 squares (3 rows of 3). Animals were evaluated 30min after a single oral dose of canola oil (vehicle) or diphenyl diselenide (0.1–100mg/kg, p.o). Each animal was placed individually at the center of the apparatus and observed for 6min 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) (Walsh and Cummins, 1976).

To verify whether the administration of diphenyl diselenide with methylene blue impairs motor abilities mice were pretreated with methylene blue (18mg/kg, i.p.) and thirty minutes after diphenyl diselenide ($(PhSe)_2$ 5mg/kg, p.o.) or canola oil was injected. Thirty minutes later, the open field was carried out.

2.3.6. Rotarod task

To avoid unspecific effects due to motor impairment, the integrity of motor system was evaluated using the rotarod test. Briefly, the rotarod apparatus consists of a rod 30cm long and 3cm in diameter that is subdivided into three compartments by discs 24cm in diameter. The rod rotates at a constant speed of 10rpm. The animals were selected 24h previously by eliminating those mice that did not remain on the bar for two consecutive periods of 60s. Animals were treated with diphenyl diselenide (0.1–100mg/kg, p.o) or with vehicle (canola oil, p.o.) and were tested in the rotarod 30min after treatment. The latency for first fall off from the rod and number of falls were noted. The cut-off time was 60s.

2.4. Statistical analysis

The results are presented as the mean±S.E.M. The statistically significant difference between groups was calculated by means of one-way or two-way analysis of variance (ANOVA) followed by Duncan's test when appropriate. Probability values less than 0.05 ($P<0.05$) were considered as statistically significant.

3. Results

3.1. Antidepressant-like effect induced by diphenyl diselenide on the FST and TST

The immobility time in the TST and FST of animals treated with diphenyl diselenide is shown in Fig. 1A and B, respectively. One-way ANOVA revealed a significant effect of diphenyl

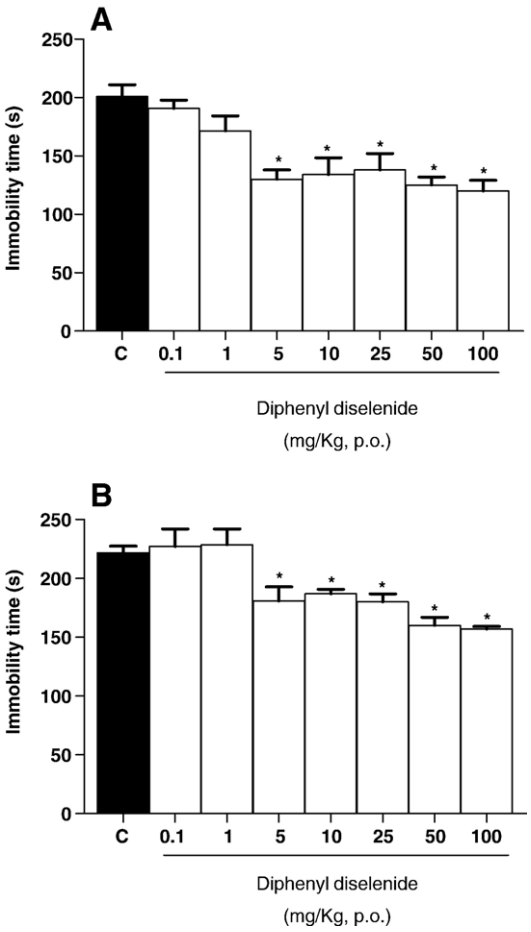

Fig. 1. Effect of acute administration of diphenyl diselenide on tail suspension test (A) and forced swimming test (B) in mice. Diphenyl diselenide (dose range: 0.1–100mg/kg) was administered orally 30min before the test. Values are expressed as mean±S.E.M. (n=9–12 mice/group-tail suspension test; n=7–9 mice/group-forced swimming test). * P<0.01 compared to the vehicle treated group (control; C).

diselenide in both TST (F(7, 60)=5.88, P<0.01) and FST (F(7, 57)=11.42, P<0.01).

3.2. Analysis of the role played by the L-arginine-nitric oxide-soluble guanylate cyclase pathway in the antidepressant-like effect of diphenyl diselenide on tail suspension test

The results depicted in Fig. 2A show that pretreatment of mice with L-arginine (750mg/kg, i.p., a NO precursor) significantly inhibited the reduction in immobility time elicited by diphenyl diselenide (5mg/kg) in TST. Two-way ANOVA revealed significant difference of pretreatment (F(1, 47)=4.97, P<0.05) and treatment (F(1, 47)=10.24, P<0.01) and pretreatment×treatment interaction (F(1, 47)=4.093, P<0.05).

Fig. 2B shows that the pretreatment of animals with methylene blue (18mg/kg i.p., an inhibitor of NO synthase and sGC) significantly inhibited the reduction in immobility time elicited by diphenyl diselenide (5mg/kg) in TST. Two-way ANOVA revealed significant difference of pretreatment (F(1, 37)=6.75, P<0.0134) and treatment (F(1, 37)=26.12, P<0.0001) and pretreatment×treatment interaction (F(1, 37)=30.99, P<0.0002).

Fig. 2C shows that the pretreatment of animals with sildenafil (5mg/kg i.p., a PDE5 inhibitor) which per se produced no effect in the TST, was able to prevent the reduction in the immobility time elicited by diphenyl diselenide (5mg/kg). Two-way ANOVA showed a significant effect of pretreatment (F(1, 40)=17.31, P<0.0002) and treatment (F(1, 40)=14.89, P<0.0004) and pretreatment×treatment interaction (F(1, 40)=23.91, P<0.0001).

These findings presented in Fig. 2 are conflicting; in fact, NOS agonists and antagonist reverse the effect of diphenyl

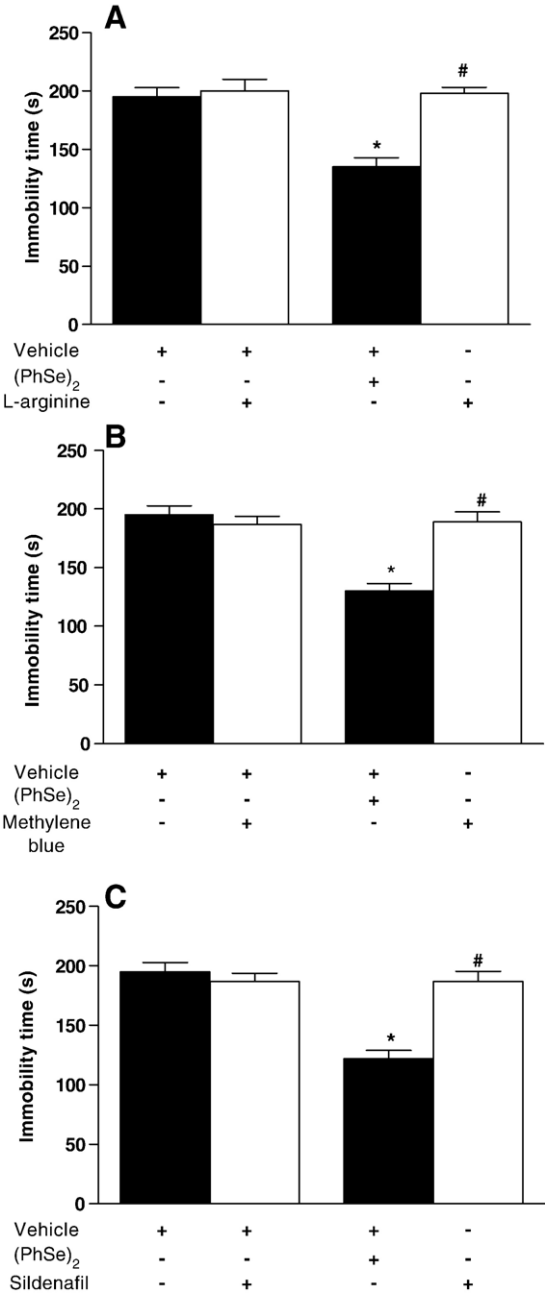

Fig. 2. Effect of pretreatment of mice with (A) L-arginine (750mg/kg, i.p.), (B) methylene blue (18mg/kg, i.p.) and (C) sildenalfil (5mg /kg, i.p.) on diphenyl diselenide (5mg/kg, p.o.)-induced reduction in immobility time in TST in mice. Values are expressed as mean±S.E.M. (n=9–12 mice/group). * P<0.05 compared to the vehicle treated group (control; C) and # P<0.05 compared to the diphenyl diselenide group pretreated with vehicle.

Table 1
Effect of diphenyl diselenide and methylene blue administration on number of crossings in the open-field test in mice

Experimental groups	Number of crossings
Vehicle	61.4±2.8
$(PhSe)_2$	52.6±3.0
Methylene blue	33.1±3.6*
$(PhSe)_2$ × Methylene blue	23.0±3.5*#

Mice were pretreated with methylene blue (18 mg/kg, i.p.) and 30 min after diphenyl diselenide ($(PhSe)_2$ 5 mg/kg, p.o.) or canola oil was injected, and 30 min later the open-field was carried out. Values are expressed as mean±S.E.M. (n=6–7 mice/group). * $P<0.05$ compared to the vehicle treated group (control) and # $P<0.05$ compared to the diphenyl diselenide group pretreated with vehicle.

diselenide in the tail suspension test. To clarify this hypothesis we verified whether methylene blue when administered in combination with diphenyl diselenide could impair motor abilities. Table 1 shows that methylene blue alone and when administered in combination with diphenyl diselenide caused a reduction in the locomotor activity assessed in the open-field. Thus, these results demonstrated that the effect caused by methylene blue in association with diphenyl diselenide in the tail suspension (Fig. 2B) occurred due to the sedative effect induced by them, suggesting that the effect on TST caused by diphenyl diselenide was a non-specific effect.

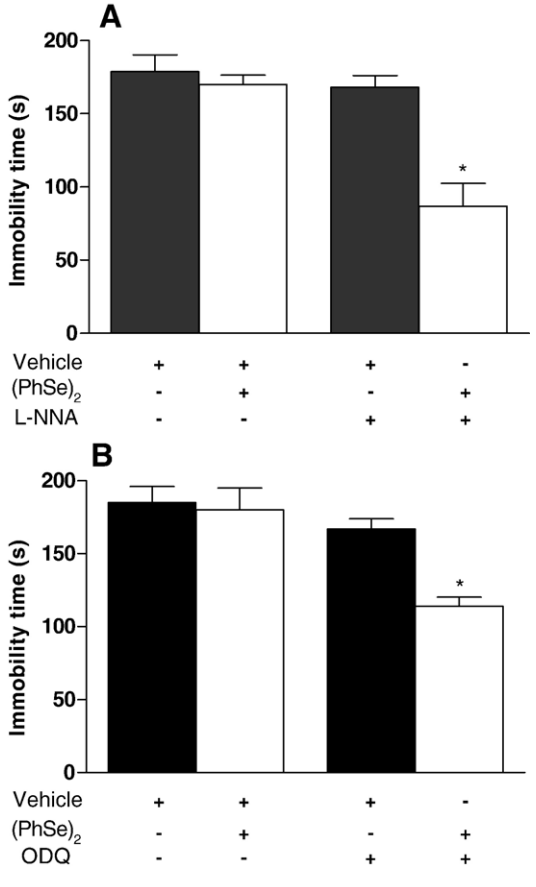

Fig. 3. Effect of (A) LNNA (0.3mg/kg, i.p.) or (B) ODQ (30 pmol/site, i.c.v) in potentiating the actions of diphenyl diselenide (0.1mg/kg, p.o.) in the TST in mice. Values are expressed as mean±S.E.M. (n=5–6 mice/group). * $P<0.05$ compared to vehicle treated group (control) and # $P<0.05$ compared to the same group pretreated with vehicle.

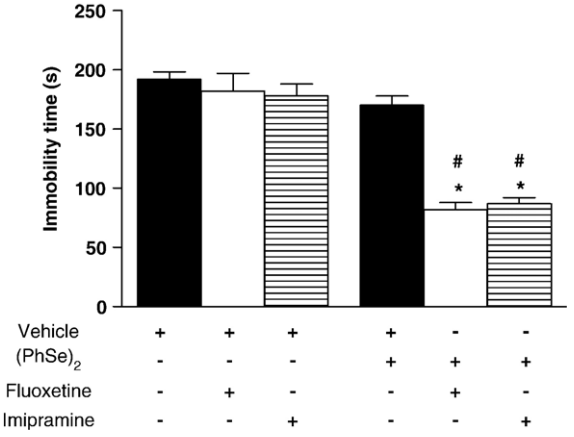

Fig. 4. Effect of pretreatment of mice with diphenyl diselenide (0.1 mg/kg, p.o.) on potentiating the actions of sub-effective doses of fluoxetine (5 mg/kg, i.p.) and imipramine (5 mg/kg, i.p.) in the TST. Values are expressed as mean±S.E.M. (n=5–7 mice/group). # $P<0.01$ compared to the diphenyl diselenide group and * $P<0.01$ compared to the antidepressant (fluoxetine or imipramine) treated group.

Fig. 3A shows that the treatment of animals with a sub-effective dose of diphenyl diselenide (0.1mg/kg, p.o.) produced a synergistic antidepressant-like effect with L-NNA (0.3mg/kg, i.p., an inhibitor of NO synthase). The two-way ANOVA showed a significant effect of pretreatment ($F(1, 18)=14.22$, $P<0.00014$) and treatment ($F(1, 18)=21.33$, $P<0.0002$) and pretreatment × treatment interaction ($F(1, 18)=14.22$ $P<0.0014$).

The results depicted in Fig. 3B show that a sub-effective dose of diphenyl diselenide (0.1mg/kg, p.o.) also produced a synergistic effect with ODQ (30pmol/site, i.c.v., a specific inhibitor sGC). These results were analyzed by two-way ANOVA, which indicated a significant effect of pretreatment ($F(1, 18)=10.36$, $P<0.0048$) and treatment ($F(1, 18)=24.33$, $P<0.0001$) and pretreatment × treatment interaction ($F(1, 18)=10.37$, $P<0.0049$).

Fig. 4. shows the influence of the pretreatment with a sub-effective dose of diphenyl diselenide (0.1mg/kg, p.o.) given in combination with a sub-effective dose of fluoxetine (5mg/kg, i.p.) or imipramine (5mg/kg, i.p.) on the immobility time in the TST. A two-way ANOVA revealed significant differences for the

Table 2
Effects of diphenyl diselenide administration on behavioural parameters in the elevated plus-maze test in mice

Experimental groups	% open arm entries	% time spent open arms
Vehicle	21.1±2.7	7.1±1.6
$(PhSe)_2$ (mg/kg)		
10	17.9±3.5	8.2±2.3
25	15.7±2.8	10.3±3.2
50	25.4±2.4	17.9±2.8*
100	33.1±5.3*	15.7±1.8*

Diphenyl diselenide ($(PhSe)_2$; 10–100 mg/kg) was administered, p.o., 30 min before the test. The effect on mouse behavioural in the elevated plus-maze test was determined by one-way ANOVA followed by Duncan's test. Data presented are mean values±S.E.M. (n=8–10 mice/group). *$P<0.05$ relative to the vehicle group (control).

Table 3
Effects of diphenyl diselenide administration on behavioural parameters in the light/dark test in mice

Experimental groups	Latency (L→D)	Transitions	Time in the light
Vehicle	18.4±6.6	11.6±1.2	108.4±15.5
$(PhSe)_2$ (mg/kg)			
10	16.9±6.7	14.1±2.3	102.3±13.4
25	8.0±2.6	8.7±1.7	108.9±18.1
50	21.5±9.0	14.0±1.3	108.0±13.4
100	47.3±9.8*	13.3±1.4	166.2±7.2*

Diphenyl diselenide ($(PhSe)_2$; 10–100 mg/kg) was administered, p.o., 30 min before the light/dark test. The effect on mouse behavioural in the light/dark test was determined by one-way ANOVA followed by Duncan's test. Data presented are mean values±S.E.M. (n=10–12 mice/group). *P<0.05 relative to the vehicle group (control).

treatment with fluoxetine (F(1, 18)=26.54, P<0.0001), pretreatment (F(1, 18)=10.89, P<0.0040) and pretreatment×treatment interaction (F(1, 18)=9.50, P<0.0064). In addition, there was a significant effect of pretreatment with imipramine (F(1, 17)=30.02, P<0.0000), treatment (F(1, 17)=17.18, P<0.007), and of treatment×pretreatment interaction (F(1, 17)=15.31, P<0.0011). Therefore, these results demonstrated that the administration of a sub-effective dose of diphenyl diselenide (0.1mg/kg, p.o.) produced a synergistic antidepressant-like effect with fluoxetine and imipramine in the TST.

3.3. Anxiolytic-like effect induced by diphenyl diselenide on the elevated-plus-maze test and light/dark box

Diphenyl diselenide administered by p.o. route demonstrated anxiolytic-like action in the elevated-plus-maze test and light/dark box. In fact, one-way ANOVA revealed a significant effect caused by diphenyl diselenide on percentage of time spent on open arms and percentage of total number of arm entries (F(4, 39)=3.26, P<0.05; F(4, 39)=4.00, P<0.01, respectively) in the elevated -plus-maze test. Post hoc analysis showed that doses of 50 and 100mg/kg of diphenyl diselenide induced a significant increase in percentage of time spent on open arms, but only at 100mg/kg diphenyl diselenide induced a significant increase in percentage of total number of arm entries (Table 2).

Table 4
Effect of diphenyl diselenide administration on behavioural parameters in the open-field test in mice

Experimental groups	Number of crossings	Number of rearings
Vehicle	64.8±3.8	26.8±1.5
$(PhSe)_2$ (mg/kg)		
0.1	66.0±6.7	23.1±3.0
1.0	53.7±4.5	19.1±1.2
5.0	61.5±4.3	29.0±3.2
10.0	55.0±7.2	33.9±2.8
25.0	57.7±7.2	25.6±2.7
50.0	52.0±2.9	25.5±2.2
100.0	54.0±5.2	29.1±3.0

Diphenyl diselenide ($(PhSe)_2$;0.1–100 mg/kg) was administered, p.o., 30 min before the test. The effect on mouse behavioural in the open-field test was determined by one-way ANOVA followed by Duncan's test. Data presented are mean values±S.E.M. (n=7–10 mice/group).

Table 5
Effect of diphenyl diselenide administration on behavioural parameters in the rotarod test in mice

Experimental groups	Number of falls	Time of latency
Vehicle	1.0±0.6	51.6±5.8
$(PhSe)_2$ (mg/kg)		
0.1	0.5±0.3	56.0±2.6
1.0	0.4±0.2	53.2±4.4
5.0	0.3±0.3	55.5±4.5
10.0	0.6±0.4	53.3±6.1
25.0	0.6±0.6	52.0±8.0
50.0	0.8±0.4	47.0±8.7
100.0	0.5±0.3	51.1±6.2

Diphenyl diselenide ((PhSe); 0.1–100 mg/kg) was administered, p.o., 30 min before the test. The effect on mouse behavioural in the rotarod test was determined by one-way ANOVA followed by Duncan's test. Data presented are mean values±S.E.M. (n=7–8 mice/group).

Moreover, the oral administration of diphenyl diselenide, at the dose of 100mg/kg, induced a significant increment of time spent by mice on the illuminated side (F(4, 68)=2.63, P<0.01) and on the latency of the first transition (F(4, 68)=3.74, P<0.01) of the light–dark apparatus. However, the number of transitions in the light–dark apparatus was not significantly different from the control group (F(4, 68)=1.74, P=0.151) (Table 3).

3.4. Effects caused by diphenyl diselenide on the open field and rotarod tests

Diphenyl diselenide given by p.o. route, at all doses tested, did not produce any change in numbers of crossings (F(7, 76)=1.14, P=0.34) and rearing (F(7, 76)=2.08, P=0.059) in the open-field test (Table 4). In this way, diphenyl diselenide did not differ statistically from the control value in the latency for the first fall (F(7, 37)=0.21, P=0.979) and in the number of falls (F(7, 37)=0.23, P=0.981) on the rotarod test (Table 5).

4. Discussion

In the present study, we demonstrated, for the first time, that diphenyl diselenide orally administrated is effective in producing significant antidepressant-like and anxiolytic-like effects, without modifying the motor performance, locomotor and exploratory activities. Diphenyl diselenide, an organoselenium compound, has been also known by its antioxidant, antinociceptive, antiinflammatory, antihyperglycemic, hepatoprotective and antiulcer actions (Rossato et al., 2002; Barbosa et al., 2006; Borges et al., 2006; Savegnago et al., 2006, 2007) and by protecting haloperidol-induced orofacial diskinesia (Burger et al., 2006). Moreover, Machado et al. (2006) reported that systemic administration of a substituted diphenyl diselenide induces an antipsychotic-like effect without affecting the open-field behavior or memory in mice. In this way, it is important to mention that the Machado findings corroborate with our results since standard antipsychotics have long been used by clinicians to treat high degrees of agitation and anxiety in non-psychotic patients with severe personality disorders (Soloff, 1987; Kaplan,

2000). Thus, antipsychotics are widely used in the treatment of affective disorders, anxiety, restlessness and agitation.

In this study, diphenyl diselenide, giving by p.o. route, caused antidepressant-like effect when assessed in two animal models predictive of antidepressant action of drugs in humans, the FST and TST. It has been argued that the TST is less stressful than FST and has greater pharmacological sensitivity (Thierry et al., 1986). The FST and TST are widely-accepted stress models of depression used to screen new antidepressant drugs, as they are sensitive to all major classes of antidepressant drugs including tricyclics, serotonin-specific reuptake inhibitors, monoamine oxidase inhibitors, and atypicals (Porsolt et al., 1977; Steru et al., 1985; Willner, 1991; Zomkowski et al., 2002). However, these tests do not have an identical neurochemical basis. Thus, the fact that diphenyl diselenide administration is active in both tests reinforces the assumption that diphenyl diselenide might play a role in the modulation of depression.

It has become generally accepted that nitric oxide (NO) plays a significant neuromodulatory role in the nervous system and pharmacological manipulation of the NO pathway may constitute a novel therapeutic approach for the treatment of depression (da Silva et al., 2000; Yildiz et al., 2000; Karolewicz et al., 2001; Volke et al., 2003).

In this study, we showed that the pretreatment of mice with L-arginine (a substrate for NOS) at doses that did not produce any effect in the TST, significantly inhibited the antiimmobility effect caused by diphenyl diselenide. Furthermore, a synergistic antidepressant-like effect was observed when diphenyl diselenide was administered with L-NNA, an inhibitor of NOS. Accordingly, some studies reported that, at least under some experimental conditions, administration of NOS inhibitors, depending on their doses, produces antidepressant-like effects in the FST and in the TST (da Silva et al., 2000; Yildiz et al., 2000; Karolewicz et al., 2001; Volke et al., 2003; Harkin et al., 2004). In fact, da Silva et al. (2000) have reported that L-NNA (0.3–10mg/kg, i.p), an inhibitor of NOS, significantly reduced the duration of immobility both in the FST and the TST. However, high dose of L-NNA (30mg/kg) did not produce any antiimmobility effect in FST and TST. Thus, these results indicate that the inhibition of NO synthesis may underlie the reduction in the immobility time in the TST elicited by diphenyl diselenide.

The assumption that the L-arginine-NO-cGMP pathway is involved in the reduction in the immobility time elicited by diphenyl diselenide in the TST is reinforced by the finding that diphenyl diselenide and ODQ (a NO-sensitive inhibitor of sGC), at sub-effective doses, produced a synergistic antidepressant-like effect. Accordingly, Heiberg et al. (2002) reported that ODQ significantly decreased the immobility time in the FST in rats, an effect that was reversed by L-arginine (a NO precursor). In fact, Kaster et al. (2005) have reported that ODQ, depending on its concentration causes a reduction in the immobility time in the FST in mice.

Another finding of the present study was the reversal of the antidepressant-like effect caused by diphenyl diselenide by pretreatment with sildenafil (a selective PDE5 inhibitor), which indicates that diphenyl diselenide exerts its effect in the TST by decreasing cGMP levels. Sildenafil is a selective PDE5 inhibitor that increases the cGMP level in target tissues (Beavo, 1995). The intracellular cGMP concentrations are regulated not only by sGC, but also by PDE, which catalyses the hydrolysis of the second messengers cAMP and cGMP. The duration and magnitude of a NO-induced cGMP signal are determined by the activity of PDE5 (Beavo, 1995). PDE5 is expressed in several brain areas, particularly in the neurons of the Purkinje cell layer in the cerebellum (Bender and Beavo, 2004) and in the pyramidal neurons of the hippocampus (Van Staveren et al., 2004).

Accordingly, the antidepressant-like effect of adenosine (Kaster et al., 2005), memantine (Almeida et al., 2006) and venlafaxine (Dhir and Kulkarni, 2007) was also potentiated by pretreatment with ODQ and/or LNNA, and prevented by sildenafil and L-arginine, suggesting that L-arginine-NO-cGMP route has an important role in the mediation of their behavioural effects (Kaster et al., 2005; Almeida et al., 2006; Dhir and Kulkarni, 2007).

Based on the considerations above, our findings clearly suggest that the acute administration of diphenyl diselenide produces antidepressant-like effect on the tail suspension test in mice by a mechanism that involves the inhibition of L-arginine-NO-cGMP pathway. In fact, antidepressant-like effect caused by diphenyl diselenide was prevented by sildenafil and L-arginine (drugs that enhance the levels of cGMP and NO, respectively) and potentiated by ODQ (a sensitive inhibitor of sGC) and LNNA (an inhibitor of NOS). The findings obtained with methylene blue together with diphenyl diselenide in the TST were clarified in the open-field test. In fact, the effect caused by methylene blue in association with diphenyl diselenide in the tail suspension occurred due to the sedative effect induced by them, suggesting that the effect on TST caused by diphenyl diselenide was a non-specific effect. Therefore, we concluded that L-arginine-nitric oxide-soluble guanylate cyclase pathway is in fact involved in the antidepressant-like effect caused by diphenyl diselenide.

Another result which reinforces that diphenyl diselenide may induce antidepressant-like effect by modulating NOS is that this compound potentiates the effects of fluoxetine (a selective serotonin (5-HT) reuptake inhibitor) and imipramine (a noradrenaline (NA) and a 5-HT reuptake inhibitor with affinity for various neurotransmitter receptors). In agreement, Harkin and coworkers (2004) have reported that NOS inhibitors augmented the behavioural effects of imipramine and fluoxetine, and that the inhibition of NOS enhanced the activity of antidepressants that work via a serotoninergic mechanism. Moreover, Harkin et al. (2003) reported that the antidepressant-like profile of NOS inhibitors is similar to that of selective inhibitors of serotonin reuptake (SSRIs). Then, the inhibition of NO synthase can result in an antidepressant-like and/or an augmented antidepressant activity.

The current study showed also that oral treatment of mice with diphenyl diselenide caused an anxiolytic-like effect in two animal models of anxiolytic action of drugs, the elevated plus-maze and light–dark box. Indeed, mice treated with diphenyl diselenide (50 and 100mg/kg, p.o.) induced a significant increase in the percentage of time spent on open arms and in the percentage of total number of arm entries in the elevated

plus-maze test, indicating anxiolytic-like effect. Our results indicated that diphenyl diselenide (100mg/kg, p.o.) induced a significant increment of time spent by mice on the illuminated side and of the latency for the first transition in the light–dark box. Accordingly, Kilfoil et al. (1989) have demonstrated that the time spent in light compartments is a more sensitive measure to indicate the anxiolytic action of drugs than the number of transitions in the light–dark apparatus.

Based on these findings, it could be supposed that diphenyl diselenide reduces the anxiety of animals exposed to these paradigms (elevated plus-maze and light/dark box) and could exert its effect through an action mechanism similar to that of the benzodiazepines (Pellow et al., 1985; Kilfoil et al., 1989; Volke et al., 2003), although there are several anxiolytic substances with different action mechanisms through noradrenergic, serotoninergic, and glutamatergic receptors (Dawson and Tricklebank, 1995).

In addition, the antidepressant-like and anxiolytic-like effects of diphenyl diselenide seem not be associated with any motor effects when assessed in the open-field test and rotarod task. Moreover, this indicates that alterations in motor activity were not involved in the action found in the TST, FST, elevated plus-maze and light/dark box and confirms the assumption that the antidepressant- and anxiolytic-like effects of diphenyl diselenide are specific.

Furthermore, it is very important to mention that diphenyl diselenide, at doses that has antidepressant-like and anxiolytic-like effects, does not induce toxic effects (Savegnago et al., 2007). Accordingly, doses in which diphenyl diselenide has potential therapeutic properties (Nogueira et al., 2004; Zasso et al., 2005; Borges et al., 2006; Barbosa et al., 2006; Savegnago et al., 2007, 2006) do not cause toxicity. On the other hand, diphenyl diselenide has potential toxicity in different experimental models, e.g., chronic exposure of mice to high doses of diphenyl diselenide affected the central nervous system, causing seizures, and impairment of glutamatergic transmission as well as liver and renal toxicity (Nogueira et al., 2003b; Brito et al., 2006). In microorganism models, diphenyl diselenide induced frameshift mutations in both *Salmonella typhimurium* and haploid yeast as well as increased crossing over and gene conversion frequencies in diploid strains of *Saccharomyces cerevisiae* (Rosa et al., 2004) and had pro-oxidant activity (Rosa et al., 2005). Recently, Rosa et al. (2007) have also reported the cytotoxic, genotoxic, and mutagenic effects of diphenyl diselenide in Chinese hamster lung fibroblasts.

The results presented here show convincing pharmacological evidence of diphenyl diselenide antidepressant-like and anxiolytic-like effects in mice. Moreover, our findings demonstrated that the antidepressant- like effect of diphenyl diselenide is mediated, at least in part, by an interaction with L-arginine/NO/cGMP pathway. However, additional research will be necessary to explain the exact mechanism underlying diphenyl diselenide exerts antidepressant-like effect.

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