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Contribution of dopaminergic and adenosinergic systems in the antinociceptive effect of *p*-chloro-selenosteroid

Marcel Henrique Marcondes Sari [a,b], Ana Cristina Guerra Souza [a,b], Suzan Gonçalves Rosa [a,b], Diego Souza [a,b], Oscar Endrigo Dorneles Rodrigues [a,b], Cristina Wayne Nogueira [a,b,*]

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

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ABSTRACT

This study investigated the antinociceptive action of *p*-chloro-selenosteroid (PCS), administered by intragastric route (i.g.) to mice against acute models. The contribution of adenosinergic, dopaminergic, serotonergic, nitric oxide and opioid systems was investigated. It was evaluated if the administration of PCS triggers toxic effect. Treatment with PCS (10 mg/kg) reduced writhing induced by acetic acid and its effect lasts up to 48 h after treatment. The compound caused an inhibition in neurogenic and inflammatory phases of nociception and in paw edema induced by formalin. The licking behavior triggered by glutamate was reduced by PCS. In the tail-immersion test, PCS elicited an increase in delta latency response. Pretreatment with caffeine (3 mg/kg, intraperitoneally [i.p.]) and SCH58261 (3 mg/kg, i.p.), antagonist at adenosinergic receptors, SCH23390 (0.05 mg/kg, i.p.) and sulpiride (5 mg/kg, i.p.), antagonist at dopaminergic receptors, caused a reduction in the antinociceptive action of PCS in the glutamate test. By contrast, pretreatment with WAY100635 (0.7 mg/kg, i.p.), ketanserin (0.3 mg/kg, i.p.), ondasentron (0.5 mg/kg, i.p.), L-arginine (600 mg/kg, i.p.) and naloxone (1 mg/kg, subcutaneous [s.c.]) did not abolish the antinociceptive effect caused by PCS (10 mg/kg, i.g.) administration. The animals treated with PCS did not show alterations in locomotor and exploratory activities, in biochemical parameters evaluated, food and water consumption, as well as in the body weight. These results clearly showed the antinociceptive action of PCS in different animal models without causing acute toxic effects in mice. Adenosinergic and dopaminergic systems seem to be related to the mechanisms by which PCS elicits antinociception.

1. Introduction

Essentially, in physiological conditions, pain is a complex phenomenon resultant of the modulation of several peripheral and central mechanisms (Olesen et al., 2012). In such context, it constitutes an alarm that has the assisting role of preserving the organism, decreasing whatever is triggering the pain and, as a result, limiting the damaging consequences (Julius and Basbaum, 2001; Le Bars et al., 2001). Despite the great importance of pain, in some cases, its presence is enduring, devastating and debilitating, which limits productivity and substantially diminishes the well-being (Millan, 1999; Olesen et al., 2012).

The existing therapeutic treatments consist in the usage of several drugs. Such treatments are, in most cases, efficient; however, there are some concerns about their safety and the unpleasant side effects (Jage, 2005; Mitchell and Warner, 2006). These facts explain the enhanced interest in the development of new drugs with improved therapeutic index, which could be applied to control and relief of pain (Kennedy, 2007; Mao, 2009).

In such context, organoselenium compounds have excelled as an interesting resource of new synthetic substances with potential therapeutic applications due to their useful pharmacological activities (Nogueira and Rocha, 2011). Among them, the antinociceptive and anti-inflammatory properties have been studied by us and others (Bruning et al., 2010; Chagas et al., 2013; Nogueira et al., 2003; Savegnago et al., 2007), suggesting that these organoselenium compounds could be relevant drugs for the management of pain.

In addition, the synthesis and studies of steroids continue to be a topic of widespread interest (Ibrahim-Ouali, 2008). Oxygenated derivatives of cholesterol, also known as oxysterols, represent a

* Corresponding author at: Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, 97105-900, RS, Brasil. Tel.: + 55 55 32208140; fax: + 55 55 3220 8978.
E-mail address: criswn@quimica.ufsm.br (C. Wayne Nogueira).

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group of biomolecules that has received more attention due to their relevant bioactivities, such as a possible regulation of cholesterol homeostasis (Gill et al., 2008), cytotoxicity (Vejux et al., 2008), and even antitumoral and antileukemic activities for inducing apoptosis (Ayala-Torres et al., 1999). The combination of oxysterols and selenium is rare, and the designed synthesis of these compounds has a great potential for the creation of a new array of molecules with biological applications (Rodrigues et al., 2010).

By virtue of the aforementioned considerations, the objectives of this study were to investigate: (a) the antinociceptive effect of *p*-chloro-selenosteroid (PCS), a selenium-oxysterol, in chemical and thermal models of acute pain in mice; (b) the contribution of adenosinergic, dopaminergic, nitric oxide, serotonergic and opioid mechanisms in the antinociceptive effect elicited by PCS; and (c) the potential acute toxicity and possible nonspecific disruptions in locomotor and exploratory behaviors caused by PCS administration in mice.

2. Materials and methods

2.1. Animals

Female adult Swiss mice (25–35 g) were used without monitoring the estrous cycle (Gomes et al., 2005), and kept in plastic boxes at controlled room temperature (22 ± 2 °C) with free access to food and water, under a 12 h light/dark cycle with lights on at 7:00 am. The animals were acclimatized at the laboratory before testing and used only once throughout the experiments. Mice were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, of 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 the minimum necessary to demonstrate the consistent effects of drug treatments. At the end of the experimental procedure, mice were killed by cervical displacement.

2.2. Drugs and reagents

PCS ($C_{34}H_{53}ClO_2Se$; Fig. 1) was prepared and characterized as previously described (Rodrigues et al., 2010). Analysis of the ^1H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. Naloxone, ondansentron, WAY100635, ketanserin, sulpiride, SCH23390, L-arginine hydrochloride (L-arginine), ω-nitro-L-arginine (L-NOArg), caffeine, and SCH58261 were obtained from Sigma-Aldrich (St. Louis, MO, USA). All the other chemicals were obtained of the highest available commercial grade.

2.3. Experimental protocol

Animals were randomly assigned into different groups, each consisting of six to eight mice for the tests. The drugs were administered in a constant volume of 10 ml/kg of body weight. PCS was dissolved in canola oil (vehicle) and administered by intragastric route (i.g.) in a single administration for the behavioral tests. Appropriate vehicle-treated groups were simultaneously assessed to discard any effect of vehicle. For each test, dose– and time–response curves were performed.

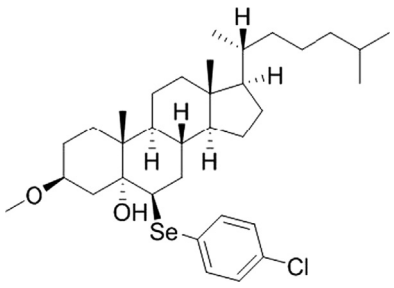

Fig. 1. Chemical structure of *p*-chloro-selenosteroid (PCS).

In order to assess the dose–response curve, animals were treated with PCS at dose range of 1–10 mg/kg (i.g.), and treatment time was chosen taking into account the time–response curve for each test with PCS at a dose of 10 mg/kg. Treatment time used was chosen based on the best effect obtained in each test. Morphine (2.5 mg/kg, subcutaneous, 15 min earlier) (Savegnago et al., 2007) was used as the standard drug in nociceptive models. Moreover, before the tests, the animals were submitted to an evaluation of locomotor and exploratory activities, assessed by the open field task to discard any alteration that could be misinterpreted as nociception.

2.4. Nociceptive tests

2.4.1. Acetic acid-induced abdominal constriction

The abdominal constrictions were induced by an intraperitoneal injection (i.p.) of acetic acid (1.6%) according to the procedures described previously (Correa et al., 1996) with some modifications (Nogueira et al., 2003). After the acetic acid injection, mice ($n=92$) were individually placed in separate boxes, and the abdominal constrictions were counted cumulatively over a period of 20 min.

2.4.2. Nociception induced by glutamate

This test was carried out to investigate if PCS has antinociceptive effect on glutamate-induced nociception. The procedure used was similar to that of described previously (Beirith et al., 2002). A volume of 20 μl of glutamate (20 μmol/paw prepared in saline solution) was injected intraplantarlly (i.pl.) into the ventral surface of the right hindpaw and the mice were observed individually for 15 min. The amount of time that animals ($n=67$) spent licking the injected paw was recorded with a chronometer and was considered as indicative of nociception.

2.4.3. Nociception and paw edema induced by formalin

The procedure was essentially the same as that prior described (Hunskaar and Hole, 1987). The mice ($n=85$) received 20 μl of a 2.5% formalin solution (0.92% of formaldehyde) i.pl. under the ventral surface of the right hindpaw and then individually placed in separate boxes and observed from 0 to 5 min (neurogenic phase) and 15 to 30 min (inflammatory phase). The time spent licking or biting the injected paw was recorded with a chronometer and considered as indicative of nociception.

The paw edema was measured by comparing the difference between the weight of the formalin-treated paw and the weight of the contralateral paw (vehicle-treated paw). After the test, animals were killed by cervical displacement, and both paws were cut at the ankle joint and immediately weighed on an analytical balance.

2.4.4. Tail-immersion induced nociception

The tail-immersion test was conducted as described previously (Janssen et al., 1963). Briefly, a 3.5 cm portion of the animals' tail ($n=68$) was marked and immersed into a cup freshly filled with water from a large constant temperature (52 °C) bath until the typical tail withdrawal response was observed (pre-drug latency) and later, after the treatment time, the same procedure was performed (pos-drug latency). A 10-s cut-off was used. Changes in tail-flick latency, Δt (seconds) latency, were calculated for each