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Further analysis of the antinociceptive action caused by *p*-methoxyl-diphenyl diselenide in mice

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ABSTRACT

The objective of this study was to extend our previous findings by investigating in greater detail the mechanisms that might be involved in the antinociceptive action of *p*-methoxyl-diphenyl diselenide, $(MeOPhSe)_2$, in mice. The pretreatment with nitric oxide precursor, L-arginine (600 mg/kg, intraperitoneal, i.p.), reversed antinociception caused by $(MeOPhSe)_2$ (10 mg/kg, p.o.) or N^G-nitro-L-arginine (L-NOARG, 75 mg/kg, i.p.) in the glutamate test. Ondansetron (0.5 mg/kg, i.p., a 5-HT_3 receptor antagonist) and SCH23390 (0.05 mg/kg, i.p.., a D_1 receptor antagonist) blocked the antinociceptive effect caused by $(MeOPhSe)_2$. Conversely, pindolol (1 mg/kg, i.p., a 5-$HT_{1A/1B}$ receptor/β adrenoceptor antagonist), WAY 100635 (0.7 mg/kg, i.p., a selective 5-HT_{1A} receptor antagonist), ketanserin (0.3 mg/kg, i.p., a selective 5-HT_{2A} receptor antagonist), prazosin (0.15 mg/kg, i.p., an α_1-adrenoreceptor antagonist), yohimbine (1.0 mg/kg, i.p., an α_2-adrenoreceptor antagonist), sulpiride (5 mg/kg, i.p., a D_2 receptor antagonist), naloxone (1 mg/kg, i.p., a non-selective opioid receptor antagonist) and caffeine (3 mg/kg, i.p., a non-selective adenosine receptor antagonist) did not change the antinociceptive effect of $(MeOPhSe)_2$. $(MeOPhSe)_2$ significantly inhibited nociception induced by intraplantar (i.pl.) injection of bradykinin (10 nmol/paw) and Des-Arg^9-bradykinin (10 nmol/paw, a B_1 receptor agonist). $(MeOPhSe)_2$ significantly inhibited phorbol myristate acetate (PMA, 0.03 μg/paw, a protein kinase C (PKC) activator)-induced licking response. These results indicate that $(MeOPhSe)_2$ produced antinociception in mice through mechanisms that involve an interaction with nitrergic system, 5-HT_3 and D_1 receptors. The antinociceptive effect is related to $(MeOPhSe)_2$ ability to interact with kinin B_1 and B_2 receptors and PKC pathway mediated mechanisms.

1. Introduction

The sensation of pain alerts us to real or impending injury and triggers appropriate protective responses. Unfortunately, pain often outlives its usefulness as a warning system and instead becomes chronic and debilitating (Julius and Basbaum, 2001). In this context, research analysis during the last decade estimated that analgesics are one of the highest therapeutic categories on which research efforts are concentrated (Elisabetsky and Castilhos, 1990). Analgesic compounds available on the market, still present a wide range of undesired effects (Katzung, 2001) leaving an open door for new and better compounds.

Under this point of view, organoselenium compounds are believed to be an important source of new chemical substances with potential therapeutic applications (Nogueira et al., 2004). Accordingly, our group of research and others have studied the antinociceptive and anti-inflammatory properties of organoselenium compounds, which could be relevant drugs for the management of pain (Parnham and Graf, 1987; Schewe, 1995; Savegnago et al., 2007a,b,c, 2008). Of particular importance, diphenyl diselenide $(PhSe)_2$ elicits antinociceptive and anti-inflammatory properties (Zasso et al., 2005; Savegnago et al., 2007a,b,c, 2008). Additionally, the mechanism of antinociceptive action caused by $(PhSe)_2$ involves the serotoninergic pathway, an interaction with nitrergic system and glutamate receptors (Zasso et al., 2005; Savegnago et al., 2007a).

Nowadays, toxicological and pharmacological studies of our research group focus on the introduction of functional groups (e.g. chloro, fluoro or methoxyl) into the aromatic ring of $(PhSe)_2$ to elucidate if the alteration in chemical structure alters $(PhSe)_2$ effects. In a toxicological point of view, the introduction of functional groups into the aromatic ring of $(PhSe)_2$ reduced or abolished the appearance of seizure episodes in mice (Nogueira et al., 2003) and did not introduce toxicity after acute exposure. Calculated LD_{50} for $(PhSe)_2$ was similar to the values obtained for disubstituted $(PhSe)_2$ after acute exposure in mice (Savegnago et al., 2007a).

In a pharmacological point of view, we reported that *p*-methoxyl-diphenyl diselenide, $(MeOPhSe)_2$, when administered by oral route in mice exerts significative antinociceptive action in several models of nociception. The mechanisms through which $(MeOPhSe)_2$ exerts its action involve, among others, an interaction with glutamatergic and GABAergic systems and protein kinase A pathway (Pinto et al., 2008).

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Based on the above considerations, the objective of this study was to extend our previous findings by investigating in greater detail the mechanisms that might be involved in the antinociceptive action of $(MeOPhSe)_2$ in mice.

2. Materials and methods

2.1. Drugs

p-Methoxyl-diphenyl diselenide, $(MeOPhSe)_2$, 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 $(MeOPhSe)_2$ (99.9%) was determined by GC/HPLC. $(MeOPhSe)_2$ was dissolved in canola oil and administered by oral route (p.o.). Mice received $(MeOPhSe)_2$ in a constant volume of 10 ml/kg of body weight. All other drugs were dissolved in saline. All chemicals were of analytical grade and obtained from standard commercial suppliers (Sigma, St. Louis, USA).

2.2. Animals

The behavioral experiments were conducted using male Swiss mice (25–35 g) maintained at 22±2 °C with free access to water and food, under a 12:12 h light/dark cycle (with lights on at 6:00 a.m.). Mice were acclimatized to the laboratory for at least 1 h before testing and were used only once through 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.

At the end of the experimental procedure mice were killed by decapitation.

2.3. Glutamate-induced nociception

To address some of the mechanisms by which $(MeOPhSe)_2$ causes antinociception in glutamate-induced nociception, animals were treated with different drugs. The doses of the drugs used were selected on the basis of the literature (Santos et al., 1999, 2005; Luiz et al., 2007; Savegnago et al., 2007a; Pinto et al., 2008).

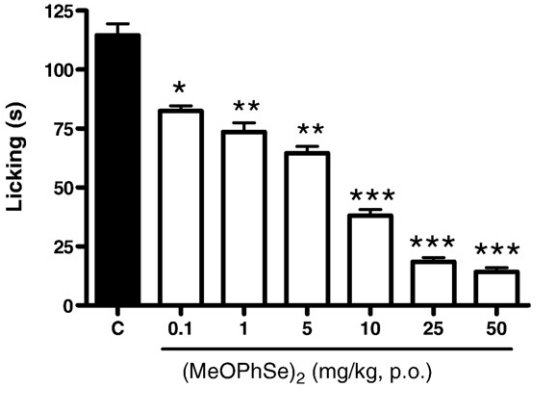

Fig. 1. Effect of $(MeOPhSe)_2$ administered orally on the licking induced by glutamate in mice. Animals were pretreated with $(MeOPhSe)_2$ at various doses (0.1–50 mg/kg) for 30 min prior to glutamate (10 μmol/paw, 20 μl). Each column represents the mean with S.E.M. for 6–8 mice in each group. Control 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):*$p<0.05$, **$p<0.01$, ***$p<0.001$.

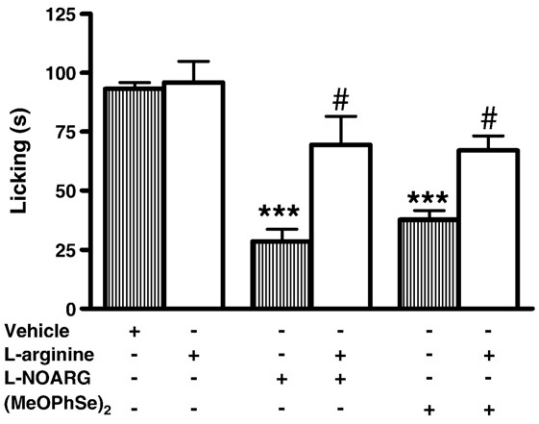

Fig. 2. Effect of pretreatment of animals with L-arginine (600 mg/kg i.p.) on the antinociceptive profiles of $(MeOPhSe)_2$ (10 mg/kg, p.o.) and L-NOARG (75 mg/kg, i.p.) against the glutamate-induced licking in mice. Each column represents the mean of 6–8 animals and vertical lines indicate the S.E.M. The symbols denote significance levels ***$p<0.001$ when compared to control groups; #$p<0.001$ when compared to L-NOARG or $(MeOPhSe)_2$ treated group by one-way ANOVA followed by Student–Newman–Keuls test.

The procedure used was similar to that described previously (Beirith et al., 2002). To this end, animals received 20 μl of glutamate solution (10 μmol/paw) injected i.pl. in the ventral surface of the

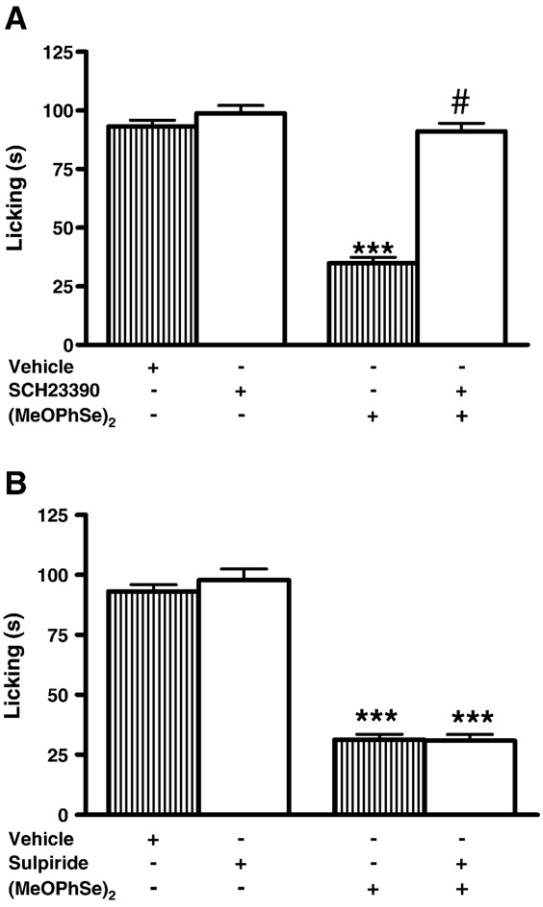

Fig. 3. Effect of pretreatment of animals with SCH 23390 (0.05 mg/kg, i.p., panel A) or with sulpiride (50 mg/kg, i.p., panel B) on the antinociceptive profiles of $(MeOPhSe)_2$ (10 mg/kg, p.o.) against the glutamate-induced licking in mice. Each column represents the mean of 6–8 animals and vertical lines indicate the S.E.M. The symbols denote the significance levels ***$p<0.001$ when compared to control groups; #$p<0.001$ when compared to $(MeOPhSe)_2$ treated group by one-way ANOVA followed by Student–Newman–Keuls test.

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 pre-treated with this compound (0.1–50 mg/kg, p.o.) or canola oil (10 ml/kg, p.o.) 30 min before glutamate injection.

2.3.1. Analysis of the mechanisms involved in the antinociceptive action caused by $(MeOPhSe)_2$ in the glutamate test

2.3.1.1. Involvement of L-arginine-nitric oxide pathway. To explore the possible contribution of L-arginine-nitric oxide pathway in the antinociception caused by $(MeOPhSe)_2$ in the glutamate test mice were pre-treated with L-arginine (600 mg/kg, i.p., a nitric oxide precursor) and after 20 min, they received $(MeOPhSe)_2$ (10 mg/kg, p.o.), N^{ω}-nitro-L-arginine (L-NOARG, 75 mg/kg, i.p., a nitric oxide synthase inhibitor) or vehicle (10 ml/kg, p.o.). The nociceptive response to glutamate was recorded 30 min after the administration of drugs. Another group of animals was pretreated with vehicle (10 ml/kg, i.p.) and after 20 min received $(MeOPhSe)_2$, L-NOARG or vehicle, 30 min before glutamate injection.

2.3.2. Involvement of dopaminergic, noradrenergic, serotonergic, adenosinergic and opioid system

To examine the possible participation of the dopaminergic system in the antinociceptive effect of $(MeOPhSe)_2$ on the glutamate test animals were pretreated with SCH23390 (0.05 mg/kg, i.p., a D_1 receptor antagonist) or sulpiride (5 mg/Kg, i.p., a D_2 receptor antagonist). To investigate the role played by the alfa adrenergic system in the antinociceptive effect caused by $(MeOPhSe)_2$ on the glutamate test, mice were pre-treated with prazosin (an α_1-adrenoreceptor antagonist, 0.15 mg/kg, i.p.) or with yohimbine (an α_2-adrenoreceptor antagonist, 1.0 mg/kg, i.p.).

The possible contribution of the serotoninergic system to the effect of $(MeOPhSe)_2$ on the glutamate test mice were pretreated with pindolol (1 mg/kg, i.p., a 5-$HT_{1A/1B}$ receptor/β adrenoceptor antagonist), WAY100635 (0.7 mg/kg, i.p., a selective 5-HT_{1A} receptor antagonist), ketanserin (0.3 mg/kg, i.p., a selective 5-HT_{2A} receptor antagonist), ondansetron (0.5 mg/kg, i.p., a 5-HT_3 receptor antagonist) or vehicle (10 ml/kg, i.p.). We investigated the possible involvement of adenosine receptors in the antinociceptive effect caused by $(MeOPhSe)_2$. To this end, mice were pretreated with caffeine (3 mg/kg, i.p., a non-selective adenosinergic receptor antagonist). After 15 min to the pretreatment with antagonists, mice received $(MeOPhSe)_2$ (10 mg/kg, p.o) or vehicle injection. Other groups of animals were pretreated with vehicle (10 ml/kg, i.p.) and after 15 min received $(MeOPhSe)_2$ or vehicle 30 min before glutamate injection.

To assess the possible participation of the opioid system in the antinociceptive effect of $(MeOPhSe)_2$ mice were pretreated with naloxone (1 mg/kg, i.p., a non-selective opioid receptor antagonist), and after 15 min the animals received an injection of $(MeOPhSe)_2$ (10 mg/kg, p.o.), morphine (5 mg/kg, s.c.) or vehicle (10 ml/kg, p.o.) 30 min before glutamate injection.

2.4. Bradykinin-induced nociception

The experiment was performed according to the method described by Ferreira et al. (2004). Mice were treated with $(MeOPhSe)_2$ (1-50 mg/kg, p.o.) or with canola oil (10 ml/kg, p.o.) 30 min before bradykinin injection (10 nmol/paw, 20 μl) in the plantar ventral surface of the right hindpaw. Animals were observed individually for 10 min following bradykinin injection. The amount of time spent licking the injected paw was recorded with a chronometer and considered as indicative of nociception.

2.5. Des-Arg^9-bradykinin-induced overt nociception

To verify a B_1 receptor-induced nociceptive response mice were pretreated with a low dose of phorbol myristate acetate (PMA, 0.5 nmol/paw; dose that did not induce overt nociception *per se*) into the plantar right hind paw (Ferreira et al., 2008). Following appropriated time points following challenge (from 0 to 45 min), the animals received an injection (20 μl) of des-Arg^9-bradykinin (10 nmol/paw) or saline into the previously PMA-treated paw. The mice were observed individually following des-Arg^9-bradykinin injection and the overt nociception was evaluated for 5 min. The time spent licking the injected paw during this period was recorded with a chronometer and considered as indicative of pain. Mice were treated with $(MeOPhSe)_2$ (1-50 mg/kg) by p.o. route, 30 min before plantar i.pl. injection of PMA. The procedure for producing algogen-induced nociception in mice was similar to that described previously (Ferreira et al., 2004).

2.6. Phorbol myristate acetate (PMA)-induced nociception

The procedure used was similar to that described previously (Siebel et al., 2004; Ferreira et al., 2005). Mice were treated with $(MeOPhSe)_2$ (1–50 mg/kg) by p.o. route, 30 min before plantar i.pl. injection of PMA (a protein kinase C (PKC) activator, 0.03 μg/paw,

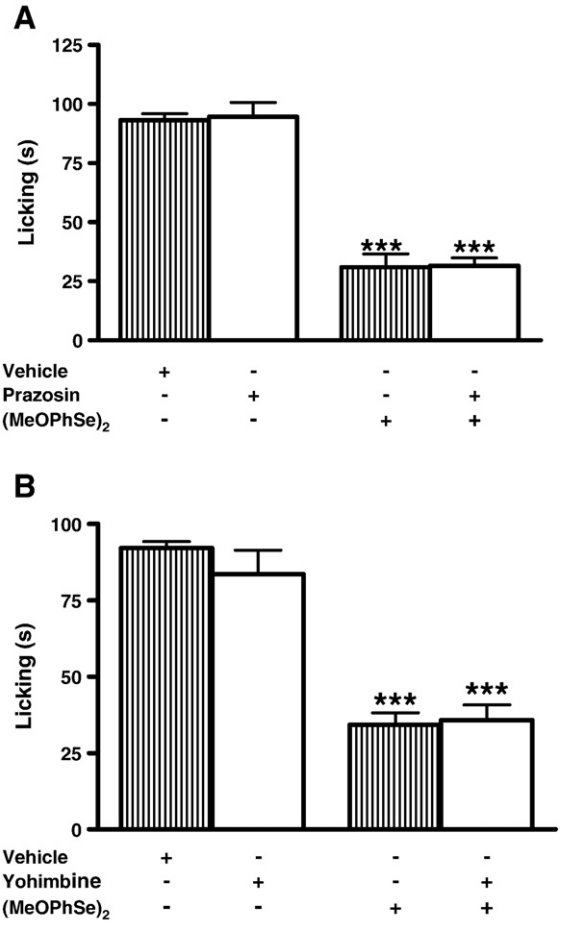

Fig. 4. Effect of pretreatment of animals with prazosin (0.15 mg/kg, i.p., panel A) or with yohimbine (1 mg/kg, i.p., panel B) on the antinociceptive profiles of $(MeOPhSe)_2$ (10 mg/kg, p.o.) against the glutamate-induced licking in mice. Each column represents the mean of 6–8 animals and vertical lines indicate the S.E.M. The symbols denote the significance levels $^{***}p<0.001$ when compared to control groups. One-way ANOVA followed by Student–Newman–Keuls test.

20 μl). After 15 min of the plantar i.pl. injection of PMA, animals were observed for a further 30 min. The time spent licking the injected paw during this period was recorded with a chronometer and considered as indicative of pain.

2.7. Statistical analysis

The results are presented as mean±S.E.M, except the ID_{50} values (i.e., the dose of compound necessary to reduce the nociceptive response by 50% relative to the control value), which are reported as geometric means accompanied by their respective 95% confidence limits. The ID_{50} value was determined by linear regression from individual experiments using linear regression GraphPad software (GraphPad software, San Diego, CA, USA). Maximal inhibition was calculated at the most effective dose using the GraphPad Prism version 3.00 for Windows. Comparisons between experimental and control groups were performed by ANOVA followed by Newman–Keuls' test when appropriated. *P* values less than 0.05 were considered as indicative of significance.

3. Results

3.1. Glutamate-induced nociception

The results presented in Fig. 1 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%.

3.2. Analysis of possible mechanism of $(MeOPhSe)_2$ action

The results presented in Fig. 2 show that the pretreatment of mice with the nitric oxide precursor, L-arginine (600 mg/kg, i.p.), given 20 min earlier, completely reversed antinociception caused by $(MeOPhSe)_2$ (10 mg/kg, p.o.) or L-NOARG (75 mg/kg, i.p.) when analyzed in the glutamate test in mice.

The results depicted in Fig. 3A show that SCH23390 (0.05 mg/kg, i.p., a D_1 receptor antagonist), given 15 min beforehand, completely reversed the antinociception caused by $(MeOPhSe)_2$ (10 mg/kg, p.o.) in glutamate induced-licking in mice. The treatment of animals with sulpiride (5 mg/kg, i.p., a D_2 receptor antagonist), given 15 min before, did not change antinociception caused by $(MeOPhSe)_2$ (10 mg/kg, p.o.) in the glutamate test in mice (Fig. 3B).

Treatment of mice with prazosin (0.15 mg/kg, i.p., an α_1-selective antagonist) (Fig. 4A) or yohimbine (1 mg/kg, i.p., an α_2-selective antagonist) (Fig. 4B) 15 min beforehand, did not reverse antinociception caused by $(MeOPhSe)_2$ (10 mg/kg, p.o.) in the glutamate test in mice.

The systemic treatment of animals with pindolol (1 mg/kg, i.p., a 5-$HT_{1A/1B}$ receptor/β adrenoceptor antagonist) (Fig. 5A), WAY100635 (0.7 mg/kg, i.p., a selective 5-HT_{1A} receptor antagonist) (Fig. 5B) and ketanserin (0.3 mg/kg, i.p., a selective 5-HT_{2A} receptor antagonist) (Fig. 5C) did not reverse antinociception caused by $(MeOPhSe)_2$ (10 mg/kg, p.o.) against glutamate-induced nociception (Figs. 5A–C). Fig. 5D shows that pretreatment of animals with ondansetron (0.5 mg/kg, i.p., a 5-HT_3 receptor antagonist) significantly reversed

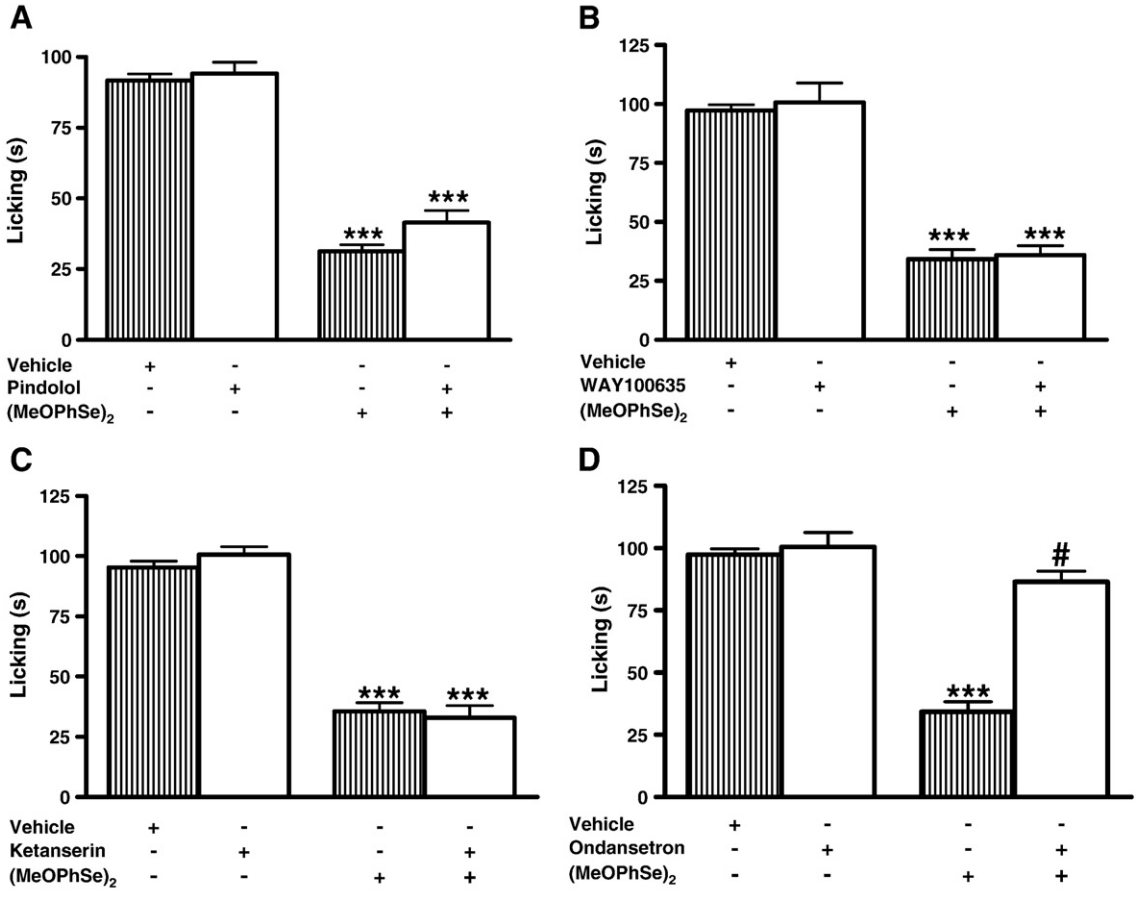

Fig. 5. Effect of pretreatment of mice with pindolol (1 mg/kg, i.p., panel A), WAY100635 (0.1 mg/kg, i.p., panel B), ketanserin (1 mg/kg, i.p., panel C) or ondansetron (1 mg/Kg, i.p., panel D) on the antinociceptive profiles of $(MeOPhSe)_2$ (10 mg/kg, p.o.) against the glutamate-induced licking in mice. Each column represents the mean of 6–8 animals and vertical lines indicate the S.E.M. The symbols denote the significance levels ***$p<0.001$ when compared to control groups; #$p<0.001$ when compared to $(MeOPhSe)_2$ treated group by one-way ANOVA followed by Student–Newman–Keuls test.

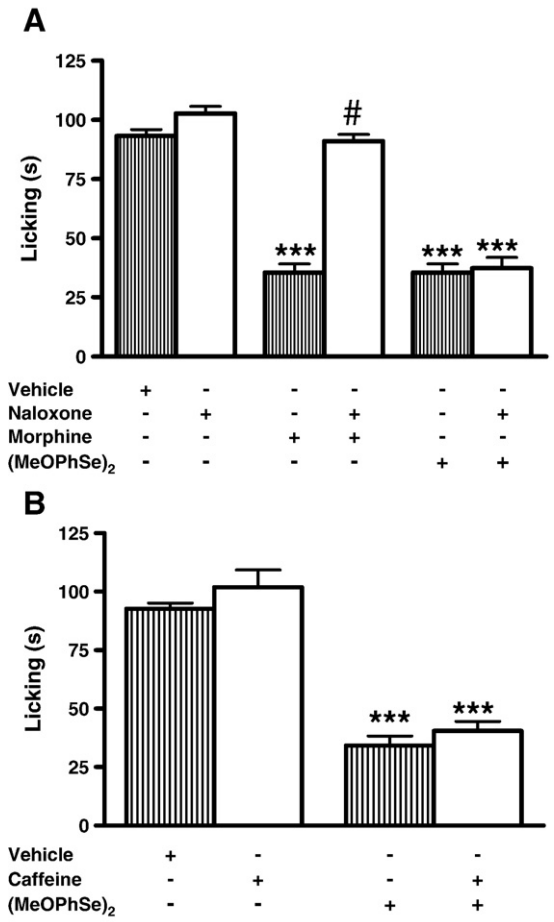

Fig. 6. Effect of pretreatment of animals with naloxone (1 mg/kg, i.p., panel A) or caffeine (3 mg/kg, i.p., panel B) on the antinociceptive profiles of $(MeOPhSe)_2$ (10 mg/kg, p.o.) against the glutamate-induced licking in mice. Each column represents the mean of 6–8 animals and vertical lines indicate the S.E.M. The symbols denote the significance levels ***$p<0.001$ when compared to control groups; #$p<0.001$ when compared to morphine treated group by one-way ANOVA followed by Student–Newman–Keuls test.

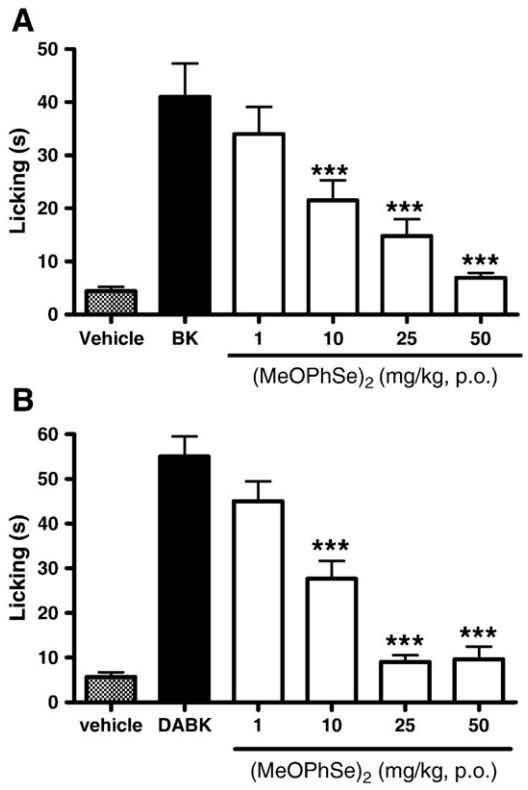

Fig. 7. Effect of $(MeOPhSe)_2$ administered orally on the licking induced by BK (A) or DABK (B) in mice. Animals were pretreated with $(MeOPhSe)_2$ at various doses (1–50 mg/kg) for 30 min prior to BK (10 nmol/paw, 20 μl) or DABK (10 nmol/paw, 20 μl). Each column represents the mean with S.E.M. for 6–8 mice in each group. Control value "C" indicates the animals injected with vehicle (canola oil). The asterisks denote the significance levels when compared to the control group (one-way ANOVA followed by Newman–Keuls test):***$p<0.001$.

antinociception caused by $(MeOPhSe)_2$ (10 mg/kg, p.o.) in the glutamate test in mice.

The results presented in Fig. 6A show that the pretreatment of mice with naloxone (1 mg/kg, i.p., a non-selective opioid receptor antagonist), given 15 min beforehand, completely reversed antinociceptive effect of morphine (5 mg/kg, s.c.) in the glutamate test. Pretreatment of mice with naloxone did not change antinociception caused by $(MeOPhSe)_2$ (10 mg/kg, p.o.) in the glutamate test (Fig. 6A). The systemic pretreatment of mice with caffeine (3 mg/kg, i.p., a non-selective adenosine receptor antagonist) did not reverse antinociception caused by $(MeOPhSe)_2$ (10 mg/kg, p.o.) against glutamate-induced nociception (Fig. 6B).

3.3. Bradykinin-induced nociception

As can be seen in Fig. 7A $(MeOPhSe)_2$ (10, 25 and 50 mg/kg, p.o.) significantly inhibited nociception induced by injection of bradykinin (10 nmol/paw). The maximal inhibition observed was 83±2% and the calculated mean ID_{50} value for this effect was 10.96 (5.09–23.60) mg/kg.

3.4. Des-Arg^9-bradykinin-induced overt nociception

The results presented in Fig. 7B show that $(MeOPhSe)_2$, given orally at the doses of 10, 25 and 50 mg/kg, caused a significant inhibition of Des-Arg^9-bradykinin-induced nociception, with a mean ID_{50} value of 10.06 (4.57–22.17.) mg/kg and maximal inhibition observed of 83±5%.

3.5. Phorbol myristate acetate (PMA)-induced nociception

Oral treatment with $(MeOPhSe)_2$ (10 and 50 mg/kg) significantly inhibited PMA-induced licking response (Fig. 8). The maximal inhibition

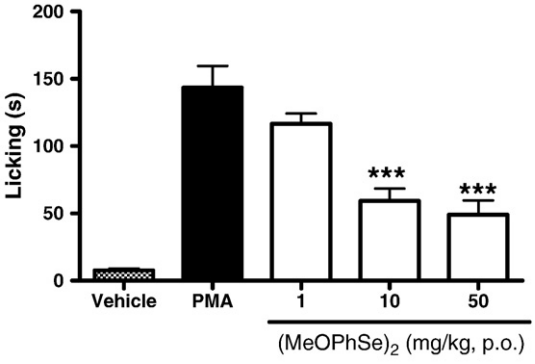

Fig. 8. Effect of $(MeOPhSe)_2$ administered orally on the licking induced by PMA in mice. Animals were pretreated with $(MeOPhSe)_2$ at various doses (1–50 mg/kg) for 30 min prior to PMA (0.03 μg/paw, 20 μl).. Each column represents the mean with S.E.M. for 6–8 mice in each group. Control value "C" 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):***$p<0.001$.

observed was 66±7% and the calculated mean ID_{50} value for this effect was 23.38 (16.04–34.08) mg/kg.

4. Discussion

In this study we demonstrated that $(MeOPhSe)_2$ causes antinociception in mice through mechanisms that involve an interaction with nitrergic system, 5-HT_3 and D_1 receptors. Our data also suggest that $(MeOPhSe)_2$ antinociceptive effect is related to its ability to interact with kinin B_1 and B_2 receptors and PKC pathway mediated mechanisms. This compound exerts antinociceptive action in several models of nociception and the mechanisms through which $(MeOPhSe)_2$ exerts its action involve an interaction with glutamatergic and GABAergic systems and protein kinase A pathway (Pinto et al., 2008). The intraplantar injection of glutamate into the mouse hindpaw produces nociceptive-like behaviors of rapid onset and short duration (about 15 min) (Beirith et al., 2002). Accumulating evidence now suggests that there is an excess of excitatory amino acids, mainly glutamate, following injury at the spinal cord or following certain inflammatory process, suggesting that excitatory amino acids might play a relevant role in sensory transmission (Jackson et al., 1995; Zhou et al., 1996; McNearney et al., 2000). In addition, glutamate acts at NMDA and non-NMDA sites, nitric oxide-cGMP pathway, capsaicin-sensitive fibers, NK_1, NK_2 and B_1 receptors (Malmberg and Yaksh, 1993; Meller et al., 1996; Beirith et al., 2002; 2003).

According to Melzack (1999) description, in the spinal cord the nociceptive information coming from gut, skin and other organs is submitted to a modulation by a great variety of transmitters that will filter and modulate the transmission of nociceptive impulses to the brain (Besson, 1999; Fürst, 1999; Millan, 2002). These modulating substances are able to act as pro- (descending facilitation) or antinociceptive (descending inhibition), depending on diverse factors, such as the type and intensity of the stimulation, the central region activated, receptor type, and others (Millan, 2002). The neurons projected by the central areas responsible for the control of the perception of pain (descending facilitation and descending inhibition) contain several transmitters, including noradrenaline, serotonin (5-HT), acetylcholine, gamma-aminobutyric acid (GABA), nitric oxide (NO), glutamate, dopamine and others (Fürst, 1999; Millan, 2002).

In view of this, we investigated the participation of the L-arginine/nitric oxide pathway in the antinociceptive effect of $(MeOPhSe)_2$. Pretreatment of animals with the substrate for NOS, L-arginine, significantly restored antinociception caused by $(MeOPhSe)_2$ and L-NOARG (a known inhibitor of NOS). To our knowledge, this is the first study indicating that L-arginine-nitric oxide pathway is, at least in part, involved in antinociception induced by $(MeOPhSe)_2$ in the glutamate test. Considerable evidence has been accumulated suggesting a role for NO as a mediator of inflammation (Lyons, 1995). NO increases the synthesis/release of pro-inflammatory mediators such as cytokines and reactive oxygen species (Marcinkiewicz et al., 1995) and prostanoids (Sautebin et al., 1995), resulting in promotion of inflammatory reaction. Beirith et al. (2002) demonstrated that nociception induced by glutamate is greatly mediated by the release of NO. This observation derives from the view that an inhibitor of nitric oxide synthase, as L-NOARG, significantly inhibits glutamate-induced nociception. The administration of L-arginine did not modify the limiar of nociception. Thus we can speculate that NO produced by L-arginine did not change the nociception. Conversely, the administration of SNAP, a NO donor, potentiated the nociception induced by glutamate. The difference is that L-arginine is the precursor of NO and to produce NO is necessary the action of the NOS and the SNAP is an NO donor and did not require the action of the NOS. It probably means that: a) $(MeOPHSe)_2$ is an "antagonist" and it is inhibiting competitively the NOS; b) L-arginine alone is not able to increase NO at systemic level; and c) L-arginine is acting as an agonist and it is displacing competitively to $(MeOPHSe)_2$.

We also investigated the possible involvement of the descending inhibitory pathways, noradrenergic and serotonergic, in the antinociceptive effect of $(MeOPhSe)_2$. The pretreatment of animals with ondansetron (a 5-HT_3 receptor antagonist) reversed the antinociceptive effect caused by oral administration of $(MeOPhSe)_2$. Conversely, pretreatment with pindolol (a 5-$HT_{1A/1B}$ receptor/β adrenoceptor antagonist), WAY 100635 (a selective 5-HT_{1A} receptor antagonist) and ketanserin (a selective 5-HT_{2A} receptor antagonist) did not promote any change in the antinociceptive effect of $(MeOPhSe)_2$. Similarly, pretreatment of animals with prazosin (an α_1-adrenoreceptor antagonist) and yohimbine (an α_2-adrenoreceptor antagonist) was ineffective in reversing the antinociceptive action of $(MeOPhSe)_2$ in the glutamate test. These results suggest that 5-HT_3-receptors participate in the antinociceptive effect of $(MeOPhSe)_2$. In this context, it is very important to mention that the analgesic action of $(PhSe)_2$, a parent compound of $(MeOPhSe)_2$, seems to be unlike the interaction with α-$_1$ and α-$_2$ adrenoceptors (Zasso et al., 2005). In this study, Zasso and collaborators (2005) showed the involvement of 5-HT_3 receptor in the antinociceptive effect induced by $(PhSe)_2$ in the formalin test in mice. Several studies have reported that the spinal serotonin system may suppress incoming noxious input to the spinal cord and inhibit pain transmission (Alhaider et al., 1991; Millan, 2002). In addition, there has been demonstrated that the nonselective serotonergic antagonist, methysergide, inhibited the nociceptive behaviors induced by formalin, substance P (i.t.) and excitatory amino acids (i.t.), such as glutamate, *N*-methyl-D-aspartic acid, -amino-3-hydroxy-5-methylisoxazole-4-propionic acid, and kainic acid (Chung et al., 2003). Several authors (Choi et al., 2003; Pietrovski et al., 2006; Luiz et al., 2007) have reported that the inhibition of nociception induced by glutamate could be attributed to the serotonergic pathway.

The antinociception caused by serotonin is partially due to a release of adenosine in the spinal cord (Sawynok and Reid, 1996). However, the antagonism of adenosine receptors did not prevent antinociceptive effect caused by $(MeOPhSe)_2$. Regarding this finding, the antinociception induced by $(MeOPhSe)_2$ is likely not related to a modulation of the adenosinergic system. Conversely, pretreatment of mice with caffeine (a non-specific adenosine receptor antagonist) and PSB1115 (an adenosine A_{2B} receptor antagonist) significantly blockades the antinociceptive effect caused by $(PhSe)_2$ in the hot-plate test (Savegnago et al., 2008).

In addition, pretreatment with SCH23390 (a D_1 receptor antagonist) but not sulpiride (a D_2 receptor antagonist) reversed the antinociceptive effect caused by oral administration of $(MeOPhSe)_2$. These results suggest that D_1-receptors participate in the antinociceptive effect of $(MeOPhSe)_2$. Diverse studies have demonstrated that dopamine exerts an important function in nociception control in several models of chronic (Jaaskelainen et al., 2001; Hagelberg et al., 2003) and acute pain (Morgan and Franklin, 1991; Zarrindast et al., 1999). When a harmful stimulation occurs, there is an increase in dopamine "turnover" in the dorsal horn of the spinal cord, suggesting an increase in the activity of descending dopaminergic pathways (Millan, 2002). Moreover, Gao et al. (2001), using carragenan as a tonic long-term nociceptive model, show that the injection of a dopamine D_1 antagonist (SCH23390) causes a decrease in algesic response. There has been reported that dopaminergic transmission in the brain is involved in the central modulation of peripheral inflammatory pain mediated by dopamine D_1 receptor. Thus, the dopamine D_1 receptor perhaps induced tonic excitation of inflammatory pain pathways, because blocking the dopamine D_1 receptor produced anti-hyperalgesia or hypoalgesia in a model of carrageenan-induced inflammatory pain (Gao et al., 2001).

The involvement of opioid system on the antinociceptive action of $(MeOPhSe)_2$ was also evaluated. Pretreatment of animals with naloxone (a non-selective opioid receptor antagonist) did not reverse the antinociceptive effect caused by oral administration of $(MeOPhSe)_2$. Correspondingly, the antinociceptive effect of $(PhSe)_2$

seems to be unlike the activation of opioid receptors (Zasso et al., 2005, Savegnago et al., 2007a).

The current study clearly indicates that the antinociceptive effect of $(MeOPhSe)_2$ is related to the release of bradykinin. In fact, $(MeOPhSe)_2$ inhibited bradykinin-induced nociception in mice. Bradykinin and its related kinins are vasoactive peptides which have an important role as inflammatory mediators and are normally released following tissue trauma or infection. Once released, bradykinin may induce pain by direct stimulation of the nociceptive fibers (Aδ and C fibers) innervating many tissues. Furthermore, bradykinin can release most inflammatory and algogenic substances, namely products derived from arachidonic acid pathways, cytokines and nitric oxide (Dray and Perkins, 1997; Calixto et al., 2000, 2001). The actions of kinins are mediated through the stimulation of two subtypes of G-protein coupled receptors, denoted B_1 and B_2 (Campos et al., 2006). The B_1 receptors are only very weakly expressed in non-traumatized tissues, but they can be strongly expressed under certain conditions, such as those following tissue injuries or in inflammatory states (Campos and Calixto, 1995; Campos et al., 1996). Ferreira et al. (2004) showed that BK-induced nociception and that this effect is related to B_2 kinin receptor activation, as the selective B_2 receptor agonist Tyr^8-BK mimicked its response. In addition, BK-induced nociception was almost abolished by the selective B_2 receptor antagonist. Recently, Ferreira et al. (2008) have demonstrated that PMA induced nociception is blocked in B_1 receptor knockout mice and that des-Arg^9 bradykinin produces nociception in paws previously treated with low doses of PMA. Our present results show that $(MeOPhSe)_2$ was effective in blocking, in a dose-dependent manner, BK and des-Arg^9-bradykinin-induced nociception, demonstrating a role of B_1 and B_2 receptors related mechanisms in the action of $(MeOPhSe)_2$.

In this study we have also demonstrated that $(MeOPhSe)_2$, orally administered, inhibited PMA-induced nociception in mice. In fact, Souza et al. (2002) have reported that an intraplantar injection of PMA, a known protein kinase C activator, induces paw licking in mice. Accordingly, Tsuchiya et al. (2005) found that nociceptive response to PMA was associated with the formation of oedema and erythema in the injected paw which was observed in a histological study. Moreover, there is a substantial amount of experimental evidence supporting the role exerted by PKC and bradykinin in the control of pain sensitivity (Ferreira et al., 2005, 2008; Savegnago et al., 2007a). Based on the results we can speculate that the antinociceptive effect caused by $(MeOPhSe)_2$ is related to its ability to interact with PKC pathway-B_1 and B_2 receptor-mediated mechanisms since $(MeOPhSe)_2$ consistently inhibited PMA-, des-Arg^9-bradykinin- and BK-mediated nociception. In addition, the effects of $(MeOPhSe)_2$ might be well explained by an interference with B_1 receptor up-regulation by PMA and with protein synthesis. The actions of kinins are mediated through the stimulation of two subtypes of G-proteins coupled receptors, denoted B_1 and B_2 (for review see: Calixto et al., 2000) and the coadministration of the selective PKC inhibitor GF109203X completely abolished BK-induced nociception (Ferreira et al., 2004).

The administration of $(MeOPhSe)_2$ exerts antinociceptive effect in the first phase of formalin test, hot plate and inhibits nociceptive response induced by glutamatergic agonists injected by intrathecal route (Pinto et al., 2008), suggesting that this organoselenium compound crosses the hemato-encephalic barrier. There has been reported that $(PhSe)_2$, a parent compound of $(MeOPhSe)_2$, crosses the hemato-encephalic barrier exerting antidepressant effect (Savegnago et al., 2007d), antinociceptive properties by act at central level (Savegnago et al., 2007b,c) and induces seizures in rats (Prigol et al., 2007). The administration of $(MeOPhSe)_2$ produces an antinociceptive effect at peripheral level, inhibits the second phase of formalin test and the increase of the oedema in formalin and glutamate tests (Pinto et al., 2008). Savegnago et al. (2007a,c) demonstrated that antinociceptive and anti-inflammatory effects of $(PhSe)_2$ in mice involved the peripheral level.

Taking into account the results presented in this study and those described previously (Pinto et al., 2008) we believe that $(MeOPhSe)_2$ may be effective in providing antinociception through mechanisms that involve an interaction with nitrergic system, 5-HT_3 and D_1 receptors. Our data also suggest that the antinociceptive effect is related to $(MeOPhSe)_2$ ability to interact with kinin B_1 and B_2 receptors and PKC pathway mediated mechanisms. Thus, $(MeOPhSe)_2$ could constitute an attractive molecule of interest for the development of new analgesic drugs to combat pain.

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