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(−)-α-Bisabolol reduces nociception and trigeminal central sensitisation in acute orofacial neuropathic pain induced by infraorbital nerve injury

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ABSTRACT

Neuropathic orofacial pain conditions represent a challenge to diagnose and treat. Natural substances are promising therapeutic options for the control of pain.

Aims: This study aimed to examine whether (−)-α-bisabolol (BISA), a natural terpene, can attenuate nociceptive behaviour and central sensitisation in a rodent model of trigeminal neuropathic pain.

Materials and methods: Infraorbital nerve transection (IONX) or sham operation was performed in adult male rats. Head withdrawal thresholds as a measure of facial mechanical sensitivity were tested with von Frey monofilaments applied bilaterally to the facial vibrissal pad pre-operatively (baseline) and then post-operatively before and at 60, 120, 240 and 360 min after administration of vehicle control *per oris* (*p.o.*) or BISA (200 mg/kg *p.o.*) (n = 8/group). Effects of BISA or vehicle on the activity of nociceptive neurons recorded in the medullary dorsal horn (MDH) were tested on post - operative day 8–10. ANOVA followed by *post-hoc* Bonferroni tested for statistically significant differences ($p < 0.05$) across study groups and time points.

Key findings: IONX animals (but not sham or naïve animals) showed post-operative facial mechanical hypersensitivity that was unaffected by vehicle. However, administration of BISA at post-operative day 7 significantly reversed the mechanical hypersensitivity in IONX rats; this effect lasted for at least 6 h. BISA also attenuated IONX-induced central sensitisation of MDH nociceptive neurons, as reflected in reversal of their reduced activation thresholds, increased responses to graded mechanical stimuli and enhanced spontaneous activity.

Significance: BISA may attenuate nociceptive behaviour and central sensitisation in a rat model of acute trigeminal neuropathic pain.

1. Introduction

Neuropathic orofacial pain conditions represent a challenge to diagnose and treat, since the orofacial region is complex, pain can arise from many sources, and there is limited understanding of the aetiology and pathogenesis of these pain disorders [1,2].

Central sensitisation is considered to be a crucial process underlying the development and maintenance of chronic pain states. It reflects an increased excitability of nociceptive neurons in the central nervous system produced by injury on inflammation of somatosensory nerves and other tissues and indeed has been documented in animal models of chronic inflammatory, or neuropathic orofacial pain (for review see [2–7]). Our previous studies have shown that central sensitisation of functionally identified nociceptive neurons in the subnucleus caudalis (which is also known as the medullary dorsal horn, MDH) of the trigeminal brainstem sensory nuclear complex occurs in association with nociceptive behaviour in acute and chronic orofacial inflammatory pain models [8–12] as well as in chronic orofacial neuropathic pain models [13–15] that include transection of the infraorbital nerve (IONX).

(−)-α-Bisabolol (BISA) is a sesquiterpene isolated from the essential oil of a variety of plants and it is a well-known ingredient used in cosmetic preparations, fine fragrances, shampoos, toilet soaps and other toiletries [16,17]. Due to its low toxicity, the Food and Drug Administration has classified BISA as 'generally regarded as safe', boosting its use as an active ingredient in commercial products [17]. Pharmacological studies have shown that BISA reduces vascular and muscle contractions through modulation of Ca^{+2} channels [18,19] and peripheral neural blockade [20], and that BISA attenuates visceral and corneal

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nociception and inflammation [21–23] in rodents [24,25]. Recently, Melo et al. [26] reported that antinociception can also be induced by BISA in rodents receiving an algesic chemical application to orofacial tissues including the temporomandibular joint.

Since the effect of BISA in orofacial neuropathic pain models has not been previously investigated, this study aimed to examine whether BISA can attenuate nociceptive behaviour and central sensitisation in a rodent model of trigeminal neuropathic pain.

2. Methods

All experiments conformed to the regulations of the Canadian Council on Animal Care and the Ontario Animals for Research Act and were approved by the University of Toronto Animal Care Committee (Protocol #20010452).

2.1. Animals and study design

Rats were housed in pairs in a 12:12 hour light–dark cycle. A hard and softened chow diet and water were available *ad libitum* pre-operatively, and soft diet post - operatively. The inclusion of only a soft diet post - operatively ensured adequate food intake after the IONX or sham surgery. Rats were monitored post - operatively to ensure that they were not experiencing discomfort leading to a disruption of feeding and loss of body weight.

A total of 24 adult male Sprague–Dawley rats (250–370 g, Charles River) were used and randomly allocated into 3 groups (n = 8/group). Rats of the IONX group underwent transection of the infraorbital nerve (ION) under general anaesthesia; rats of the sham group received the same general anaesthesia and surgical procedure as rats of the IONX group but without actual nerve transection. Naïve rats received no general anaesthesia and no surgical treatment.

In the behavioural experiments, head withdrawal thresholds following punctate stimulation of the facial vibrissal pads were measured to quantify facial mechanical sensitivity. We have previously documented that the facial hypersensitivity reflecting nociceptive behaviour in IONX rats peaks around post - operative day 5–7 [13]. Thus, to test the drugs' effects on peak facial mechanical sensitivity, punctate withdrawal thresholds were measured on pre-operative days 1, 2, and 3 to obtain a mean baseline value, and then at post - operative days 3, 5 and 7. On post - operative day 5, punctate withdrawal thresholds were measured before and at 2, 4 and 6 h after the administration of vehicle control, and on post - operative day 7, punctate withdrawal thresholds were measured before and at 2, 4 and 6 h after the administration of BISA. All groups of animals in the behavioural experiments received a single administration of vehicle (isotonic saline and 10% Cremophor A25) at post - operative day 5, and BISA at post - operative day 7.

Rats of the naïve and IONX groups used in the behavioural experiments were also used for the neuronal recording experiments to test the effects of BISA or vehicle on the activity of wide dynamic range (WDR) nociceptive neurons in the MDH, as detailed below (Section 2.5). The activity of 8 MDH WDR neurons (one neuron/animal) was recorded in 8 rats of the IONX group, before and after intravenously (*i.v.*) infusion of BISA (*i.e.*, IONX/BISA group, n = 8), and the activity of an additional 8 MDH WDR neurons (one/animal) was recorded in 8 rats of the naïve group before and after *i.v.* infusion of vehicle solution (*i.e.*, naïve/vehicle group, n = 8). Because of the well-documented difficulty of maintaining a neuron's recording > 60 min [11,27], BISA or vehicle was administered only to IONX or naïve groups, respectively. Also, only one WDR neuron per animal was tested for any effects of BISA or vehicle because of the possibility of residual effects that might affect baseline properties of subsequently recorded neurons if these neurons were tested in the same animals. These studies were carried out on post - operative day 8, 9 or 10 which are close to the peak time of post - operative facial mechanical hypersensitivity [13]. Sham - operated animals were not used in the neuronal recording experiments due to limited animal availability and since the behavioural experiments revealed no statistically significant differences between naïve and sham - operated rats after the 5th post - operative day.

2.2. Drug treatment

(−)-α-Bisabolol (BISA, Sigma - Aldrich, USA) was dissolved in sterilized isotonic saline and Cremophor (10%, A25 - Sigma - Aldrich) and administered by oral gavage (200 mg/kg *per oris* - *p.o.*) to naïve, sham and IONX rats studied in the behavioural testing experiments. This dose of BISA was guided by our previous studies showing that doses between 50 and 200 mg/kg were effective in attenuating acute orofacial nociceptive behaviour [26]. In the neuronal recording experiments, BISA was dissolved in sterilized isotonic saline and Cremophor. BISA (30 mg/kg) or vehicle (isotonic saline and 10% Cremophor A25) was administered intravenously (*i.v.*). This dose of BISA was based on findings from a preliminary study in which we carried out dose - response experiments of BISA given at *i.v.* doses of 10, 20 or 30 mg/kg in 18 rats; we found 30 mg/kg was most efficacious in attenuating the nociceptive jaw - opening reflex evoked in the anterior digastric muscle by tooth pulp stimulation, and so this was the *i.v.* dose chosen for the neuronal recording experiment.

2.3. Behavioural testing

As previously described [13], a testing chamber was used to acclimatize and test the facial mechanical sensitivity while the rats remained in the testing chamber and placed their snout through a hole in the chamber. Head withdrawal thresholds as a measure of facial mechanical sensitivity were tested, as previously described [3,5,14,15], by applying graded forces (0.16–10 g) with von Frey monofilaments (North Coast Medical, Inc., Gilroy, CA) to the ipsilateral and contralateral facial vibrissal pads. Head withdrawal responses to the mechanical stimulation were demonstrated as a sudden backward escape movement of the head. The head withdrawal threshold was defined as the lowest filament force that evoked three or more escapes responses out of five stimulation trials with inter-trial intervals of 10 s. The filament exerting 10 g forces was used as the cut - off value because higher forces could move the rat head before the von Frey filament bent [13,14].

2.4. Infraorbital nerve transection and sham operation

Standardised aseptic surgical procedures were carried out under isoflurane anaesthesia (5% induction, 2–2.5% maintenance). As previously described [13,14], an intra - oral incision (2 mm) was made in the oral mucosa of the left front - lateral maxillary vestibule, the left ION was exposed at its entry into the infraorbital foramen and cut with scissors to produce a complete ION transection (IONX). Care was taken not to damage facial nerve branches. After the surgery, wounds were allowed to heal spontaneously with no suturing. Sham operation involved the same general anaesthesia and surgical procedures as in the IONX experimental rats but without actual transection of the ION.

2.5. Neuron recording and facial stimulation procedures

The methods used for animal preparation, facial stimulation, neuronal recording, and classification of neurons were similar to those described previously in detail [11,13,27,28] and so they will only be briefly outlined here.

Experiments were carried out on post - operative day 8–10. Each rat was anaesthetized with intraperitoneal urethane (1 g/kg) and α - chloralose (50 mg/kg), and the trachea and left external jugular vein were cannulated. Then, the rat was placed in a stereotaxic apparatus, and the dura and subarachnoid membrane were removed. A supplemental dose of urethane (200–300 mg/kg *i.v.*) was delivered by an

infusion pump just prior to the neuronal recording session. Throughout the experimental period, rats were artificially ventilated, and their level of anaesthesia was maintained by an *i.v.* infusion of 70% urethane (0.2 g/mL) at a rate of 0.2 to 0.3 mL/h [13,29]. A deep level of anaesthesia was judged periodically by the lack of spontaneous movements and responses to a noxious paw pinching. Heart rate (330–430 beats/minute), percentage expired CO_2 (3.5–4.5%), and rectal temperature (37.0–37.5 °C) were constantly monitored and maintained at physiological levels [13,29].

The activity of single neurons was extracellularly recorded from the deep laminae of the MDH with a glass - insulated tungsten microelectrode (1–5 MΩ, 10–20 μM exposed tip, 125 μm shank diameter and 300 μm outer diameter, Alpha - Omega Engineering, Nazareth, Israel). Signals were amplified and displayed on oscilloscopes and digitized *via* an analogue – to - digital converter. The data were collected and analysed with Spike 2 software (Cambridge Electronic Design, Cambridge, UK) [11,15,30]. A wide range of mechanical (brush, pressure, pinch) and noxious thermal (radiant heat, 51 °C to 53 °C) stimuli were applied to classify MDH neurons as WDR. The cutaneous tactile mechanoreceptive field (RF) of the MDH WDR neurons was determined by applying low - threshold mechanical stimuli produced by brushing the skin with a camel - hair brush (force < 2 g). The cutaneous pinch component of the RF of the WDR neurons was established by using electronic graded non - serrated force - monitoring forceps. Consistent with previous studies [11–13,27,28,30], the RF was found to occupy a relatively large orofacial skin area (> 10 mm^2) with clear boundaries, and pinch stimulation of the RF produced long - lasting neuronal discharges.

The average spontaneous activity (spikes/min) of the WDR neuron was determined over the initial 5 - minute recording period before drug or vehicle administration, as outlined in our previous studies [11–13,30]. The responses were quantified as number of spikes per minute during the 5 - minute recording.

The neuronal responses to a series of graded stimuli applied to the craniofacial RF of each WDR neuron were established by using the electronic graded non - serrated force - monitoring forceps and gradually increasing the applied force (40, 60, 80 g); each force stimulus was applied for 3 s at an interval of > 60 s. The responses were quantified as the sum of spikes during the 3 s of stimulation. Only WDR neurons were tested for the effects of BISA *versus* vehicle control on MDH neuronal properties in the IONX and naïve rats. Further, our previous studies have shown that vehicle administration does not affect MDH neuronal properties in rat models of trigeminal neuropathic pain, and so because of this and limited animal availability, its effect was not tested in IONX rats.

Graded mechanical stimuli in an ascending order were also applied to the centre of the RF by means of Von Frey filaments at non - noxious force levels (0.4, 1, and 2 g) and at noxious levels (6, 15, 26, 60, and 100 g), each for a 2 - second duration and intervals of > 45 s. These graded mechanical stimuli were used to determine the mechanical activation thresholds evoked by the graded mechanical stimuli [11,13,30]. The responses were quantified as the sum of spikes during the 2 s of stimulation.

After the 5 - minute recording of baseline values for each of the WDR neurons tested, any change in the spontaneous activity threshold to a mechanical pinch stimulus, and mechanical activation threshold were assessed following *i.v.* injection of BISA in the IONX rats and vehicle in the naïve rats. Starting at 3 min after BISA application, the neuronal properties were re - assessed at 10 - minute intervals throughout a 50 - minute observation period [13,14]. Neuronal responses were recorded and stored in a microcomputer and the neuronal responses were analysed offline using the Spike2 software (CED 1401 Plus; Cambridge Electronic Design).

2.6. Data and statistical analysis

Our published studies indicate that orofacial injury produces a large effect size ($f = 0.25$) across study groups in primary outcomes. According to G*Power calculation, the minimum sample size required to obtain statistically significant results for ANOVA test with 80% power and $\alpha = 0.05$ is 8 rats per group. Data were tested for normality (Kolmogorov–Smirnov test) and equal variance. Data are reported as mean ± standard error of mean (SEM). For analyses, the baseline was normalised and all values, post - BISA or post - vehicle administration (*p.o.* or *i.v.*) were expressed as percentage (%) change from baseline.

For the behavioural tests, for each study group (Naïve, Sham, IONX), differences in head withdrawal thresholds between baseline values (average of the 3 preoperative day values) and each of the post - operative days were tested by one - way analysis of variance (ANOVA) followed by the Bonferroni *post - hoc* test. The effects over time of BISA *versus* vehicle (control) on head withdrawal thresholds in the IONX/BISA and naïve/vehicle groups were tested with two - way repeated measures ANOVA followed by the Bonferroni *post - hoc* test.

For the neuronal recordings, differences in spontaneous neuronal activity, responses to the series of graded mechanical stimuli, and mechanical activation threshold between the baseline values (*i.e.*, before BISA or vehicle administration) of the IONX and naïve groups were treated by one - way ANOVA followed by *post - hoc* Bonferroni. The effects of BISA over time *versus* vehicle on the neuronal spontaneous activity, responses to a mechanical pinch stimulus, and mechanical activation threshold in the IONX/BISA and naïve/vehicle groups were tested with two - way repeated measures ANOVA followed by the Bonferroni *post - hoc* test. Calculations were carried out by means of SPSS 19.0 (SPSS Inc., Chicago, IL). $p < 0.05$ was considered to reflect statistical significance.

3. Results

3.1. Effects of BISA on mechanical hypersensitivity in IONX rats

The mechanical head - withdrawal thresholds to punctate stimulation of the ipsilateral and contralateral facial vibrissal pads were used to measure facial mechanical sensitivity before and after IONX and test the effects of BISA *versus* vehicle control on these measures. Compared to baseline values, IONX rats showed decreases in mechanical head - withdrawal thresholds for both the ipsilateral and contralateral sides.

The decreased thresholds in the IONX rats were significant at post - operative day 3 (ipsilateral - 1-way ANOVA $p = 0.002$, Bonferroni $p < 0.001$; contralateral 1-way ANOVA, $p < 0.05$, Bonferroni $p = 0.02$) compared to baseline, and the thresholds were still significantly decreased at post - operative day 7 (Fig. 1A, B).

Compared to naïve or sham rats, IONX rats showed significantly decreased head - withdrawal thresholds at post - operative days 3 to 7 (ipsilateral: 2 - way ANOVA, $p = 0.001$, day 3 Bonferroni $p = 0.001$; day 5, Bonferroni $p = 0.001$ and day 7 Bonferroni $p = 0.001$; contralateral: 2-way ANOVA, $p = 0.003$, day 3 Bonferroni $p = 0.04$; day 5 Bonferroni $p = 0.05$; day 7, Bonferroni $p = 0.05$).

The thresholds of sham - operated rats decreased at post - operative day 3 and 5 only for the ipsilateral side (2 - way ANOVA, $p = 0.003$; day 3 Bonferroni $p = 0.001$; day 5 Bonferroni $p = 0.006$) but not for the contralateral side (2 - way ANOVA, $p = 0.31$; day 3 Bonferroni $p = 0.7$; day 5 Bonferroni $p = 1.0$). There were no significant changes in thresholds in the naïve group (Ipsilateral: 2 - way ANOVA, $p = 0.228$; day 3 Bonferroni $p > 0.05$. contralateral: 2-way ANOVA, $p = 0.375$; day 3 Bonferroni $p > 0.05$).

The oral administration of vehicle on post - operative day 5 had no significant effect on the withdrawal thresholds in the IONX, naïve or sham groups (Fig. 1A, B). In contrast, at post - operative day 7, the oral administration of BISA in the IONX group was associated with significant reversal of the decreased thresholds for the ipsilateral and

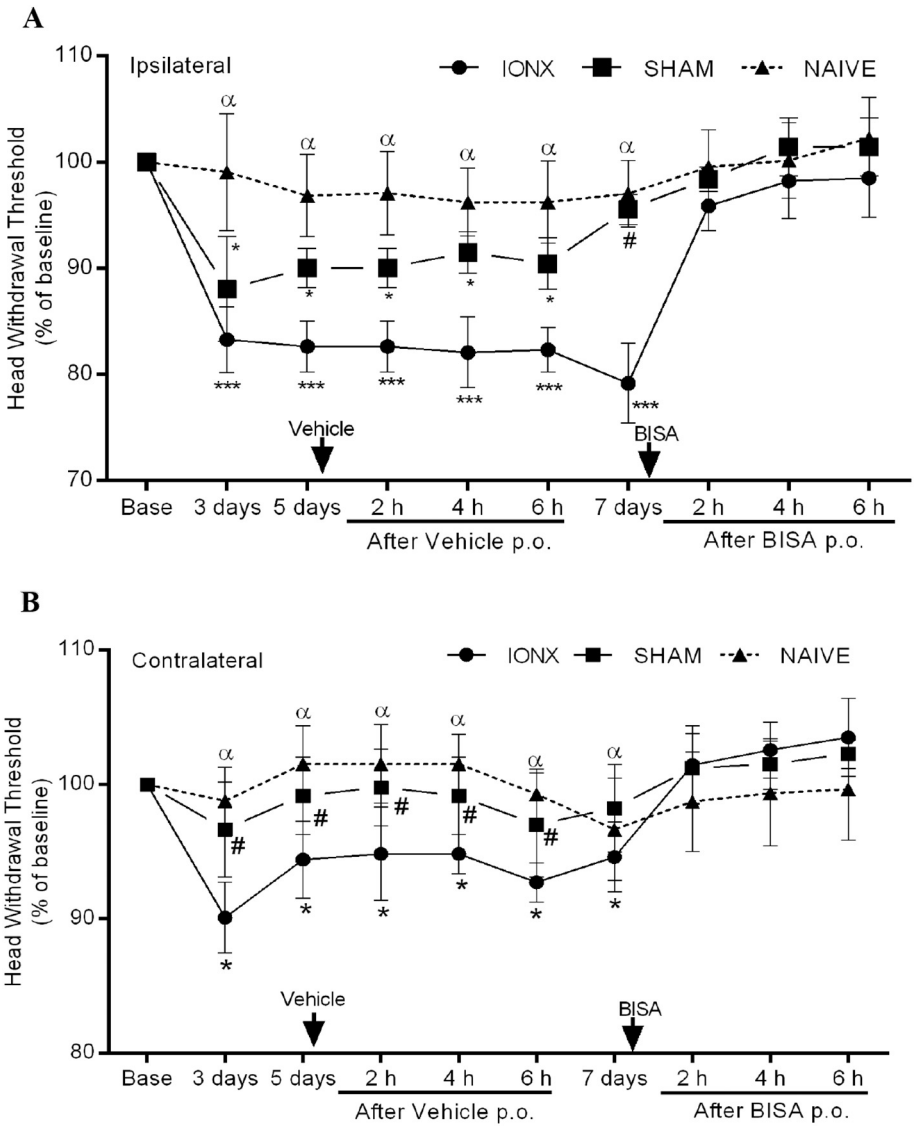

Fig. 1. A, B. Head withdrawal thresholds (% change of baseline, mean ± SEM – 8 animals/group) in response to graded punctate (von Frey) stimulation of the ipsilateral (A) and contralateral (B) facial vibrissal pads as a measure of facial mechanical sensitivity. Thresholds were measured before (baseline) and after infraorbital nerve transection (IONX) or sham operation (Sham) and then before and after *p.o.* administration of vehicle or BISA (arrows). Naïve rats were also used. ANOVA, *post - hoc* Bonferroni: *$p < 0.05$ and ***$p < 0.001$ for comparisons between baseline and different post - IONX time points; ANOVA, *post - hoc* Bonferroni: α $p < 0.05$ for comparisons between IONX and naïve groups at different post - IONX time points; # $p < 0.05$ for comparisons between IONX and sham groups at the different time points; and *$p < 0.05$ for comparisons between baseline at different post-Sham time points. Differences between the normalised baseline values (*i.e.*, before BISA or vehicle administration) of each group were expressed as percent change (%) from baseline (control, 100%). BISA: (−)-α-bisabolol; vehicle: isotonic saline and 10% Cremophor A25.

contralateral sides. For the ipsilateral side, the BISA effect was evident by 2 h after administration and lasted for at least 6 h ($p = 0.1$). For the contralateral side, the BISA effect was evident at 2 h after administration and lasted for an additional 4 h ($p = 0.04$). BISA had no significant effects on the withdrawal thresholds for naïve and sham groups for either the ipsilateral side (sham: 2-way ANOVA, $p = 0.38$, and naïve: 2-way ANOVA $p = 0.41$) or contralateral side (sham: Two-way ANOVA $p = 0.45$ and naïve: 2 - way ANOVA, $p = 0.31$).

3.2. Effects of BISA on central sensitisation of MDH nociceptive neurons induced by IONX

Sixteen functionally identified WDR neurons (n = 8/group) responding to ipsilateral craniofacial tactile and noxious stimulations were studied at post - operative day 8–10.

3.2.1. Neuronal properties

In comparison with the WDR neuronal properties of naïve rats, the WDR neurons at 8–10 days following IONX showed evidence of central sensitisation as reflected by increased spontaneous activity (Fig. 2), larger evoked responses to graded mechanical stimuli (Fig. 3), and in a decreased mechanical activation threshold (Fig. 4).

At baseline, prior to BISA (or vehicle) administration, the spontaneous activity of WDR neurons in the IONX rats was significantly higher ($p = 0.05$) than that in the naïve rats (Fig. 2). In the naïve group, administration of vehicle had no significant effect on the spontaneous activity of the WDR neurons ($p = 0.7$). However, the elevated spontaneous neuronal activity of the IONX group shifted downward significantly ($p = 0.001$) after 3 min of BISA administration, and this effect lasted for the remainder of the 53 - minute observation period.

At baseline, the WDR neuronal responses evoked by the graded mechanical stimuli were also significantly ($p < 0.001$) elevated in the IONX group compared to those of the naïve group (Fig. 3). Within 3 min, BISA significantly ($p = 0.01$) reduced the elevated evoked responses in the IONX group and this effect lasted for the rest of the observation period. In the naïve group, administration of vehicle had no significant effect ($p = 0.72$) on the WDR neuronal responses which remained stable throughout the observation period.

At baseline, the mechanical activation threshold of the WDR neurons in the IONX group was significantly decreased ($p < 0.001$) compared to that in the naïve group (Fig. 4). Within 13 min of BISA administration, there was a significant ($p = 0.001$) reversal of the decreased thresholds in the IONX group and this effect lasted for the rest of the observation period (Fig. 4); the small but significant "overshoot" in neuronal mechanical activation threshold was noted at the longer time points after BISA administration In the naïve group, administration of vehicle had no significant effect on the mechanical activation threshold ($p = 0.5$) (Fig. 4).

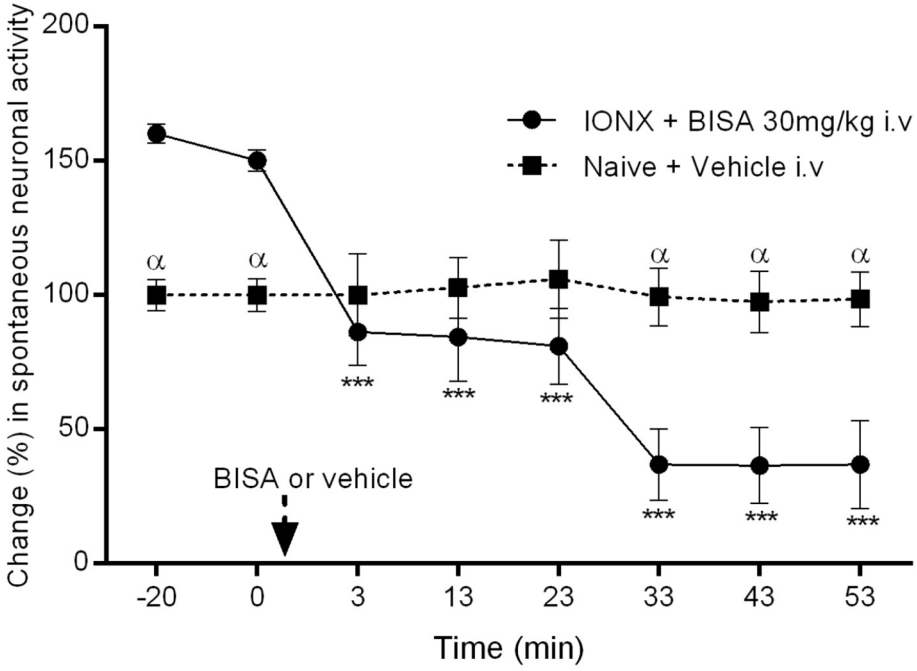

Fig. 2. Change (%) in spontaneous neuronal activity in MDH neurons of IONX/BISA group and naïve/vehicle group (mean ± SEM – 8 animals/group). ***p < 0.001 for comparisons between baseline and different time - points in the IONX group. *α* for comparisons between IONX and naïve groups at different time points (baseline to 53 - minute observation period). Differences between the normalised baseline values (*i.e.*, before BISA or vehicle administration) of each group were expressed as percent change (%) from baseline (control, 100%). Arrow indicates the time of vehicle or BISA administration (*i.v.*). IONX, infraorbital nerve transection; (−)-α-bisabolol (BISA); vehicle (isotonic saline and 10% Cremophor A25).

4. Discussion

This is the first study to document that (−)-α-bisabolol (BISA) is effective in attenuating sustained facial mechanical hypersensitivity and central sensitisation in an orofacial neuropathic pain model that involves transection of the infraorbital nerve (ION). To the authors' knowledge, the present study is also the first to test BISA effects in any neuropathic pain model and to show its effectiveness. The findings of the effectiveness of BISA in this orofacial neuropathic pain model are consistent with recent reports that BISA reduces nociception in other pain models, including orofacial pain models [18–26], as noted below.

Several models of trigeminal neuropathic pain have been established to investigate the neural mechanisms involved in orofacial neuropathic pain [2–4,13,31]. For example, previous studies in orofacial neuropathic pain models have shown that trigeminal nerve injury including IONX induces bilateral facial mechanical hypersensitivity indicative of mechanical allodynia that is accompanied by neuroplastic changes reflecting central sensitisation in functionally identified nociceptive neurons in the MDH [2,4,13,15,28,32]. The present study confirmed that IONX produces bilateral mechanical hypersensitivity by post - operative day 3; complete transection of ION produced a similar degree of mechanical hypersensitivity to that seen in our previous studies using partial transection of ION [13,14,28]. We also found that nociceptive MDH neurons in IONX animals manifesting mechanical allodynia exhibited central sensitisation as reflected in significantly increased spontaneous activity and responses evoked by graded mechanical stimuli and a decreased activation threshold, consistent with the previous above - mentioned studies of the effects of trigeminal nerve injury on MDH neuronal properties.

The present study has documented that around the peak time of

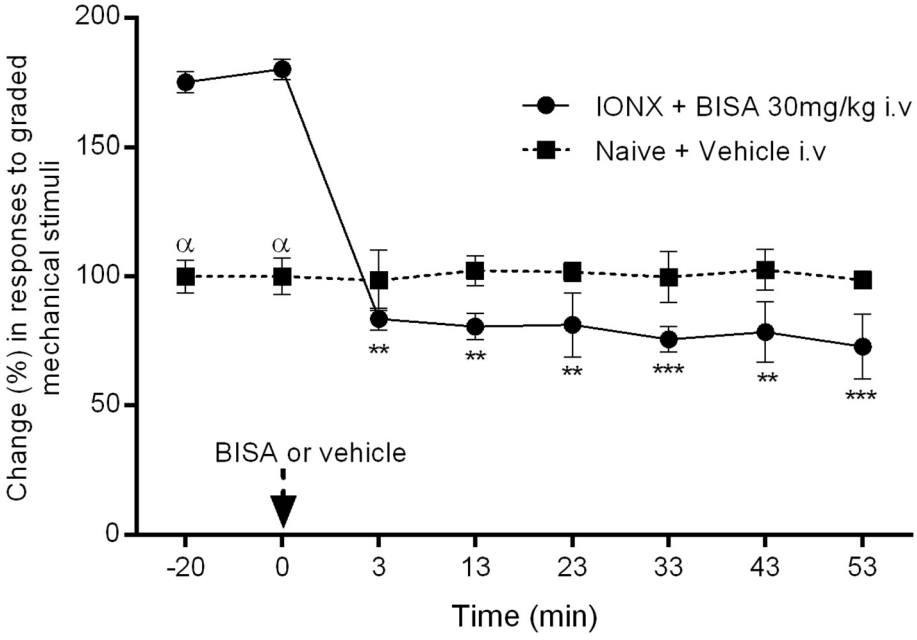

Fig. 3. Change (%) in responses to graded mechanical stimuli in MDH neurons of IONX/BISA group and naïve/vehicle group (mean ± SEM – 8 animals/group). **p < 0.01 and ***p < 0.001 for comparisons between baseline and different time - points in the IONX group. *α* for comparisons between IONX and naïve groups at different time points (baseline to 53 - minute observation period). Differences between the normalised baseline values (*i.e.*, before BISA or vehicle administration) of each group were expressed as percent change (%) from baseline (control, 100%). Arrow indicates the time of vehicle or BISA administration (*i.v.*). IONX, infraorbital nerve transection; (−)-α-bisabolol (BISA); vehicle (isotonic saline and 10% Cremophor A25).

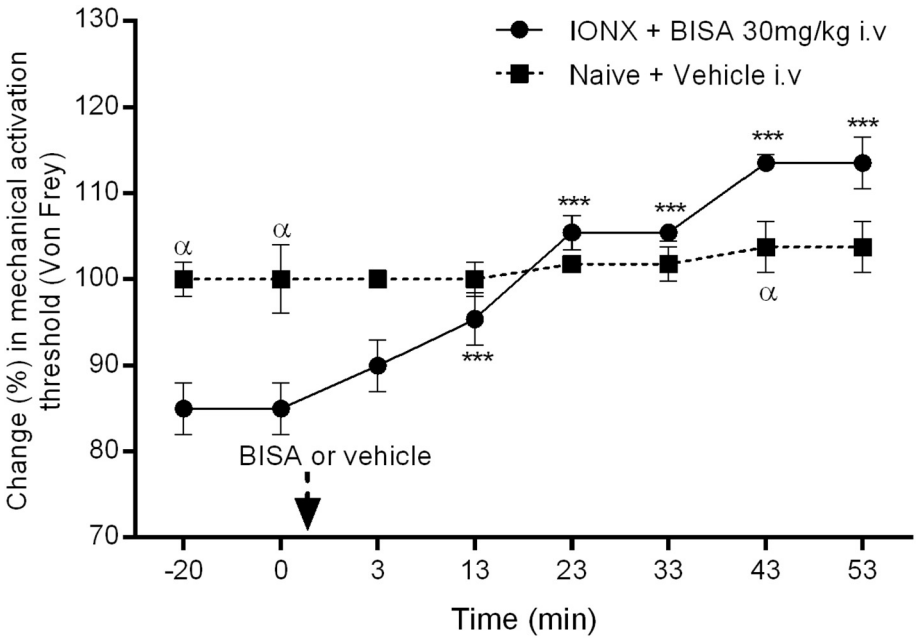

Fig. 4. Change (%) in mechanical activation thresholds (Von - Frey) in MDH neurons of IONX/BISA group and naïve/vehicle group (mean ± SEM – 8 animals/group). ***p < 0.001 for comparisons between baseline and different time - points in the IONX group. *α* for comparisons between IONX and naïve groups at different time points (baseline to 53 - minute observation period). Differences between the normalised baseline values (*i.e.*, before BISA or vehicle administration) of each group were expressed as percent change (%) from baseline (control, 100%). Arrow indicates the time of vehicle or BISA administration (*i.v.*). IONX, infraorbital nerve transection; (−)-α-bisabolol (BISA); vehicle (isotonic saline and 10% Cremophor A25).

facial mechanical allodynia in the IONX neuropathic pain model, BISA administration had no effect on the facial mechanical sensitivity of naïve and sham - operated animals. But it could attenuate the facial mechanical allodynia seen in the IONX rats. BISA also attenuated the accompanying central sensitisation in the IONX rats by reducing neuronal spontaneous activity and the evoked responses to graded mechanical stimuli and by reversing the lowered mechanical activation threshold of WDR neurons in the MDH. These novel findings suggest that the attenuation by BISA of the facial mechanical allodynia established within 3 days after trigeminal nerve injury may at least in part be explained by the drug's action on nociceptive neurons in the MDH which is the major brainstem site for transmission of nociceptive signals in the trigeminal somatosensory system [2,6,7,33].

While there have been no previous studies of possible BISA effects in orofacial neuropathic pain models, topical as well as oral administration of BISA in other studies using rodent models of orofacial pain have shown its effectiveness in attenuating nociceptive behaviour [24–26]. A previous study using a corneal nociception model [34] showed that topical pre - treatment with BISA (200 mg/mL) produced a significant reduction in nociceptive behaviour and also revealed an interaction between BISA and TRPV1 receptors [24,25]. Another study showed that oral administration of BISA was effective for several hours in reducing nociceptive behaviour induced by formalin, capsaicin or glutamate in rodent models of orofacial pain; this study also showed that BISA has an anti - inflammatory effect which is associated with a significant reduction in the pro-inflammatory cytokine TNF-α but not IL - 1β [35]. Nociceptive behaviour induced by application of formalin or cinnamaldehyde to orofacial tissues including the temporomandibular joint and cornea has also been shown to be significantly decreased by oral administration of BISA [26]. A recent clinical study has shown that BISA mouthwashes reduced pain and promoted healing in post-operative maxillofacial surgeries [36].

Pharmacological studies have shown that BISA reduces vascular and muscle contractions through modulation of Ca^{+2} channels [18,19] and peripheral neural blockade [20]. BISA shows a high binding affinity for TRPV1 [22] and TRPA1 [26] in molecular docking and *in vivo* experiments, but blockers of opioid receptors, NO synthesis, and K + ATP channels have been shown to not inhibit the effect of BISA [26].

Others have reported the effect of BISA to be associated with the inhibitory activity of COX [37] but not suppression of central processes [20]. BISA has also been reported to be effective in reducing neural excitability by blockade of voltage - dependent sodium channels [20]. Importantly, BISA did not interfere with motor activities when tested in mice in open field and rotarod tests at doses ranging between 50 and 400 mg/kg (*p.o.*) [22,38]. The dose of BISA used in the present behavioural experiments fell within this range.

The present study has provided novel insights into the early time - course of effects of BISA in a rat model of orofacial neuropathic pain. While these findings indicate that BISA is effective in attenuating ongoing nociceptive behaviour and associated central sensitisation, further studies are needed to determine the full time - course of its effects and also whether it is effective in preventing the development of the nociceptive process in neuropathic pain. There is also the need to examine its effect in other forms of neuropathic as well as inflammatory models of orofacial pain.

In conclusion, the present study has provided the first documentation that systemic administration of BISA reduces facial mechanical hypersensitivity and central sensitisation in a trigeminal neuropathic pain model and thereby provides support to recent findings pointing to the potential usefulness of BISA in the treatment of orofacial pain. More studies are required to test if it also has an effect in chronic models of neuropathic pain and if it can prevent the development of chronic pain.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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