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Research Paper

Mechanisms involved in the antinociceptive and anti-inflammatory effects of bis selenide in mice

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Abstract

Objectives The present study examined the mechanisms involved in the antinociceptive effects of bis selenide [(*Z*)-2,3-bis(4-chlorophenylselanyl)prop-2-en-1-ol].

Methods The effects of oral bis selenide were tested against licking behaviour and oedema in mice induced by formalin, serotonin, histamine, glutamate, phorbol 12-myristate 13-acetate (PMA), 8-bromoadenosine 3′,5′-cyclic monophosphate (8-BrcAMP) and prostaglandin E_2. The effects of a variety of receptor antagonists on the antinociceptive activity were tested to determine the likely mechanism of action of bis selenide.

Key findings Bis selenide caused antinociception on the first and second phases of the formalin test, with mean ID50 values of 34.21 (29.66–39.45) and 15.86 (12.17–20.67) mg/kg and maximal inhibition of 65 ± 3% and 90 ± 1%, respectively. At 50 mg/kg bis selenide significantly inhibited (31 ± 2%) paw oedema induced by intraplantar injection of formalin. At 25 mg/kg given 5 min after the formalin injection, bis selenide caused a significant inhibition (42 ± 5%) in the second phase of the formalin test, whereas the prophylactic treatment caused more intense inhibition (64 ± 3%). Oral administration of bis selenide reduced licking and paw oedema induced by serotonin, histamine, glutamate, PGE_2, PMA and 8-BrcAMP. The antinociceptive effect of bis selenide (25 mg/kg, p.o.) on the formalin test was reversed by i.p. administration of p-chlorophenylalanine methyl ester (an inhibitor of serotonin synthesis), ketanserin (a selective 5-HT_{2A} receptor antagonist), ondansetron (a 5-HT_3 receptor antagonist) and ranitidine (a histamine H_2-receptor antagonist).

Conclusions Glutamatergic, prostaglandin E_2, serotonergic (5-HT_{2A} and 5-HT_3) and histamine H_2 receptors are involved in the antinociceptive effects of bis selenide in mice. The interaction of bis selenide with protein kinase C and A signalling pathways was also demonstrated.

Keywords antinociception; bis selenide; inflammation; mice

Introduction

Acute pain results from direct thermal, mechanical or chemical activation of particular subsets of primary afferent neurons (nociceptors).[1] The persistent component of the pain response – acute pain – is associated with the production and release of multiple inflammatory factors, including neurotransmitters, eicosanoids and hydrogen ions.[2] These act in concert not only to maintain activity of primary afferent nociceptors and sustain pain but also to heighten nociceptor sensitivity, such that innocuous stimuli produce pain. Moreover, tissue injury generates endogenous factors that heighten our sense of pain by increasing the response of sensory nerve endings to noxious stimuli.[3] Thus, inflammation results in the production of a soup of cytokines and inflammatory mediators, including serotonin (5-hydroxytryptamine; 5-HT), bradykinin, histamine, glutamate, prostaglandin E_2 (PGE_2), interleukin-1 and tumor necrosis factor α (TNFα), and activates signalling routes, including protein kinase A (PKA), protein kinase C (PKC), calcium/calmodulin-dependent protein kinase, nitric oxide and mitogen-activated protein kinases (MAPKs).[4] Taking this into account, substances able to counteract these signalling pathways at either a peripheral or central level might be promising compounds to control pain.

The selenium organic compound, ebselen, is a classic antioxidant and is well known as a glutathione peroxidase mimetic agent.[5] In addition to its peroxidase-like activity, ebselen has show anti-inflammatory activity in different models of inflammation,[6,7] which may be related at least in part to its ability to scavenge peroxynitrite, a potent inflammatory

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mediator.[8] The mechanism(s) underlying the anti-inflammatory activity of ebselen is still not completely understood but is linked to inhibition of NADPH-oxidase, PKC, nitric oxide synthase and lipoxygenases, most likely by interacting with critical thiol/disulfide groups in these enzymes.[9] In addition, we have reported that diphenyl diselenide, a simple diaryl diselenide, is a safe drug when administered acutely to mice in pharmacological doses.[10–12] The mechanism underlying the antinociception of diphenyl diselenide involves the serotonergic pathway, an interaction with the nitrergic system and interaction with the redox modulatory site of glutamate receptors.[11,12] Interestingly, the antinociceptive and anti-inflammatory potency of diphenyl diselenide was higher than that of ebselen.[10] Diphenyl diselenide is also more active as a glutathione peroxidase mimetic than ebselen.[10]

Recently we have reported that bis-selenide alkene derivatives injected subcutaneously elicit significant antinociception when assessed in acetic acid, capsaicin and tail-immersion behavioural tests.[13] We demonstrated in the same study that the antinociceptive action of bis-selenide alkene derivatives is not influenced by opioidergic mechanisms.[13]

Based on a previous study demonstrating that subcutaneous injection of bis-selenide alkene derivatives in mice elicits antinociceptive properties,[13] the purpose of the present study was to further evaluate the effects of oral administration of bis selenide [(*Z*)-2,3-bis(4-chlorophenylselanyl)prop-2-en-1-ol] on the nociceptive responses induced by intraplantar injection of formalin, serotonin, histamine, glutamate, PGE_2, phorbol, 12-myristate-13-acetate (PMA; a PKC activator) and 8-bromoadenosine 3′,5′-cyclic monophosphate (8-BrcAMP; a PKA activator). We also carried out experiments to determine the possible involvement of the serotonergic and histaminergic systems in the antinociception induced by bis selenide in the formalin test in mice.

Materials and Methods

Animals

Behavioural experiments were conducted using Swiss male mice (25–35 g) from our own breeding colony. The mice were kept in separate animal rooms in a 12 h light–dark cycle, at room temperature (22 ± 1°C) and with free access to food and water. Animals (male and female mice distributed evenly between groups) were acclimatised to the laboratory for at least 1 h before testing and were used only once.

To record licking behaviour, animals were placed individually in chambers (transparent glass cylinders, 20 cm diameter) and were acclimatised for at least 20 min before the test.

Animals handling was according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, the Federal University of Santa Maria, Brazil and the ethical guidelines for investigations of experimental pain in conscious animals.[14] The number of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate consistent effects of drug treatments.

Drugs

Bis selenide was prepared and characterised in our laboratory by the method described by Moro and colleagues.[15] Analysis of ^1H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of bis selenide (99.9%) was determined by GC/HPLC.

Bis selenide was dissolved in canola oil and administered p.o. The mice received bis selenide in a constant volume of 10 ml/kg body weight.

Formalin was purchased from Merck (Darmstadt, Germany). p-Chlorophenylalanine methyl ester (PCPA), WAY100635, ketanserin, ondansetron, pyrilamine, ranitidine, thioperamide, serotonin, histamine, glutamate, PGE_2, phorbol 12-myristate 13-acetate (PMA) and 8-bromoadenosine 3′,5′-cyclic monophosphate (8-BrcAMP) were purchased from Sigma-Aldrich (St Louis, MO, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers. All drugs except bis selenide were dissolved in saline.

Effect of bis selenide on formalin-induced licking behaviour and paw oedema

The formalin test was carried out as described by Hunskaar & Hole.[16] Intraplantar injections of 2.5% formalin (20 μl; 0.92% formaldehyde) were made into the ventral right hindpaw. Animals were given bis selenide (1–50 mg/kg p.o.) 30 min before formalin injection. This time of pretreatment (30 min) was chosen based on time-course experiments, in which bis selenide administered orally exhibited peak antinociceptive effects in the formalin test at 30 min. Control animals received a similar volume of vehicle (canola oil, 10 ml/kg p.o.). After intraplantar injection of formalin, the animals were observed from 0 to 5 min (neurogenic phase) and 15 to 30 min (inflammatory phase), and the time spent licking the injected paw, indicative of nociception, was timed with a chronometer.

In order to assess whether the antinociceptive activity produced by bis selenide in formalin-induced nociception was associated with inhibition of oedema formation, we measured paw oedema by comparing the difference between the weight of the formalin-treated paw and the weight of the contralateral paw (non-treated paw). Animals were euthanised 30 min after formalin injection by cervical dislocation, and both paws were cut at the ankle joint and weighed on an analytical balance.

In a separate set of experiments, we investigated the antinociceptive effect of bis selenide given after formalin injection. The challenge test was evaluated only in the second phase of the formalin test (15–30 min after formalin injection). For this purpose, mice were given an intraplantar injection of formalin followed by bis selenide (25 mg/kg p.o.) either 10 min (post-administered) or 30 min (pre-administered) before the second phase of the formalin test. These different timings were necessary to elucidate if bis selenide has therapeutic or prophylactic effects (pre- and post-administered, respectively). Control experiments using only the vehicle (canola oil, p.o.) were carried out in parallel.

Analysis of the possible mechanism of bis selenide action in the formalin test

To address some of the mechanisms by which bis selenide inhibits formalin-induced nociception, mice were treated with different drugs. Doses of each drug were chosen on the basis of data in the literature. The formalin test was chosen for this purpose because of the specificity and sensitivity in nociception transmission that this model provides.[17]

Involvement of the serotonergic system

The participation of the serotonergic system in the antinociceptive action of bis selenide was investigated using WAY100635 (0.1 mg/kg i.p.), a selective 5-HT_{1A} receptor antagonist, ketanserin (1 mg/kg i.p.), a selective 5-HT_{2A} receptor antagonist, ondansetron (0.5 mg/kg i.p.), a 5-HT_3 receptor antagonist, or vehicle (10 ml/kg i.p.) given 15 min before bis selenide (25 mg/kg p.o.) or canola oil, which was administered 30 min before the intraplantar formalin injection.

In a separate series of experiments to investigate the possible contribution of endogenous serotonin to the antinociceptive effect of bis selenide, animals were pretreated with PCPA (100 mg/kg i.p.), an inhibitor of serotonin synthesis, or vehicle, once a day for four consecutive days. Animals were given bis selenide (25 mg/kg p.o.) or vehicle 15 min after the last PCPA or vehicle injection and were tested using the formalin test 30 min later.[18]

Involvement of the histaminergic system

To investigate the role played by the histaminergic system in the antinociception caused by bis selenide, mice were pretreated with pyrilamine (3 mg/kg i.p.), a histamine H_1 receptor antagonist, ranitidine (3 mg/kg i.p.), an H_2 receptor antagonist, thioperamide (5 mg/kg i.p), an H_3 receptor antagonist, or vehicle (10 mg/kg i.p.) according to the methods of Girard and colleagues[19] and Farzin & Nosrati,[20] with minor modifications. These drugs were administered 15 min before mice were given bis selenide (25 mg/kg p.o.) or vehicle, which was followed 30 min later by intraplantar formalin injection.

Serotonin-induced licking behaviour and paw oedema

The experiment was performed according to the method described by Inagaki and colleagues.[21] Mice were treated with bis selenide (5–50 mg/kg p.o.) or vehicle (canola oil, 10 ml/kg p.o.) 30 min before intraplantar serotonin injection (20 ng/paw, 20 μl) into the ventral surface of the right hindpaw. Animals were observed individually for 60 min following serotonin injection and the amount of time spent licking the injected paw was recorded with a chronometer. Paw oedema was measured 60 min after serotonin injection, as described above.

Histamine-induced licking behaviour and paw oedema

The experiment was performed according to the method described by Shinmei and colleagues.[22] Mice were treated with bis selenide (1–50 mg/kg p.o.) or vehicle (canola oil 10 ml/kg p.o.) 30 min before intraplantar histamine injection (100 nmol/paw, 20 μl) into the ventral surface of the right hindpaw. Licking time was recorded for 60 min and paw oedema measured as described above.

Glutamate-induced licking behaviour and paw oedema

The procedure used was similar to that described previously by Beirith and colleagues.[23] Mice were treated with bis selenide (1–50 mg/kg p.o.) or vehicle (canola oil 10 ml/kg p.o.) 30 min before glutamate injection. Glutamate solution (10 μmol/paw in 20 μl saline) was injected into the ventral surface of the right hindpaw. The mice were observed individually for 15 min after glutamate injection and the amount of time spent licking the injected paw recorded. Paw oedema was measured at the end of the experiment.

PGE_2-induced licking behaviour and paw oedema

The procedure used was similar to that described previously by Kassuya and colleagues.[24] Mice were given intraplantar injections of PGE_2 (3 nmol/paw in 20 μl) 30 min before administration of bis selenide (1–50 mg/kg p.o.) or vehicle (canola oil 10 ml/kg p.o.). After challenge, the mice were observed individually for 15 min. The amount of time spent licking the injected paw was recorded and paw oedema was measured at the end of the experiment.

PMA-induced licking behaviour and paw oedema

The procedure used was similar to that described previously by Ferreira and colleagues.[25] Mice were treated with bis selenide (5–50 mg/kg p.o.) 30 min before intraplantar injection of PMA (0.03 μg/paw in 20 μl). Animals were observed for 30 min, starting 15 min after PMA injection. The time spent licking the injected paw during this period was recorded. Paw oedema was measured 45 min after PMA injection.

8-BrcAMP-induced licking behaviour and paw oedema

The procedure used was similar to that described by Otuki and colleagues.[26] Mice were treated with bis selenide (5–50 mg/kg p.o.) 30 min before intraplantar injection of 8-BrcAMP (10 nmol/paw in 20 μl). Animals were observed for 10 min after 8-BrcAMP injection. The amount of time spent licking the injected paw was recorded, and paw oedema was measured at the end of the experiment.

Rotarod task

To test for non-specific effects due to motor impairment, the integrity of the motor system was evaluated using the rotarod test. Briefly, the rotarod apparatus consists of a rod 30 cm long and 3 cm in diameter that is subdivided into three compartments by discs 24 cm in diameter. The rod rotates at a constant speed of 10 rpm. The animals were selected 24 h previously by eliminating those that did not remain on the bar for two consecutive periods of 60 s. Animals were treated with bis selenide (1–50 mg/kg p.o.) or vehicle (canola oil p.o.)

and were tested on the rotarod 30 min after treatment. The latency for the first fall off the rod and the number of falls were noted. The cut-off time was 60 s.[18]

Statistical analysis

The results are presented as means ± SEM from 7–9 mice, except the ID50 values (i.e. the dose of bis selenide reducing the pain response by 50% in relation to control group values), which 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. The statistical significance of differences between groups was determined by analysis of variance followed by the Newman–Keul's test. *P* values less than 0.05 were considered significant.

Results

Effect of bis selenide on formalin-induced licking behaviour and paw oedema

Table 1 shows that bis selenide (5–50 mg/kg p.o.) caused significant inhibition in the first (0–5 min) and second (15–30 min) phases of the formalin test. The mean ID50 for these effects were: 34.21 (29.66–39.45) and 15.86 (12.17–20.67) mg/kg, respectively, and the inhibition observed was 65 ± 3% and 90 ± 1%, respectively. Furthermore, bis selenide (50 mg/kg p.o.) significantly inhibited paw oedema induced by intraplantar injection of formalin (31 ± 2%; Table 1).

Post-administered bis selenide (25 mg/kg p.o., given 10 min before the second phase) caused a significant inhibition (42 ± 5%) of inflammatory nociception. Prophylactic treatment with bis selenide (25 mg/kg p.o., given 30 min before the second phase), produced a significant inhibition (64 ± 3%) of nociception in the second phase of the formalin test (Figure 1).

Table 1 Effect of bis selenide on formalin-induced licking and oedema in mice. Mice were pre-treated orally with bis selenide 30 min before intraplantar formalin injection. Total time spent licking the hindpaw was measured in the first (0–5 min) and second phases (15–30 min) of the formalin test. Oedema was measured at the end of the second phase

Dose of bis selenide (mg/kg)	Formalin test		Oedema (mg)
	First phase (s)	Second phase (s)	
Control	62.00 ± 3.83	150.3 ± 8.13	73.33 ± 2.68
1	56.56 ± 3.05	141.7 ± 7.58	68.44 ± 1.54
5	47.80 ± 3.31**	103.4 ± 7.79**	66.00 ± 2.24*
10	43.40 ± 2.37**	64.38 ± 6.23***	61.56 ± 1.60***
25	26.38 ± 2.43***	24.38 ± 4.28***	54.38 ± 0.70***
50	22.02 ± 1.89***	14.38 ± 1.48***	50.88 ± 1.28***

Values are means ± SEM of 6–8 mice. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs control group (one-way ANOVA followed by Newman–Keul's test).

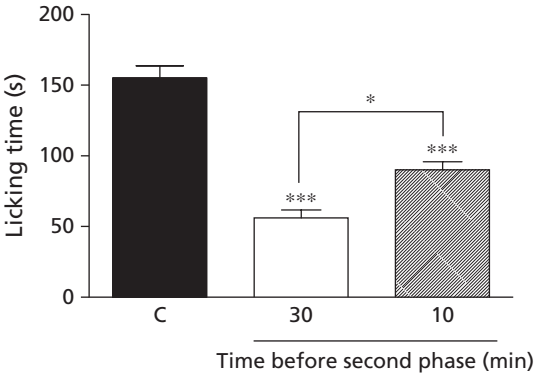

Figure 1 Effect of orally administered bis selenide on licking induced by formalin in mice. Bis selenide (25 mg/kg p.o.) was administered 10 min (post-treatment) or 30 min (pre-treatment) before the second phase of the formalin challenge test. Control mice (C) received vehicle only (canola oil p.o.). Data are means ± SEM of 7–9 animals. *$P < 0.05$; ***$P < 0.001$ vs control (one-way ANOVA followed by Newman–Keul's test).

Serotonin-induced licking behaviour and paw oedema

As shown in Table 2, treatment with bis selenide (10–50 mg/kg) significantly inhibited serotonin-induced licking behaviour. The maximal inhibition was 64 ± 4% and the calculated mean ID50 for this effect was 30.31 (25.68–35.79) mg/kg. Bis selenide (50 mg/kg) also produced a significant inhibition of paw oedema formation (35 ± 4%) (Table 2).

Histamine-induced licking behaviour and paw oedema

Table 2 shows that oral bis selenide (10–50 mg/kg) inhibited histamine-induced licking behaviour. The maximal inhibition was 62 ± 5%. The calculated mean ID50 for this effect was 27.26 (19.00–39.11) mg/kg. Furthermore, bis selenide (50 mg/kg) inhibited 22 ± 4% of paw oedema formation induced by histamine (Table 2).

Glutamate-induced licking behaviour and paw oedema

As shown in Table 3, oral bis selenide (5–50 mg/kg) significantly inhibited glutamate-induced nociception, with a mean ID50 of 16.58 (13.72–20.02) mg/kg and inhibition of 78 ± 3%. Furthermore, one-way ANOVA revealed that oral treatment with bis selenide (25–50 mg/kg) inhibited paw oedema induced by intraplantar injection of glutamate (Table 3). The maximum inhibition observed was 24 ± 2%.

PGE_2-induced licking behaviour and paw oedema

As shown in Table 3, bis selenide (5–50 mg/kg p.o.) significantly inhibited nociception induced by intraplantar injection of PGE_2. The maximal inhibition observed was 69 ± 3% and the mean ID50 was 24.83 (20.74–29.74) mg/kg. Bis selenide (10–50 mg/kg p.o.) caused significant inhibition of paw oedema induced by intraplantar injection of PGE_2 (31 ± 4% at 50 mg/kg; Table 3).

Table 2 Effects of bis selenide on the licking and oedema induced by intraplantar injection of serotonin or histamine in mice. Mice were pre-treated orally with bis selenide 30 min before intraplantar injection of serotonin (20 ng/paw) or histamine (100 nmol/paw)

Dose of bis selenide (mg/kg)	Serotonin		Histamine	
	Licking (s)	Oedema (mg)	Licking (s)	Oedema (mg)
Control	89.20 ± 4.53	44.60 ± 2.01	108.21 ± 8.75	76.50 ± 2.56
5	79.50 ± 5.57	45.40 ± 1.69	98.43 ± 5.25	74.83 ± 3.03
10	60.83 ± 3.79***	43.20 ± 2.88	67.01 ± 3.64***	68.50 ± 1.80
25	37.17 ± 3.22***	39.40 ± 2.73	35.67 ± 4.80***	59.60 ± 2.67**
50	29.17 ± 3.89***	29.17 ± 1.70***	41.50 ± 4.86***	61.83 ± 3.11**

Values are means ± SEM of 6–8 mice. $^{**}P < 0.01$, $^{***}P < 0.001$ vs control group (one-way ANOVA followed by Newman–Keul's test).

Table 3 Effect of bis selenide on the licking and oedema induced by prostaglandin E_2 (PGE_2) or glutamate in mice. Mice were pre-treated orally with bis selenide 30 min before intraplantar injection of PGE_2 (3 nmol/paw) or glutamate (10 μmol/paw)

Dose of bis selenide (mg/kg)	PGE_2		Glutamate	
	Licking (s)	Oedema (mg)	Licking (s)	Oedema (mg)
Control	95.83 ± 3.97	74.67 ± 2.07	86.00 ± 4.01*	67.22 ± 1.97
1	86.86 ± 2.83	73.86 ± 3.18	79.86 ± 4.76	71.43 ± 2.43
5	69.71 ± 2.79**	65.83 ± 2.72	59.00 ± 4.15**	68.10 ± 2.019
10	56.67 ± 3.63***	62.67 ± 1.90*	42.82 ± 3.03***	64.25 ± 1.79
25	35.71 ± 3.84***	55.00 ± 2.21***	26.40 ± 3.08***	57.27 ± 1.79**
50	29.17 ± 2.89***	51.50 ± 3.28***	17.00 ± 2.67***	51.90 ± 1.47***

Values are means ± SEM of 6–8 mice. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs control group (one-way ANOVA followed by Newman–Keul's test).

PMA-induced licking behaviour and paw oedema

As shown in Table 4, pre-treatment with bis selenide (10, 25 and 50 mg/kg) significantly inhibited PMA-induced nociception, with a maximum inhibition of 86 ± 3%. The mean ID50 was 25.49 (22.62–28.73) mg/kg. Bis selenide at a dose of 50 mg/kg significantly inhibited mouse paw oedema induced by intraplantar injection of PMA (18 ± 2%; Table 4).

8 BrcAMP-induced licking behaviour and paw oedema

Oral treatment with bis selenide (10–50 mg/kg) significantly inhibited 8 BrcAMP-induced licking response (Table 4). The maximal inhibition was 54 ± 4% and the mean ID50 was 36.86 (29.05–46.78) mg/kg. Treatment of animals with bis selenide did not modify the paw oedema formation induced by intraplantar injection of 8 BrcAMP (Table 4).

Involvement of the serotonergic system

Pre-treatment of animals with PCPA (100 mg/kg i.p. for 4 consecutive days) produced significant inhibition of the antinociception caused by bis selenide (25 mg/kg p.o.) in the formalin test in mice (Table 5).

The results in Table 5 show that the previous treatment of mice with WAY100635, a selective 5-HT_{1A} receptor antagonist, did not reverse the antinociception caused by bis selenide (25 mg/kg, p.o.) in the first (0–5 min) or second (15–30 min) phases of the formalin test. Moreover, pre-treatment of mice with ketanserin, a selective 5-HT_{2A} receptor antagonist, or ondansetron, a selective 5-HT_3 receptor antagonist, significantly reversed the antinociceptive action induced by bis selenide (25 mg/kg p.o.) in both phases of the formalin test (Table 5).

Table 4 Effect of bis selenide on licking and oedema induced by 12-myristate-13-acetate (PMA; a PKC activator) and 8-bromoadenosine 3′,5′-cyclic monophosphate (8-BrcAMP; a PKA activator) in mice. Mice were pre-treated orally with bis selenide 30 min before intraplantar injection of PMA (0.03 μg/paw) or 8 BrcAMP (10 nmol/paw)

Dose of bis selenide (mg/kg)	PMA		8 BrcAMP	
	Licking (s)	Oedema (mg)	Licking (s)	Oedema (mg)
Control	150.41 ± 12.33	47.40 ± 1.80	90.02 ± 6.96	30.57 ± 0.99
5	161.34 ± 3.70	44.75 ± 1.93	88.71 ± 5.16	28.71 ± 1.12
10	104.37 ± 6.05***	42.71 ± 1.47	63.13 ± 3.82 ***	30.75 ± 1.33
25	53.80 ± 4.06***	42.00 ± 1.30	41.83 ± 3.34 ***	32.33 ± 1.92
50	22.67 ± 3.88***	39.33 ± 1.11**	41.57 ± 3.26 ***	31.89 ± 1.09

Values are means ± SEM of 5–7 mice. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs control group (one-way ANOVA followed by Newman–Keul's test).

Table 5 Effect of pre-treatment of animals with p-chlorophenylalanine methyl ester (PCPA; 100 mg/kg i.p. for 4 consecutive days), WAY100635 (0.1 mg/kg i.p.), ketanserin (0.3 mg/kg i.p.) or ondansetron (0.5 mg/kg, i.p.) 15 min before administration of bis selenide on formalin-induced licking in mice. The total time spent licking the hindpaw was measured in the first (0–5 min) and second phases (15–30 min) of the formalin test

Saline	Antagonist	Canola oil	Bis selenide	First phase (s)	Second phase (s)
+	–	+	–	65.44 ± 3.81	152.21 ± 15.66
+	–	–	+	26.38 ± 2.43***	36.38 ± 5.41***
–	PCPA	+	–	65.22 ± 3.96	129.12 ± 15.55
–	PCPA	–	+	52.44 ± 3.66†††	82.44 ± 4.98†††
–	WAY100635	+	–	62.22 ± 4.33	149.03 ± 10.02
–	WAY100635	–	+	23.88 ± 3.03***	35.78 ± 5.12***
–	Ketanserin	+	–	60.67 ± 4.19	134.05 ± 6.45
–	Ketanserin	–	+	40.88 ± 3.66††	96.56 ± 7.42††
–	Ondansetron	+	–	65.13 ± 3.76	161.12 ± 6.98
–	Ondansetron	–	+	61.38 ± 4.62†††	146.62 ± 11.20†††

Values are means ± SEM (5–7 mice). ***$P < 0.001$ vs control group; $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs bis selenide (one-way ANOVA followed by Newman–Keul's test).

Involvement of the histaminergic system

Table 6 shows that pretreatment with pyrilamine, an H_1 receptor antagonist, and thioperamide, an H_3 receptor antagonist, did not change the antinociceptive effect caused by bis selenide (25 mg/kg p.o.) on the formalin test. However, pretreatment with ranitidine, an H_2 receptor antagonist, given 15 min beforehand, prevented the antinociception caused by this dose of bis selenide in both phases of the formalin test (Table 6).

Rotarod test

Oral administration of bis selenide (1–50 mg/kg) did not alter the latency for the first fall and the number of falls in the rotarod test (data not shown).

Discussion

The results of the present study demonstrate that bis selenide administered orally elicits a significant antinociceptive action in mice, as assessed in the formalin test. It produced graded inhibition against neurogenic (65 ± 3%) and inflammatory (90 ± 1%) pain responses caused by formalin injection. The neurogenic pain (first phase, 0–5 min) is caused by direct activation of nociceptive nerve terminals, while the inflammatory pain (second phase, 15–30 min) is mediated by a combination of peripheral input and spinal cord sensitisation.[3] In addition, bis selenide (50 mg/kg p.o.) significantly inhibited mouse paw oedema (31 ± 2%) induced by intraplantar injection of formalin. Thus, these results suggest that bis selenide is more effective against the inflammatory pain of the formalin test and that its antinociceptive effect is associated with its anti-inflammatory action.

The results of the current study also demonstrate that bis selenide (25 mg/kg p.o.) produced both prophylactic and therapeutic effects in the formalin test (i.e. pre-emptive antinociception when pre-administered and effective when post-administered). These findings may have implications for the development of new drugs to treat inflammatory pain.

Putative inflammatory mediators such as histamine, serotonin, glutamate and prostaglandins induce nociceptive behaviour when injected into the paw.[27,28] The current study demonstrates that, when given orally, bis selenide inhibits the nociceptive response and paw oedema induced by serotonin. Application of serotonin to peripheral tissues produces pain in humans[29] and nociceptive behaviours and hyperalgesia in rodents.[30] Furthermore, serotonin participates in the nociception induced by formalin or carrageenan,[31] and induces excitation and sensitisation of primary afferent fibres.[32] We report here that depletion of endogenous serotonin with the

Table 6 Effect of pre-treatment with pyrilamine (3 mg/kg i.p.), ranitidine (3 mg/kg i.p.) or thioperamide (5 mg/kg i.p.) 15 min before injection of bis selenide on formalin-induced licking in mice. The total time spent licking the hindpaw was measured in the first (0–5 min) and second phases (15–30 min) of the formalin test

Saline	Antagonists	Canola oil	Bis selenide	First phase (s)	Second phase (s)
+	+	+	–	66.03 ± 4.71	142.71 ± 12.08
+	+	–	+	24.02 ± 5.56***	34.56 ± 2.80***
–	Pyrilamine	+	–	62.22 ± 7.07	159.37 ± 10.37
–	Pyrilamine	–	+	23.01 ± 5.70***	31.33 ± 3.62***
–	Ranitidine	+	–	66.68 ± 4.07	141.57 ± 8.70
–	Ranitidine	–	+	57.75 ± 5.08†††	106.71 ± 7.42†††
–	Thioperamide	+	–	62.15 ± 6.06	154.07 ± 11.17
–	Thioperamide	–	+	24.38 ± 5.30***	27.05 ± 4.57***

Values are means ± SEM (5–7 mice). ***$P < 0.001$ vs control group; $^{\dagger\dagger\dagger}P < 0.001$ vs bis selenide (one-way ANOVA followed by Newman–Keul's test).

tryptophan hydroxylase inhibitor PCPA, at a dose known to decrease the cortical content of serotonin and to significantly reverse morphine antinociception, largely antagonises the antinociceptive action of bis selenide in the formalin model of pain. Moreover, the selective 5-HT_{2A} and 5-HT_3 receptor antagonists, namely ketanserin and ondansetron, respectively, consistently reversed the antinociception caused by systemic administration of bis selenide. Importantly, peripheral injection of serotonin evokes acute pain that is attenuated by relatively selective 5-HT_2 and 5-HT_3 antagonists.[33] In marked contrast, 5-HT_{1A} receptors sensitive to WAY100635 appear not to account to any large extent for the antinociceptive action of bis selenide.

The current study also demonstrated that oral bis selenide inhibited nociceptive responses and paw oedema induced by histamine. Histamine is one of the local inflammatory mediators known to be involved in both peripheral and central mechanisms.[27] Pre-treatment with the H_2-receptor antagonist ranitidine prevented antinociception induced by bis selenide in the formalin test. The involvement of histamine H_2 receptors in antinociception has been reported in thermal, mechanical and chemical nociceptive tests.[34] H_2 receptors are linked, via the α-subunits of the Gs family, to the stimulation of adenylyl cyclase and thus to the activation of cAMP–PKA in target cells.[35] Here we present pharmacological evidence to corroborate these results by demonstrating that bis selenide inhibits nociception induced by intraplantar injection of the PKA activator 8 BrcAMP. Conversely, pre-treatment with the H_1-receptor antagonist pyrilamine and the H_3-receptor antagonist thioperamide did not change antinociception induced by bis selenide.

Orally administered bis selenide also produced significant inhibition of licking and paw oedema caused by intraplantar injection of glutamate into the mouse hindpaw. We recently reported that the glutamatergic system, more specifically the interaction with kainate and trans-1-aminocyclopentane-trans-1,3-dicarboxylic acid (ACPD) receptors, cytokines and kinins, plays a critical role in the antinociceptive effect caused by bis-selenide in mice.[36] The nociceptive response induced by intraplantar glutamate in the mouse paw is primarily mediated by release of neuropeptides from sensory fibres, namely neurokinins and kinins.[23]

In this study we also demonstrated that orally administered bis selenide inhibited PGE_2-induced licking behaviour and paw oedema in mice. In fact, during an inflammatory event, pain generation is a consequence of complex interactions between a number of inflammatory mediators, including prostaglandins, some of which (notably PGE_2) are known to have a critical role in the generation and maintenance of the nociceptive response (see Samad and colleagues[37] for review). Some studies have demonstrated that the mechanical hyperalgesia caused by peripheral PGE_2 injection in rats is mediated by cAMP–PKA pathways.[38] In contrast, thermal hyperalgesia produced by peripheral injection of PGE_2 is only marginally reduced in mice with a targeted mutation of the type-I regulatory subunit of PKA, suggesting that other intracellular pathways could also be involved in PGE_2-induced nociceptive effects.[39] In fact, the activation of prostanoid EP receptors by PGE_2 can stimulate other protein kinases, including PKC and MAPKs, both of which exert a critical role in nociceptor excitation and sensitisation.[24]

Of particular interest, our data show that bis selenide blocks the nociceptive response caused by intraplantar injection of the PKC activator PMA. Several lines of evidence suggest that PKC phosphorylates cellular components involved in a number of inflammatory and persistent pain models,[40] including enzymes, ion channels and membrane-bound receptors (for review see Ji & Woolf[4]). There is also growing evidence for PKC-mediated release of the pro-inflammatory cytokines interleukin-1 and -6 and TNFα, and prostaglandins, suggesting that the role of peripheral PKC in bis selenide-induced antinociception might involve the inhibition or modulation of inflammatory cytokines and/or prostaglandins.

The results of this study indicate that bis selenide inhibits the nociceptive response caused by the intraplantar injection of the PKA activator 8 BrcAMP in mice. Pharmacological as well as genetic inhibition of PKA results in a reduction of inflammatory mediator-induced hyperalgesic behaviour,[39] reduced nociceptor discharge[41] and attenuated stimulus-induced peptide release.[42] While the exact mechanisms of these PKA-mediated effects are not fully understood, mutation of PKA phosphorylation sites on effector ion channels, such as the primary afferent nociceptor-specific ion channel and the ligand-gated ion channel ($TRPV_1$),[43] results in ablation of channel modulation by PKA. In addition, we have convincing evidence demonstrating that the antinociceptive action of bis selenide involves the activation of ATP-sensitive voltage-gated potassium ion channels and the inhibition of vanilloid receptors.[13,36]

Conclusions

Bis selenide inhibited the licking behaviour and paw oedema induced by serotonin, histamine, glutamate and PGE_2 in mice. The involvement of peripheral PKA and PKC in the antinociceptive effect induced by oral administration of bis selenide in mice was demonstrated, as was the involvement of serotonergic 5-HT_{2A} and 5-HT_3 receptors and histamine H_2 receptors in the antinociception induced by bis selenide. Together, these results using different pharmacological models of pain and receptor antagonists indicate that bis selenide has potential for the development of new clinically relevant drugs for the management of inflammatory conditions in humans.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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