Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 88 (2008) 487–496
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Evidence for the involvement of glutamatergic and GABAergic systems and protein kinase A pathway in the antinociceptive effect caused by *p*-methoxy-diphenyl diselenide in mice

Larissa Garcia Pinto, Cristiano Ricardo Jesse, Cristina Wayne Nogueira, Lucielli Savegnago *

Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil

Received 9 August 2007; received in revised form 17 October 2007; accepted 17 October 2007
Available online 23 October 2007

Abstract

The present study investigated the antinociceptive effect of *p*-methoxy-diphenyl diselenide $(MeOPhSe)_2$, a simple organochalcogenide, in chemical and thermal behavioural models of nociception in mice, without accompanying changes in ambulation when assessed in an open field. This compound given by oral route (p.o.) produced antinociception when assessed on acetic acid-induced visceral nociception, with mean ID_{50} value of 9.64 (3.28–28.35) mg/kg. In addition, the per oral administration of $(MeOPhSe)_2$ exhibited significant inhibition of the neurogenic nociception induced by intraplantar (i.pl.) injection of capsaicin, with mean ID_{50} value of 16.29 (11.43–23.22) mg/kg. $(MeOPhSe)_2$ showed an antinociceptive effect when measured by the tail-immersion and hot-plate tests. Likewise, compound inhibited both neurogenic and inflammatory phases of the overt nociception caused by i.pl injection of formalin, with mean ID_{50} values of 22.32 (17.84–27.92) and 19.65 (13.67–28.24) mg/kg, respectively. $(MeOPhSe)_2$ reduced the nociception produced by i.pl. injection of glutamate and 8-bromo-cAMP (8-Br-cAMP, a protein kinase A [PKA] activator), with mean ID_{50} values of 11.05 (7.12–17.15) and 8.72 (5.42–14.02) mg/kg, respectively. $(MeOPhSe)_2$ also reduced formalin-, glutamate-, induced paw oedema formation. A marked inhibition of the biting behaviour induced by intrathecal (i.t.) injection of glutamate, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and (±)-1 aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD) was caused by $(MeOPhSe)_2$. However, $(MeOPhSe)_2$ completely failed to affect the nociception induced by i.t. injection of *N*-methyl-D-aspartate (NMDA; 450 pmol/site) and kainate (110 pmol /site). The antinociceptive effect caused by $(MeOPhSe)_2$ was blocked by picrotoxin (a chloride ion channel blocker) and bicucculine (a specific $GABA_A$ receptor antagonist) but not by phaclofen (a specific $GABA_B$ receptor antagonist) in the hot-plate test. Together, these results indicate that $(MeOPhSe)_2$ produces antinociception in several models of nociception through mechanisms that involve an interaction with glutamatergic and GABAergic systems, as well as the inhibition of protein kinase A pathway.

Keywords: Selenium; Organoselenium compounds; Antinociceptive; Glutamatergic and GABAergic systems

1. Introduction

Pain is one of the most pervasive problems in our society and has high social costs due to the significant impairment or permanent disabling of millions of people.

Tissue damage can result in activation of nociceptors through the release of several mediators, including excitatory amino acids, peptides, protons, lipids and cytokines, which bind to receptors and activate signaling pathways, among these protein kinases A and C, calcium/calmodulin-dependent protein kinase, and mitogen-activated protein kinases (MAPKs) (Ji and Strichartz, 2004). Taking this into account, pain can be subject to multiple levels of biochemical and pharmacological controls, involving a diversity of cell types and soluble mediators (Basbaum and Jessell, 2000; Julius and Basbaum, 2001; Ji and Strichartz, 2004). Thus, compounds that present antinociceptive

* Corresponding author. Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil. Tel.: +55 55 32208140; fax: +55 55 32208978.
E-mail address: luciellisavegnago@yahoo.com.br (L. Savegnago).

0091-3057/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2007.10.006

and/or antiinflammatory effects are of potential therapeutic interest for the treatment of human and animal pain.

Under this point of view, our group of research and others have widely studied the antinociceptive and antiinflammatory properties of organoselenium compounds, which could be relevant drugs for the management of pain. Of particular importance, ebselen (an organoselenium compound) has antiinflammatory activity in different models of inflammation (Parnhan and Graf, 1987; Schewe, 1995), which may be related at least in part to its capability to scavenge peroxynitrite, a potent inflammatory mediator (Sies and Arteel, 2000). Moreover, this compound is a classical antioxidant and well known as an important glutathione peroxidase mimetic agent (Muller et al., 1984; Daiber et al., 2000).

Taking this into account, our group of research reported that the systemic administration of diphenyl diselenide, an organoselenium compound, elicits antinociceptive and antiinflammatory properties (Zasso et al., 2005; Savegnago et al., 2007a). Additionally, the mechanism of antinociception action caused by diphenyl diselenide [$(PhSe)_2$] involves the serotoninergic pathway, an interaction with nitrergic system and glutamate receptors (Zasso et al., 2005; Savegnago et al., 2007a). Interestingly, the antinociceptive, antiinflammatory and antioxidant effect caused by $(PhSe)_2$ was higher than that of ebselen (Nogueira et al., 2003b). It is very important, in view of the fact that a good antioxidant agent is a potential candidate for antiinflammatory drugs research (Nogueira et al., 2003a; Meotti et al., 2004).

Actually, toxicological and pharmacological studies have been performed in our laboratory to verify whether the introduction of functional groups (e.g. chloro, fluor or methoxyl) in the aromatic ring of $(PhSe)_2$ alter its effect. This fact is important in view of the data showing that administration of $(PhSe)_2$ in mice presented tonic–clonic seizures and the introduction of a functional groups in the aromatic ring of $(PhSe)_2$ (*p*-chloro-diphenyl diselenide, *m*-trifluoromethyl-diphenyl diselenide, *p*-methoxy-diphenyl diselenide) reduced or abolished the appearance of seizure episodes (Nogueira et al., 2003a).

Therefore, based on the considerations above, the introduction of functional groups (e.g. chloro, fluor or methoxyl) in the aromatic ring of diphenyl diselenide can provide alternatives to current therapeutic agents. However, pharmacological studies on these compounds still are scarce. For this reason, the purpose of the present study was to examine whether *p*-methoxy-diphenyl diselenide, $(MeOPhSe)_2$ induces antinociceptive activity in chemical and thermal models of nociception in mice and to investigate some possible mechanisms involved in the antinociceptive activity caused by $(MeOPhSe)_2$.

2. Materials and methods

2.1. Drugs

Diaryl diselenide disubstituide, *p*-methoxy-diphenyl diselenide, $(MeOPhSe)_2$ (Fig. 1) 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 $(MeOPhSe)_2$ (99.9%) was determined by GC/HPLC. $(MeOPhSe)_2$ was dissolved in canola oil and administered by oral route (p.o.). The mice received the $(MeOPhSe)_2$ in a constant volume of 10 ml/kg body weight. All other drugs used were dissolved in a saline, with the exception of capsaicin, which was prepared in absolute ethanol and the final concentration of ethanol did not exceed 0.5% and did not cause any detectable effect per se. All other chemicals were of analytical grade and obtained from standard commercial suppliers (Sigma, St. Louis, USA).

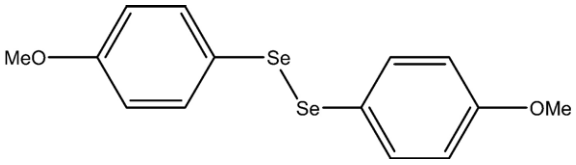

Fig. 1. Chemical structure of $(MeOPhSe)_2$.

2.2. Intrathecal (i.t.) injection

The i.t. injections were performed in accordance to the method described by Hylden and Wilcox (1980). The conscious animals received, using a microsyringe connected to polyethylene tubing, a volume of 5 μl of saline (control) or drugs which were injected directly between the subdural spaces of the L5–L6 spinal segments. Injections were given over a period of 5 s.

2.3. Animals

The behavioural experiments were conducted using female Swiss mice (25–35 g) maintained at 22–25 °C with free access to water and food, under a 12:12 h light/dark cycle (with lights on at 6:00a.m.). Mice were acclimatized to the laboratory for at least 1 h before testing and were used only once throughout the experiments. 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 and the ethical guidelines for investigations of experimental nociception in conscious animals (Zimmermann, 1983). The number of animals and intensities of noxious stimuli used were minimum necessary to demonstrate the consistent effects of the drug treatments.

2.4. Acetic acid-induced abdominal constriction

The abdominal constriction was induced according to procedures described previously by Corrêa et al. (1996) and modified by Nogueira et al. (2003b) and resulted in contraction of the abdominal muscle together with a stretching of the hind limbs in response to an intraperitoneal injection (i.p.) of acetic acid (1.6%) at time of the test. Mice were pretreated with $(MeOPhSe)_2$ (1–50 mg/kg) by oral route (p.o), 30 min before the irritant injection. Control animals received a similar volume of vehicle (10 ml/kg, canola oil).

After the challenge, mice were individually placed in separate boxes and the abdominal constrictions were counted cumulatively over a period of 20 min. Antinociceptive activity was expressed as the reduction in the number of abdominal

constrictions, i.e. the difference between control animals (mice pretreated with vehicle) and animals pretreated with the drug.

To assess time-course of the antinociceptive effect of $(MeOPhSe)_2$, mice were pretreated with compound (50 mg/kg, p.o.) 0.5–6 h before i.p. of acetic acid.

2.5. Capsaicin-induced nociception

To evaluate the possible antinociceptive effect caused by $(MeOPhSe)_2$ on neurogenic nociception, we investigated whether this compound antagonizes capsaicin-induced nociception in the mouse paw. The procedure was similar to that described by Sakurada et al. (1993). After an adaptation period, 20 µl of capsaicin (1.6 µg/paw) was injected intraplantarly (i.pl) in the ventral surface of the right hindpaw. Animals were observed individually for 5 min following capsaicin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. Animals were treated with vehicle (canola oil) or $(MeOPhSe)_2$ (0.1–50 mg/kg, p.o) 30 min before capsaicin injection.

2.6. Tail-immersion test

The tail-immersion test was carried out as described by Janssen et al. (1963). The lower 3.5 cm portion of tail was marked and the animals were then injected with $(MeOPhSe)_2$ (1–50 mg/kg, p.o) or vehicle (canola oil, p.o, 10 ml/kg), 30 min before the test. The reaction was determined by immersing the lower 3.5 cm of the tail into a cup freshly filled with water from a large constant temperature (55 °C) bath until the typical tail withdrawal response was observed. A 7s cut-off was imposed to avoid tail damage by heat. Changes in tail-flick latency, Δt (s), was calculated for each animal according to the formula: Δt (s)= postdrug latency − predrug latency (Pinardi et al., 2003).

2.7. Hot-plate test

The hot-plate test was used to measure the response latencies according to the method described previously by Woolfe and MacDonald (1944), with minor modifications. In these experiments, the hot plate (model-DS 37; Ugo Basile) was maintained at 55±1 °C. Animals were placed into a glass cylinder with 24 cm diameter on the heated surface, and the time between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. An automatic 45-s cut-off was used to prevent tissue damage. Each animal was tested before administration of drugs to obtain the baseline. Control or pretreated with $(MeOPhSe)_2$ (1–50 mg/kg, p.o) animals were injected 30 min earlier. The latency, Δt (s), was calculated for each animal according to the formula: Δt (s)=postdrug latency − predrug latency.

2.7.1. Analysis of the mechanisms involved in the antinociceptive action caused by $(MeOPhSe)_2$ in the hot-plate test

To explore the possible contribution of GABAergic system in the antinociception caused by $(MeOPhSe)_2$ in the hot-plate test, mice were pretreated with picrotoxin (0.25 µg/site, i.t.; a chloride ion channel blocker), bicuculline (0.5 µg /site, i.t.; a specific $GABA_A$ receptor antagonist), or with phaclofen (10 µg/site, i.t.; a specific $GABA_B$ receptor antagonist), and after 15 min they received $(MeOPhSe)_2$ (10 mg/kg, p.o.) or vehicle (canola oil). Other groups of animals received saline (5 µl/site, i.t.) 15 min prior to the administration of $(MeOPhSe)_2$ or vehicle. The nociceptive response to hot plate was recorded 30 min after the administration of $(MeOPhSe)_2$. The dose of picrotoxin and bicuculline were based on study described by Rady and Fujimoto (1995) and phaclofen was accordingly to by Aran and Hammond (1991).

2.8. Effect of $(MeOPhSe)_2$ on formalin-induced nociception

The formalin test was carried out as described by Hunskaar and Hole (1987). Animals received 20 µl of 2.5% formalin solution (0.92% of formaldehyde), injected i.pl. in the ventral right hindpaw. Animals were pretreated with $(MeOPhSe)_2$ by oral route (0.1–50 mg/kg, p.o.), 30 min before formalin injection. Control animals received a similar volume of vehicle (canola oil; 10 ml/kg; p.o.). After i.pl. injection of formalin, the animals were observed from 0–5 min (neurogenic phase) and 15–30 min (inflammatory phase) and the time spent licking the injected paw was timed with a chronometer and considered as indicative of nociception.

In order to assess whether the antinociceptive activity produced by $(MeOPhSe)_2$ in formalin-induced nociception was associated with development of oedema formation, we measured the paw oedema by comparing the difference between the weight of the formalin-treated paw and the weight of the contralateral paw (nontreated paw). For this purpose, animals were sacrificed 30 min after formalin injection by cervical dislocation, and both paws were cut at the ankle joint and weighed on an analytical balance.

2.9. Glutamate-induced nociception and paw oedema

The procedure used was similar to that described previously (Beirith et al., 2002). In an attempt to provide more direct evidence concerning the interaction of $(MeOPhSe)_2$ with glutamatergic system, we separately investigated whether or not $(MeOPhSe)_2$ was able to antagonize glutamate-induced licking of the mouse paw. Of particular importance, $(PhSe)_2$ caused antinociceptive effect against glutamate-induced nociception and paw oedema.

To this end, animals received 20 µl of glutamate solution (10 µmol/paw) injected i.pl. in the ventral surface of the right hindpaw. The mice were observed individually for 15 min following glutamate injection and the amount of time spent licking the injected paw was recorded with a chronometer and was considered as indicative of nociception. To assess the systemic action of $(MeOPhSe)_2$, animals were treated with this compound (0.1–50 mg/kg, p.o) or with canola oil (10 mg/ml, p.o.), 30 min before glutamate injection.

In order to verify whether the antinociceptive activity produced by $(MeOPhSe)_2$ in glutamate-induced nociception was associated with development of oedema formation, we measured the paw oedema by comparing the difference between the weight of the glutamate-treated paw and the weight of the contralateral paw (nontreated paw). For this purpose, animals were sacrificed

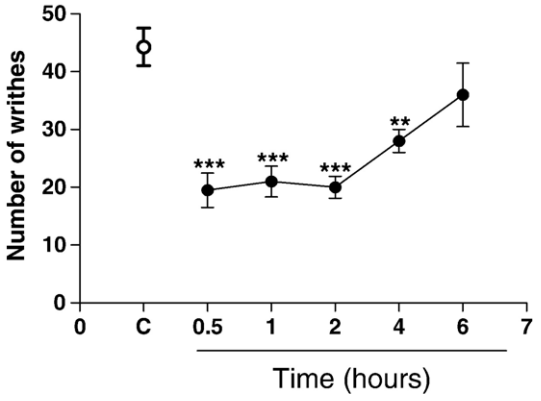

Fig. 2. Time-course of the antinociceptive effect of $(MeOPhSe)_2$ (50 mg/kg) on acetic acid-induced writhing in mice. $(MeOPhSe)_2$ was administered 0.5, 1, 2, 4, and 6 h before acetic-acid injection. Each line represents the mean with S.E.M. for 6–12 mice in each group. Control value "C" indicates the animals injected with vehicle (canola oil). The asterisks denote the significance levels when compared with control group (one-way ANOVA followed by Newman–Keuls test): $^{**}p<0.01$; and $^{***}p<0.001$.

15 min after glutamate injection by cervical dislocation, and both paws were cut at the ankle joint and weighed on an analytical balance.

2.10. Spinal excitatory amino acids induced nociception-related behaviour in mice

To test the hypothesis that excitatory amino acids (EAA) might be involved in the antinociceptive effect caused by $(MeOPhSe)_2$, we assessed the effect of $(MeOPhSe)_2$ (10 mg/kg, p.o.) 30 min before i.t. injection of 5 μl of the EAA induced biting response in mice. The nociceptive response was elicited by glutamate (an excitatory amino acid, 175 nmol/site, i.t.), NMDA (a selective agonist of NMDA-subtype of glutamatergic ionotropic receptors, 450 pmol/site, i.t.) (Urca et al., 1998), AMPA (a selective agonist of AMPA-subtype of glutamatergic ionotropic receptors, 135 pmol/site, i.t.) (Brambilla et al., 1996), kainate (a selective agonist of kainate-subtype of glutamatergic ionotropic receptors, 110 pmol/site, i.t.) *trans*-ACPD (a metabotropic glutamate agonist, 50 nmol/site, i.t.) (Boxall et al., 1998). A group of mice received only vehicle (saline solution) by i.t. route. Immediately after i.t injection of each agonist, mice were placed individually in observation chambers, and the amount of time (s) the animal spent biting itself was evaluated: glutamate (3 min); AMPA (1 min); NMDA (5 min); kainate (4 min) and *trans*-ACPD (15 min) A bite was defined as a single head movement directed at the flanks or hind limbs, resulting in contact of the animal's snout with the target organ.

2.11. 8-Bromo-cAMP, a PKA activator, induced nociception

To test the hypothesis that the PKA pathway is involved in the peripheral neurotransmission of nociception, we investigate the involvement of peripheral PKA in the antinociceptive effect caused by $(MeOPhSe)_2$. To this end, mice were treated with $(MeOPhSe)_2$ (1–25 mg/kg) by p.o. route, 30 min before i.pl. injection of 8-Br-cAMP (a protein Kinase A (PKA) activator, 10 nmol/paw, 20 μl). After that, animals were observed individually for 10 min and the amount of time spent licking the injected paw timed with a chronometer was considered indicative of nociception. The procedure used was similar to that described previously (Otuki et al., 2005).

2.12. Open-field task

Mice were evaluated 30 min after oral exposure with canola oil or $(MeOPhSe)_2$ (50 mg/kg, p.o.). Each animal was placed individually at the center of the apparatus and observed for 6 min to record the spontaneous ambulation (number of segments crossed with the four paws) and exploratory activity (expressed by the number of time rearing on the hind limbs) (Walsh and Cummins, 1976). The open field was made of plywood and was 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 9 squares (3 rows of 3).

2.12. Statistical analysis

The results are presented as mean ± S.E.M., except the ED_{50} or ID_{50} values (i.e., the dose of $(MeOPhSe)_2$ producing half maximal antinociceptive or the dose necessary to reduce the nociceptive response by 50% relative to the control value). The ED_{50} or ID_{50} values were determined by graphical interpolation from individual experiments and are reported as geometric means accompanied by their respective 95% confidence limits (GraphPad software, San Diego, CA, USA). Comparisons between experimental and control groups were performed by ANOVA followed by Newman–Keuls' test when appropriated. *P* values less than 0.05 ($P<0.05$) were considered as indicative of significance.

3. Results

3.1. Acetic acid-induced abdominal constriction

A time-course analysis of the antinociceptive profile of $(MeOPhSe)_2$ was accomplished. The antinociceptive effect of $(MeOPhSe)_2$ reached it peak 30 min and remained significant

Table 1
Effect of $(MeOPhSe)_2$ administered orally against acetic-acid induced writhing movements in mice

Experimental groups	Acetic acid (1.6%)
	Number of abdominal constriction
Control	42.6±3.0
$(MeOPhSe)_2$ (mg/kg)	
1	31.2±2.9
5	26.3±4.8*
10	16.9±4.2***
50	19.0±3.2***

Animals were pretreated orally with $(MeOPhSe)_2$ at various doses (from 1 to 50 mg/kg) for 30 min prior to the acetic-acid (1.6%, i.p.). Each column represents the mean with S.E.M. for 6–12 mice in each group. Control value indicates the animals injected with vehicle (canola oil). The asterisks denote the significance levels when compared with control group (one-way ANOVA followed by Newman–Keuls test): $^{*}p<0.05$ and $^{***}p<0.001$.

Table 2
Effect of $(MeOPhSe)_2$ administered orally against capsaicin-induced licking in mice

Experimental groups	Capsaicin
	Licking (s)
Control	71.7±4.6
$(MeOPhSe)_2$ (mg/kg)	
0.1	65.8±6.6
1	46.5±11.2*
5	47.7±10.2*
10	44.0 ±6.0*
25	19.4±2.7***
50	13.6 ±2.1***

Animals were pretreated orally with $(MeOPhSe)_2$ at various doses (from 0.1 to 50 mg/kg) for 30 min prior to capsaicin (1.6 μg/paw). Each column represents the mean with S.E.M. for 8–12 mice in each group. The asterisks denote the significance levels when compared with control group (one-way ANOVA followed by Newman–Keuls test): *$p<0.05$; and ***$p<0.001$. (s=seconds).

up to 4 h (Fig. 2). Thus, the time point (30 min) of the maximum effect of $(MeOPhSe)_2$ was chosen for all further studies.

The results of antinociceptive effect of $(MeOPhSe)_2$ on acetic acid-induced abdominal constriction response in mice are presented in Table 1. It can be seen that $(MeOPhSe)_2$, given (30 min earlier) by oral route, produced an inhibition of the acetic acid-induced abdominal constriction in mice with the mean ID_{50} value (and their respective 95% confidence limits) of 9.64 (3.28–28.35) mg/kg and maximal inhibitory effect of 68±7%.

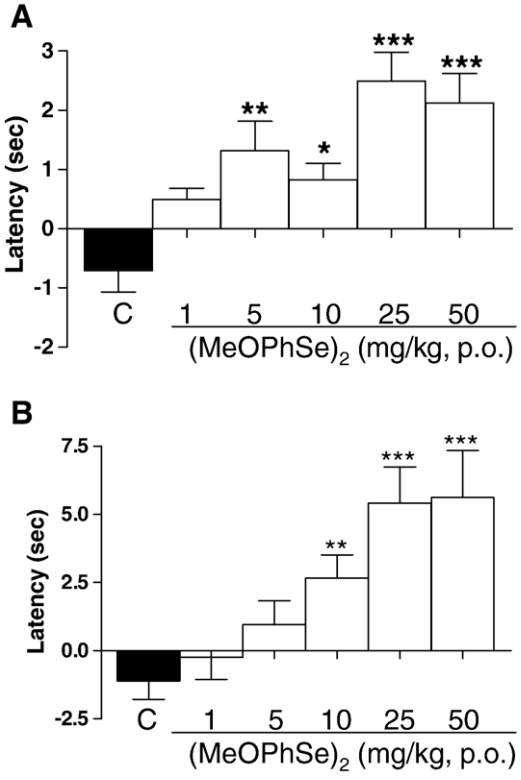

Fig. 3. Effect of $(MeOPhSe)_2$ administered orally on the tail-immersion test (A) or hot-plate tests (B) in mice. Animals were pretreated orally with $(MeOPhSe)_2$ at various doses (from 1 to 50 mg/kg) for 30 min prior to tail-immersion or hot-plate test at 55 °C. Each column represents the mean with S.E.M. for 6–10 mice in each group. Control value "C" indicates the animals injected with vehicle (canola oil). The asterisks denote the significance levels when compared with control group (one-way ANOVA followed by Newman–Keuls test): *$p<0.05$; **$p<0.01$; and ***$p<0.001$.

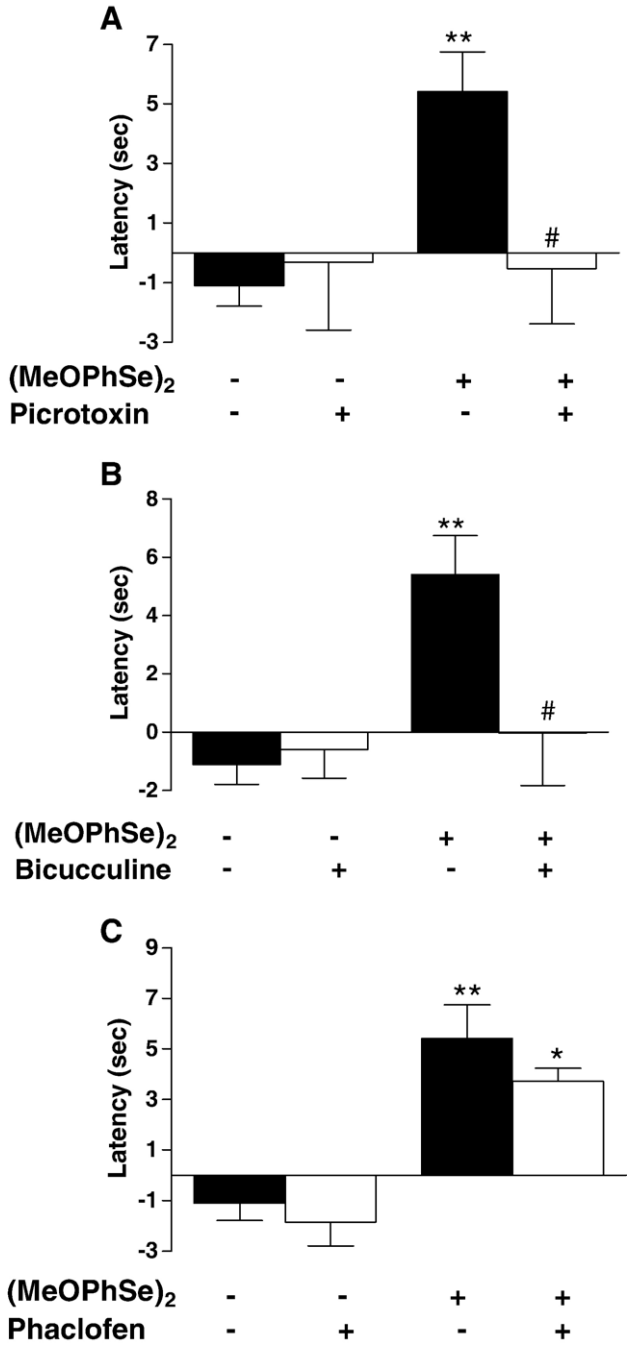

Fig. 4. Effects of picrotoxin (A), bicucculine (B) and phaclofen (C) injected i.t. on inhibition of the hot-plate response induced by $(MeOPhSe)_2$ administered orally. Picrotoxin, bicucculine or phaclofen was pretreated i.t. for 15 min, before oral administration of vehicle or $(MeOPhSe)_2$ (10 mg/kg). The hot-plate response was measured 30 min after $(MeOPhSe)_2$ or vehicle treatment. Each column represents the mean with S.E.M. for 8–12 mice in each group. *$p<0.05$; and **$p<0.01$ compared with control (one-way ANOVA followed by Newman–Keuls test), $^{\#}p<0.01$ as compared to the $(MeOPhSe)_2$ group pretreated with vehicle.

3.2. Capsaicin-induced nociception

The p.o. administration of $(MeOPhSe)_2$ produced attenuation of capsaicin-induced neurogenic nociception (Table 2). The mean ID_{50} value obtained was 16.29 (11.43–23.22) mg/kg and the observed maximal inhibitory effect was 81±3%.

3.3. Tail-immersion-induced nociception

As demonstrated in Fig. 3A, $(MeOPhSe)_2$ administrated at doses of 5–50 mg/kg caused a significant increase in tail-flick response latency time as compared to control animals. The calculated mean ED_{50} value (and the 95% confidence limits) for $(MeOPhSe)_2$ was 6.9 (−0.6 to 5.6) mg/kg and percentage in the latency times was increased 99± 10% in the higher dose used (50 mg/kg).

3.4. Hot-plate test induced nociception

In the hot-plate test, oral treatment with $(MeOPhSe)_2$ at doses of 10–50 mg/kg increased the latency time as compared to the control group (Fig. 3B). The calculated mean ED_{50} value (and the 95% confidence limits) for $(MeOPhSe)_2$ was 9.24 (−3.6 to 15.9) mg/kg and the percentage in the latency times was increased 210±13% in the higher dose used (50 mg/kg).

We also examined the possible involvement of GABAergic system in the $(MeOPhSe)_2$-induced antinociception in the hot-plate test. The blockade of $GABA_A$ receptors with picrotoxin attenuated $(MeOPhSe)_2$-induced inhibition of the hot-plate response (Fig. 4A). Similarly, $(MeOPhSe)_2$-induced inhibition of the hot-plate response was decreased when mice were pretreated with bicucculine (a $GABA_A$ receptor antagonist) i.t. (Fig. 4B). However, the blockade of $GABA_B$ receptors with phaclofen did not affect the inhibition of the hot-plate response induced by $(MeOPhSe)_2$ (Fig. 4C).

Table 4
Effect of $(MeOPhSe)_2$ administered orally on the licking and oedema induced by glutamate in mice

Experimental groups	Glutamate	
	Licking (s)	Oedema (mg)
Control	126.1±8.4	72.8±4.3
$(MeOPhSe)_2$ (mg/kg)		
0.1	96.8±14*	69.5±1.9
1	81.8±6.4**	68.4±4.5
5	76.5±11.1**	66.4±3.8
10	39.8±4.0***	67.1±3.0
25	13.8±4.7***	59.0±3.5
50	8.0±1.1***	53.8±3.2*

Mice were pretreated with $(MeOPhSe)_2$ at various doses (from 0.1–50 mg/kg) for 30 min prior to glutamate (10 μmol/paw, 20 μL). Each column represents the mean with S.E.M. for 6–10 mice in each group. Control value indicates the animals injected with vehicle (canola oil). The asterisks denote the significance levels when compared with control group (one-way ANOVA followed by Newman–Keuls test): *$p<0.05$; **$p<0.01$; and ***$p<0.001$.

3.5. Formalin-induced nociception

Table 3 shows that $(MeOPhSe)_2$ caused significant inhibition of both neurogenic (0 to 5 min) and inflammatory (15 to 30 min) phases of formalin-induced licking. The calculated mean ID_{50} values for neurogenic and inflammatory nociception were: 22.32 (17.84–27.92) and 19.65 (13.67–28.24) mg/kg and the maximal inhibitory effects observed were 82±2% and 95±1%, respectively.

$(MeOPhSe)_2$ 50 mg/kg was significantly effective in inhibiting the mouse paw oedema induced by i.pl. injection of formalin (percentage of inhibition of 40±8%) (Table 3).

Table 3
Effect of $(MeOPhSe)_2$ given by oral route on the licking and oedema induced by formalin in mice

Experimental groups	Formalin		
	First phase licking (s)	Second phase licking (s)	Oedema (mg)
Control	89.3±6.8	138.0±12.3	79.5±5.3
$(MeOPhSe)_2$ (mg/kg)			
0.1	78.6±11.3	131.8±17.9	62.1±4.3
1	63.4±6.8*	93.8±14.9*	68.5±5.9
5	53.7±4.9***	83.8±11.3**	63.2±3.8
10	40.3±7.7***	69.0±5.4***	64.8±7.5
50	18.3±2.6***	9.3±1.8***	54.6±4.1*

Animals were pretreated orally with $(MeOPhSe)_2$ at various doses (from 0.1 to 50 mg/kg) for 30 min prior to formalin. The total time spent licking the hindpaw was measured in the first (0–5 min, panel A) and the second (15–30 min, panel B) phases after intraplantar injection of formalin. The oedema was measured at the end of second phase of formalin test. Each column represents the mean of 7 to 10 animals and vertical lines indicate the S.E.M. Control value indicates the animals injected with vehicle (canola oil). The asterisks denote the significance levels when compared with control group (one-way ANOVA followed by Newman–Keuls test): *$p<0.05$; **$p<0.01$; and ***$p<0.001$.

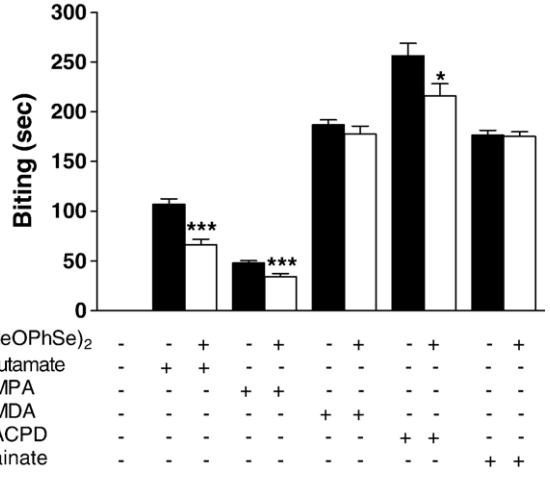

Fig. 5. Effect of $(MeOPhSe)_2$ (10 mg/kg) administered orally on the biting response caused by i.t. injection of glutamate, AMPA, NMDA, t-ACPD and kainate in mice. Each column represents the mean with S.E.M. for 6–10 mice in each group. Control value "C" indicates the animals injected with vehicle (canola oil). The asterisks denote the significance levels when compared with control group (one-way ANOVA followed by Newman–Keuls test): *$p<0.05$; and ***$p<0.001$.

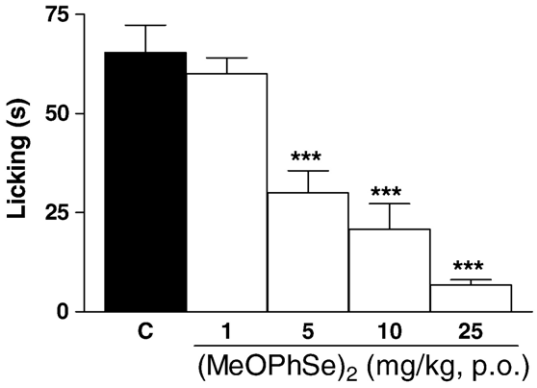

Fig. 6. Effect of $(MeOPhSe)_2$ administered orally on the licking induced by 8-Br-cAMP in mice. Animals were pretreated with $(MeOPhSe)_2$ at various doses (1–25 mg/kg) for 30 min prior to 8-Br-cAMP (10 nmol/paw, 20 μL). Each column represents the mean with S.E.M. for 6–10 mice in each group. Control value "C" indicates the animals injected with vehicle (canola oil). The asterisks denote the significance levels when compared with control group (one-way ANOVA followed by Newman–Keuls test): ***$p<0.001$.

3.6. Glutamate-induced nociception and paw oedema

The results presented in Table 4 show that $(MeOPhSe)_2$, given orally, caused a significant inhibition of the glutamate-induced nociception, with a mean ID_{50} value of 11.05 (7.12–17.15) mg/kg and maximal inhibitory effect of 94±1%. Furthermore, one-way ANOVA revealed that the p.o treatment of the animals with $(MeOPhSe)_2$ (50 mg/kg) resulted in an inhibition of the paw oedema formation induced by i.pl. injection of glutamate (Table 4). The maximal inhibitory effect observed was of 27±5%.

3.7. Spinal excitatory amino acids induced nociception-related behaviour in mice

The results presented in Fig. 5 show that $(MeOPhSe)_2$ (10 mg/kg), given orally, caused significant inhibition of the nociceptive response induced by spinal injections of glutamate, AMPA and *trans*-ACPD, with maximal inhibitory effect of 38±5, 28±6 and 27±5%, respectively. In contrast, $(MeOPhSe)_2$ had no significant effect against NMDA and kainate mediated biting response in mice.

3.8. 8-Bromo-cAMP, a PKA activator, induced nociception"

As revealed in Fig. 6, oral treatment with $(MeOPhSe)_2$ (1–25 mg/kg) significantly inhibited 8-bromo-cAMP-induced nociceptive response. The maximal inhibitory effect was 89±2% and the calculated mean ID_{50} value was 8.72 (5.42–14.02) mg/kg.

3.9. Effect of (MeOPhSe)2 on the open-field task

Mice treated with $(MeOPhSe)_2$ at 50 mg/kg did not cause changes in the numbers of crossings and rearings when compared to the control group in the open-field test (data not shown).

4. Discussion

The present study demonstrated, for the first time, the antinociceptive effect caused by $(MeOPhSe)_2$ in chemical and thermal models of nociception in mice without modifying the locomotor and exploratory activities of these animals in the open-field test.

The results presented in this study revealed that $(MeOPhSe)_2$, orally administered, elicited an inhibition of the acetic acid-induced visceral nociceptive response in mice. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which then excite the pain nerve endings. In addition, this model of nociception has long been used to screen for both peripherally and centrally acting agents (Vinegar et al., 1979; Tjolsen and Hole, 1997). Of particular interest, $(PhSe)_2$ also reduced visceral nociception induced by acetic acid with ID_{50} value of about 4.3-fold higher than $(MeOPhSe)_2$ (compare ID_{50} of 39.72 to 9.64 mg/kg for $(PhSe)_2$ and $(MeOPhSe)_2$, respectively), suggesting that the introduction of an electron-donating substituent bounded to the aromatic ring could explain the best antinociceptive effect of $(MeOPhSe)_2$ in this model of nociception (Savegnago et al., 2007a). It is possible that the good antinociceptive effect caused by $(MeOPhSe)_2$ can be due to the changes in its chemical structure which might be responsible for an improvement of pharmacokinetic parameters.

We have also shown, in the present study, that $(MeOPhSe)_2$ produced significant antinociceptive effect on the capsaicin-induced neurogenic paw licking response. These results are consistent with our previous work, in which, $(PhSe)_2$ caused significant antinociception against capsaicin-induced licking (Nogueira et al., 2003b; Savegnago et al., 2007a). In addition, $(PhSe)_2$ and $(MeOPhSe)_2$ caused similar effect against capsaicin-induced nociceptive response (compare ID_{50} of 22.5 to 16.29 mg/kg for $(PhSe)_2$ and $(MeOPhSe)_2$, respectively) (Savegnago et al., 2007a). It has been proposed that capsaicin-induced nociception is brought about by activation of the capsaicin receptor, also known as the vanilloid receptor (TRPV), termed TRPV subtype 1 (TRPV1), ligand-gated nonselective cation channel in primary sensory neurons (Caterina et al., 1997; Tominaga et al., 1998; Szallasi and Bluemberg, 1999). It has been widely documented that capsaicin evokes the release of neuropeptides, excitatory amino acids (glutamate and aspartate), nitric oxide and pro-inflammatory mediators in the periphery and transmits nociceptive information to the spinal cord (Santos and Calixto, 1997; Sakurada et al., 1996, 2003).

One interesting finding in the present study was the observation that $(MeOPhSe)_2$ orally injected caused a prolonged latency, indicating an increase of nociceptive threshold in two thermal models of nociception: tail-immersion and hot plate. Nociceptive pathways activated in the tail-flick and hot-plate tests are not the same (Bodnar and Habjinarkou, 2002; Schaidle and Richter, 2004). In fact, tail-immersion is considered to be a spinal reflex, but the mechanism of response could also involve higher neural structures (Jensen and Yaksh, 1986) and the hot-plate test produces, at constant temperature, two kinds of behavioural response, which are paw licking and

jumping. Both of these are considered to be supraspinally-integrated responses (Chapman et al., 1985).

Based on the results obtained above, we investigated some of the mechanisms through which $(MeOPhSe)_2$ exerts its anti-nociceptive action in the hot-plate model of nociception in mice.

We reported here, for the first time, the involvement of supraspinal GABAergic system in the antinociceptive effect caused by $(MeOPhSe)_2$. This conclusion derives from the fact that pretreatment of animals with picrotoxin (a chloride ion channel blocker) and bicucculine (a specific $GABA_A$ receptor antagonist) attenuated $(MeOPhSe)_2$-induced inhibition of the hot plate response. However, phaclofen (a specific $GABA_B$ receptor antagonist) did not affect the inhibition induced by $(MeOPhSe)_2$ in the hot-plate test. Thus, collectively these results suggest a major participation of the $GABA_A$ receptor associated chloride channels in the antinociceptive effect caused by $(MeOPhSe)_2$ in the hot-plate test. In this line, a considerable body of evidence has been accumulated demonstrating that neurotransmission involving the GABAergic system plays a key role in the modulation of nociception pathways (Andrews and Johnston, 1979; Dickenson et al., 1997).

Of particular interest, previous studies from our research group demonstrated that diphenyl diselenide, an analogue of $(MeOPhSe)_2$, given subcutaneously or orally caused antinociception against formalin and tail-immersion tests by mechanism that did not involve the opioid system (Nogueira et al., 2003b; Savegnago et al., 2007a). However, in this study we did not verify if the effect caused by $(MeOPhSe)_2$ in thermal models of nociception is reverted by pretreatment with naloxone. Here, we investigated some mechanisms involved in the antinociceptive action caused by $(MeOPhSe)_2$, but additional experiments would help to verify whether effects caused by $(MeOPhSe)_2$ can be reverted by treating animals with naloxone.

Another interesting finding in the present study is the demonstration that $(MeOPhSe)_2$ elicited a significant antinociceptive action in mice, when assessed in the formalin model. The formalin nociception test is very useful for evaluating the mechanism of nociception and analgesia. In the formalin test, the response to formalin is biphasic with an early and a late phase involving different mechanisms of nociception. The first phase is due to a direct effect of formalin on nociceptors and the second due to inflammation (Hunskaar and Hole, 1987). Drugs which act mainly centrally, such as narcotic analgesics, inhibit both phases of nociception in this model while peripherally acting drugs, such as acetylsalicylic acid or indomethacin, only inhibit the late phase. $(MeOPhSe)_2$ inhibited both phases of the formalin-induced nociception with a similar potency in the first and second phases (ID_{50} of 22.32 to 19.65 mg/kg for the first and second phases, respectively). On the contrary, $(PhSe)_2$ was more inhibitory in the second phase than in the first one (compare ID_{50} of 25.55 to 6.45 mg/kg for the first and the second phases, respectively) (Savegnago et al., 2007a). In addition, we observed that the effect caused by $(MeOPhSe)_2$ in the paw oedema caused by intraplantar injection of formalin into the mouse hindpaw was not dose related. In fact, distinct mechanisms are involved in the formalin test. The neurogenic and inflammatory aspects of pain are mediated by different mechanisms. The noxious stimulus is an injection of dilute formalin under the skin of the surface of the right hindpaw. The response is the amount of time the animals spend licking the injected paw. Two distinct periods of high licking activity can be identified, an early phase, lasting the first 5 min, and a late phase, lasting from 20 to 30 min after the injection of formalin. It is suggested that the early phase is due to a direct effect on nociceptors and that prostaglandins do not play an important role during this phase. The late phase seems to be an inflammatory response with inflammatory pain that can be inhibited by antiinflammatory drugs. In agreement, it has been demonstrated that nociceptive response caused by the intraplantar injection of formalin together with formation of oedema has been associated with release of several inflammatory mediators, including prostaglandins (Tjølsen et al., 1992).

Another notable piece of evidence was that $(MeOPhSe)_2$ administered orally, produced a significant inhibition in the licking and the paw oedema caused by intraplantar injection of glutamate into the mouse hindpaw. $(MeOPhSe)_2$ caused a similar antinociceptive effect against the licking induced by glutamate when compared to $(PhSe)_2$ (compare ID_{50} of 11.05 to 14.2 mg/kg for $(MeOPhSe)_2$ and $(PhSe)_2$, respectively). The nociceptive response induced by glutamate, when injected i.pl. into the mouse paw, is primarily mediated by release of neuropeptides from sensory fibers, namely neurokinins (NK) and kinins. On the other hand, the paw oedema associated with glutamate response appears to be mediated via stimulation of capsaicin-sensitive fibers and activation of NK_2 receptors (Beirith et al., 2002).

In order to provide more evidence of glutamatergic system involvement in antinociception caused by $(MeOPhSe)_2$, we attempted to determine whether or not $(MeOPhSe)_2$ administered by oral route was capable of inhibiting nociceptive response caused by selective glutamatergic receptor agonists. In agreement with findings obtained, $(MeOPhSe)_2$ caused significant inhibition of glutamate (Glu), AMPA and *trans*-ACPD induced biting response, but did not inhibit the biting response caused by intrathecal injection of kainate and NMDA. Thus, taking together the present findings strongly suggest that the antinociceptive action caused by $(MeOPhSe)_2$ depends on its selective interaction with AMPA and *trans*-ACPD receptor. On the contrary, previous findings suggest that the antinociceptive action caused by $(PhSe)_2$ depends on its selective interaction with NMDA receptor (Savegnago et al., 2007a,b).

The current study also indicates that the antinociceptive effect of $(MeOPhSe)_2$, given by p.o. route, inhibited 8-Br-cAMP-induced nociception. In fact, 8-Bromo-cAMP, a known protein kinase A activator, induced paw licking in mice. Moreover, PKA phosphorylates calcium channels, glutamate receptors, and the cAMP response element-binding protein (Dash et al., 1991; Blackstone et al., 1995; Hell et al., 1995). It is well known that peripheral tissue injury can lead to pain and inflammation, and increase evidence shows that protein kinases, such as cyclic AMP (cAMP)-dependent protein kinase A (PKA) and diacylglycerol (DAG)-protein kinase C (PKC), are involved in these events (Coderre, 1992; Lin et al., 1996, 2002; Woolf and Salter, 2000; Julius and Basbaum, 2001; Ji and Woolf, 2001;Willis, 2001). Moreover, several studies have also demonstrated peripheral PKA and PKC involvement in a

number of persistent pain models (Cunha et al., 1999; Khasar et al., 1999; Dina et al., 2001, 2005; Souza et al., 2002; Joseph and Levine, 2003a,b; Cunha et al., 2004). In addition, it has been revealed that serine/theonine phosphorylation of glutamate receptors is regulated by PKA on certain amino acid residues (Fang et al., 2002, 2003; Zou et al., 2002). These including that NR1 subunits of NMDA receptor and GluR1 subunits of AMPA receptor are phosphorylated by a PKA-mediated regulation mechanism.

Taken together the results of the present study show that $(MeOPhSe)_2$ exerts significative antinociceptive action in several models of nociception and the precise mechanisms through which $(MeOPhSe)_2$ exerts its action seems to involve an interaction with glutamatergic and GABAergic systems and protein kinase A pathway. However, additional studies are now in progress in order to further explore precise mechanism of action caused by $(MeOPhSe)_2$.

Acknowledgements

The financial support by UFSM (FIPE), FAPERGS, CAPES and CNPq is gratefully acknowledged. L.S., C.R.J. and C.W.N. are the recipients of CNPq fellowships.

References

Andrews PR, Johnston GAR. Commentary GABA agonists and antagonists. Biochem Pharmacol 1979;28:2697–702.

Aran S, Hammond DL. Antagonism of baclofen-induced antinociception by intrathecal administration of phaclofen or 2-hydroxy-saclofen, but not g-aminovaleric acid in rats. J Pharmacol Exp Ther 1991;257:360–8.

Basbaum AI, Jessell TM. In: Kandel ER, Schwartz JH, Jessell TM, editors. Principles of neuroscience. New York: McGraw-Hill; 2000. p. 472–91.

Beirith A, Santos ARS, Calixto JB. Mechanisms underlying the nociception and paw oedema caused by injection of glutamate into the mouse paw. Brain Res 2002;924:219–28.

Blackstone C, Murphy TH, Moss SJ, Baraban JM, Huganir RL. Cyclic AMP and synaptic activity dependent phosphorylation of AMPA preferring glutamate receptors. J Neurosci 1995;14:7585–93.

Bodnar RJ, Habjinarkou MM. Endogenous opiates and behavior: 2001. Peptides 2002;23:2307–65.

Boxall SJ, Berthele A, Tolle TR, Zieglgansberger W, Urban L. mGIuR activation reveals a tonic NMDA component in inflammatory hyperalgesia. Neuroreport 1998;9:1201–3.

Brambilla A, Prudentino A, Grippa N, Borsini F. Pharmacological characterization of AMPA-induced biting behaviour in mice. Eur J Pharmacol 1996;305:115–7.

Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 1997;389:816–24.

Chapman CR, Casey KL, Dubner R, Foley DM, Graceley RH, Reading AE. Pain measurement: an overview. Pain 1985;22:1–31.

Coderre TJ. Contribution of protein kinase C to central sensitization and persistent pain following tissue injury. Neurosci Lett 1992;140:181–4.

Corrêa CR, Kyle DJ, Chakravarty S, Calixto JB. Antinociceptine profile of the pseudopeptide B2 bradykinin receptor antagonist NPC 18688 in mice. Br J Pharmacol 1996;117:552–8.

Cunha FQ, Teixeira MM, Ferreira SH. Pharmacological modulation of secondary mediator systems – cyclic AMP and cyclic GMP – on inflammatory hyperalgesia. Br J Pharmacol 1999;127:671–8.

Cunha JM, Rae GA, Ferreira SH, Cunha FQ. Endothelins induce ETB receptor-mediated mechanical hypernociception in rat hindpaw: roles of cAMP and protein kinase C. Eur J Pharmacol 2004;501:87–94.

Daiber A, Zou M, Bachschmid M, Ullrich V. Ebselen as a peroxynitrite scavenger in vitro and ex vivo. Biochem Pharmacol 2000;59:153–60.

Dash PK, Karl KA, Colicos MA, Prywes R, Kandel ER. cAMP response element-binding protein is activated by Ca^{2+}/calmodulin as well as cAMP-dependent protein kinase. Proc Natl Acad Sci USA 1991;88:5061–5.

Dickenson AH, Chapman V, Green GM. The pharmacology of excitatory and inhibitory amino acid-mediated events in the transmission and modulation of pain in spinal cord. Gen Pharmacol 1997;28:633–8.

Dina OA, Chen X, Reichling D, Levine JD. Role of protein kinase Cepsilon and protein kinase A in a model of paclitaxel-induced painful peripheral neuropathy in the rat. Neuroscience 2001;108:507–15.

Dina OA, Hucho T, Yeh J, Malik-Hall M, Reichling DB, Levine JD. Primary afferent second messenger cascades interact with specific integrin subunits in producing inflammatory hyperalgesia. Pain 2005;115:191–203.

Fang L, Wu J, Lin Q, Willis WD. Calcium-calmodulin-dependent protein kinase II contributes to spinal cord central sensitization. J Neurosci 2002;22:4196–204.

Fang L, Wu J, Lin Q, Willis WD. Protein kinases regulate the phosphorylation of the GluR1 subunit of AMPA receptors of spinal cord in rats following noxious stimulation. Brain Res Mol Brain Res 2003;118:160–5.

Hell JW, Yokoyama CT, Breeze LJ, Chavkin C, Catterall WA. Phosphorylation of presynaptic and postsynaptic calcium channels by cAMP dependent protein kinase in hippocampal neurons. EMBO J 1995;14:3036–44.

Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 1987;30:103–14.

Hylden JL, Wilcox GL. Intrathecal morphine in mice: a new technique. Eur J Pharmacol 1980;67:313–6.

Janssen PAJ, Niemegeers CJE, Dony JHG. The inhibitory effect of fentanyl and other morphine-like analgesics on the warn water induced tail withdrawal reflex in rats. Arzeneimittelforschung 1963;13:502–7.

Jensen TS, Yaksh TL. Comparison of antinociceptive action of morphine in the periaqueductal gray, medial and paramedial medullal in rat. Brain Res 1986;363:99–113.

Ji RR, Strichartz G. Cell signaling and the genesis of neuropathic pain. Science 2004;252:1–19.

Ji RR, Woolf CJ. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. Neurobiol Dis 2001;8:1–10.

Joseph EK, Levine JD. Sexual dimorphism in the contribution of protein kinase C isoforms to nociception in the streptozotocin diabetic rat. Neuroscience 2003a;120:907–13.

Joseph EK, Levine JD. Sexual dimorphism for protein kinase c épsilon signaling in a rat model of vincristine-induced painful peripheral neuropathy. Neuroscience 2003b;119:831–8.

Julius D, Basbaum AI. Molecular mechanisms of nociception. Nature 2001;413:203–10.

Khasar SG, Lin YH, Martin A, Dadgar J, McMahon T, Wang D, et al. A novel nociceptor signaling pathway revealed in protein kinase C epsilon mutant mice. Neuron 1999;24:253–60.

Lin Q, Peng YB, Willis WD. Possible role of protein kinase C in the sensitization of primate spinothalamic tract neurons. J Neurosci 1996;16:3026–34.

Lin Q, Wu J, Willis WD. Effects of protein kinase a activation on the responses of primate spinothalamic tract neurons to mechanical stimuli. J Neurophysiol 2002;88:214–21.

Meotti FC, Stangherlin E, Zeni G, Nogueira CW, Rocha JBT. Protective role of aryl and alkyl diselenide on lipid peroxidation. Environ Res 2004;94:276–82.

Muller A, Cadenas E, Graf P, Sies H. A novel biologically active selenoorganic compound I. Glutathione peroxidase-like activity in vitro and antioxidant capacity of PZ51 (Ebselen). Biochem Pharmacol 1984;33:3235–9.

Nogueira CW, Meotti FC, Curte E, Pilissão C, Zeni G, Rocha JBT. Investigations into the potential neurotoxicity induced by diselenides in mice and rats. Toxicology 2003a;183:29–37.

Nogueira CW, Quinhones EB, Jung EAC, Zeni G, Rocha JBT. Anti-inflammatory and antinociceptive activity of diphenyl diselenide. Inflamm Res 2003b;52:56–63.

Otuki MF, Ferreira J, Lima FV, Meyre-Silva C, Malheiros A, Muller LA, Cani GS, Santos ARS, Yunes RA, Calixto JB. Antinociceptive properties of mixture of α-amyrin and β-amyrin triterpenes: evidence for participation of protein kinase C and protein kinase A pathways. J Pharmol Exp Ther 2005;313:310–8.

Parnham MJ, Graf E. Seleno-organic compounds and the therapy of hydroperoxide-linked pathological conditions. Biochem Pharmacol 1987;36:3095–102.

Paulmier C. Selenoorganic functional groups. In: Paulmier C, editor. Selenium reagents and intermediates in organic synthesis. 1 ed. Oxford: Pergamon Press; 1986. p. 25–51.

Pinardi G, Sierralta F, Miranda HF. Atropine reverses the antinociception of nonsteroidal anti-inflammatory drugs in the tail-flick test of mice. Pharm Biochem Behav 2003;74:603–8.

Rady JJ, Fujimoto JM. Spinal GABA receptors mediate brain delta opioid analgesia in Swiss webster mice. Pharm Biochem Behav 1995;51:655–9.

Sakurada T, Katsumata K, Yogo H, Tan-No K, Sakurada S, Kisara K. Antinociception induced by CP 96345, a non-peptide NK-1 receptor antagonist, in the formalin and capsaicin tests. Neurosci Lett 1993;151:142–5.

Sakurada T, Sugiyama A, Sakurada C, Tanno K, Sakurada S, Kisara K, Hara A, Abiko Y. Involvement of nitric oxide in spinally mediated capsaicin- and glutamate-induced behavioural responses in the mouse. Neurochem Int 1996;29: 271–8.

Sakurada T, Matsumura T, Moriyama T, Sakurada C, Ueno S, Sakurada S. Differential effects of intraplantar capsazepine and ruthenium red on capsaicin-induced desensitization in mice. Pharmacol Biochem Behav 2003;75:115–21.

Santos A, Calixto JB. Ruthenium red and capsazepine antinociceptive effect in formalin and capsaicin models of pain in mice. Neurosci Lett 1997;235:73–6.

Savegnago L, Pinto LG, Jesse CR, Alves D, Rocha JBT, Nogueira CW, Zeni G. Antinociceptive properties of diphenyl diselenide: evidences for the mechanism of action. Eur J Pharmacol 2007a;555:129–38.

Savegnago L, Pinto LG, Jesse CR, Rocha JBT, Nogueira CW, Zeni G. Spinal mechanisms of antinociceptive action caused by diphenyl diselenide. Brain Res 2007b;1162:32–7.

Schaidle HJ, Richter F. Pathophysiology of pain. Langenbeck's Arch Surg 2004;389:337–43.

Schewe T. Molecular actions of ebselen—an anti-inflammatory antioxidant. Gen Pharmacol 1995;26:1153–69.

Sies H, Arteel GE. Interaction of peroxynitrite with selenoproteins and glutathione peroxidase mimics. Free Radic Biol Med 2000;28:1451–5.

Souza AL, Moreira FA, Almeida KR, Bertollo CM, Costa KA, Coelho MM. In vivo evidence for a role of protein kinase C in peripheral nociceptive processing. Br J Pharmacol 2002;135:239–47.

Szallasi A, Blumberg PM. Vanilloid (capsaicin) receptors and mechanisms. Phamacol Rev 1999;51:159–212.

Tjølsen A, Berge O-G, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992;51:5–17.

Tjolsen A, Hole K. Animal models of analgesia. In: Dieckson A, Besson J-M, editors. The pharmacology of pain 130/I. Berlin: Springer-Verlag; 1997. p. 1–20.

Tominaga M, Caterina MJ, Malmberg AB, Rosen TA, Gilbert H, Skinner K, Raumann BE, Basbaum AI, Julius D. The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 1998;21:531–43.

Vinegar R, Truax JF, Selph JL, Jonhston PR. Antagonism of pain and hyperalgesia. Anti-inflammatory drugs. In: Vane JR, Ferreira SH, editors. Handbook of experimental pharmacology, 50/II. Berlin: Springer-Verlag; 1979. p. 208–12.

Urca G, Raigorodsky G. Behavioral classification of excitatory amino acid receptors in mouse spinal cord. Eur J Pharmacol 1998;153:211–20.

Walsh RN, Cummins RA. The open-field test: a critical review. Psychol Bull 1976;83:482–504.

Willis WD. Role of neurotransmitters in sensitization of pain responses. Ann NY Acad Sci 2001;933:142–56.

Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. Science 2000;288:1765–9.

Woolfe G, MacDonald AD. The evaluation of the analgesic action of pethidine hydrochloride (demerol). J Pharmacol Exp Ther 1944;80:300–7.

Zasso FB, Gonçales CEP, Jung EAC, Araldi D, Zeni G, Rocha JBT, Nogueira CW. On the mechanisms involved in antinociception induced by diphenyl diselenide. Environ Toxicol Pharmacol 2005;19:283–9.

Zimmermann M. Ethical guidelines for investigations of experimental pain in conscious animals. Pain 1983;16:109–10.

Zou X, Lin Q, Willis WD. Role of protein kinase A in phosphorylation of NMDA receptor 1 subunits in dorsal horn and spinothalamic tract neurons after intradermal injection of capsaicin in rats. Neuroscience 2002;115:775–86.