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Antinociceptive effect of butyl (2-phenylethynyl) selenide on formalin test in mice: Evidences for the involvement of serotonergic and adenosinergic systems

Cristiane Luchese, Marina Prigol, Carmine Inês Acker, Cristina Wayne Nogueira *

Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil

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ABSTRACT

The present study investigated the effect of per oral (p.o.) administration of butyl (2-phenylethynyl) selenide (1–50 mg/kg) on formalin-induced nociception in mice. The involvement of serotonergic, adenosinergic, muscarinic cholinergic and opioid mechanisms in the antinociceptive effect was also investigated. Butyl (2-phenylethynyl) selenide inhibited both neurogenic (at doses equal or higher than 10 mg/kg) and inflammatory (at doses equal or higher than 25 mg/kg) phases of the nociception caused by intraplantar (i.pl.) injection of 2.5% formalin solution (20 μl), with ID_{50} values of 36.7 (29.28–46.0) and 20.37 (15.74–26.36) mg/kg, respectively. This compound reduced the formalin-induced paw oedema formation (55 ± 4%) at doses equal or higher than 25 mg/kg. The antinociceptive effect of compound (25 mg/kg, p.o.) was reversed by ondansetron (0.5 mg/kg, a 5-HT_3 receptor antagonist) and caffeine (3 mg/kg, a nonselective adenosine receptor antagonist), but not by atropine (0.1 mg/kg, a non selective muscarinic antagonist), WAY100635 (0.1 mg/kg, a selective 5-HT_{1A} receptor antagonist), ritanserin (1 mg/kg, a 5-HT_2 receptor antagonist) and naloxone (1 mg/kg, a non selective opioid receptor antagonist). These results indicate that butyl (2-phenylethynyl) selenide produced antinociception in the formalin test through mechanisms that involve an interaction with serotonergic (5-HT_3) and adenosinergic systems.

1. Introduction

Several drugs induce antinociception by interfering with neuronal pathways involved in the receipt and transmission of nociceptive information from the periphery to supra-spinal sites in the central nervous system (Furst, 1999). Serotonin (5-HT) is known to play an important role in central and peripheral mechanisms of nociception (Millan, 2002). Atropine, a cholinergic muscarinic antagonist, significantly antagonized non-steroidal anti-inflammatory drugs (NSAID)-induced antinociception. This effect suggests a possible muscarinic receptor-mediated mechanism for the antinociceptive activity of NSAIDs (Pinardi et al., 2003). Moreover, opioid and adenosinergic mechanisms have been shown to be involved in the modulation of nociception (Abo-Salem et al., 2004; Lopes et al., 2009).

The interest in organoselenium biochemistry and pharmacology has increased in the last two decades due to a variety of organoselenium compounds that show biological activity (Nogueira et al., 2004). Accordingly, a number of novel pharmaceutical agents which are selenium-based or which target specific aspects of selenium metabolism are under development (May, 1999; Nogueira et al., 2004). In addition to their antioxidant properties (Nogueira et al., 2004; Luchese et al., 2009; Prigol et al., 2009), selenium compounds show neuroprotective (Porciúncula et al., 2001; Rossato et al., 2002), antihypertensive, anticancer, antiviral, immunosuppressive, antimicrobial, antinociceptive and anti-inflammatory properties (Schewe, 1995; May, 1999; Nogueira et al., 2004; Savegnago et al., 2006).

Alkynylselenoalcohols are organoselenium compounds that possess an alkyl or aryl group at the triple bond and a carbon chain between the selenium atom and the hydroxyl group (Okoronkwo et al., 2008). A previous study of our research group revealed that these compounds presented an *in vitro* antioxidant activity on liver and brain of rats (Acker et al., 2009). Our research group has also demonstrated that modifications in the molecular structure of alkynylselenoalcohols affect their antioxidant profile. The absence of the hydroxyl group at the carbon chain of alkynylselenoalcohol improves the *in vitro* antioxidant activity of these compounds (Acker et al., 2009).

Reactive oxygen species have been proposed to contribute to and/or maintain conditions of chronic pain (Wang et al., 2004). Free radical molecules and radical-derived reactive oxygen species, including hydrogen peroxide, superoxide, hydroxyl radicals and nitric oxide have been implicated in tissue injury and enhanced nociceptive response (Khalil and Khodr, 2001). In fact, recent reports suggest that antioxidants are effective analgesics in neuropathic and inflammatory pain models (Hacimuftuoglu et al., 2006; Erdemoglu et al., 2009).

In view of the above considerations and based on the previous screening for antioxidant activity of alkynylselenoalcohols (Acker et al., 2009), butyl (2-phenylethynyl) selenide, an alkynylselenoalcohol

* Corresponding author. Departamento de Química, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil. Tel.: +55 55 3220 8140; fax: +55 55 3220 8978.
E-mail address: criswn@quimica.ufsm.br (C.W. Nogueira).

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without the hydroxyl group at the carbon chain, was chosen for evaluating the antinociceptive activity in the formalin-induced pain model in mice. We also examined the involvement of serotonergic, adenosinergic, muscarinic cholinergic and opioid mechanisms in the antinociceptive effect of this compound.

2. Materials and methods

2.1. Drugs

Butyl (2-phenylethynyl) selenide (Fig. 1) was prepared and characterized in our laboratory. Analysis of the ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of butyl (2-phenylethynyl) selenide (99.9%) was determined by gas chromatography/high performance liquid chromatography (GC/HPLC). Butyl (2-phenylethynyl) selenide was dissolved in canola oil and administered by oral route (p.o.). The mice received the compound in a constant volume of 10 ml/kg body weight. All other drugs used were dissolved in saline. All other chemicals were of analytical grade and obtained from standard commercial suppliers (Sigma, St. Louis, USA).

2.2. Animals

Male adult Swiss mice, weighing 25–35 g, were obtained from a local breeding colony. The animals were kept in a separate animal room, on a 12 h light/dark cycle, in an air conditioned room (22 ± 2 °C). Commercial diet (GUABI, RS, Brazil) and tap water were supplied *ad libitum*. We applied strict ethical criteria in our study to minimize the pain and suffering. 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.

2.3. Formalin test

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 intraplantarly (i.pl.) in the ventral right hind paw. Animals were pretreated with butyl (2-phenylethynyl) selenide by gavage (1–50 mg/kg, p.o.). Control animals received a similar volume of vehicle (canola oil, 10 ml/kg, p.o.). To assess time–course of the antinociceptive effect of butyl (2-phenylethynyl) selenide, mice were pretreated with compound (25 mg/kg, p.o.) 15–240 min before i.pl. injection of formalin. After formalin injection, the animals were observed from 0 to 5 min (first phase, neurogenic phase) and 15–30 min (second phase, inflammatory phase) and the time spent licking or biting the injected paw was recorded with a chronometer and considered as indicative of nociception.

In order to assess whether the antinociceptive activity produced by butyl (2-phenylethynyl) selenide 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 (non treated paw). For this purpose, animals were euthanized 30 min after formalin injection by cervical dislocation, and both paws were cut at the ankle joint and immediately weighed on an analytical balance (Santos and Calixto, 1997).

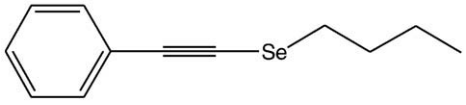

Fig. 1. Chemical structure of butyl (2-phenylethynyl) selenide.

2.4. Antagonism studies

To address some of the mechanisms by which butyl (2-phenylethynyl) selenide causes antinociception in the formalin test, animals were treated with different drugs. All antagonists were used at subeffective doses as previously reported (Santos et al., 2005; Lopes et al., 2009).

In those experiments designed to evaluate the involvement of cholinergic, serotonergic and adenosinergic mechanisms in butyl (2-phenylethynyl) selenide-induced antinociception, animals were pre-injected by intraperitoneal (i.p.) route with atropine (a non selective muscarinic antagonist, 0.1 mg/kg, a subeffective dose), ondansetron (a 5-HT_3 receptor antagonist, 0.5 mg/kg, a subeffective dose), *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridyl)cyclohexanecarboxamide (WAY100635) (a selective 5-HT_{1A} receptor antagonist, 0.1 mg/kg, a subeffective dose), ritanserin (a 5-HT_2 receptor antagonist, 1 mg/kg, a subeffective dose), caffeine (a non selective adenosine receptor antagonist, 3 mg/kg, a subeffective dose) or vehicle (saline 0.9%, 10 ml/kg). Twenty minutes following administration of each antagonist, mice received a single oral dose of butyl (2-phenylethynyl) selenide (25 mg/kg, p.o.) or vehicle (canola oil, 10 ml/kg, p.o.), 30 min before formalin i.pl. injection.

To assess the participation of the opioid system, mice were pretreated (i.p.) with naloxone (a non selective opioid receptor antagonist, 1 mg/kg, a subeffective dose). Twenty minutes following administration of naloxone, animals received butyl (2-phenylethynyl) selenide (25 mg/kg, p.o.) or vehicle (canola oil, 10 ml/kg, p.o.), 30 min before formalin administration. Another group of mice was pretreated with vehicle (saline 0.9%, 10 ml/kg, i.p.) and after 20 min received butyl (2-phenylethynyl) selenide or vehicle (canola oil, 10 ml/kg, p.o.), 30 min before saline administration.

2.5. Statistical analysis

The results are presented as means ± S.E.M. The statistical difference between groups was calculated by means of one-way analysis of variance (ANOVA) followed by the Duncan's test when appropriate. Probability values less than 0.05 ($P<0.05$) were considered as statistically significant. The ID_{50} values (i.e. the dose of butyl (2-phenylethynyl) selenide which reduced the pain response by 50% in relation to control group values) were determined by linear regression from individual experiments using "GraphPad Software" (GraphPad software, San Diego, CA, USA) and are reported as geometric means accompanied by their respective 95% confidence limits. Maximal inhibition values were calculated at the most effective dose used.

3. Results

3.1. Effect of butyl (2-phenylethynyl) selenide on formalin-induced nociception

A time–course analysis of the antinociceptive profile of butyl (2-phenylethynyl) selenide was accomplished. The antinociceptive effect of butyl (2-phenylethynyl) selenide reached its peak at 30 min and remained significant up to 45 min after compound administration (25 mg/kg) in the first phase (Fig. 2A). In the second phase and in the paw oedema, the time point of the maximum effect of butyl (2-phenylethynyl) selenide was 15 min and remained significant up to 60 min after compound administration (Fig. 2A and B, respectively). Thus, the time chosen for all further studies of the antinociceptive profile of butyl (2-phenylethynyl) selenide was 30 min after compound administration.

The effect of butyl (2-phenylethynyl) selenide on the time spent licking the injected paw during the first (0–5 min) and second phases (15–30 min) of the formalin test is depicted in Table 1. Butyl (2-phenylethynyl) selenide at doses equal and greater than 10 mg/kg