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Antinociceptive and anti-allodynic effects of 3-alkynyl selenophene on different models of nociception in mice

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ABSTRACT

In this study, antinociceptive and anti-hyperalgesic effects of 3-alkynyl selenophene (3-ASP) were evaluated in mice. Acute toxicity of 3-ASP (1–50 mg/kg, per oral) was investigated in mice. 3-ASP neither caused toxicity nor affects locomotor activity in the rota-rod test. 3-ASP did not change plasma aspartate (AST) and alanine aminotransferase (ALT) activities, urea and creatinine levels. 3-ASP caused a significant increase in tail-immersion and hot-plate response latencies time. 3-ASP inhibited early and late phases of nociception caused by intraplantar (i.pl.) injection of formalin. 3-ASP reduced nociception produced by i.pl. injection of glutamate, bradykinin, phorbol myristate acetate (PMA) and capsaicin in mice. Mechanical hyperalgesia induced by Freund's Complete Adjuvant (CFA) was attenuated by 3-ASP administration to mice (maximal inhibition of 42 ± 11%). The anti-hyperalgesic effect of 3-ASP was maintained for up to 6 h. The antinociceptive effect of 3-ASP was not abolished by naloxone (5 mg/kg), discarding the involvement of opioidergic mechanism in this effect. These results indicate that 3-ASP at a dose range of 5–50 mg/kg was especially potent and produced systemic anti-hyperalgesic and antinociceptive actions in mice.

1. Introduction

Chronic persistent pain impacts negatively on quality of life, affecting several aspects of health and well-being including relationships, cognitive abilities and the capacity to work (Rustøen et al., 2008). 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" (IASP Task Force on Taxonomy, 1994). Although there is an arsenal of effective and widely used analgesics, there is some concern regarding their safety and side-effects, making their clinical use problematic (Jage, 2005).

The nociception involves the activation of specific primary sensory neuron subpopulations that transmit the nociceptive information to the spinal cord from where it is relayed to supra spinal levels (Millan, 1999). Through the tissue damage may occur by activation of nociceptors through the release of several mediators, including excitatory amino acids, peptides, protons, lipids and cytokines, which bind to receptors and activate signaling pathways (Gilchrist et al., 1996). Thus, pain can be a subject to multiple levels of biochemical and pharmacological controls, involving a diversity of cell types and soluble mediators (Ji and Strichartz, 2004). As a result, compounds that present antinociceptive effect are of potential therapeutic interest for the treatment of human and animal pain.

Under this point of view, organoselenium chemistry is a very broad and exciting field, with many opportunities for research and development of applications. Organoselenium compounds have become attractive synthetic targets because of their chemo-, regio-, and stereo-selective reactions, and their useful biological activities (Alves et al., 2008). These compounds have been reported as neuroprotective, antiulcer, cytoprotective, chemopreventive, antidepressant, anti-inflammatory and antinociceptive agents (Sies, 1993; Schewe, 1995; Yamagushi et al., 1998; Combs and Gray, 1998; Nogueira et al., 2004). They have been also successfully utilized in haloperidol-induced orofacial dyskinesia (Burger et al., 2006) and apomorphine-induced stereotypy in mice (Machado et al., 2006).

The antinociceptive properties of organoselenium compounds, which could be relevant drugs for the management of pain, have been reported by our research group (Savegnago et al., 2007a,b,c; Jesse et al., 2008a,b; Pinto et al., 2008). In fact, diphenyl diselenide $(PhSe)_2$, a simple diaryl diselenide, attenuates chemical-induced nociception, including visceral pain induced by acetic acid, licking behavior induced by intraplantar injection of glutamate, formalin, capsaicin, bradykinin and phorbol 12-myristate 13-acetate (PMA) in mice (Nogueira et al., 2003; Zasso et al., 2005; Savegnago et al., 2007b). Recently, bis-selenide alkene derivatives were demonstrated to produce antinociception when

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assessed in acetic acid, capsaicin and tail-immersion behavioral tests and this effect is not influenced by opioidergic mechanism (Savegnago et al., 2006).

In addition to organoselenium compounds described above, chalcogenophenes are compounds widely studied (Hudson et al., 1989; Gonçales et al., 2005; Meotti et al., 2004; Wilhelm et al., 2009a,b). Among chalcogenophenes, selenophenes play an important role in organic synthesis because of their electrical properties and stability (Alves et al., 2008). Besides, in previous studies we demonstrated that 3-alkynyl selenophene (3-ASP), the selenophene studied in this paper, presents anticonvulsant and antioxidant effects in 21-day-old rats in a pilocarpine model of seizures. This study confirmed the anticonvulsant activity of 3-ASP and the drug's ability in reducing the oxidative stress in the pilocarpine model (Wilhelm et al., 2009a). 3-ASP has hepatoprotective effect against acute liver injury induced by D-galactosamine and lipopolysaccharide in rats by the mechanism that involves its antioxidant activity (Wilhelm et al., 2009b).

Based on the results described here, the aims of the present study were to evaluate: (i) the antinociceptive effect of 3-ASP on different models of acute nociception; (ii) the possible involvement of opiod system in 3-ASP antinociceptive effect; and (iii) the anti-hyperalgesic effect of 3-ASP in mechanical hyperalgesia induced by Freund's Complete Adjuvant in mice.

2. Materials and method

2.1. Drugs

3-alkynyl selenophene (1-(2,5-diphenylselenophen-3-yl)-3-methylpent-1-yn-3-ol; 3-ASP; Fig. 1) was prepared according to the literature method (Alves et al., 2008). Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of 3-ASP (99.9%) was determined by GC/HPLC. 3-ASP was dissolved in canola oil and administered by intragastric gavage as a single oral dose (p.o.). All other drugs were dissolved in a saline solution (0.9%), with the exception of capsaicin, which was prepared in absolute ethanol. The final concentration of ethanol did not exceed 0.5% and did not cause any detectable effect per se.

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 experiments were performed after protocol approval by the Institutional Ethics Committee and were carried out in accordance with current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals as specified (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 the mice were killed by decapitation.

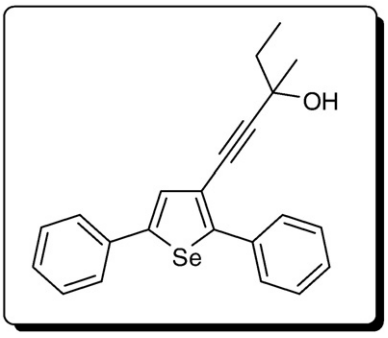

Fig. 1. Chemical structure of 3-ASP.

2.3. Chemical models of nociception

2.3.1. Formalin test

The formalin test was carried out as described by Hunskaar and Hole (1987). Animals received the intraplantar (i.pl.) administration of formalin (2.5%/paw, 20 µl) (v/v) into the dorsal right hind paw. After formalin injection, mice were returned to the experimental cage and the time spent licking or biting the injected paw was recorded during the periods of 0–5 min (early phase) and 15–30 min (late phase). The effect of 3-ASP (1–50 mg/kg, p.o.) was determined by pretreatment with this compound and the injection of formalin (20 µl) into the dorsal right hind paw (ipsilateral) of the mice. 3-ASP at different doses or vehicle (canola oil, p.o., 10 ml/kg) was administrated 30 min previous to formalin administration.

2.3.2. Capsaicin-induced nociception

The capsaicin test was performed as described by Sakurada et al. (1993). After an adaptation period, the mice were pretreated with vehicle (canola oil, p.o., 10 ml/kg) or 3-ASP (1–50 mg/kg, p.o.) 30 min before the i.pl. administration of capsaicin (1.6 µg/paw, 20 µl) in the ventral surface of the right hind paw. The amount of time spent licking the injected paw was recorded with a chronometer for 5 min following capsaicin injection and was considered as a nociceptive behaviour.

2.3.3. Bradykinin-induced nociception (BK)

The experiment was performed according to the method described by Ferreira et al. (2004). Mice were treated with 3-ASP (1–50 mg/kg, p.o.) or vehicle (canola oil, p.o., 10 ml/kg) 30 min before i.pl. injection of BK (10 nmol/ paw, 20 µl) in the right hind paw. Animals were observed individually for 10 min following bradykinin injection and the amount of time spent licking the injected paw was recorded with a chronometer and was considered as a nociceptive behaviour.

2.3.4. Glutamate-induced nociception

The procedure used was similar to that described previously (Beirith et al., 2002). Mice were treated with 3-ASP (1–50 mg/kg, p.o.) or vehicle (canola oil, p.o., 10 ml/kg) 30 min before i.pl. injection of glutamate (10 µmol/paw, 20 µl) on the right hind paw. The mice were observed individually for 15 min following glutamate injection and the amount of time spent licking the injected paw was recorded with a chronometer and was considered as a nociceptive behaviour.

2.3.5. Phorbol myristate acetate (PMA)-induced nociception

The procedure used was similar to that described previously (Ferreira et al., 2005). Mice were treated with 3-ASP (1–50 mg/kg, p.o.) or vehicle (canola oil, p.o., 10 ml/kg), 30 min before i.pl. injection of PMA (a protein kinase C (PKC) activator, 0.03 µg/paw, 20 µl). Fifteen minutes after i.pl. injection of PMA, the animals were observed for a further 30 min. The time spent licking the injected paw during this period was recorded with a chronometer and was considered as a nociceptive behaviour.

2.4. Thermal models of nociception

2.4.1. Tail-immersion-induced nociception

The tail-immersion test was carried out as described by Janssen et al. (1963). The lower 3.5 cm portion of the tail was marked and animals were then injected with 3-ASP (1–50 mg/kg, p.o.) or vehicle (canola oil, p.o., 10 ml/kg), 30 min before the test. The test was

determined 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 in this measure. Changes in tail-flick latency, Δt (s), were calculated for each animal according to the formula: Δt (s) = post-drug latency − pre-drug latency (Pinardi et al., 2003).

2.4.2. Hot-plate test

The hot-plate test was carried out as described by Woolfre 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. Mice were treated with 3-ASP (1–50 mg/kg, p.o.) or vehicle (canola oil, p.o., 10 ml/kg). The change in thermal withdrawal latencies was recorded before treatment and at 30 min after treatment with 3-ASP. The delta of latency, Δt (s), was calculated for each animal according to the formula: Δt (s) = post-drug latency − pre-drug latency. In order to avoid damage to the paws of the animals, the time standing on the plate was limited to 30 s.

Opioids have been reported to cause analgesia in rodents in the hot-plate test (Ankier, 1974; Hunskaar et al., 1986). Thus, in a separate series of experiments, we also investigated the possible participation of the opioid system in the antinociceptive effect caused by 3-ASP in the hot-plate test. To this end, the mice were pre-treated with naloxone (5 mg/kg, s.c.) or saline (0.9% NaCl) 15 min before administration of vehicle (canola oil, p.o., 10 ml/kg), morphine (2.5 mg/kg, s.c.) or 3-ASP (50 mg/kg, p.o.).

2.5. Reversal of inflammation-induced mechanical hyperalgesia

We investigated the effect caused by 3-ASP on inflammatory pain model, through immunologic reaction induced by i.pl. injection of CFA-complete Freund's adjuvant. The mechanical hyperalgesia was measured as described by Bortolanza et al. (2002). In this experiment we evaluated the time–course of the antinociceptive effect of 3-ASP at the doses of 25 and 50 mg/kg (p.o.). Briefly, the mice were lightly anesthetized and received the i.pl. injection of CFA (1 mg/ml of heat killed *Mycobacterium tuberculosis* in 85% paraffin oil and 15% mannide monoleate, 20 μl) on the right hind paw. Excepting that, the inflammatory response was verified 24 h after CFA injection in the intraplantar surface of the right hind paw in mice. Therefore, the mice that presented hyperalgesia received vehicle (canola oil, p.o., 10 ml/kg) or 3-ASP (25 and 50 mg/kg, p.o.) and the withdrawal response frequency in VHF was recorded after (0.5, 1, 2, 4, 6 and 8 h) 3-ASP treatment. The frequency of withdrawal was determined before (baseline) CFA injection, in order to obtain data purely derived from the treatments in CFA allodynia. Mechanical hyperalgesia in mice with CFA was measured by using a calibrated nylon von Frey filament of 0.6 g.

2.6. Acute toxicity

To investigate the potential acute toxicity caused by 3-ASP, the mice received a single oral dose of 3-ASP (1–50 mg/kg, p.o.) or vehicle (canola oil, p.o., 10 ml/kg). After administration of 3-ASP, the animals were observed up to 72 h to determine the general toxicity. After this time of exposure, the mice were anesthetized for blood collection by heart puncture in tubes containing heparin.

Plasma was obtained by centrifugation at 2000 ×*g* for 10 min (hemolyzed plasma was discarded) and used for biochemical assays. Plasma aspartate (AST) and alanine aminotransferase (ALT) activities, used as the biochemical markers for the early acute hepatic damage, were determined by the colorimetric method of Reitman and Frankel (1957). Renal function was analyzed by determining plasma urea (Mackay and Mackay, 1927) and creatinine levels (Jaffe, 1886).

2.7. Locomotor activity

The rota-rod test was carried out to determine if the antinociceptive effect of 3-ASP, in chemical and thermal models of nociception, could be related to nonspecific disturbances in the locomotor activity of the animal. Briefly, the rota-rod apparatus consists of a rod 30 cm long and 3 cm in diameter that is subdivided into three compartments by discs 24 m in diameter. The rod rotates at a constant speed of 10 rpm. The animals were selected 24 h previously by eliminating those mice that did not remain on the bar for two consecutive periods of 60 s. The animals were treated with 3-ASP (1–50 mg/kg, p.o.) or vehicle (canola oil, p.o., 10 ml/kg) and were retested 30 min after. Time they remained on the rotating bar (maximum 60 s) was recorded (Santos et al., 1999).

2.8. Statistical analysis

The results are presented as means ± SEM, except the ID_{50} values (i.e., the dose of 3-ASP need to reduce the nociceptive response by 50% relative to the control value), which are reported as geometric means accompanied by their respective 95% confidence limits. Comparisons between experimental and control groups were performed by ANOVA followed by Newman–Keuls' test when appropriated. *P* values less than 0.05 ($p<0.05$) were considered as indicative of significance. Maximal inhibition values were calculated at the most effective dose used.

3. Results

3.1. Effect of 3-ASP on nociception in mice

Fig. 2A and B show that 3-ASP caused significant inhibition of both early (0–5 min) and late (15–30 min) phases of formalin-induced

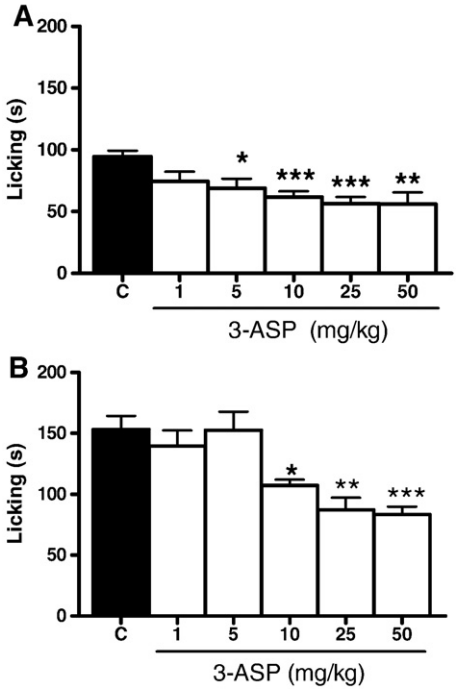

Fig. 2. Effect of 3-ASP on the licking induced by formalin in mice. Animals were pretreated orally with 3-ASP at a range dose of 1–50 mg/kg for 30 min prior to formalin (2.5%/paw, 20 μl). The total time spent licking the hind paw was measured (A) in the first (0–5 min) phase and (B) in the second (15–30 min) phase after intraplantar injection of formalin. Each column represents the mean with S.E.M. for 6–10 mice in each group. Control (C) value indicates the animals administered with vehicle (canola oil). The asterisks denote the significance levels when compared with the control group (one-way ANOVA followed by Newman–Keuls test): $^{*}p<0.05$; $^{**}p<0.01$; and $^{***}p<0.001$.

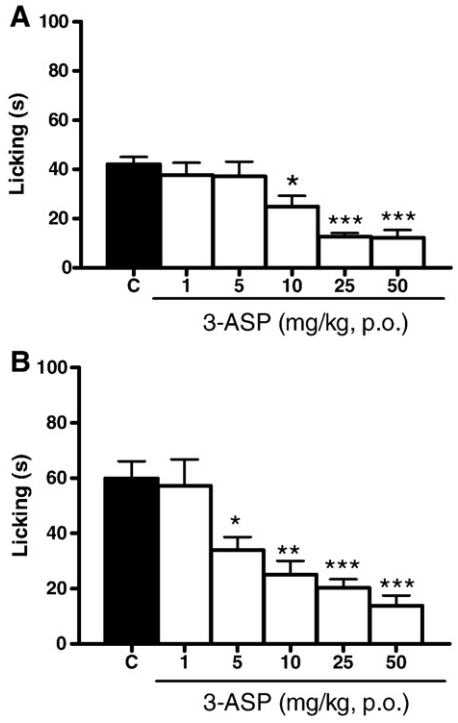

Fig. 3. Effect of 3-ASP against (A) capsaicin- and (B) bradykinin-induced licking in mice. Animals were pretreated orally with 3-ASP at various doses (from 1 to 50 mg/kg) for 30 min prior to capsaicin (1.6 μg/paw, 20 μl) or bradykinin (10 nmol/ paw, 20 μl). Each column represents the mean with S.E.M. for 6–10 mice in each group. Control (C) indicates the animal administered with vehicle (canola oil). The asterisks denote the significance levels when compared to the control group (one-way ANOVA followed by Newman–Keuls test): $^{*}p<0.05$; $^{**}p<0.01$; and $^{***}p<0.001$.

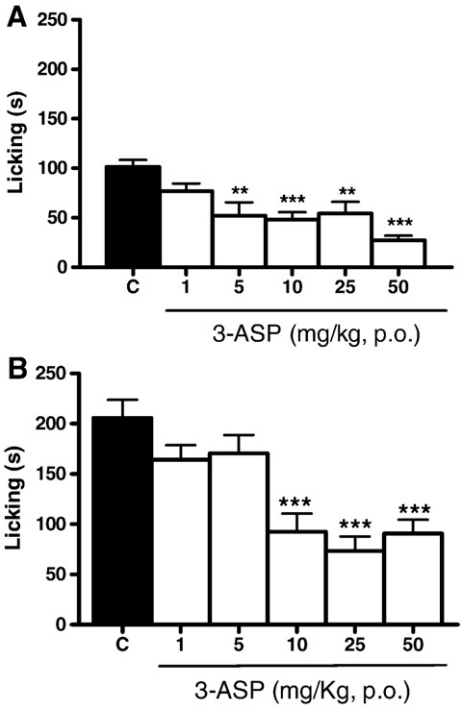

Fig. 4. Effect of 3-ASP against (A) glutamate- and (B) phorbol myristate acetate (PMA)-induced nociception in mice. Animals were pretreated orally with 3-ASP at various doses (from 1 to 50 mg/kg) for 30 min prior to glutamate (10 μmol/paw, 20 μl) or PMA 0.03 μg/paw, 20 μl). Each column represents the mean with S.E.M. for 6–10 mice in each group. Control (C) indicates the animal administered with vehicle (canola oil). The asterisks denote the significance levels, when compared to the control group (one-way ANOVA followed by Newman–Keuls test): $^{**}p<0.01$; and $^{***}p<0.001$.

licking. The maximal inhibition values observed were 41 ± 10% and 46 ± 4% for the first and second phases, respectively.

3-ASP (10–50 mg/kg) caused an inhibition of the capsaicin-induced licking response (Fig. 3A), with a maximal inhibition value of 71 ± 7%. As can be seen in Fig. 3B, 3-ASP (5–50 mg/kg, p.o.) significantly inhibited nociception induced by i.pl. injection of BK. The maximal inhibition observed was 77 ± 6%.

The results presented in Fig. 4A show that 3-ASP (5–50 mg/kg), given orally, resulted in a significant inhibition of glutamate-induced nociception, with an inhibition value of 73 ± 5%. As revealed in Fig. 4B, oral treatment with 3-ASP (10–50 mg/kg) significantly inhibited PMA-induced licking response. The maximal inhibition observed was 56 ± 7%.

In the tail-immersion test, 3-ASP, at doses of 10–50 mg/kg, caused a significant increase in the reaction time to thermal stimuli as compared to the control group (Fig. 5A). In the hot-plate test, oral treatment with 3-ASP at doses of 25 and 50 mg/kg increased the latency time as compared to the control group (Fig. 5B).

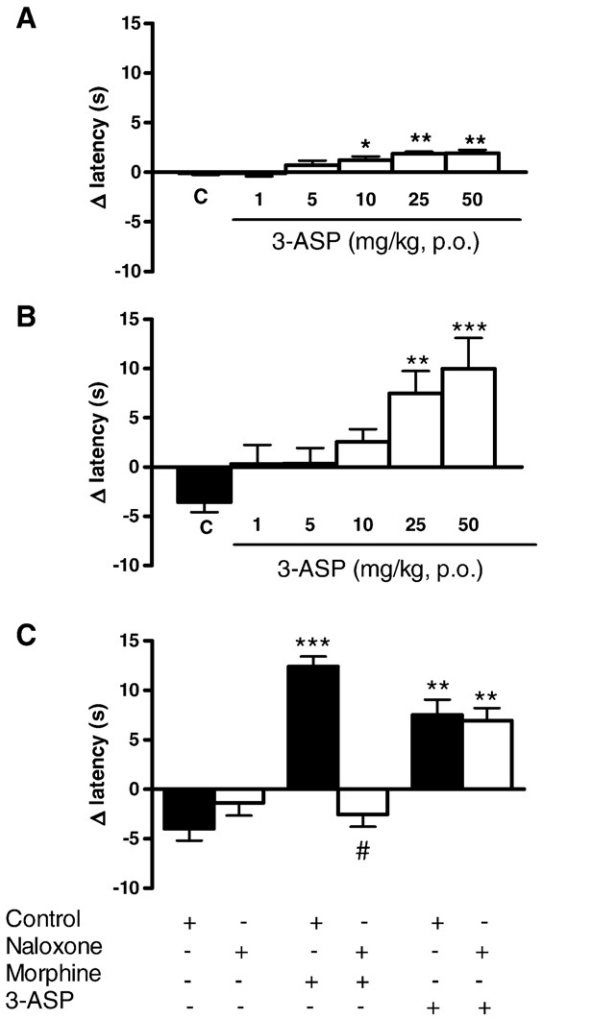

Fig. 5. Effect of 3-ASP on the tail-immersion (A) and hot-plate tests (B) in mice. Animals were pretreated orally with 3-ASP at various doses (from 1 to 50 mg/kg) for 30 min prior to tail-immersion or hot-plate test at 55 °C. (C) Effect of naloxone (5 mg/kg, s.c) or 0.9% saline, 15 min before administration of vehicle (canola oil, p.o., 10 ml/kg), morphine (2.5 mg/kg, s.c.) or 3-ASP (50 mg/kg, p.o.) in the hot-plate test in mice. Each column represents the mean with S.E.M. for 6–10 mice in each group. Control value (C) indicates the animals administered with vehicle (canola oil). The asterisks denote the significance levels when compared with the control group (one-way ANOVA followed by Newman–Keuls test): $^{*}p<0.05$; $^{**}p<0.01$; and $^{***}p<0.001$.

The possible involvement of the opioid system in the antinociceptive effect of 3-ASP was examined. Pretreatment with naloxone (5 mg/kg, s.c.), an opioid receptor antagonist, abolished the antinociceptive effect of morphine (positive control) at the dose of 2.5 mg/kg (s.c). The antinociceptive effect of 3-ASP (50 mg/kg, p.o.) was not protected by pretreatment with naloxone (Fig. 5C).

3.2. Reversal of inflammation-induced mechanical hyperalgesia

The effect caused by 3-ASP on inflammatory pain model, through immunological reaction induced by i.pl. injection of CFA, was investigated. The i.pl. injection of CFA produced a mechanical hyperalgesia, which were kept during all test.

The animals that received 3-ASP (25 and 50 mg/kg, p.o.) demonstrated a reduction on mechanical hyperalgesia induced by CFA. This hypersensitivity reduction started 30 min after 3-ASP administration and was maintained for up to 6 h (Fig. 6A).

The maximal inhibition observed was 42 ± 11%, at 8 h the sensitivity of animals was similar to baseline values (Fig. 6A). No significant difference was observed in the contralateral paw when compared to the basal (Fig. 6B).

3.3. Acute toxicity and locomotor activity

No mortality of mice was observed after acute administration of 3-ASP. Oral administration of 3-ASP to mice, at all doses tested, did not change plasma AST and ALT activities as well as urea and creatinine levels when compared to the control group (Table 1). No alteration in the locomotor activity in the animals treated with 3-ASP, at all doses, was observed (Table 1).

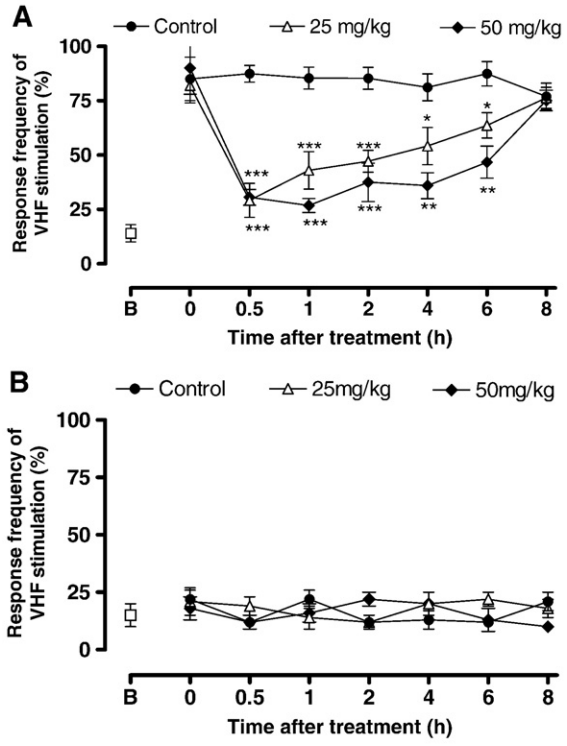

Fig. 6. Effect of 3-ASP administered orally (25 and 50 mg/kg) on mechanical hyperalgesia in response to 10 applications of 0.6 g VFH induced by CFA in the ipsilateral paw (A) and in the contralateral paw (B) in mice. The animals received vehicle (●) or 3-ASP 25 mg/kg (Δ) or 3-ASP 50 mg/kg (♦) 24 h after CFA injection. The baseline (□) was recorded before CFA injection. The measure that follows was 24 h after CFA-injection (0) and (0.5, 1, 2, 4, 6 and 8 h) subsequent to 3-ASP (25 and 50 mg/kg, p.o.) treatment. The results represent the means ± S.E.M. of eight animals. The symbols denote significant difference $^{*}p<0.05$; $^{**}p<0.01$; and $^{***}p<0.001$ between vehicle treated and 3-ASP treated mice by one-way analysis of variance (ANOVA), followed by Student–Newman–Keuls test.

Table 1
Effect of 3-ASP administered orally on biochemical parameters and the locomotor activity in mice.

Groups (mg/kg)	AST (U/l)	ALT (U/l)	Urea (mg/dl)	Creatinine (mg/dl)	Rota-rod (s)
Control	71.75 ± 4.04	27.00 ± 3.10	53.00 ± 3.58	0.295 ± 0.02	59.40 ± 0.50
1	62.20 ± 2.70	25.60 ± 1.96	48.60 ± 2.24	0.308 ± 0.01	59.17 ± 0.83
5	70.60 ± 2.73	30.40 ± 1.66	47.40 ± 2.33	0.296 ± 0.02	58.83 ± 0.80
10	64.60 ± 3.93	28.40 ± 1.28	51.80 ± 2.42	0.312 ± 0.01	58.83 ± 0.66
25	65.20 ± 4.33	28.00 ± 1.58	50.20 ± 2.01	0.320 ± 0.01	59.17 ± 0.83
50	66.40 ± 4.70	29.00 ± 2.34	54.60 ± 3.34	0.324 ± 0.01	59.33 ± 0.65

Data are reported as means ± S.E.M of six animals.

4. Discussion

The present study demonstrates that 3-ASP administered orally did not produce acute toxicity and induced antinociception in chemical and thermal models of nociception in mice, without modifying the locomotor activity of mice in the rota-rod test.

We have found that 3-ASP decreased the nociception in both phases of the formalin test, with a greater potency in the inflammatory phase. Morphine (Shibata et al., 1989) and non-steroidal anti-inflammatory drugs (NSAIDs) (Martindale et al., 2001) inhibit nociception in both phases of the formalin test. The effect of 3-ASP in the formalin test was similar to that of caused by $(PhSe)_2$ (Savegnago et al., 2008a).

We have also demonstrated, in the present study, that 3-ASP caused a significant antinociceptive effect on capsaicin-induced biting behavior (Imax of 71 ± 7%). These results are consistent with other findings of our research group, in which, $(PhSe)_2$ caused significant antinociception against capsaicin-induced licking (Nogueira et al., 2003; Savegnago et al., 2007b). In addition, $(PhSe)_2$ and 3-ASP caused similar effects against capsaicin-induced nociceptive response (Savegnago et al., 2007b). It has been previously demonstrated that celecoxib (260 μmol/kg, p.o.), a well-known pyrazole compound, reduced nociception induced by the TRPV1 agonist capsaicin (Oliveira et al., 2009) with an Imax of 51 ± 6%.

Based on these results, we sought to determine whether or not 3-ASP inhibits nociceptive response caused by intraplantar injection of glutamate into the mouse hind paw. Glutamate is the major excitatory amino acid neurotransmitter present in the central nervous system, where it participates in a great diversity of biological functions, such as learning and memory, neurodegenerative diseases and neuronal death. Apart from these actions, glutamate is found in sensory C fibres where it is believed to play a relevant role in transmission of nociceptive mechanisms at the spinal cord (Hudspith, 1997). In this study, we showed that the antinoceptive effect of 3-ASP (5–50 mg/kg) on glutamate-induced biting behavior (Imax of 73 ± 5%) is similar to that of caused by $(PhSe)_2$ (Imax of 95 ± 1%) in this test. Thus, similar to $(PhSe)_2$ (Savegnago et al., 2007b) 3-ASP, at least in part, presents antinociceptive action due to an interaction with capsaicin receptor (TRPV1) and the glutamatergic system. Aspirin (100 mg/kg, p.o.) and acetaminophen (200 mg/kg, p.o.) demonstrated antinociceptive effect in the glutamate test (Choi et al., 2001). Thus, 3-ASP (50 mg/kg) showed an effective antinociceptive effect when compared to these drugs.

The current study clearly indicates that the antinociceptive effect of 3-ASP, given by p.o. route, strongly inhibited BK-induced biting in mice (Imax of 77 ± 6%). Oliveira et al. (2008) have reported that celecoxib (260 μmol/kg, p.o.) presents antinociceptive effect on BK test, with an Imax of 77 ± 8%. The peptide BK has long been established as an important peripheral mediator of pain (Calixto et al., 2001). Once released, BK may induce pain by direct stimulation

of the nociceptors (Aδ and C fibers) innervating many tissues. Furthermore, BK can release most inflammatory and algogenic substances, namely products derived from arachidonic acid pathways, cytokines and nitric oxide (Calixto et al., 2001). Moreover, both functional and neurochemical evidences have been accumulated that BK can excite primary sensory neurons and evoke release of neuropeptides, including calcitonin gene-related peptide and substance P (Vasko et al., 1994). Accordingly, organoselenium compounds inhibit nociception induced by intraplantar injection of BK (Savegnago et al., 2007b; Jesse et al., 2008a).

We have also demonstrated that 3-ASP (50 mg/kg), orally administered, inhibited PMA-induced nociception in mice (Imax: 56 ± 7%). In accordance with these results are those obtained with $(PhSe)_2$ (Savegnago et al., 2007b) and *p*-methoxyl-diphenyl diselenide (Jesse et al., 2008b) in the same model of nociception in mice. Souza et al. (2002) have reported that an intraplantar injection of PMA, a known PKC activator, induces paw licking in mice. It is now well recognized that PKC activation is an important step for the nociceptive effects caused by numerous stressful stimuli, including that caused by inflammatory mediators. PKC is known to phosphorylate several cellular components, including enzymes, ion channels and membrane-bound receptors, all that are key regulators in the processes of nociceptor excitation and sensitization (Ji and Woolf, 2001). For instance, PKC activation is associated with the excitation and sensitization of nociceptors in response to bradykinin or histamine in vitro (Dray and Perkins, 1997). Moreover, PKC inhibitors are able to inhibit the in vivo nociception produced by bradykinin, by epinephrine, or by the inflammatory substance carrageenan, in mice and rats (Ferreira et al., 2004). Tsuchiya et al. (2005) suggested that capsaicin-sensitive fibres (mainly nonmyelinated C-fibres) exert a relevant effect on PMA induced nociception. These fibers are the source of certain mediators involved in the nociceptive effect induced by PMA, including glutamate and substance P (Szallasi and Blumberg, 1999).

Another interesting finding in the present study is the demonstration that 3-ASP was effective against CFA-induced inflammatory nociception. It is now well recognized that persistent nociception resulting from peripheral injection of CFA leads to the release of multiple inflammatory and nociceptive mediators, resulting in increased long-lasting discharge of primary sensory fibers that modifies neuronal, neuro-glial and neuro-immune cell phenotype and function in the central nervous system. These alterations can occur at translational or post-translational levels and affect receptors, ion channels, soluble mediators and other molecules involved in cell signaling (Ji and Stricharstz, 2004). In this context, valuable effects of 3-ASP in counteracting CFA-induced inflammatory nociception are probably associated with its ability to interfere in cell signaling, particularly that related to PKC, NO, Ca^{2+} and K^{+} pathways. In addition, the anti-hyperalgesic effect of 3-ASP appeared 30 min administration and was maintained for up to 6 h with the maximal inhibition of 42 ± 11%. Different from the results obtained with 3-ASP, the attenuation of neuropathic pain by $(PhSe)_2$ is persistent for up to 4 h (Savegnago et al., 2007a). Dipyrone (1.0 mmol/kg, p.o) is effective up to 4 h after its administration, while morphine (0.026 mmol/kg, p.o) produces an anti-hyperalgesic effect that is maintained for up to 6 h (Milano et al., 2008).

The tail-immersion and hot-plate tests have been widely used as experimental models to measure nociception, especially for the screening of analgesic drugs (Dewey et al., 1969). In the present study we demonstrated that 3-ASP orally administered prolonged the latency in both thermal tests of nociception. Tail-immersion is considered to be a spinal reflex, but the mechanism of response could also involve higher neural structures (Jensen and Yaksh, 1986). The hot-plate test produces, at constant temperature, two kinds of behavioural response, which are paw licking and jumping. Both of these are considered to be supraspinally integrated responses (Chapman et al., 1985). Organoselenium compounds prolonged the latency in thermal tests of nociception (Pinto et al., 2008; Jesse et al., 2008b; Savegnago et al., 2008b).

In this study, morphine was more potent than 3-ASP, however, several other studies, that show the antinociceptive effect of novel drugs, have reported drugs with less potency than morphine in the tail-flick and hot-plate tests (Silbert et al., 1991; Vaz et al., 1996). The involvement of opioid system on the antinociceptive action of 3-ASP was evaluated in the hot-plate test. Pretreatment of animals with naloxone (a nonselective opioid receptor antagonist) did not reverse the antinociceptive effect caused by oral administration of 3-ASP demonstrating that opioid system is not involved in this effect. In the same way, the antinociceptive effect of $(PhSe)_2$ seems to be unlike the activation of opioid receptors (Zasso et al., 2005; Savegnago et al., 2007a).

Together, the present results indicate that 3-ASP might be of potential interest in the development of new clinically relevant drugs for the management of pain. Additional studies are, however, necessary to confirm this hypothesis and to investigate the exact mechanism involved in the antinociceptive and anti-hyperalgesic effects.

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