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Antinociceptive action of diphenyl diselenide in the nociception induced by neonatal administration of monosodium glutamate in rats

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ABSTRACT

Monosodium glutamate (MSG) is a neuroexcitatory amino acid commonly used as flavoring of foods. MSG neonatal administration to animals leads to behavioral and physiological disorders in adulthood, including increased pain sensitivity. This study aimed to investigate the effect of diphenyl diselenide $(PhSe)_2$, an organoselenium compound with pharmacological properties already documented, on nociception induced by MSG. Newborn Wistar rats received 10 subcutaneous injections of MSG at a dose of 4.0 g/kg or saline (once daily). At the 60th day of life, the rats were daily treated with $(PhSe)_2$ (1 mg/kg) or vehicle (canola oil) by the intragastric route for 7 days. The behavioral tests (locomotor activity, hot plate, tail-immersion and mechanical allodynia) were carried out. *Ex vivo* assays were performed in samples of hippocampus to determine Na^+, K^+-ATPase and Ca^{2+}-ATPase activities, cytokine levels and [^{3}H]glutamate uptake. The results demonstrated that MSG increased nociception in the hot plate test and in the mechanical allodynia stimulated by Von-Frey hair but did not alter the tail immersion test. $(PhSe)_2$ reversed all nociceptive behaviors altered by MSG. MSG caused an increase in Na^+,K^+-ATPase and Ca^{2+}-ATPase activities and in pro-inflammatory cytokine levels and a decrease in the anti-inflammatory cytokine and in the [^{3}H]glutamate uptake. $(PhSe)_2$ was effective in reversing all alterations caused by MSG. The results indicate that $(PhSe)_2$ had a potential antinociceptive and anti-inflammatory action in the MSG model.

1. Introduction

The human diet has changed greatly during the past decades and with this the introduction of industrialized foods in diet of children widely increased, because processed foods are more palatable by the use of food additives to preserve flavor and enhance taste. One of the most commonly consumed food additives is monosodium glutamate (MSG), a neuroexcitatory amino acid used as a flavoring agent (McCabe and Rolls, 2007).

Earlier studies have demonstrated that MSG has some adverse effects in humans and experimental animals. In rodents, the administration of high doses of MSG during early stages of brain development induces destruction of sites in the hypothalamus (Abe et al., 1990), which causes neuroendocrine abnormalities. These abnormalities can result in animal functional and behavioral disorders in adulthood, including obesity, hyperexcitability, impairment of memory, anxiogenic-like and depressive-like behaviors, pain-sensitivity and changes in analgesic responses (Badillo-Martinez et al., 1984; Collison et al., 2010; Lopez-Perez et al., 2010; Quines et al., 2014; van den Buuse et al., 1985).

Even though little is known about the mechanism of action by which MSG induces nociception, studies have demonstrated that neonatal exposure to MSG leads to degeneration in hippocampal CA1 pyramidal cells (Beas-Zarate et al., 2002; Ishikawa et al., 1997). In addition, previous studies indicate that the hippocampal formation is involved in emergence of nociception (Klamt and Prado, 1991; Mckenna and Melzack, 1992) as well as the intensity of nociceptive stimulation is positively related to amplitude of excitatory postsynaptic potentials in hippocampal CA1 pyramidal cells (Wei et al., 2000). Thus, studies focus on the involvement of hippocampus in the nociception induced by MSG and the search for compounds which are effective in blocking nociceptive signaling pathways implicated in this process are of great importance.

Organoselenium compounds have roused interest due to their pharmacological activities (Nogueira et al., 2004). Besides their antioxidant action (Bortolatto et al., 2013; Bruning et al., 2012) these compounds have shown to have neuroprotective (Abdel-Hafez and

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Abdel-Wahab, 2008), anti-inflammatory and antinociceptive properties (Chagas et al., 2014; Marcondes Sari et al., 2014). Diphenyl diselenide $(PhSe)_2$ is an organoselenium compound that has been documented as an attractive candidate for therapeutic agent in experimental models of nociception and inflammation (da Rocha et al., 2013a; Luchese et al., 2012). The mechanism of action by which $(PhSe)_2$ elicits the antinociceptive action involves the modulation of serotonergic, nitrergic and glutamatergic systems (Savegnago et al., 2007b; Zasso et al., 2005). Regarding the pharmacokinetic properties, $(PhSe)_2$ shows a wide tissue distribution profile in rodents (Prigol et al., 2012).

Considering what was mentioned before, the present study was designed to investigate the effect of $(PhSe)_2$ on nociception induced by MSG in rats. We also investigated the possible mechanisms of action by which $(PhSe)_2$ causes antinociception.

2. Materials and methods

2.1. Animals

The experiments were carried out using newborn Wistar rats from our own breeding colony. The animals were kept in a separate animal room, on a 12 h light/dark cycle; the lights were turned on every day at 7:00 a.m., at room temperature (22 ± 1 °C) with free access to water and food. The experiments were performed according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, (#027/2014). All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2. Drugs

Diphenyl diselenide $(PhSe)_2$ (Fig. 1) was prepared in our laboratory according to a previous described method (Paulmier, 1986). The chemical purity (99.9%) was determined by gas chromatography–mass spectrometry (GC/MS). Analysis of ^1H and ^{13}C Nuclear Magnetic Resonance Spectroscopy showed analytical and spectroscopic data in full agreement with its assigned structure. $(PhSe)_2$ was diluted in canola oil.

Monosodium glutamate (MSG) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

2.3. Experimental design

The newborn Wistar rats received 10 subcutaneous injections of MSG at a dose of 4.0 g/kg or saline (0.9%) in a similar volume (1 ml/kg), once a day starting at the first postnatal day (Klingberg et al., 1987). The pups were weaned at the 21st day of life. At the 60th day of life, the female rats were divided into four groups. MSG and saline groups were treated with $(PhSe)_2$ (1 mg/kg) or vehicle (canola oil, 1 ml/kg) by the intragastric (i.g.) route once a day for 7 days. Thirty minutes after the last treatment with $(PhSe)_2$, the animals were submitted to the locomotor activity monitor and nociceptive tests (hot plate, tail-immersion and mechanical allodynia). Behavioral tests were carried out after 30 min of the last treatment with $(PhSe)_2$ by different groups of animals following the order; locomotor activity and one of the nociceptive tests for each group of animals. Immediately after the nociceptive test, rats were killed by cervical dislocation and hippocampi, a target structure for neonatal MSG action (Beas-Zarate et al., 2002), were excised from the brain and frozen (−80 °C) to perform the *ex vivo* assays.

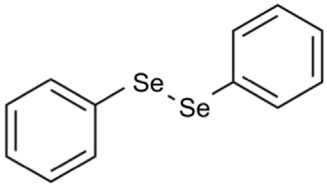

Fig. 1. Chemical structure of diphenyl diselenide $(PhSe)_2$.

Repeated treatment with a dose of 1 mg/kg was chosen based on the fact that this is a sub-effective antinociceptive dose of $(PhSe)_2$ in an acute nociceptive model in rats (Da Rocha et al., 2013b). The 7 days regimen of $(PhSe)_2$ administration was selected based on a previous pharmacological study (Bortolatto et al., 2015).

2.4. Behavioral tests

2.4.1. Spontaneous locomotor activity

With the purpose of excluding sedative or motor abnormality, spontaneous locomotor activity of rats was performed in the locomotor activity monitor. The locomotor activity monitor is a Plexiglas box (45 cm × 45 cm × 45 cm) surrounded by a frame consisting of 32 photocells mounted on opposite walls (16 L × 16 W, spaced 2 cm apart) that continuously tracks the animal's movement. Animals were placed in the center of the apparatus and allowed to freely explore the arena during 4 min. Motor activity was monitored with the Insight® Monitor Activity System and the rat position in the chamber is detected by breaks of the photocell beams. The number of crossings and rearings, average velocity (mm/s) and total distance traveled (dm) were recorded.

2.4.2. Hot plate test

The hot-plate test was carried out according to the method described previously (Woolfe and MacDonald, 1944). In this test, the animals were placed in a glass cylinder on a heated metal plate maintained at 55 ± 1 °C. The latency of nociceptive responses, such as licking or shaking one of the paws or jumping, was recorded as the reaction time. In order to avoid damage to the paws of the animals, the time standing on the plate was limited to 60 s.

2.4.3. Tail-immersion test

The tail-immersion test was conducted as described previously (Janssen et al., 1963). The test was performed 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 7 s cut-off was imposed.

2.4.4. Mechanical allodynia test

The mechanical allodynia was measured as described before (Bortalanza et al., 2002). Rats were further acclimatized in individual clear Plexiglas boxes (9 cm × 7 cm × 11 cm) on an elevated wire mesh platform to allow access to the ventral surface of the hind paw. The withdrawal response frequency of the left hind paw was measured following 10 applications (duration of 1–2 s each) of 2 g Von Frey hairs (VFH; Stoelting, Chicago, IL, USA).

2.5. Ex vivo assays

2.5.1. Tissue preparation

The hippocampus samples of all animals were homogenized in 50 mM Tris/HCl at pH 7.4. The homogenate was centrifuged at 2.500 g for 10 min at 4 °C to yield a low-speed supernatant fraction (S1). Freshly prepared S1 was used for the determination of Na^+, K^+-ATPase and Ca^{2+}-ATPase activities and for the measurement of cytokine levels.