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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).

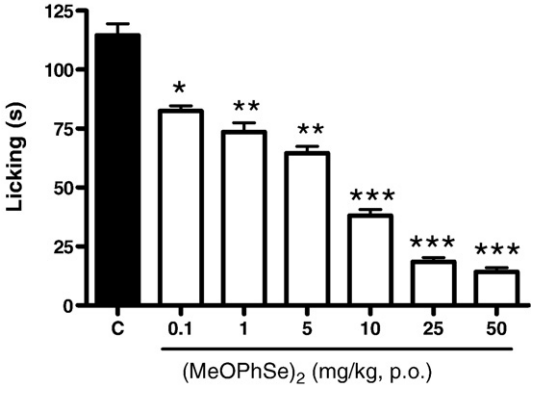

Fig. 1. Effect of $(MeOPhSe)_2$ administered orally on the licking induced by glutamate in mice. Animals were pretreated with $(MeOPhSe)_2$ at various doses (0.1–50 mg/kg) for 30 min prior to glutamate (10 μmol/paw, 20 μl). Each column represents the mean with S.E.M. for 6–8 mice in each group. Control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

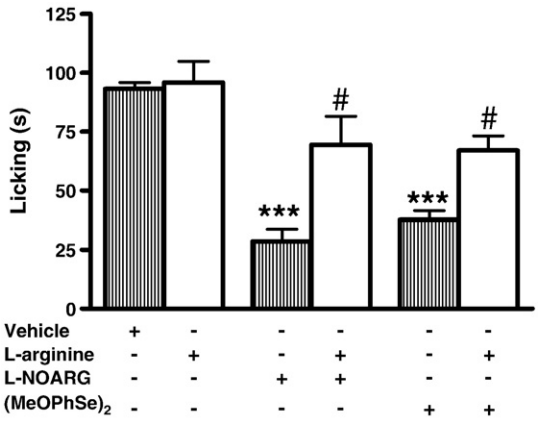

Fig. 2. Effect of pretreatment of animals with L-arginine (600 mg/kg i.p.) on the antinociceptive profiles of $(MeOPhSe)_2$ (10 mg/kg, p.o.) and L-NOARG (75 mg/kg, i.p.) against the glutamate-induced licking in mice. Each column represents the mean of 6–8 animals and vertical lines indicate the S.E.M. 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

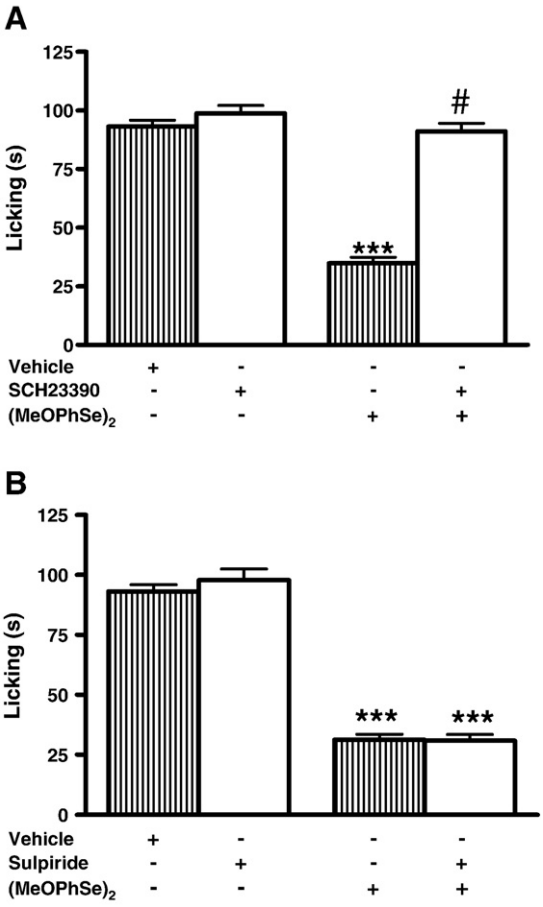

Fig. 3. Effect of pretreatment of animals with SCH 23390 (0.05 mg/kg, i.p., panel A) or with sulpiride (50 mg/kg, i.p., panel B) on the antinociceptive profiles of $(MeOPhSe)_2$ (10 mg/kg, p.o.) against the glutamate-induced licking in mice. Each column represents the mean of 6–8 animals and vertical 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.