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Original article

Contribution of dopaminergic and noradrenergic systems in the antinociceptive effect of α-(phenylalanyl) acetophenone

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ABSTRACT

Background: This study evaluated the antinociceptive action of α-(phenylalanyl) acetophenone (PSAP) in mice.
Methods: Evaluated whether the serotonergic, adrenergic and dopaminergic systems are involved in PSAP antinociceptive activity. PSAP was administered intragastrically (*ig*) 30 min prior to formalin or glutamate test and compared with a standard drug, meloxicam (10 mg/kg, *ig*).
Results: The treatment with PSAP (10–50 mg/kg) caused inhibition in the neurogenic phase and reduced the paw oedema caused by intraplantar (*ipl*) injection of formalin. PSAP (1–50 mg/kg) decreased the nociceptive response in the inflammatory phase of the formalin test and in licking behaviour triggered by glutamate at doses of 0.1–50 mg/kg. The antinociceptive effect of PSAP (1 mg/kg) was abolished when the animals were pre-treated with prazosin (α_1-adrenergic antagonist receptor, 0.15 mg/kg, intraperitoneally, *ip*), yohimbine (α_2-adrenergic antagonist receptor, 1 mg/kg, *ip*) and sulpiride (D_2/D_3 dopamine antagonist, 5 mg/kg, *ip*). The antinociceptive effect of PSAP (1 mg/kg) was not abolished by WAY100635 (5-HT_{1A}-selective serotoninergic antagonist, 0.7 mg/kg, *ip*), ketanserin (selective antagonist of serotonergic 5-$HT_{2A/2C}$, 0.3 mg/kg, *ip*), ondansetron (5-HT_3 selective serotoninergic antagonist, 0.5 mg/kg, *ip*) or SCH23390 (D_1 dopamine receptor antagonist, 0.05 mg/kg, *ip*) in the glutamate test. No changes in locomotor activity were observed in the animals treated with PSAP and/or antagonists in the open field test.
Conclusion: These results showed the antinociceptive action of PSAP in formalin and glutamate tests and the involvement of the dopaminergic and adrenergic systems in its antinociceptive activity.
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Introduction

Chronic, persistent pain has a negative impaction on quality of life, affecting several aspects of health and well-being, including relationships, cognitive abilities and work capacity [1]. The International Association for the Study of Pain (IASP) defines pain as 'an unpleasant sensory or emotional experience associated with actual or potential tissue damage, or described in terms of such damage' [2].

Under the condition of pain, an increase in neural activity occurs due to neuronal excitability, leading to higher utilization of metabolic substrates and increased production of reactive oxygen and nitrogen species (RS) [3]. RS, which include free radicals and peroxides, are normally formed during the cell respiration process and play an important role in both physiologic and pathologic conditions [4].

In this context, the scope of application of organoselenium compounds in therapeutics has increased due to demonstration of their playing an important role in a variety of conditions which are involved with free radical release [5]. Organoselenium compounds also present many pharmacological activities [6] such as antinociceptive and anti-inflammatory effects [7,8].

Among organoselenium compounds, arylselenyl acetophenones are a class with a range of interesting biological activities.

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Studies have shown that α-(phenylalanyl) acetophenone (PSAP) exhibits glutathione peroxidase-like activity and capacity to inhibit tumour promoter-induced down-regulation in intercellular communication between liver epithelial cells, *via* gap junctions. Also, PSAP has antioxidant activity and protects against lipid peroxidation [9,10].

Thus, the aim of this study was to assess the antinociceptive activity of PSAP in the formalin and glutamate tests, as well as to investigate the contribution of the noradrenergic, dopaminergic and serotonergic systems to PSAP's antinociceptive effect.

Materials and methods

Animals

The experiments were conducted using male adult Swiss mice (25–35 g) from our own breeding colony. The animals were kept in a separate animal room, on a 12 h light/dark cycle with lights on at 7:00 a.m., at room temperature (22 ± 1 °C) with free access to water and food. All experimental procedures were conducted in accordance with the guidelines of the Committee of Ethics in Research (number 3301-2015).

Drugs

PSAP (Fig. 1) was prepared and characterized in the Laboratory of Clean Organic Synthesis (LASOL) according to the method previously described [11]. PSAP was dissolved in canola oil. WAY100635, ketanserin, ondansetron, sulpiride, SCH23390, yohimbine and prazosin were obtained from Sigma–Aldrich (St. Louis, MO, USA) and were dissolved in saline solution (0.9%). Formalin solution (0.92% formaldehyde) was dissolved in saline solution (0.9%) and sodium L-glutamate 1-hydrate (20 μmol) was dissolved in distilled water. All other chemicals were obtained at the highest available commercial grade.

Chemical models of nociception

Nociception and paw oedema induced by formalin

The formalin test was carried out as described by [12]. Animals received 20 μl of 2.5% formalin solution (0.92% of formaldehyde), injected intraplantarly (*ipl*) in the ventral right hind paw. Animals were treated with PSAP by intragastric route (0.1–50 mg/kg, ig), vehicle (canola oil, 10 ml/kg, ig) or a standard drug, meloxicam (10 mg/kg, ig) [13], 30 min before formalin administration. The literary data show that organoselenium compounds have a time of action beginning after approximately 30 min [14].

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 and biting the injected paw was recorded with a chronometer and considered as indicative of nociception (Fig. 2).

In order to assess if PSAP inhibits oedema associated with inflammatory pain, paw oedema was measured by comparing the difference between the weight of the formalin-treated paw and the weight of the contralateral paw treated with saline solution. For this purpose, animals were euthanized 30 min after formalin injection and both paws were cut at the ankle joint and immediately weighed on an analytical balance [14].

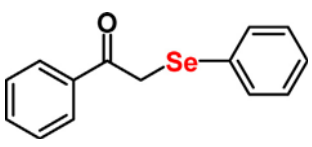

Fig. 1. Chemical structure of α-(phenylselanyl) acetophenone (PSAP).

Nociception induced by glutamate

Mice were treated with PSAP (0.1–50 mg/kg, *ig*) or vehicle (canola oil, 10 ml/kg, *ig*) 30 min before receiving the injection of sodium L-glutamate 1-hydrate (20 μmol, 20 μl/paw, *ipl*) in the ventral right hind paw (Fig. 3). Mice were observed individually for 15 min following glutamate injection and the amount of time spent licking and biting the injected paw was recorded and considered as nociception-response behaviour. Meloxicam (10 mg/kg, *ig*), given 30 min before the test, was used as a standard drug in this test model [15].

Analysis of possible mechanisms involved in the action of PSAP

Fifteen minutes after antagonist administration, PSAP (1 mg/kg, *ig*) or vehicle was administered. Thirty minutes later, mice performed the open field test (see 'Open field test' section in 'Materials and methods'), to assess if the interaction between drug and PSAP could cause any alterations in locomotor activity. All antagonists were used at sub-effective doses as previously reported [16].

Involvement of the dopaminergic system

To evaluate the possible contribution of the dopaminergic system in the antinociceptive action of PSAP, animals were pre-treated with SCH23390 (0.05 mg/kg, *ip*, a selective antagonist at the dopamine D_1 receptor), sulpiride (5 mg/kg, *ip*, an antagonist at dopamine D_2 and D_3 receptors) or vehicle (saline solution).

Involvement of the noradrenergic system

To investigate the role played by the α-adrenergic system in the antinociceptive effect caused by PSAP on the glutamate test, mice were pre-treated with prazosin (0.15 mg/kg, *ip*, α_1-adrenoreceptor antagonist) or yohimbine (1 mg/kg, *ip*, α_2-adrenoreceptor antagonist) or vehicle (saline solution).

Involvement of the serotoninergic system

In an effort to assess the participation of the serotonergic system in the antinociceptive action of PSAP, it was investigated using WAY100635 (0.7 mg/kg, *ip*, a selective antagonist at the 5-HT_{1A} receptor), ketanserin (0.3 mg/kg, *ip*, a selective antagonist at the 5-$HT_{2A/2C}$ receptors) and ondansentron (0.5 mg/kg, a selective antagonist at the 5-HT_3 receptor) or respective vehicle (saline solution).

Open field test

Spontaneous locomotor (number of segments crossed with the four paws) behaviour was assessed using the open field test. The open field was made of plywood and surrounded by walls 30 cm in height. The floor of the open field was divided by masking tape markers into nine squares (3 rows of 3), 45 cm in length and 45 cm in width. Animals were evaluated 30 min after vehicle or PSAP (0.01–50 mg/kg, *ig*) or 45 min after the administration of antagonists WAY100635 (0.7 mg/kg, *ip*), ketanserin (0.3 mg/kg, *ip*), ondansentron (0.5 mg/kg, *ip*), SCH23390 (0.05 mg/kg, *ip*), sulpiride (5 mg/kg, *ip*), prazosin (0.15 mg/kg, *ip*) and yohimbine (1 mg/kg, *ip*). Each animal was placed individually at the centre of the apparatus and observed for 6 min to record locomotion (number of segments crossed with the four paws) [17].

Statistical analysis

All experiment results are given as the mean ± standard error of the mean (SEM). Results obtained from behavioural nociceptive tests were statistically analyzed by one-way ANOVA and, when

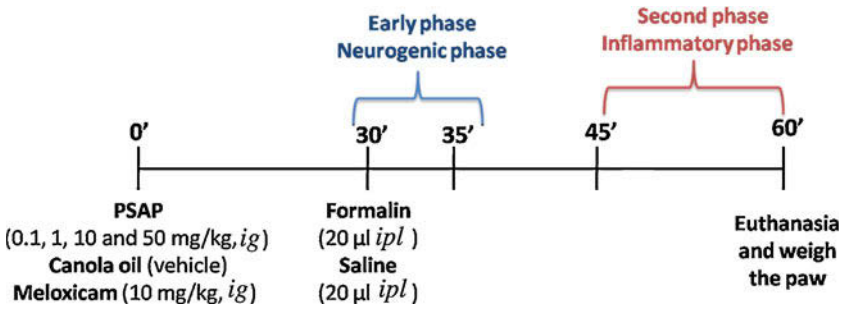

Fig. 2. Experimental protocol of nociception induced by formalin test in mice and possible reversal of the same by the administration of PSAP. Neurogenic phase (0–5 min) comprises nociception process, while the inflammatory phase (15–30 min) comprises the process of activation of inflammatory cascades in response to a noxious stimulus. *Abbreviations: ig* = intragastric; *ipl* = intraplantar; PSAP = α-(phenylselanyl) acetophenone.

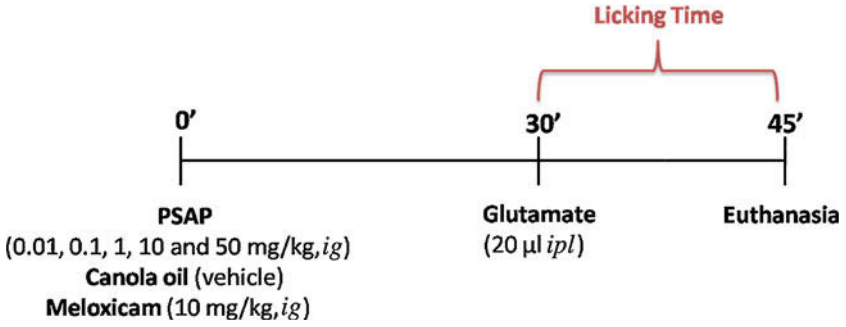

Fig. 3. Experimental protocol of nociception induced by glutamate test in mice and possible reversal of the same by the administration of PSAP. *Abbreviations: ig* = intragastric; *ipl* = intraplantar; PSAP = α-(phenylselanyl) acetophenone.

appropriate, subjected to the consecutive application of *post hoc* Newman–Keuls test. Information related to the involvement of different systems in the antinociceptive action caused by PSAP (antagonists × PSAP) was evaluated by two-way ANOVA and, if necessary, followed by the *post hoc* Newman–Keuls test. A value of $p < 0.05$ was considered significant. All results were assessed using Graph Pad software (Graph Pad Software, San Diego, CA, USA) and are reported as median effective dose accompanied by their respective 95% confidence limits.

Results

Effect of PSAP on animal model of nociception

Nociception and paw oedema induced by formalin

In the first phase (neurogenic) (Fig. 4A) PSAP at doses of 10 and 50 mg/kg produced a significant inhibition of the licking and biting behaviour, 51.4% and 86% respectively, in the formalin test; the same was observed for meloxicam at a dose of 10 mg/kg (31.77% inhibition).

On the other hand, the second phase (inflammatory) PSAP at doses of 1, 10 and 50 mg/kg inhibited the licking and biting time by 98–100% ($p < 0.001$) in the formalin test (Fig. 4B). This decrease in licking and biting time was also observed for meloxicam (52% inhibition) (standard drug, 10 mg/kg).

In Fig. 4C, it can be seen that PSAP (10 and 50 mg/kg) effectively inhibited mice paw oedema formation by *ipl* injection of formalin. However, meloxicam was not effective in inhibiting paw oedema.

Nociception induced by glutamate

The results presented in Fig. 5 show that PSAP (0.01–50 mg/kg, *ig*) and meloxicam (10 mg/kg, *ig*) produced a significant reduction of licking behaviour induced by *ipl* injected glutamate. The dose of 0.1 and 50 mg/kg (PSAP), presenting an inhibitory effect of 23.33% and 100% respectively. This reduction in paw-licking and biting time was also observed in the standard drug, meloxicam (10 mg/kg, *ig*), with an inhibitory effect of 50%.

Analysis of the possible antinociceptive mechanism of PSAP action

Involvement of the dopaminergic system

The results illustrated in Fig. 6A show that pre-treatment of the mice with SCH23390 (0.05 mg/kg, *ip*, a selective antagonist at the dopamine D_1 receptor) was not effective in blocking the antinociceptive effect of PSAP (1 mg/kg, *ig*) in the glutamate test. Two-way ANOVA revealed a significant main effect of PSAP (1 mg/kg, *ig*) [$F_{1,28} = 257.39$, $p < 0.0001$], but did not reveal an effect of SCH23390 (0.05 mg/kg, *ip*) [$F_{1,28} = 1.93$, $p = 0.1753$] or SCH23390 × PSAP interaction [$F_{1,28} = 0.28$, $p = 0.6026$].

Pre-treatment of mice with sulpiride (5 mg/kg, *ip*, an antagonist at the dopamine D_2 and D_3 receptors) reduced the antinociceptive effect caused by PSAP administration in the glutamate test (Fig. 6B). Two-way ANOVA of data demonstrated significant main effects caused by systemic treatment of animals with PSAP (1 mg/kg, *ig*) [$F_{1,30} = 93.53$, $p < 0.0001$] and PSAP × sulpiride interaction [$F_{1,30} = 16.93$, $p = 0.0003$] but not sulpiride only (5 mg/kg, *ip*) [$F_{1,30} = 0.34$, $p = 0.5615$].

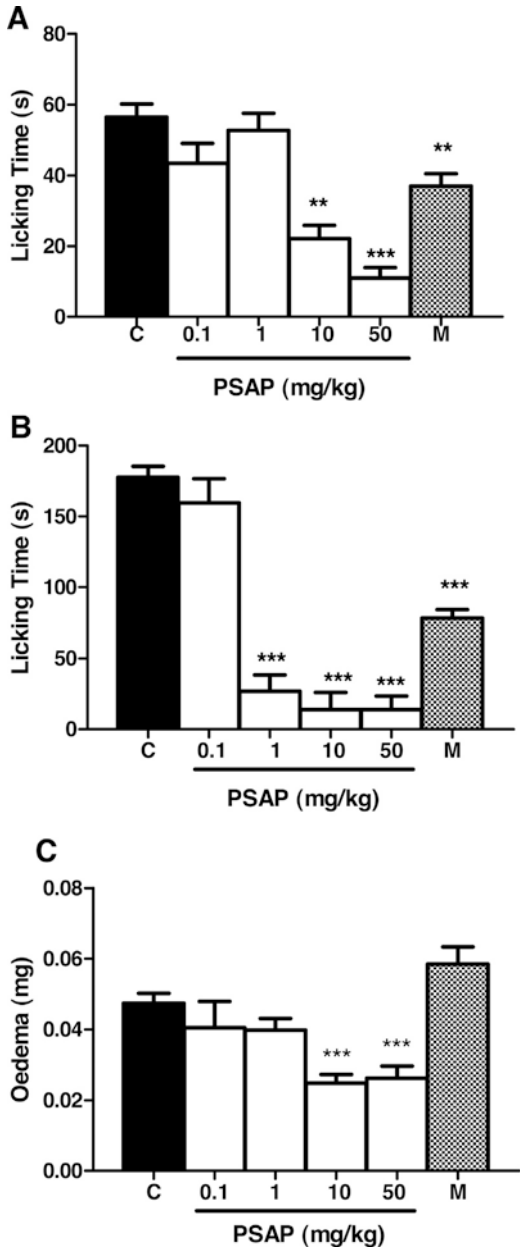

Fig. 4. Effect of PSAP on licking and biting behaviour induced by formalin in mice. (A) Neurogenic, first (0–5 min) phase and (B) inflammatory, second (15–30 min) phase of the formalin test. (C) Effect of PSAP on the paw oedema. Each column represents the mean ± SEM. *Abbreviations:* (C) Control indicates animals treated with canola oil and (M) indicates animals treated with the standard drug, meloxicam (10 mg/kg, *ig*; 60 min of treatment). Asterisks denote significance levels when compared to the control group (one-way ANOVA followed by the Newman–Keuls' test). (**) $p < 0.01$ and (***) $p < 0.001$.

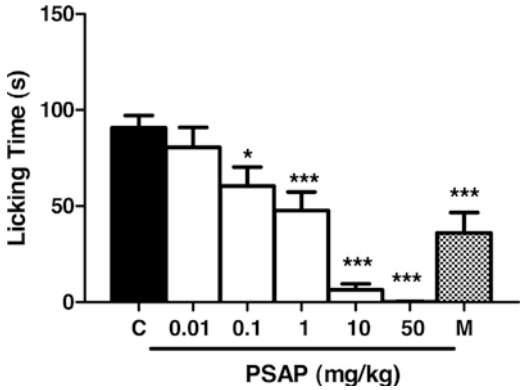

Fig. 5. Effect of PSAP on licking and biting behaviour induced by glutamate in mice. Mice were treated with PSAP at doses 0.01–50 mg/kg (*ig*) 30 min before the glutamate test. Each column represents the mean ± SEM. *Abbreviations:* (C) Control indicates animals treated with canola oil and (M) indicates animals treated with the standard drug, meloxicam (10 mg/kg, *ig*; 60 min of treatment). Asterisks denote significance levels when compared to the control group (one-way ANOVA followed by the Newman–Keuls' test); (*) $p < 0.05$ and (***) $p < 0.001$.

Involvement of the noradrenergic system

Fig. 7A shows that pre-treatment of mice with prazosin (0.15 mg/kg, *ip*, an α_1-selective antagonist), given 15 min beforehand, blocked the antinociception caused by PSAP (1 mg/kg, *ig*) in glutamate-induced licking and biting in mice. Two-way ANOVA of data demonstrated significant main effects caused by systemic treatment of animals with PSAP (1 mg/kg, *ig*) [$F_{1,24} = 25.20$, $p < 0.0001$] and PSAP × prazosin interaction [$F_{1,24} = 4.96$, $p = 0.0355$], but not prazosin pre-treatment [$F_{1,24} = 5.05$, $p = 0.0340$] in glutamate-induced licking and biting in mice.

The results depicted in Fig. 7B show that the pre-treatment of mice with yohimbine (1 mg/kg, *ip*, an α_2-selective antagonist) also blocked the effect caused by PSAP (1 mg/kg, *ig*) in the glutamate test. Two-way ANOVA revealed significant main effects of PSAP (1 mg/kg, *ig*) [$F_{1,22} = 59.35$, $p < 0.0001$] and PSAP × yohimbine (1 mg/kg, *ip*) interaction [$F_{1,22} = 15.62$, $p = 0.0007$], but not yohimbine alone (1 mg/kg, *ip*) [$F_{1,22} = 7.05$, $p = 0.0145$].

Involvement of the serotoninergic system

The results depicted in Fig. 8 show that pre-treatment with WAY100635 (0.7 mg/kg, *ip*, a selective antagonist at the 5-HT_{1A} receptor), ketanserin (0.3 mg/kg, *ip*, a selective antagonist at the 5-$HT_{2A/2C}$ receptors) and ondansentron (0.5 mg/kg, *ip*, a selective antagonist at the 5-HT_3 receptor) did not reverse the antinociceptive effect caused by PSAP (1 mg/kg, *ig*) in the glutamate test. Two-way ANOVA revealed a significant main effect of PSAP (1 mg/kg, *ig*) [$F_{1,28} = 41.41$, $p < 0.0001$], but not of WAY100635 (0.7 mg/kg, *ip*) [$F_{1,28} = 0.17$, $p = 0.680$] or PSAP × WAY100635 interaction [$F_{1,28} = 0.09$, $p = 0.762$]. Likewise, two-way ANOVA of data showed a significant main effect of PSAP (1 mg/kg, *ig*) [$F_{1,29} = 94.31$; $p < 0.0001$], but not of ketanserin (0.3 mg/kg, *ip*) [$F_{1,29} = 0.49$, $p = 0.488$] or PSAP × ketanserin interaction [$F_{1,29} = 1.17$, $p = 0.288$] (Fig. 8B).

Two-way ANOVA revealed a significant main effect of PSAP (1 mg/kg, *ig*) [$F_{1,31} = 55.16$, $p < 0.0001$] but did not present one for ondansetron (0.5 mg/kg, *ip*) [$F_{1,31} = 0.19$, $p = 0.668$] or PSAP × ondansetron interaction [$F_{1,31} = 0.03$, $p = 0.869$] (Fig. 8C).

Open field test

Treatment of mice with PSAP did not cause any significant change in the number of crossings when compared to the control group (Table 1) in the open field test. In Table 2, it can be seen that one-way ANOVA revealed no significant effect for antagonists and antagonist × PSAP interaction in the number of crossings in the open field test.

Discussion

In this study, we extended our results on the antinociceptive action of PSAP and provided behavioural evidence for involvement of the dopaminergic, noradrenergic and serotoninergic systems in this action. The administration of PSAP reduced the nociceptive

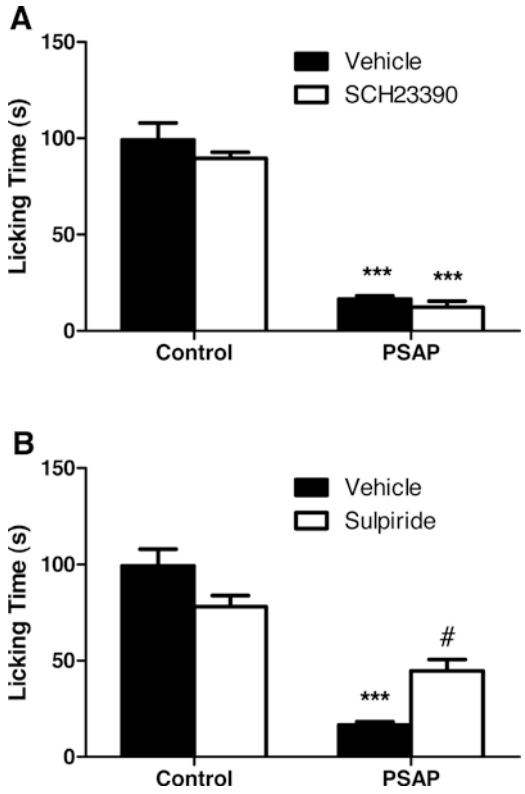

Fig. 6. Effect of pre-treatment of mice with (A) SCH23390 (0.05 mg/kg, *ip*) and (B) sulpiride (5 mg/kg, *ip*) in the antinociceptive effect of PSAP (1 mg/kg, *ig*) against the glutamate-induced paw licking and biting. Each column represents to mean ± SEM. Statistical analysis was performed by two-way ANOVA followed by Student Newman–Keuls' test; (***) $p < 0.001$ as compared with the vehicle treated (control). (#) $p < 0.05$ compared to PSAP pre-treated with vehicle.

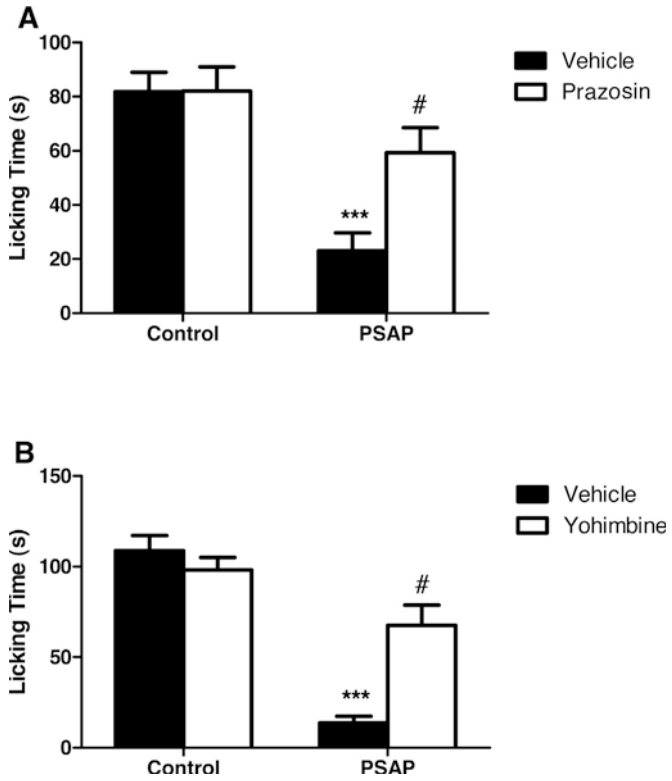

Fig. 7. Effect of pre-treatment of mice with (A) prazosin (0.15 mg/kg, *ip*) or (B) yohimbine (1 mg/kg, *ip*) in the antinociceptive effect of PSAP (1 mg/kg, *ig*) against the glutamate-induced paw licking and biting. Each column represents to mean ± SEM. Statistical analysis was performed by two-way ANOVA followed by Student Newman–Keuls' test; (***) $p < 0.001$ as compared with the vehicle treated (control); (#) $p < 0.05$ compared to PSAP pre-treated with vehicle.

response induced by glutamate and formalin injection (*ipl*), and antagonists (dopaminergic and noradrenergic) blocked the effect of PSAP.

Pain is a debilitating condition and even with the wide range of analgesic drugs available on the market, there is still not a drug that provides a completely effective treatment without the occurrence of adverse effects [18]. Therefore, the search for new, safe and effective drugs which may be used in the treatment of pain and inflammation is of great importance.

According to [19], the formalin-induced pain model evaluates two distinct phases: the first phase, which produces an intense stimulation of nociceptores that will mediate the phase of acute pain, and the second phase, which is characterized by a release of pro-inflammatory mediators such as histamine, prostaglandins and leukotrienes, leading to local oedema formation and hyperalgesia [19]. Our results also indicate that PSAP at low doses elicited a reduction of the formalin-induced nociceptive behaviour in both phases, neurogenic and inflammatory, as well as preventing paw oedema.

In the formalin test, the standard drug, meloxicam (10 mg/kg, *ig*) reduced licking and biting behaviour; this can be related to the fact that meloxicam is a nonsteroidal anti-inflammatory drug (NSAID) and the mechanism of action involves the inhibition of prostaglandin biosynthesis [20]. Regarding the inflammatory phase, PSAP was effective in reducing licking and biting time when compared with the control group, acting similarly to meloxicam. PSAP was also able to reduce paw oedema, but the same was not observed in the standard drug. Meloxicam had no activity in reducing oedema formation induced by formalin in this study; maybe this occurred due to the low dose used [19].

Another test performed in this study, the induction of nociception by glutamate injection, demonstrated that PSAP reduced licking and biting time in nociception in this test. An important observation is that PSAP did not alter the locomotion activity at any dose in the open field test, a classical animal model used to evaluate general activity of animals [20].

Glutamate is the major excitatory amino acid neurotransmitter present in the central nervous system and in the peripheral endings of small diameter afferent fibres and can contribute to the development and maintenance of pain. The nociception induced by glutamate causes release of NO inflammatory mediators and neuropeptides involved in the transmission of pain [20,21].

In previous studies of our research group, it was demonstrated that the oral administration of high doses of PSAP (400 mg/kg, *ig*), *ex vivo*, did not alter the levels of thiobarbituric acid reactive substances (TBARS), δ-aminolevulinate dehydratase (δ-ALA-D) or catalase activity, which indicates that PSAP at high doses does not

Table 1
Effect of administration of PSAP (0.01–50 mg/kg, *ig*) administered in mice 30 min before the open field test in mice.

Experimental groups	Number of crossings
Control (canola oil)	88.00 ± 2.78
PSAP (0.01 mg/kg)	103.55 ± 7.73
PSAP (0.1 mg/kg)	88.82 ± 5.13
PSAP (1 mg/kg)	87.38 ± 7.57
PSAP (10 mg/kg)	62.00 ± 5.92
PSAP (50 mg/kg)	62.25 ± 0.07

The effect of treatment with PSAP behaviour of mice in the open-field test was determined by one-way ANOVA followed by Newman–Keuls test. Data presented are mean values ± SEM.

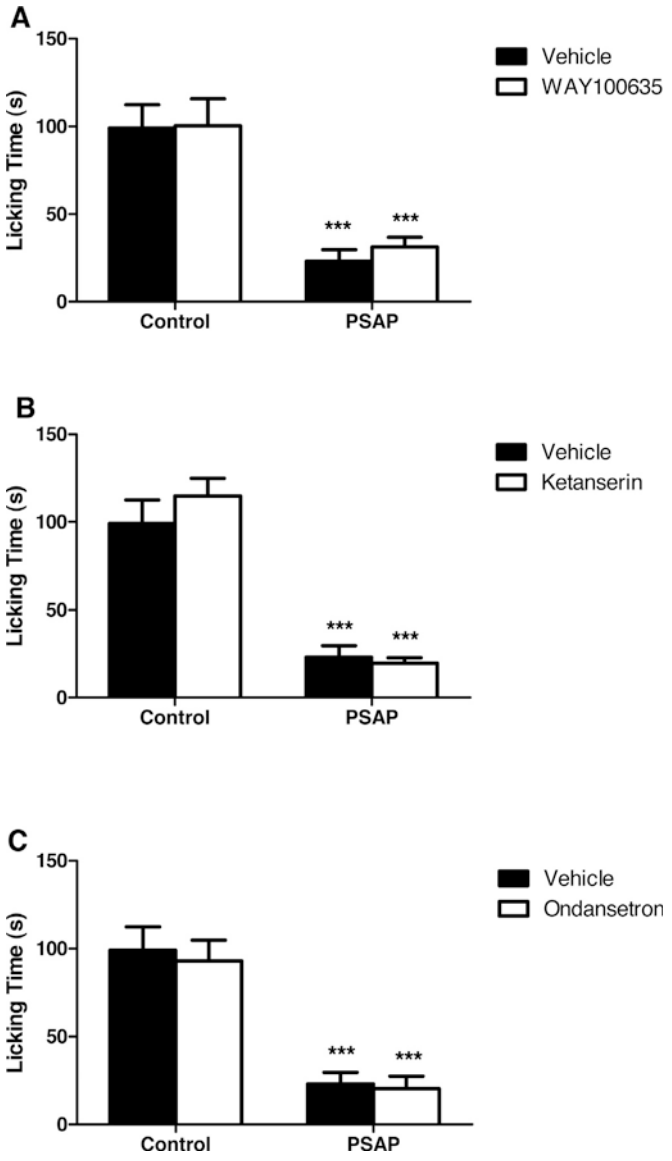

Fig. 8. Effect of pre-treatment of mice with (A) WAY100635 (0.7 mg/kg, *ip*), (B) ketanserin (0.3 mg/kg, *ip*) or (C) ondansetron (0.5 mg/kg, *ip*) in the antinociceptive effect of PSAP (1 mg/kg, *ig*) against the glutamate-induced paw licking and biting. Each column represents to mean ± SEM. Statistical analysis was performed by two-way ANOVA followed by Student Newman–Keuls' test. (***) $p < 0.001$ as compared with the vehicle treated (control).

lead to acute toxicity in mice. PSAP was evaluated in four test systems (DPPH, ABTS, FRAP and inhibition of lipid peroxidation). PSAP showed potent antioxidant activity and protected against lipid peroxidation [10].

Our research group has also investigated the *in vitro* toxicity of PSAP on Chinese hamster ovary cells (through the MTT assay). Besides that, PSAP genotoxicity was also analyzed using the comet assay in mice leukocytes after acute and chronic treatments, along with biochemical analyses. The results demonstrated that the oral administration of PSAP in acute and chronic did not cause genotoxicity. Moreover, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) tests were performed in liver, kidney and brain of mice given chronic treatment of PSAP, the plasmatic levels were not altered [22].

In previous studies, we observed that PSAP is a promising compound, since it does not lead to acute or chronic toxicity in

Table 2
Effect of administration of PSAP and antagonists on behaviour parameter in the open field test in mice.

Experimental groups	Number of crossings
Vehicle (saline 0.9%)	87.14 ± 8.63
PSAP (1 mg/kg)	89.23 ± 5.25
WAY100635 (0.7 mg/kg)	116.3 ± 11.30
WAY100635 + PSAP	91.50 ± 11.20
Ketanserin (0.3 mg/kg)	106.0 ± 9.80
Ketanserin + PSAP	90.86 ± 6.36
Ondansetron (0.5 mg/kg)	116.3 ± 6.71
Ondansetron + PSAP	95.43 ± 12.18
Vehicle (saline 0.9%)	98.57 ± 13.07
PSAP (1 mg/kg)	86.29 ± 11.84
SCH23390 (0.05 mg/kg)	63.83 ± 7.80
SCH23390 + PSAP	63.00 ± 4.789
Sulpiride (5 mg/kg)	97.75 ± 9.77
Sulpiride + PSAP	85.75 ± 8.11
Vehicle (saline 0.9%)	103.6 ± 6.67
PSAP (1 mg/kg)	96.00 ± 6.29
Prazosin (0.15 mg/kg)	120.3 ± 6.78
Prazosin + PSAP	106.8 ± 5.85
Yohimbine (1 mg/kg)	84.63 ± 6.53
Yohimbine + PSAP	82.17 ± 4.62

The effect of treatment with PSAP behaviour of mice in the open-field test was determined by one-way ANOVA followed by Newman–Keuls test. Data presented are mean values ± SEM.

tests. Therefore, the possible involvement of the serotoninergic, noradrenergic and dopaminergic systems in the antinociceptive effect of PSAP was also investigated. The selective antagonists of 5-HT_{1A}, 5-HT_{2A} and 5-HT_3 receptors, WAY100635, ketanserin and ondansetron, respectively, did not block the reduction in paw-licking response elicited by PSAP (1 mg/kg, *ig*) in the glutamate test, and did not change the locomotor activity of animalseither. This result indicates that the serotonergic system is not involved in he antinociceptive action of PSAP. Accordingly, the activation of spinal cord and subtype 5-HT_{1A} peripheral serotonin receptors has been reported to reduce nociception [23,24]. By contrast, the role of 5-HT_2 receptors in the control of nociception is sometimes controversial. Some authors reported that the activation of 5-HT_2 receptors at a peripheral level produces nociceptive responses [25], while at a spinal level, activation of 5-HT_2 receptors causes antinociception [26,27]. None of the serotonergic antagonists tested was effective in abolishing the antinociceptive action of PSAP, suggesting that PSAP action is not related to an interaction with the serotoninergic system.

In parallel with the serotoninergic system, the involvement of the noradrenergic system is also suggested in the pathophysiology of nociception. In the spinal cord, norepinephrine released from descending pathways suppresses pain by its inhibitory action on α_2-adrenoceptors in the central terminals of primary afferent nociceptors (presynaptic inhibition), by direct α_2-adrenergic action on pain-relay neurons (postsynaptic inhibition) and by α_1-adrenoceptor-mediated activation of inhibitory interneurons [28]. In this study it was possible to observe that the adrenergic antagonists prazosin (α_1-adrenoreceptor antagonist) and yohimbine (α_2-adrenoreceptor antagonist) blocked the analgesic effect of PSAP. These results suggest the involvement of adrenergic α_1 and α_2 receptors in PSAP action to reduce nociception induced by glutamate.

The dopaminergic system seems to be related to nociception control in some models of pain [29]. It is believed that when a harmful stimulation occurs, suggesting augmentation in the activity of descending dopaminergic pathways, there is an increase in dopamine 'turnover' in specific nervous system regions [3]. Even though some studies confirm the involvement of this system in nociceptive behaviour, contradictory data have been reported. Whereas the stimulation of D_1-like (D_1/D_5) receptors (excitatory G-

protein coupled receptors) leads to an increase in neuronal activity, activation of D_2-like ($D_2/D_3/D_4$) receptors (inhibitory G-protein coupled receptors) results in the opposite effect, leading to an inhibition of neuronal activity. Both events directly influence the transmission of stimulus [30]. Additionally, some studies also indicate that both kinds of receptor are simultaneously implicated in the modulation of nociceptive effects under different conditions of pain [31]. This divergence among studies may be due to varied experimental conditions and procedures and the animal models used, as well as the brain areas investigated [32]. Pre-treatment with SCH23390 (a selective antagonist of the dopamine D_1 receptor) did not abolish PSAP antinociceptive action, and sulpiride (an antagonist at the dopamine D_2 and D_3 receptors) reversed the PSAP antinociceptive effect, suggesting the involvement of the dopaminergic receptors D_2 and D_3 in its action.

In conclusion, the results of this study show that PSAP has an antinociceptive effect on nociception-induced tests (formalin and glutamate tests). PSAP presented the ability to reduce paw oedema caused by formalin. Also, this compound did not cause any changes in the locomotor activity of animals. A contribution of the noradrenergic and dopaminergic systems in the antinociceptive action of PSAP was demonstrated. Further studies will allow understanding of the exact mechanisms involved in PSAP action to support its beneficial role in the treatment of pain.

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References

[1] Rustøen T, Stubhaug A, Eidsmo I, Westheim A, Paul SM, Miaskowski C. Pain and quality of life in hospitalized patients with heart failure. J Pain Symptom Manage 2008;36:497–504.
[2] International Association for the Study of Pain (IASP). IASP task force on taxonomy. Classification of chronic pain, vol. 2. Seattle: IASP Press; 1994. p. 209–14 http://www.iasp-pain.org/.
[3] Hjornevik T, Jacobsen LM, Qu H, Bjaalie JG, Gjerstad J, Willoch F. Metabolic plasticity in the supraspinal pain modulating circuitry after noxious stimulus-induced spinal cord LTP. Pain 2008;140:456–64.
[4] Von Hehn CA, Baron R, Woolf CJ. Deconstructing the neuropathic pain phenotype to reveal neural mechanisms. Neuron 2012;73:638–52.
[5] Nogueira CW, Zeni G, Rocha JBT. Organosellenium and organotellurium compounds: pharmacology and toxicology. Chem Rev 2004;104:6255–86.
[6] Brod LMP, Fronza MG, Wilhelm EA, Luchese C, Vargas JP, Lüdtke S, Savegnago L. Involvement of monoaminergic system in the antidepressant-like effect of (octylseleno)-xylofuranoside in the mouse tail suspension test. Prog Neuropsychopharmacol Biol Psychiatry 2016;65:201–7.
[7] Brüning CA, Gai BM, Soares SM, Martini F, Nogueira CW. Serotonergic systems are implicated in antinociceptive effect of m-trifluoromethyldiphenyl diselenide in the mouse glutamate test. Pharmacol Biochem Behav 2014;125:15–20.
[8] Donato F, Pavin NF, Goes ATR, Souza LC, Soares LC, Rodrigues OED, Jesse CR, Savegnago L. Antinociceptive and anti-hyperalgesic effects of bis(4-methylbenzoyl) diselenide in mice: evidence for the mechanism of action. Pharm Biol 2015;53:395–403.
[9] Cotgreave IA, Moldéus P, Brattsand R, Hallberg A, Andersson CM, Engman L. α-(Phenylselenenyl) acetophenone derivatives with glutathione peroxidase-like activity: a comparison with ebselen. Biochem Pharmacol 1992;43:793–802.
[10] Gerzson M, Victoria F, Radatz C, Gomes M, Boeira S, Jacob R, Alves D, Jesse C, Savegnago L. In vitro antioxidant activity and in vivo antidepressant-like effect of α-(phenylselanyl) acetophenone in mice. Pharm Biochem Behav 2012;102:21–9.
[11] Victoria FN, Radatz CS, Sachini M, Jacob RG, Perin G, Silva WP, Lenardão EJ. KF/Al_2O_3 and PEG-400 as a recyclable medium for the selective α-selenation of aldehydes and ketones. Preparation of potential antimicrobial agents. Tetrahedron Lett 2009;50:6761–3.
[12] Hunskaar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain 1987;30:103–14.
[13] Aguirre-Bañuelos P, Granados-Soto V. Evidence for the participation of the nitric oxide-cyclic GMP pathway in the antinociceptive action of meloxicam in the formalin test. Eur J Pharmacol 2000;395:9–13.
[14] Santos ARS, Calixto JB. Further evidence for the involvement of tachykinin receptor subtypes in formalin and capsaicin models of pain in mice. Neuropeptides 1997;37:381–9.
[15] Beirith A, Santos AR, Calixto JB. Mechanisms underlying the nociception and paw oedema caused by injection of glutamate into the mouse paw. Brain Res 2002;924:219–28.
[16] Santos ARS, Gadotti VM, Oliveira GL, Tibola D, Paszcuk AF, Neto A, Spindola HM, Souza MM, Rodrigues ALS, Calixto JB. Mechanisms involved in the antinociception caused by agmatine in mice. Neuropharmacology 2005;48:1021–34.
[17] Walsh RN, Cummins RA. Open-field test critical review. Psychol Bull 1976;83:482–504.
[18] Mendell JR, Sahenk Z. Painful sensory neuropathy. New Engl J Med 2003;348:1243–55.
[19] Gonçalves JC, de Oliveira FS, Benedito RB, de Sousa DP, de Almeida RN, de Araujo DA. Antinociceptive activity of (−)-carvone: evidence of association with decreased peripheral nerve excitability. Biol Pharm Bull 2008;31:1017–20.
[20] Shibata M, Ohkubo T, Takahashi H, Inoki R. Modified formalin test: characteristic biphasic pain response. Pain 1989;38:347–458.
[21] Miller KE, Hoffman EM, Sutharshan M, Schechter R. Glutamate pharmacology and metabolism in peripheral primary afferents: physiological and pathophysiological mechanisms. Pharmacol Ther 2011;130:283–309.
[22] Casaril AM, Martinez DM, Ricordi VG, Alves D, Lenardão EJ, Schultze E, Collares T, Seixas FK, Savegnago L. Evaluation of the toxicity of α-(phenylselanyl) acetophenone in mice. Regul Toxicol Pharmacol 2015;73:868–74.
[23] Millan MJ. Descending control of pain. Prog Neurobiol 2002;66:355–474.
[24] Jeong HJ, Mitchell VA, Vaughan CW. Role of 5-HT_1 receptor subtypes in the modulation of pain and synaptic transmission in rat spinal superficial dorsal horn. Br J Pharmacol 2012;165:1956–65.
[25] Cervantes-Duran C, Pineda-Farias JB, Bravo-Hernandez M, Quinonez-Bastidas GN, Vidal-Cantu GC, Barragan-Iglesias P. Evidence for the participation of peripheral 5-HT(2A), 5-HT(2B), and 5-HT(2C) receptors in formalin-induced secondary mechanical allodynia and hyperalgesia. Neuroscience 2012;232:169–81.
[26] Bardin L, Lavarenne J, Eschalier A. Serotonin receptor subtypes involved in the spinal antinociceptive effect of 5-HT in rats. Pain 2000;86:11–8.
[27] Seyrek M, Kahraman S, Deveci MS, Yesilyurt O, Dogrul A. Systemic cannabinoids produce CB1-mediated antinociception by activation of descending serotonergic pathways that act upon spinal 5-HT_7 and 5-HT_{2A} receptors. Eur J Pharmacol 2010;649:183–94.
[28] Pertovaara A. Noradrenergic pain modulation. Prog Neurobiol 2006;80:53–83.
[29] Zarrindast MR, Nassiri-Rad S, Pazouki M. Effects of dopaminergic agents on antinociception in formalin test. Gen Pharmacol 1999;32:517–22.
[30] Sheng HY, Qu CL, Huo FQ, Du JQ, Tang JS. D-2-like but not D-1-like dopamine receptors are involved in the ventrolateral orbital cortex-induced antinociception: a GABAergic modulation mechanism. Exp Neurol 2009;215:128–34.
[31] Altier N, Stewart J. Dopamine receptor antagonists in the nucleus accumbens attenuate analgesia induced by ventral tegmental area substance P or morphine and by nucleus accumbens amphetamine. J Pharmacol Exp Ther 1998;285:208–15.
[32] Altier N, Stewart J. The role of dopamine in the nucleus accumbens in analgesia. Life Sci 1999;65:2269–87.