Nitric Oxide 93 (2019) 90–101

Contents lists available at ScienceDirect

Nitric Oxide

journal homepage: www.elsevier.com/locate/yniox

Propargylglycine decreases neuro-immune interaction inducing pain response in temporomandibular joint inflammation model

Emanuela G. Garattini[a], Bruna M. Santos[b], Daniele P Ferrari[a], Camila P Capel[a], Heloísa D.C. Francescato[b], Terezila M. Coimbra[b], Christie R.A. Leite-Panissi[c], Luiz G.S. Branco[a,b,**], Glauce C. Nascimento[a,b,*]

[a] Department of Basic and Oral Biology, Dental School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil
[b] Department of Physiology, Medical School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil
[c] Psychobiology Graduate Program, School of Philosophy, Science and Literature of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil

ARTICLE INFO

Keywords:
Hydrogen sulfide
Inflammation
Orofacial nociception
Cytokines
Glia

ABSTRACT

The mechanisms underlying temporomandibular disorders following orofacial pain remain unclear. Hydrogen sulfide (H_2S), a newly identified gasotransmitter, has been reported to modulate inflammation. Cystathionine γ-lyase (CSE) is responsible for the systemical production of H_2S, which exerts both pro- and antinociceptive effects through inflammation. In the current study, we investigated whether the endogenous H_2S production pathway contributes to arousal and maintenance of orofacial inflammatory pain, through the investigation of the effects of a CSE inhibitor, propargyglycine (PAG), in a rat CFA (Complete Freund Adjuvant)-induced temporomandibular inflammation model to mimic persistent pain in the orofacial region. For this, rats received either CFA or saline in the temporomandibular joints (TMJs), and after 3 or 14 days, they received a single injection of PAG or saline and were evaluated for nociception with the von Frey and formalin test. Also, pro-inflammatory cytokines, tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) were analyzed in TMJs and trigeminal ganglion (TG). In this last one, glial cells reactivity was also verified. Endogenous H_2S production rate were measured in both, TMJ and TG. Our results indicated decreased allodynia and hyperalgesic responses in rats submitted to CFA after injection of PAG. Moreover, PAG inhibited leucocyte migration to temporomandibular synovial fluid after 3 and 14 days of inflammation. PAG was able to reduce levels of CBS, CSE, TNF-α, and IL-1β in the TMJ and TG, after 13 days of CFA injection. The observed increased activation of glial cells in the trigeminal ganglia on the 14th day of inflammation can be prevented by the highest dose of PAG. Finally, CBS and CSE expression, and endogenous H_2S production rate in the TMJ and TG was found higher in rats with persistent temporomandibular inflammation compared to rats injected with saline and PAG was able to prevent this elevation. Our results elucidated the molecular mechanisms by which H_2S exerts its pro-inflammatory and pro-nociceptive role in the orofacial region by alterations in both local tissue and TG.

1. Introduction

Chronic orofacial pain is a challenging clinical condition due to its multifactorial etiology and, therefore, difficult diagnosis and complex treatment. Likewise, its undefined molecular mechanisms also contribute to the complexity of chronic orofacial pain [1]. Temporomandibular dysfunctions (TMDs) associated or not with muscular symptoms comprise the major cause of chronic states of pain in orofacial structures [2,3]. Besides their high incidence, the pathophysiology of temporomandibular joint (TMJ) pain remains unclear even with the notable evolution already made towards the clarification of their pathogenesis [4]. Classical animal models of inflammatory pain allow us to examine these disorders and their potential therapeutics having remarkable relevance to translational science. In fact, CFA (Complete Freund Adjuvant)-rat is a reliable model that promotes acute and chronic states of inflammatory pain. Numerous studies have been

* Corresponding author. Department of Morphology, Physiology and Basic Pathology, Dental School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, 14040-904, Brazil.
** Corresponding author. Department of Morphology, Physiology and Basic Pathology, Dental School of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, 14040-904, Brazil.
E-mail addresses: branco@forp.usp.br (L.G.S. Branco), glauce.nascimento@usp.br (G.C. Nascimento).

https://doi.org/10.1016/j.niox.2019.10.001
Received 28 February 2019; Received in revised form 22 September 2019; Accepted 2 October 2019
Available online 08 October 2019
1089-8603/ Published by Elsevier Inc.

using this model to analyze systemic [5,6] and orofacial pain [7–9].

The closely mutual neuro-immune interaction contributes to the pain response [10]. The release of cytokines [such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β)] within inflamed joints by many types of cells [11,12] induces pain by the direct activation of their receptors in the terminal nerve or indirectly by increasing the expression and production of others pain-related mediators [10]. TNF-α and IL-1β are strongly expressed in the joints affected by TMJ disorders [11,13]. Many studies have shown substantial levels of both in the synovial fluid of patients suffering from this condition [14]. In chronic pain conditions, there is also an increase in the release of cytokines by the nociceptors neurons activating satellite glial cells and concomitantly facilitating central pain sensitization [15]. Accordingly, TG also has an increase in microglial activation [16] and an increase in TNF-α production [17] in CFA-model.

Hydrogen sulfide (H_2S) is an endogenous gaseous element synthesized from the metabolism of L-cysteine by the handling of cystathionine γ-lyase (CSE – mainly expressed in peripheral tissues) or cystathionine β-synthase (CBS – mainly expressed in the brain) [18]. Numerous efforts have been made to clarify the pathophysiology of this gas since its discovery [19,20]. About pain states, it has been shown that H_2S exerts pro- and antinociceptive effects [21–23] which appear to result from various interrelated mechanisms dependent upon the distinct molecular targets. Among them, H_2S mediates local inflammation-induced pain through mechanisms involving neutrophil migration, cytokine production and edema formation [24,25]. Moreover, a potential therapeutic role of H_2S in systemic and orofacial inflammatory pathologies has been proposed [26–29].

It is a well-acknowledged fact, particularly about orofacial pain, that the TMJ inflammation-induced hyperalgesia produces an upregulated intraarticular CBS gene expression [8]. Additionally, a recent finding from our research group pointed out the anti-nociceptive effect of CSE inhibition on temporomandibular inflammatory pain. This study reveals the acute and persistent anti-nociceptive effect of local H_2S inhibition, while the systemic injection caused only a persistent antinociceptive effect [29]. It has also been demonstrated that stimulation of H_2S signaling increases neuronal excitability of trigeminal neurons [30]. The trigeminal system has been plentifully studied in mammals and birds being responsible for motor coordination, sensory and cognitive oral functions [31–33]. The main feature of this system is the presence of two distinct primary afferent neuronal groups: trigeminal ganglion (TG) and mesencephalic trigeminal nucleus (MTN). Cell bodies of these primary afferent neurons are present in TG [34], and a few are located in the MTN. MTN is involved mainly in proprioception [35,36]. Regarding the TG, the dorsomedial part is involved in nociception, thermoreception, and proprioception while its ventrolateral part is involved in mechanoreception [33]. Signals from the trigeminal system are transmitted by second order neurons into the brainstem and ascend to different regions of central nervous system (CNS) [34].

Considering TG is an essential relaying station firstly activated in the processing of painful orofacial information, the study of the role of H_2S in this region may be a promising therapeutic strategy by decreasing neuro-immune interactions inducing pain response. Notably, the doses chosen to our study are described to do not change body core temperature [37] or systolic blood pressure [38] under basal or health conditions, indicating that PAG might be a possible therapeutic target for persistent orofacial pain without side effects. This question is relevant in the context of the necessity to develop novel analgesics since some patients do not respond to analgesics such as opioids and non-steroidal anti-inflammatory drugs and others patients might suffer from their major adverse effects.

Hence, the present study investigated the unexplored anti-nociceptive and anti-inflammatory effects of a CSE inhibitor, propargylglycine (PAG) in different doses on the CFA-induced inflammatory hypernociception (acute and persistent states) in the TMJ of rats by evaluating the leucocytes infiltrate in synovial fluid of the joint and IL-1β and TNF-α levels, CBS and CSE expression in TMJ tissue and trigeminal ganglia. Moreover, the activation of satellite glial cells on ganglion was also investigated, since glial plasticity is part of modifications in the peripheral nervous system during the development of chronic states of pain and inflammation, and it was measured H_2S production rate in both structures. This pioneering investigation was undertaken to test the hypothesis that the modulatory effect of PAG on the orofacial nociception is regulated by H_2S in the TMJ and TG and it may provide insights into chemical events that initiate and maintain chronic inflammatory pain in TMJ.

2. Methods

2.1. Animals

Male Wistar rats (n = 6 per group; 160–220 g) were housed in standard plastic cages, they had access to food and water ad libitum and were maintained in a temperature-controlled room (23 ± 2 °C) with a 12/12-h light-dark cycle. This study was conducted in accordance with the local Institutional Animal Care and with the approval of the local ethical committee (2016.1.415.58.5) and we designed it to reduce animal suffering and the number of animals.

2.2. Drugs

The drugs used in this study - Complete Adjuvant of Freund (CFA, temporomandibular inflammation inducer) and Propargylglycine (PAG, an inhibitor of CSE) - were purchased from Sigma-Aldrich and dissolved in sterile saline (vehicle; 0.9%). The doses of PAG were based according to reports in the literature [39].

2.3. CFA-induced inflammatory hypernociception on temporomandibular region

Initially, rats were anesthetized with an intramuscular injection of ketamine 10% (75 mg/kg) and xylazine 4% (10 mg/kg) followed by bilateral intraarticular administration with 50 μg of CFA (*Mycobacterium tuberculosis*) suspended in a 50 μL paraffin oil (Sigma Aldrich) or 0.9% saline solution (SAL). This dose was based on previous reports [40]. A 26 G ½ needle attached to a 1 mL plastic syringe was used for the injection. To locate the TMJ for the injection, we palpated the zygomatic arch and the condyle. The needle was inserted immediately below the posteroinferior border of the zygomatic arch and advanced anteriorly to contact the edge of the posterolateral condyle [41].

2.4. Experimental protocols

On Fig. 1, it is possible to see an experimental design evidencing the experimental protocols of this work. After 3 and 14 days of CFA injection inducing acute or persistent inflammation, respectively, in the temporomandibular joints, concomitantly or not with local PAG treatment, behavioral tests (orofacial mechanical and chemical nociception), euthanasia and sample collection (synovial fluid, tissue joint, and trigeminal ganglion) and inflammatory analysis (cell counting and ELISA) were all performed.

2.5. Evaluation of orofacial hypernociception

2.5.1. Mechanical threshold evaluation – Von Frey test

Inflammatory hypernociception in the TMJ was evaluated by measuring the threshold of force needed to be applied to the TMJ region until the head withdrawal occurred. The measurements were performed by a blinded examiner who used a digital device (Insight, Brazil) that consisted of a rigid filament linked to an electronic device – automatic Von Frey anesthesiometer, which in turn measures the response

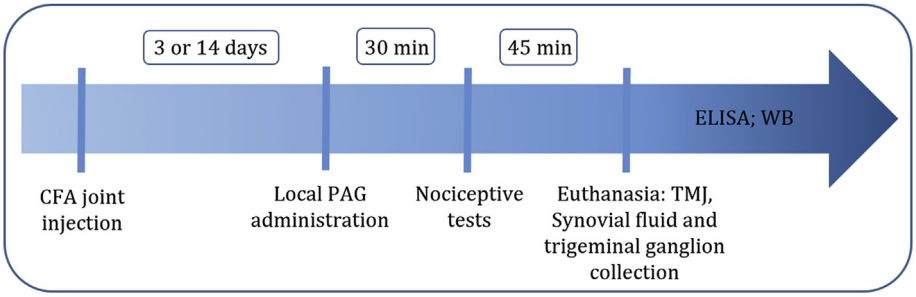


Fig. 1. Experimental line.

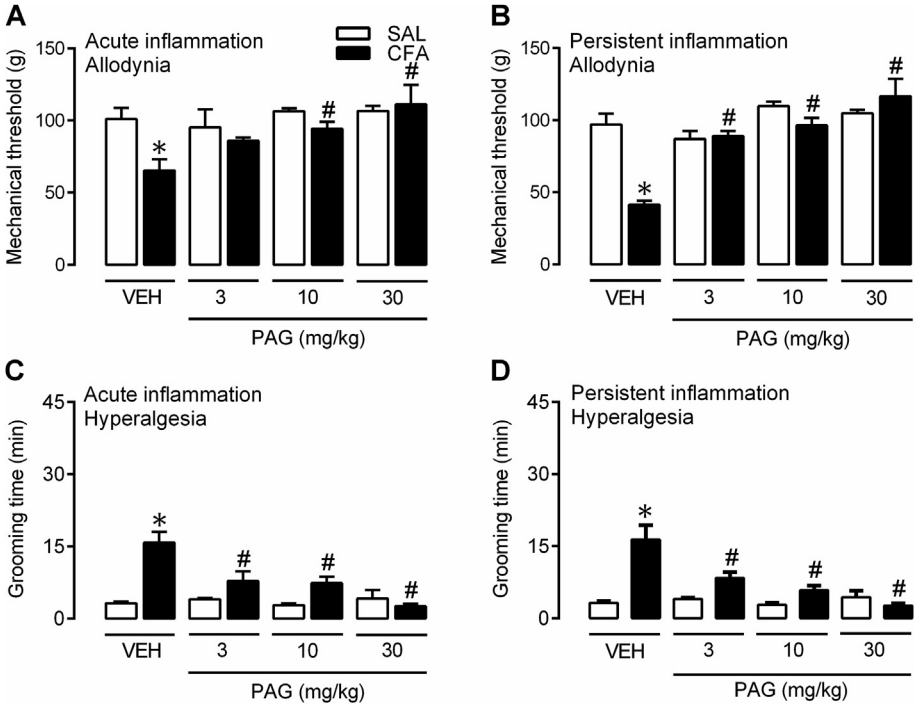


Fig. 2. PAG prevents mechanical alodinia and orofacial hyperalgesia induced by CFA in ATMs. **A:** Mechanical nociception threshold (in grams) of the rats submitted to 3 days of temporomandibular inflammation by CFA or injected with Saline. **B:** Mechanical nociception threshold (in grams) of rats submitted to 14 days of temporomandibular inflammation by CFA or injected with saline (SAL). **C:** Orofacial grooming time after formalin injection (1.5%) in the region of vibrissae of rats submitted to 3 days of temporomandibular inflammation by CFA or injected with saline (SAL). **D:** Orofacial grooming time after formalin injection (1.5%) in the region of vibrissae of rats submitted to persistent temporomandibular inflammation by CFA or injected with Saline. Data are expressed as mean ± standard error of the mean. *P < 0.05, vs. control group (SAL) at the same dose of PAG. #P < 0.05, vs. CFA treated with vehicle of PAG group. The pharmacological treatment consisted of PAG injected locally into the joint at different doses (3, 10, 30 mg/kg) or its vehicle (VEH; saline injection).

Table 1
Quantification of leucocytes on synovial fluid of TMJs from rats submitted to acute (3 days after CFA injection) or persistent (14 days after CFA injection) temporomandibular inflammation or 3 or 14 days after saline injection (SAL). Data are expressed as mean ± standard error of the mean. *P < 0.05, compared to SAL and #P < 0.001, compared to CFA inducing acute temporomandibular inflammation. The pharmacological treatment consisted of PAG injected locally into the joint at different doses (3, 10, 30 mg/kg) or its vehicle (VEH; saline injection).

Leucocytes count in synovial fluid (x 10^3/ul)				
	Saline 3 days	Saline 14 days	CFA 3 days	CFA 14 days
Vehicle	50.3 ± 1	50.2 ± 2.1	115.3 ± 1.3*	210 ± 1.6*#
PAG 3 mg	48.8 ± 0.3	52.3 ± 1.4	106 ± 1.6*+	93.2 ± 0.9*#
PAG 10 mg	52.8 ± 1.3	52.2 ± 1.8	92.8 ± 0.7*+	82.8 ± 0.7*#
PAG 30 mg	53.3 ± 2	49.2 ± 0.4	53.2 ± 1.6+	64.8 ± 1.3*#

threshold in grams (g) when the filament is applied to the surface of the tested region [29]. The facial areas to be tested around the TMJ were shaved before the experimental procedure, and the animals were placed in individual plastic cages 45 min before the tests. The animals underwent conditioning sessions in the testing room for 4 consecutive days. On day five, the basal force threshold value was recorded three times, and the average of these values was calculated.

2.5.2. Formalin test

To evaluate a tonic chemogenic pain response of rats in the orofacial region, we performed an orofacial formalin test. For the administration of saline or formalin solutions, the rats were allowed to adapt to a testing chamber for 20 min. The experimental room had little human activity, and a controlled temperature of 25 ± 1 °C. The animals were removed from the box and a volume of 50 µL of 2% formalin or 0.9% saline solution (control group) was injected subcutaneously into the orofacial region between the nose and the upper lip (vibrissae area). A 30 G 1/2" needle attached to a plastic syringe of 1 mL was used for the injections. Injections were performed as quickly as possible to avoid prolonged handling that could interfere with the results of this study. Immediately after the injection, rats were returned to the testing chamber, and the number of seconds they spent rubbing the ipsilateral face was recorded. According to Grabow and Dougherty [42], the orofacial formalin test can be characterized by two phases. Phase 1 is the first interval of vibrissal rubbing (0–3 min), and phase 2 is defined as the period of vibrissal rubbing from min 15 to 45 of the test period. In general, the peak of the vibrissal rubbing in phase 2 was observed during interval 7 (min 21 to 24) and diminished before interval 15 (min 43 to 45).

2.6. Euthanasia

Rats were deeply anesthetized with urethane (1.5 g/kg, Sigma

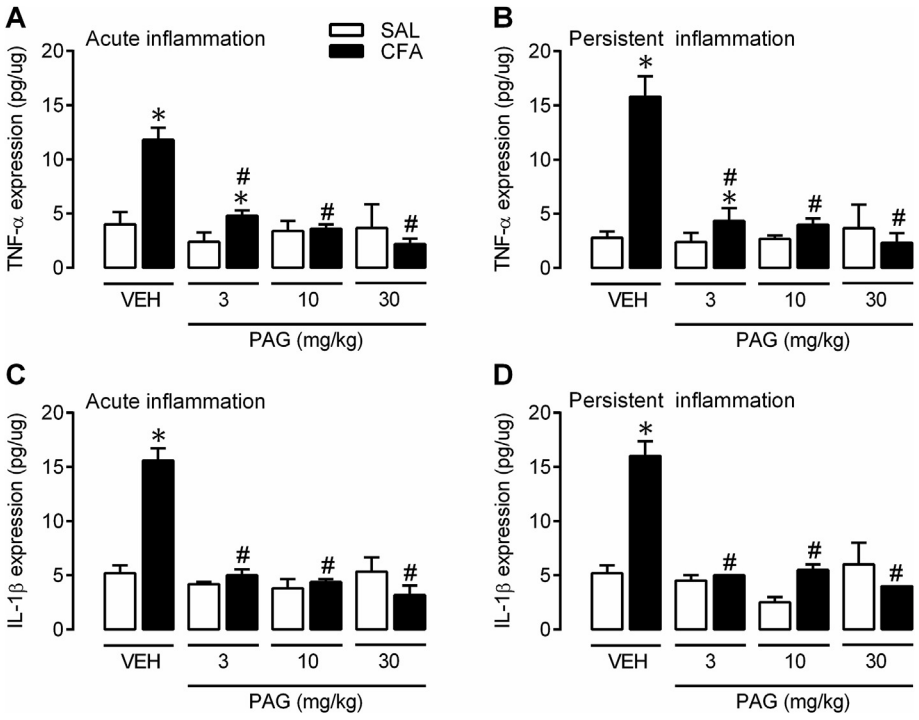


Fig. 3. PAG reduces proinflammatory cytokines in TMJ after 3 (acute inflammation) and 14 days (persistent inflammation) of CFA-induced temporomandibular inflammation. **A:** Quantification of TNF-α from rats submitted to acute temporomandibular inflammation by CFA or injected with Saline. **B:** Quantification of TNF-α from rats submitted to persistent temporomandibular inflammation by CFA or injected with Saline. **C:** Quantification of IL-1β from rats submitted to acute temporomandibular inflammation by CFA or injected with Saline. **D:** Quantification of IL-1β from rats submitted to persistent temporomandibular inflammation by CFA or injected with saline (SAL). Data are expressed as mean ± standard error of the mean. *P < 0.05, vs. control group (SAL) at the same dose of PAG. #P < 0.05, vs. CFA treated with vehicle of PAG group. The pharmacological treatment consisted of PAG injected locally into the joint at different doses (3, 10, 30 mg/kg) or its vehicle (VEH; saline injection).

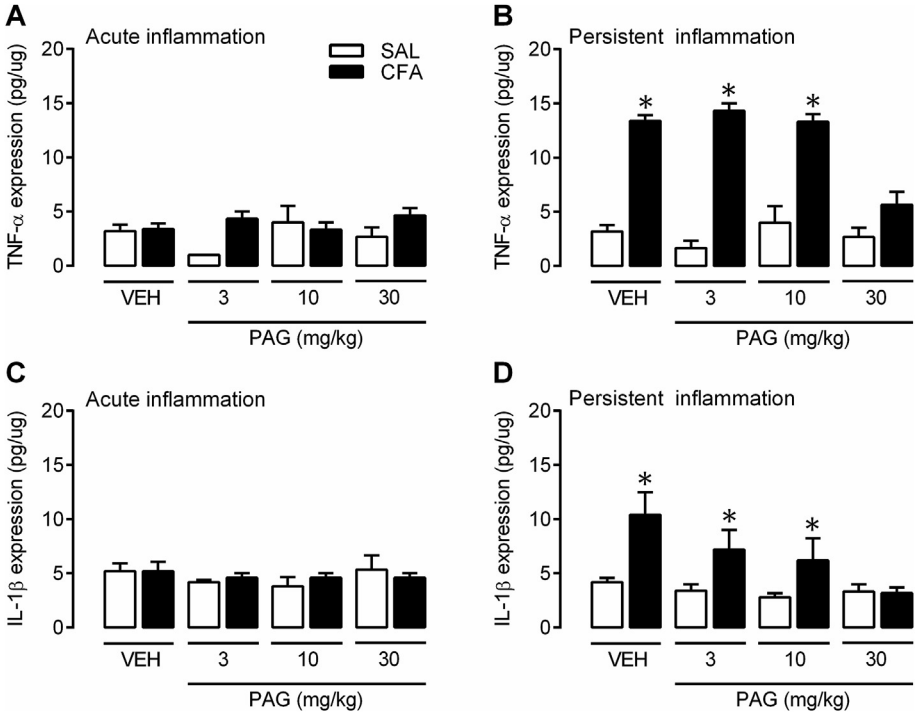


Fig. 4. The major dose of PAG (30 mg/kg) reduces pro-inflammatory cytokines in the trigeminal ganglia after 14 days of CFA-induced persistent temporomandibular inflammation. **A:** Quantification of TNF-α from rats submitted to acute temporomandibular inflammation by using CFA or saline (SAL). **B:** Quantification of TNF-α from rats submitted to persistent temporomandibular inflammation by CFA or injected with saline (SAL). **C:** Quantification of IL-1β from rats submitted to acute temporomandibular inflammation by CFA or injected with saline (SAL). **D:** Quantification of IL-1β from rats submitted to 14 days of temporomandibular inflammation by CFA or injected with saline (SAL). Data are expressed as mean ± standard error of the mean. *P < 0.05, vs. control group (SAL) at the same dose of PAG. #P < 0.05, vs. CFA treated with vehicle of PAG group. The pharmacological treatment consisted of PAG injected locally into the joint at different doses (3, 10, 30 mg/kg) or its vehicle (VEH; saline injection).

Aldrich) and were rapidly euthanized by means of decapitation. To relieve any suffering, decapitation was performed by an experienced person in the area and in a quiet environment. The remaining animals were kept in a separate environment to avoid any influence of the odor of blood. In addition, after each decapitation, the materials were washed and cleaned with 70% alcohol. Brains were immediately removed for TMJ and TG dissection.

2.7. Sample collection

2.7.1. Synovial fluid

The superficial tissues were dissected, and the TMJ cavity was washed two times to collect the synovial fluid by the pumping and aspiration technique using 0.05 mL of EDTA (1.77 mg EDTA/mL PBS). These samples were used for leucocyte count.

2.7.2. Joint tissue and trigeminal ganglion

Temporomandibular joint and trigeminal ganglion were dissected with the help of a magnifying lens (Leica Zoom 2000) and removed

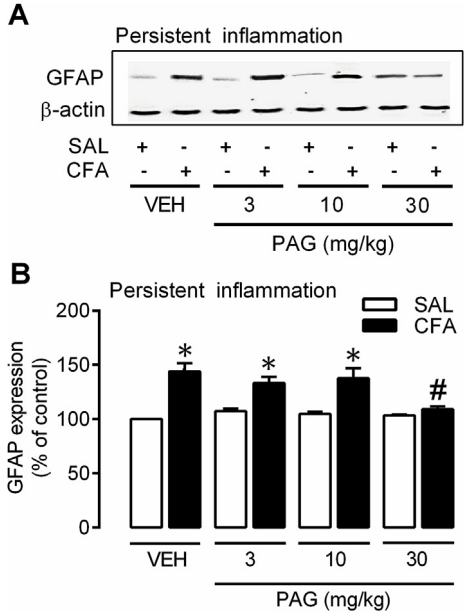


Fig. 5. The persistent state of CFA-induced temporomandibular inflammation (14 days) produced an increased expression of reactive SGCs in trigeminal ganglia and PAG (30 mg/kg) was able to reverse this change. **A:** Representative gel from WB technique indicating expression of GFAP protein in TG from rats submitted to 14 days of temporomandibular inflammation by CFA or injected with Saline and β-actin expression as a control. B: Percentage of GFAP expression in TG from rats submitted to persistent temporomandibular inflammation by CFA or injected with saline (SAL). Data are expressed as mean ± standard error of the mean. *P < 0.05, vs. control group (SAL) at the same dose of PAG. #P < 0.05, vs. CFA treated with vehicle of PAG group. The pharmacological treatment consisted of PAG injected locally into the joint at different doses (3, 10, 30 mg/kg) or its vehicle (VEH; saline injection).

according to Ref. [9]. All joints were weighted and stored in a freezer at −80 °C. These samples were used for ELISA and WB techniques.

2.8. Synovial lavage collection and cell counting

3 or 14 days after CFA injections inducing acute or persistent inflammation, in the temporomandibular joints, respectively, the rats were sacrificed under anesthesia and the synovial fluid from TMJs was collected. The present protocol was based in previously described studies [43]. For the determination of the total number of white cells in the synovial lavage, a total leucocyte count was performed in a Neubauer chamber using 20 µl of articular lavage solution diluted in 380 µl Turk (1:20 dilution).

2.9. TNF-α and IL-1β ELISA assays

The TMJ tissue and the trigeminal ganglion were excised 3 or 14 days after the CFA-injection inducing acute or persistent temporomandibular inflammation, respectively, in rats and were homogenized in a solution of RIPA Lysis Buffer System (Santa Cruz Biotechnology). The samples were centrifuged at 10,000 rpm for 15 min at 4 °C. The supernatants were stored at −80 °C for posterior analysis to evaluate the protein levels of TNF-α and IL-1β in the TMJ tissue and the trigeminal ganglion. The following kits quantified the cytokine levels: TNF-α–Rat TNF-alpha/TNFSF1A Quantikine ELISA Kit (R&D Systems, catalog number RTA00); and IL-1β–Rat IL-1 beta/IL-1F2 Quantikine ELISA Kit (R&D Systems, catalog number DY501). The absorbance was measured at 450 nm. IL-1β and TNF-α concentrations were expressed as pg/µg.

2.10. Western blot (WB) analysis

Trigeminal ganglion (TG) samples were stored at −80 °C until use. The sample was homogenized on ice in 200 µL of tissue in sterile saline using a Polytron® PT 1200 handheld homogenizer (Kinematica Inc). The homogenate was used for WB measurement. For the immunoblot analysis, the protein was isolated from the TGs of both controls and experimental rats. Samples were treated with boiling lysis buffer (1% sodium dodecyl sulfate, 1.0 mM sodium orthovanadate, 10 mM Tris, pH 7.4). Equal amounts (30 µg) of total protein were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (10%) and transferred to polyvinylidene difluoride membranes. Immunostaining of the blots was performed using four primary antibodies, rabbit polyclonal antibody to GFAP, CBS (1:1.000; Millipore and Cell Signaling, respectively) and mouse monoclonal antibody to CSE and anti-β-actin (1:1,000 and 1:10,000; Sigma-Aldrich and Abnova Corporation, respectively). Membranes were then incubated with peroxidase-coupled secondary antibodies (1:2,000; Millipore) for 1 h at room temperature. Blots were developed using the Amersham ECL Prime western blotting detection reagent (GE Healthcare). Densitometric analysis was performed using the Eagle Eye TMII Still Video System (Stratagene).

2.11. Measurements of H_2S production rate in the TMJ and TG

To test our hypothesis that H_2S production is altered in TMJ and TG, we measured H_2S production rate in homogenates of these tissues of animals with or without temporomandibular inflammation. TMJs and TGs samples were homogenized in potassium phosphate buffer (100 mM; pH 7.4) using a microprocessor (VirTis, Gardiner). Each sample (50% w/v; 100 µl) contained L-cysteine (10 mM; 20 µl), pyridoxal 5′-phosphate (2 mM; 20 µl) and PBS (30 µl). The reaction was performed in eppendorf tubes sealed with parafilm, and started by transferring the tubes from ice to bath at 37 °C. After 2 h incubation, zinc acetate (1% w/v; 100 µl) was included to trap evolved H_2S followed by tricloroacetic acid (10% w/v; 100 µl) to precipitate proteins and thus finalize the reaction. After centrifugation, *N*,*N*-dimethyl-p-phenylenediamine sulfate (20 mM; 50 µl) in HCl 7.2 M followed by $FeCl_3$ (30 mM; 50 µl) in HCl 1.2 M was then added to 50 µl of the supernatant, and optical density was measured at 670 nm. The H_2S concentration of each sample was calculated against a calibration curve of absorbance of Na_2S solutions (0.1–100 µg/mL). To quantified the protein content of the samples, the pellets were diluted in 4 mL of sodium hydroxide (0.1 N) and the solution was then assayed by using a protein dye reagent (Bio-Rad Laboratories; code number: 500–0006). NaSH (0–250 µM). The results were expressed as nmol/mg proteins per 1 h.

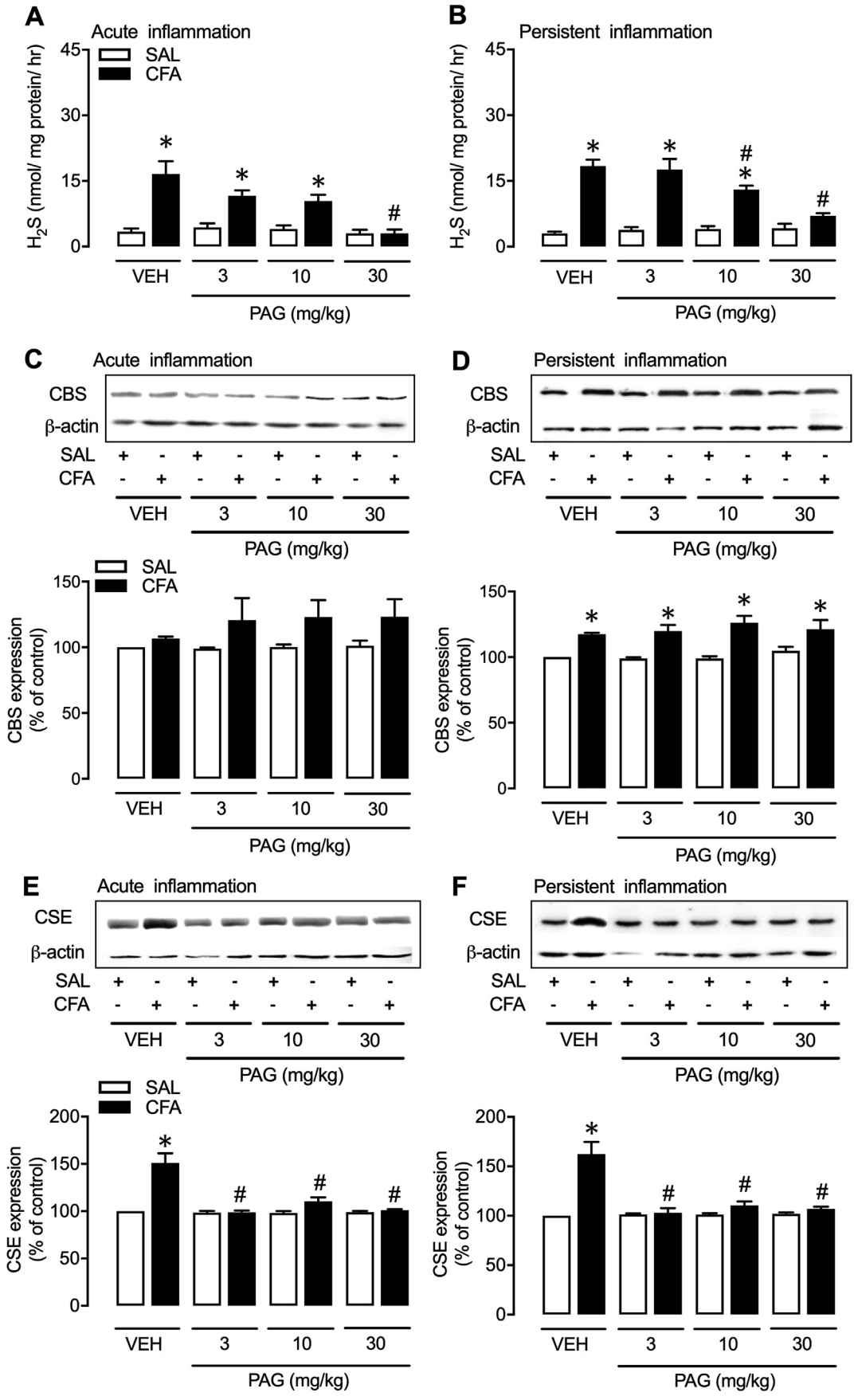
2.12. Statistical analysis

The data are presented as the mean ± SEM when appropriate. Differences between means were compared using two-way ANOVA followed by the Student-Newman-Keuls post-test. A value of $p < 0.05$ indicated significant differences.

3. Results

3.1. Endogenous H_2S mediates mechanical orofacial acute and persistent inflammatory hypernociception

Recent studies have shown a dual role of H_2S in inflammatory hypernociception. Pro- and anti-nociceptive functions have been recognized for this endogenous gas until now [39]. Notably, on the orofacial region, evidence has indicated H_2S exerting a pro-nociceptive effect [28,29,44]. The present study evaluated the effect of the pretreatment of rats with PAG (3–30 mg/kg) on the nociceptive mechanical threshold (Fig. 2A and B), as well as chemical nociception (Fig. 2C and D) in the orofacial region. These data show decreased hyperalgesia



(caption on next page)

Fig. 6. Acute and persistent state of CFA-induced temporomandibular inflammation (3 and 14 days) and the H_2S production rate (A, B), CBS (C, D) and CSE (E, F) expression in the TMJ. Data are expressed as mean ± standard error of the mean. Representative gel from WB technique indicating expression of each protein in the TMJ is shown at the top of its respective graph. β-actin expression was used as a control.*P < 0.05, vs. control group (SAL) at the same dose of PAG. #P < 0.05, vs. CFA treated with vehicle of PAG. The pharmacological treatment consisted of PAG injected locally into the joint at different doses (3, 10, 30 mg/kg) or its vehicle (VEH; saline injection).

and allodynia in CFA-induced acute or persistent temporomandibular inflammation rats treated with PAG. The only exception was on the mechanical threshold of animals that received 3 mg/kg of PAG acutely, since there was no difference between its control (SAL) at same dose of PAG.

3.2. Leucocyte migration seems to be involved in the pro-nociceptive role of endogenous H_2S

Research groups have shown that neutrophils and leucocytes [39,45–47] play an important role in the genesis of inflammatory hypernociception. Since endogenous H_2S production has been shown to be involved in the recruitment of inflammatory cells [27,48] during the inflammatory disease, we tested the hypothesis that leucocyte recruitment mediates the inflammatory hypernociception also in orofacial processes. Our data consistently show that PAG (3–30 mg/kg) pretreatment inhibited CFA-induced leucocyte migration to temporomandibular synovial fluid (Table 1). This effect was observed in the acute and persistent phase of the inflammation.

3.3. Endogenous H_2S inhibition reduce joint tissue TNF-α and IL-1β in both, acute and persistent phases of temporomandibular pain

Based on the TMJ-leucocyte role in the effect of H_2S inhibition as an anti-nociceptive mechanism and considering also previous results about plasmatic extravasation on TMJ mediating this reaction [29], we now investigate the role of the main inflammatory cytokines related to inflammatory hypernociception on pro-nociceptive H_2S function. The data evidenced on TNF-α and IL-1β on temporomandibular tissue from CFA-induced inflamed rats after treatment with PAG, in acute and persistent inflammation development in all doses tested (Fig. 3).

3.4. Endogenous H_2S inhibition reduce TNF-α and IL-1β in trigeminal ganglion in persistent phase of temporomandibular pain

Considering that H_2S exerts an effect on the periphery and also on the nervous system [39], it is important to evaluate if, in our temporomandibular inflammation model, there is some indication of neural plasticity. The first structure on the nervous system related to the processing of orofacial nociceptive information is the trigeminal ganglion, which contains the bodies of the primary neurons participating in this modulation. There are significant findings of H_2S effects on neuron excitability [30,49], receptors [44] and enzymes (CBS and CSE [8]) on trigeminal ganglion. However, the present study is the pioneer in analyzing inflammatory cytokines in this structure after H_2S inhibition (Fig. 4). Our data support the idea that PAG is able to reduce the production of TNF-α and IL-1β on trigeminal ganglion in a persistent temporomandibular inflammation in a dose of 30 mg/kg. The other doses had no significant effect. Remarkably, the highest dose of PAG was able to block the CFA-induced IL-1β increase at 14 days of inflammation, since it was not observed statistical difference between its control (SAL) at the same dose of PAG (Fig. 4D).

3.5. The reduction of TNF-α and IL-1β in the TG in the persistent phase of temporomandibular pain is accompanied by reduction activation of satellite glial cells (SGCs) in trigeminal ganglion induced by endogenous H_2S inhibition

Following an abnormally intense pain elicited by noxious stimuli, increased levels of Anti-Glial Fibrillary Acidic Protein (GFAP), a marker of activated glial cells, can be detected [50]. This effect occurs in the CNS as well as in the peripheral nervous system on nervous ganglia, as TG [51]. This glial activation leads to pro-inflammatory cytokines like TNF-α and IL-1β [52]. Fig. 5B precisely shows increased activation of SGCs in TG on the 14th day of temporomandibular inflammation. Interestingly, the highest dose of PAG (30 mg/kg) was able to prevent this stimulation, since, after the therapy; the levels of GFAP protein expression on TG are in basal conditions, found by Western blotting analysis (Fig. 5).

3.6. Endogenous H_2S inhibition reduces TMJ and TG H2S production rate in both, acute and persistent phases of temporomandibular pain

The production rate of H_2S was measured in TMJ and TG samples of Wistar rats at 3 and 10 days of inflammation treated or not with PAG (Fig. 6A and B; 7A, B, respectively). Fig. 6A and B precisely show increased H_2S production rate in the TMJs after 3 and 14 days of CFA induced inflammation and specific doses of PAG inhibiting this H2S elevation in both acute inflammation (PAG – 30 mg/kg) and persistent process (PAG – 10 and 30 mg/kg). In the same manner, Fig. 7A and B presents an increased H_2S production rate in the TG after 3 and 14 days of CFA-induced inflammation and all doses of PAG inhibiting this H_2S elevation in both acute inflammation and persistent process.

3.7. CSE (but not CBS) expression is increased in the TMJ in the acute phase of temporomandibular pain

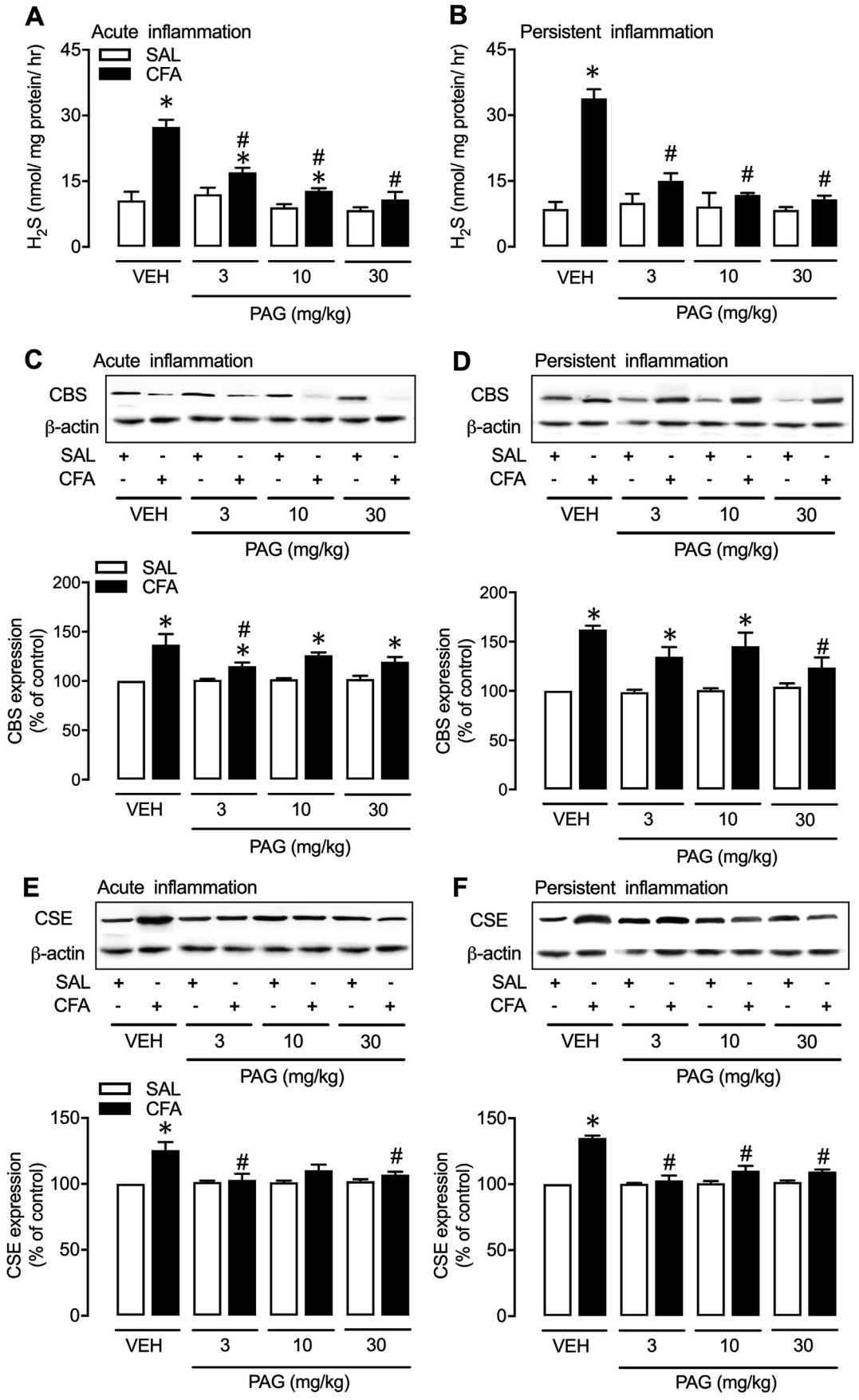
CSE and CBS expression were measured in TMJ samples of Wistar rats at 3 and 10 days (acute and persistent inflammation, respectively) of inflammation treated or not with PAG (Fig. 6). It was observed that the inflammatory response and consequently the acute pain observed at the initial days of this model is only related to an increase in the expression of CSE in the TMJ and this response was abolished using PAG (Fig. 6C, E).

3.8. CSE and CBS expression are increased in the TMJ in the persistent phase of temporomandibular pain

Interestingly, CSE expression was maintained increased (Fig. 6F), but an increase in CBS expression in the TMJ was observed at the persistent phase of inflammation (Fig. 6D). This result indicates that CBS overexpression in the TMJ is a consequence of the neuro-immune interaction at the persistent phase of this inflammation model. Unfortunately, PAG was not capable of decrease the expression of CBS in the TMJ of rats with persistent inflammation.

3.9. TG CSE and CBS expression are increased in both, acute and persistent phases of TMJ pain and PAG treatment caused a decrease in the expression of CBS and CSE in the TG

As expected [8], the overexpression of CBS in the TG was observed at the acute and persistent phase of inflammation induced in the TMJ area (Fig. 7C and D). Likewise, CSE expression was also increased in the TG of Wistar rats in both phases of inflammation indicating its role in orofacial inflammatory response (Fig. 7E and F). The peripheral treatment with PAG caused a decrease not only in the expression of CSE in the TMJ (Fig. 6E and F) but also in the TG, indicating its action centrally. Also, the highest dose of PAG used here caused a decrease in CBS



(caption on next page)

Fig. 7. Acute and persistent state of CFA-induced temporomandibular inflammation (3 and 14 days) and the H_2S production rate (A, B), CBS (C, D) and CSE (E, F) expression in the TG. Data are expressed as mean ± standard error of the mean. Representative gel from WB technique indicating expression of each protein in the TMJ is shown at the top of its respective graph. β-actin expression was used as a control.*P < 0.05, vs. control group (SAL) at the same dose of PAG. #P < 0.05, vs. CFA treated with vehicle of PAG. The pharmacological treatment consisted of PAG injected locally into the joint at different doses (3, 10, 30 mg/kg) or its vehicle (VEH; saline injection).

expression in the TMJ (Fig. 7D), highlighting the importance of CBS in the TMJ inflammatory-induced pain.

4. Discussion

We recently reported that a CSE inhibitor was able to produce an anti-nociceptive effect induced by temporomandibular inflammation which was accompanied by the decrease in joint region-plasmatic extravasation [29]. Since local injection has shown consistent results in this nociception model, PAG was injected on TMJs in distinct doses. We demonstrate here that the endogenous H_2S could upregulate cytokine production in both, TMJ and TG tissue, contributing to TMJ inflammatory hypernociception. These results are based on the PAG effect in local tissue (TMJ) and trigeminal ganglion (TG). Moreover, this study is pioneer in detecting molecularly H_2S production rate in TMJ and TG of rats with CFA-induced temporomandibular inflammation treated with PAG at distinct doses, as well as, in two phases of orofacial pain (acute and persistent).

The periods of 3 and 14 days after CFA injection used for analysis were established based on previous evidences with CFA-induced inflammation [53]. The two periods chosen for the analysis of this study show a peak of the acute phase of the CFA effect as an inflammatory agent and an established chronic inflammatory phase of the painful state, respectively. According to reports in the literature, CFA as an inflammatory inducing agent may be active within 42 days of experimentation [54], and this model is one of the major types to show long-lasting chronic pain [55]. In general, the CFA model stability starts to represent severe hypersensitivity to mechanical and thermal stimulation from one to three days [53]. Importantly, this model also presents an advantage to produce neuroplasticity in diverse parts from the central and the peripheral nervous system [56]. In fact, besides cellular alterations [57], synaptic transmission is altered in critical cortical areas for nociceptive and chronic pain processing after a CFA-induced peripheral inflammation [53].

Our results show an acute and persistent nociceptive profile in rats injected with CFA in the temporomandibular joints. These results are consistent with previous studies showing the greater effectiveness of CFA compared to other irritants inducing articular inflammation in various periods of time [58,59]. Evidently, PAG was able to reduce the nociceptive behavior, in both phases of pain, suggesting an acute and chronic pro-nociceptive role of H_2S in orofacial inflammatory pain.

Physiological gaseous mediators have been proposed to induce, inhibit and regulate the inflammatory process [60]. We believe that the antinociceptive effect of inhibition of H_2S synthesis is directly related to its anti-inflammatory action. The number of leucocytes and proinflammatory cytokines on TMJ of CFA-inflamed rats were reduced by PAG injection. Accordingly, Andruski [61] used an acutely inflamed knee joints model to show an H_2S effect on leucocyte-endothelium interactions. The authors reveal that H_2S has the propensity to interfere in the generation of mediators necessary for leucocyte recruitment. Again, the absence of leucocyte trafficking was attributed to an alteration in the expression of adhesion molecules and their associated ligands in the synovial microvasculature. Considering our results about leucocytes count on synovial fluid and an inflamed joint model used for us, it is possible that PAG effects on leucocytes are related to these impairments on the infiltration of leukocytes into the temporomandibular joint.

Leucocyte growth and differentiation are stimulated by cytokines in an inflammatory process [62]. The proinflammatory cytokines tumor necrosis factor (TNF) α, and interleukin (IL) 1β have been strongly implicated in the pathogenesis of chronic pain [63]. In addition, TNF-α and IL-1β are mediators associated with structural changes found in osteoarthritis or other arthritic conditions of the TMJ [64,65]. In agreement, these cytokines reduce the expression of type II collagen and aggrecan by the increased secretion of matrix metalloproteinases by chondrocytes, favoring joint destruction [66]. We observed increased levels of TNF-α and IL-1β in TMJ tissue on the 3rd and 14th days after inflammation induction. Consistent with our results, other studies showed statistically higher concentrations of these proinflammatory mediators upon induction of articular inflammation in acute [40], intermediate [67] and chronic phases [58,68]. The inhibition of H_2S synthesis caused a reduction of these levels in both phases of pain and the three doses of PAG were effective. In agreement, the literature shows this ability of PAG in decreasing these cytokines during inflammatory states [27,69]. In the data, nothing was known in relation to the temporomandibular joint or orofacial structures, which reinforces the novelty of the present study.

Moreover, we observed a reduction in TNF-α and IL-1β in the trigeminal ganglion (TG) after PAG treatment in rats from 14 days of CFA-induced temporomandibular inflammation. This effect was found when 30 mg/kg of PAG was administered. In the trigeminal ganglia, TNF- α is mostly produced by the satellite reactive glial cells which increases BDNF expression suggesting its role in neuroplasticity [70]. Reconciling the neuroplastic effect of TNF- α with our data, we suggest that there is a process of neuroplasticity installed in the structure. This process includes morphological, physiological and neurochemical modifications of the cells that make up the neural tissue, including both neurons and glial ones [71]. Satellite glial cells (SGCs) are the main type of glia in sensory ganglia, such as TG, and SGCs can proliferate under pathological conditions [72], upregulating glial fibrillary acidic protein (GFAP) and interleukin-1β, augmenting intercellular coupling to increase gap junctions [73] as evidenced by upregulation of gap junction connexin 43 [73], and altering electrophysiological properties [74]. Such changes suggest that glial cell activation in peripheral ganglia participates in nociception [75,76]. Furthermore, TNF-α expression increases in dorsal root ganglion which has been immunoreactively detected in SGCs and neuronal bodies after neuropathic hyperalgesia [77,78]. In fact, according to the literature, glial cells are able to produce pro-inflammatory substances in the face of insults, such as a nociceptive state [79,80]. They become reactive through proliferation, conformational change, and production of these pro-inflammatory agents. These events participate in the neuroplasticity process and normally occur in chronic conditions of injury with the goal of adjusting the tissue to the ongoing condition and ensure the system is sensitized [81].

The pro-inflammatory changes observed in our work in TG occurred after 14 days of inflammation that is indicative of the neuroplasticity installed in this structure. PAG was effective in controlling the inflammation and pain evoked by CFA on TMJs, not only blocking local alterations, but also modulating the information transmitted by peripheral sensory nerve endings. This proposal is further supported by the data found in the analysis of GFAP expression in TG. Our results evidenced that the increase of pro-inflammatory cytokines in this region induced by the inflammatory state in the TMJs at 14 days. This response was accompanied by an increase in activated SCGs. Similarly; the robust reduction in pro-inflammatory response caused by PAG locally was also reproduced for glial cells. In fact, elegant studies have suggested that SGCs are also involved in the peripheral mechanisms of pain facilitation. Morphologically, the cell bodies of TG neurons are completely surrounded by several SGCs, forming distinct functional

units [82]. Following an abnormally intense pain elicited by noxious stimuli, increased levels of GFAP, a marker of activated glia cells, can be detected [50]. The activated SGCs may directly influence neuronal activity by releasing inflammatory mediators such as IL-1β and TNF-α, contributing to the development and maintenance of allodynia and hyperalgesia responses [15]. Our suggestion is that the local H_2S acts on neuro-immune interactions inducing painful states from orofacial regions since its action was not restricted to local effects, but it also increases central pain sensitization by increasing the production of IL-1β and TNF-α in the TG and facilitating glial activation. This suggestion is sustained not only by the increase overproduction rate of H_2S, by also by the overexpression of CSE and CBS in both tissues.

Miao [8] observed that CBS overexpression in the trigeminal ganglia contributes to inflammatory pain in the TMJ. Here, we add the importance of TMJ CBS/H_2S pathway in the induction of persistent TMJ pain. It is also described in the literature that H_2S production is increased after inflammatory stimuli and thus, stimulates TRPV1 receptors which are intrinsically involved in central pain sensitization [15,83]. Our hypothesis is that CFA induces inflammation and, consequently, can increase H_2S production in the TMJ. This increase in H_2S levels activate TRPV1 receptors in the nerve sensory endings facilitating central pain sensitization leading to CBS and CSE overexpression in the trigeminal ganglia.

The analysis of H_2S production rate, CBS and CSE expression in both tissues, TMJ and TG are firstly described by the present data. Our evidences indicate an increased H_2S production rate in both structures after the temporomandibular inflammation stimulation and interestingly, PAG treatment prevented this elevation, strongly suggesting a modulation of H_2S as one of the mechanisms for temporomandibular nociception and indicating H_2S as a proinflammatory and pro-nociceptive endogenous gas for orofacial conditions.

5. Conclusion

For the first time, we elucidated one of the molecular mechanisms, acting in the nervous system, both locally and peripherally (TG), by which H_2S plays a role as a pro-inflammatory and pro-nociceptive endogenous gas for orofacial conditions. The pathophysiologic relevance of our findings is consistent in deepening the search for new therapeutic targets for TMDs.

Funding

This work was supported by the FAPESP [grant numbers #15/03053-3; #16/17681-9; #17/03645-3 and #16/09364-3, São Paulo Research Foundation]; CNPq and Capes.

Declaration of competing interest

We have no conflict of interest to declare.

Acknowledgements

We are grateful for Grant Thomas Harris' English writing support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.niox.2019.10.001.

References

[1] L.N. Melek, M. Devine, T. Renton, The psychosocial impact of orofacial pain in trigeminal neuralgia patients: a systematic review, Int. J. Oral Maxillofac. Surg. 47 (2018) 869–878, https://doi.org/10.1016/j.ijom.2018.02.006.

[2] M.J. Racich, Occlusion, temporomandibular disorders, and orofacial pain: an evidence-based overview and update with recommendations, J. Prosthet. Dent. 120 (2018) 678–685, https://doi.org/10.1016/j.prosdent.2018.01.033.

[3] O.C. Eller-Smith, A.L. Nicol, J.A. Christianson, Potential mechanisms underlying centralized pain and emerging therapeutic interventions, Front. Cell. Neurosci. 12 (2018) 35, https://doi.org/10.3389/fncel.2018.00035.

[4] M.M. Sperry, M.E. Ita, S. Kartha, S. Zhang, Y.-H. Yu, B. Winkelstein, The interface of mechanics and nociception in joint pathophysiology: insights from the facet and temporomandibular joints, J. Biomech. Eng. 139 (2017), https://doi.org/10.1115/1.4035647.

[5] M. V Hamity, R.Y. Walder, D.L. Hammond, Increased neuronal expression of neurokinin-1 receptor and stimulus-evoked internalization of the receptor in the rostral ventromedial medulla of the rat after peripheral inflammatory injury, J. Comp. Neurol. 522 (2014) 3037–3051, https://doi.org/10.1002/cne.23564.

[6] M. Llorián-Salvador, S. González-Rodríguez, A. Lastra, M.T. Fernández-García, A. Hidalgo, L. Menéndez, A. Baamonde, Involvement of CC chemokine receptor 1 and CCL3 in acute and chronic inflammatory pain in mice, Basic Clin. Pharmacol. Toxicol. 119 (2016) 32–40, https://doi.org/10.1111/bcpt.12543.

[7] H. Imbe, K. Iwata, Q.Q. Zhou, S. Zou, R. Dubner, K. Ren, Orofacial deep and cutaneous tissue inflammation and trigeminal neuronal activation. Implications for persistent temporomandibular pain, Cells Tissues Organs 169 (2001) 238–247, https://doi.org/10.1159/000047887.

[8] X. Miao, X. Meng, G. Wu, Z. Ju, H.-H. Zhang, S. Hu, G.-Y. Xu, Upregulation of cystathionine-β-synthetase expression contributes to inflammatory pain in rat temporomandibular joint, Mol. Pain 10 (2014) 9, https://doi.org/10.1186/1744-8069-10-9.

[9] G.C. do Nascimento, C.R.A. Leite-Panissi, Time-dependent analysis of nociception and anxiety-like behavior in rats submitted to persistent inflammation of the temporomandibular joint, Physiol. Behav. 125 (2014) 1–7, https://doi.org/10.1016/j.physbeh.2013.11.009.

[10] A.D. Cook, A.D. Christensen, D. Tewari, S.B. McMahon, J.A. Hamilton, Immune cytokines and their receptors in inflammatory pain, Trends Immunol. 39 (2018) 240–255, https://doi.org/10.1016/j.it.2017.12.003.

[11] S.F. Ahmad, M.A. Ansari, K.M.A. Zoheir, S.A. Bakheet, H.M. Korashy, A. Nadeem, A.E. Ashour, S.M. Attia, Regulation of TNF-α and NF-κB activation through the JAK/STAT signaling pathway downstream of histamine 4 receptor in a rat model of LPS-induced joint inflammation, Immunobiology 220 (2015) 889–898, https://doi.org/10.1016/j.imbio.2015.01.008.

[12] N. Yeremenko, K. Zwerina, G. Rigter, D. Pots, J.E. Fonseca, J. Zwerina, G. Schett, D. Baeten, Brief report: tumor necrosis factor and interleukin-6 differentially regulate dkk-1 in the inflamed arthritic joint, Arthritis Rheum. 67 (2015) 2071–2075, https://doi.org/10.1002/art.39183.

[13] S.V. Kellesarian, A.A. Al-Kheraif, F. Vohra, A. Ghanem, H. Malmstrom, G.E. Romanos, F. Javed, Cytokine profile in the synovial fluid of patients with temporomandibular joint disorders: a systematic review, Cytokine 77 (2016) 98–106, https://doi.org/10.1016/j.cyto.2015.11.005.

[14] J.W. Park, J.W. Chung, Inflammatory cytokines and sleep disturbance in patients with temporomandibular disorders, J. Oral Facial Pain Headache 30 (2016) 27–33, https://doi.org/10.11607/ofph.1367.

[15] F.A. Pinho-Ribeiro, W.A. Verri, I.M. Chiu, Nociceptor sensory neuron–immune interactions in pain and inflammation, Trends Immunol. 38 (2017) 5–19, https://doi.org/10.1016/j.it.2016.10.001.

[16] G. Villa, S. Ceruti, M. Zanardelli, G. Magni, L. Jasmin, P.T. Ohara, M.P. Abbracchio, Temporomandibular joint inflammation activates glial and immune cells in both the trigeminal ganglia and in the spinal trigeminal nucleus, Mol. Pain 6 (2010) 89, https://doi.org/10.1186/1744-8069-6-89.

[17] Q. Bai, S. Liu, H. Shu, Y. Tang, S. George, T. Dong, B.L. Schmidt, F. Tao, TNFα in the trigeminal nociceptive system is critical for temporomandibular joint pain, Mol. Neurobiol. 56 (2019) 278–291, https://doi.org/10.1007/s12035-018-1076-y.

[18] G.K. Kolluru, X. Shen, S.C. Bir, C.G. Kevil, Hydrogen sulfide chemical biology: pathophysiological roles and detection, Nitric Oxide Biol. Chem. 35 (2013) 5–20, https://doi.org/10.1016/j.niox.2013.07.002.

[19] L. Li, M. Bhatia, Y.Z. Zhu, Y.C. Zhu, R.D. Ramnath, Z.J. Wang, F.B.M. Anuar, M. Whiteman, M. Salto-Tellez, P.K. Moore, Hydrogen sulfide is a novel mediator of lipopolysaccharide-induced inflammation in the mouse, FASEB J. 19 (2005) 1196–1198, https://doi.org/10.1096/fj.04-3583fje.

[20] C. Szabó, Hydrogen sulphide and its therapeutic potential, Nat. Rev. Drug Discov. 6 (2007) 917–935, https://doi.org/10.1038/nrd2425.

[21] S. Fiorucci, S. Orlandi, A. Mencarelli, G. Caliendo, V. Santagada, E. Distrutti, L. Santucci, G. Cirino, J.L. Wallace, Enhanced activity of a hydrogen sulphide-releasing derivative of mesalamine (ATB-429) in a mouse model of colitis, Br. J. Pharmacol. 150 (2007) 996–1002, https://doi.org/10.1038/sj.bjp.0707193.

[22] M. Matsunami, T. Tarui, K. Mitani, K. Nagasawa, O. Fukushima, K. Okubo, S. Yoshida, M. Takemura, A. Kawabata, Luminal hydrogen sulfide plays a pronociceptive role in mouse colon, Gut 58 (2009) 751–761, https://doi.org/10.1136/gut.2007.144543.

[23] J.L. Wallace, J.G.P. Ferraz, M.N. Muscara, Hydrogen sulfide: an endogenous mediator of resolution of inflammation and injury, Antioxidants Redox Signal. 17 (2012) 58–67, https://doi.org/10.1089/ars.2011.4351.

[24] M. Bhatia, J. Sidhapuriwala, S.M. Moochhala, P.K. Moore, Hydrogen sulphide is a mediator of carrageenan-induced hindpaw oedema in the rat, Br. J. Pharmacol. 145 (2005) 141–144, https://doi.org/10.1038/sj.bjp.0706186.

[25] T.M. Cunha, W.A. Verri, Hydrogen sulfide, is it a promise analgesic drug or another inflammatory pain mediator? Pain 130 (2007) 300–302, https://doi.org/10.1016/j.pain.2007.04.036.

[26] G. Yang, L. Wu, B. Jiang, W. Yang, J. Qi, K. Cao, Q. Meng, A.K. Mustafa, W. Mu, S. Zhang, S.H. Snyder, R. Wang, H2S as a physiologic vasorelaxant: hypertension in

mice with deletion of cystathionine gamma-lyase, Science 322 (2008) 587–590, https://doi.org/10.1126/science.1162667.
[27] H. Zhang, L. Zhi, S.M. Moochhala, P.K. Moore, M. Bhatia, Endogenous hydrogen sulfide regulates leukocyte trafficking in cecal ligation and puncture-induced sepsis, J. Leukoc. Biol. 82 (2007) 894–905, https://doi.org/10.1189/jlb.0407237.
[28] A.F. Donatti, R.M. Araujo, R.N. Soriano, L.U. Azevedo, C.A. Leite-Panissi, L.G.S. Branco, Role of hydrogen sulfide in the formalin-induced orofacial pain in rats, Eur. J. Pharmacol. 738 (2014) 49–56, https://doi.org/10.1016/j.ejphar.2014.05.023.
[29] B.M. Santos, E.G. Garattini, L.G.S. Branco, C.R.A. Leite-Panissi, G.C. Nascimento, The therapeutic potential of cystathionine gamma-lyase in temporomandibular inflammation-induced orofacial hypernociception, Physiol. Behav. 188 (2018) 128–133, https://doi.org/10.1016/j.physbeh.2018.02.007.
[30] X. Feng, Y.-L. Zhou, X. Meng, F.-H. Qi, W. Chen, X. Jiang, G.-Y. Xu, Hydrogen sulfide increases excitability through suppression of sustained potassium channel currents of rat trigeminal ganglion neurons, Mol. Pain 9 (2013) 4, https://doi.org/10.1186/1744-8069-9-4.
[31] K.G. Usunoff, E. Marani, J.H. Schoen, The trigeminal system in man, Adv. Anat. Embryol. Cell Biol. 136 (I–X) (1997) 1–126.
[32] E. Marani, K.G. Usunoff, The trigeminal motonucleus in man, Arch. Physiol. Biochem. 106 (1998) 346–354, https://doi.org/10.1076/apab.106.5.346.4364.
[33] N.E. Lazarov, The mesencephalic trigeminal nucleus in the cat, Adv. Anat. Embryol. Cell Biol. 153 (2000) 1–103 iii–xiv.
[34] N.E. Lazarov, Comparative analysis of the chemical neuroanatomy of the mammalian trigeminal ganglion and mesencephalic trigeminal nucleus, Prog. Neurobiol. 66 (2002) 19–59 http://www.ncbi.nlm.nih.gov/pubmed/11897404 , Accessed date: 17 September 2019.
[35] N.F. Copra, K.V. Anderson, R.C. Atkinson, Localization and morphometric analysis of masticatory muscle afferent neurons in the nucleus of the mesencephalic root of the trigeminal nerve in the cat, Cells Tissues Organs 122 (1985) 115–125, https://doi.org/10.1159/000145992.
[36] Y. Shigenaga, M. Sera, T. Nishimori, S. Suemune, M. Nishimura, A. Yoshida, K. Tsuru, The central projection of masticatory afferent fibers to the trigeminal sensory nuclear complex and upper cervical spinal cord, J. Comp. Neurol. 268 (1988) 489–507, https://doi.org/10.1002/cne.902680403.
[37] R.N. Soriano, S.P. Braga, J.S.C. Breder, M.E. Batalhao, G.R. Oliveira-Pelegrin, L.F.R. Ferreira, M.J.A. Rocha, E.C. Carnio, L.G.S. Branco, Endogenous peripheral hydrogen sulfide is propyretic: its permissive role in brown adipose tissue thermogenesis in rats, Exp. Physiol. 103 (2018) 397–407, https://doi.org/10.1113/EP086775.
[38] X. Zhou, S. Tang, K. Hu, Z. Zhang, P. Liu, Y. Luo, J. Kang, L. Xu, dl-Propargylglycine protects against myocardial injury induced by chronic intermittent hypoxia through inhibition of endoplasmic reticulum stress, Sleep Breath. 22 (2018) 853–863, https://doi.org/10.1007/s11325-018-1656-0.
[39] T.M. Cunha, D. Dal-Secco, W.A. Verri, A.T. Guerrero, G.R. Souza, S.M. Vieira, C.M. Lotufo, A.F. Neto, S.H. Ferreira, F.Q. Cunha, Dual role of hydrogen sulfide in mechanical inflammatory hypernociception, Eur. J. Pharmacol. 590 (2008) 127–135, https://doi.org/10.1016/j.ejphar.2008.05.048.
[40] R.P. Harper, C.A. Kerins, J.E. McIntosh, R. Spears, L.L. Bellinger, Modulation of the inflammatory response in the rat TMJ with increasing doses of complete Freund's adjuvant, Osteoarthr. Cartil. 9 (2001) 619–624, https://doi.org/10.1053/joca.2001.0461.
[41] Q. Zhou, H. Imbe, R. Dubner, K. Ren, Persistent Fos protein expression after orofacial deep or cutaneous tissue inflammation in rats: implications for persistent orofacial pain, J. Comp. Neurol. 412 (1999) 276–291, https://doi.org/10.1002/(sici)1096-9861(19990920)412:2<276::aid-cne7>3.0.co;2-9.
[42] T.S. Grabow, P.M. Dougherty, Gabapentin produces dose-dependent antinociception in the orofacial formalin test in the rat., Reg. Anesth. Pain Med. 27 (n.d.) 277-283. http://www.ncbi.nlm.nih.gov/pubmed/12016601 (accessed September 18, 2019).
[43] F.F.B. de Oliveira, J.C.B. de Araújo, A.F. Pereira, G.A.C. Brito, D.V. Gondim, R. de A. Ribeiro, I.R.A. de Menezes, M.L. Vale, Antinociceptive and anti-inflammatory effects of Caryocar coriaceum Wittm fruit pulp fixed ethyl acetate extract on zymosan-induced arthritis in rats, J. Ethnopharmacol. 174 (2015) 452–463, https://doi.org/10.1016/j.jep.2015.08.017.
[44] K. Koroleva, A. Mustafina, A. Yakovlev, A. Hermann, R. Giniatullin, G. Sitdikova, Receptor mechanisms mediating the pro-nociceptive action of hydrogen sulfide in rat trigeminal neurons and meningeal afferents, Front. Cell. Neurosci. 11 (2017), https://doi.org/10.3389/fncel.2017.00226.
[45] A.T.G. Guerrero, W.A. Verri, T.M. Cunha, T.A. Silva, I.R.S. Schivo, D. Dal-Secco, C. Canetti, F.A.C. Rocha, C.A. Parada, F.Q. Cunha, S.H. Ferreira, Involvement of LTB$_4$ in zymosan-induced joint nociception in mice: participation of neutrophils and PGE$_2$, J. Leukoc. Biol. 83 (2008) 122–130, https://doi.org/10.1189/jlb.0207123.
[46] J.D. Levine, J. Gooding, P. Donatoni, L. Borden, E.J. Goetzl, The role of the polymorphonuclear leukocyte in hyperalgesia, J. Neurosci. 5 (1985) 3025–3029 http://www.ncbi.nlm.nih.gov/pubmed/2997412 , Accessed date: 17 September 2019.
[47] T.M. Cunha, W.A. Verri, I.R. Schivo, M.H. Napimoga, C.A. Parada, S. Poole, M.M. Teixeira, S.H. Ferreira, F.Q. Cunha, Crucial role of neutrophils in the development of mechanical inflammatory hypernociception, J. Leukoc. Biol. 83 (2008) 824–832, https://doi.org/10.1189/jlb.0907654.
[48] M. Bhatia, Hydrogen sulfide as a vasodilator, IUBMB Life 57 (2005) 603–606, https://doi.org/10.1080/15216540500217875.
[49] V. Wild, K. Messlinger, M.J.M. Fischer, Hydrogen sulfide determines HNO-induced stimulation of trigeminal afferents, Neurosci. Lett. 602 (2015) 104–109, https://doi.org/10.1016/j.neulet.2015.06.056.
[50] S. Matsuura, K. Shimizu, M. Shinoda, K. Ohara, B. Ogiso, K. Honda, A. Katagiri, B.J. Sessle, K. Urata, K. Iwata, Mechanisms underlying ectopic persistent tooth-pulp pain following pulpal inflammation, PLoS One 8 (2013), https://doi.org/10.1371/journal.pone.0052840.
[51] R. Ito, M. Shinoda, K. Honda, K. Urata, J. Lee, M. Maruno, K. Soma, S. Okada, N. Gionhaku, K. Iwata, Tumor necrosis factor Alpha signaling in trigeminal ganglion contributes to mechanical hypersensitivity in masseter muscle during temporomandibular joint inflammation., J. Oral Facial Pain Headache. 32 (n.d.) 75–83. doi:10.11607/ofph.1854.
[52] H. Zhang, L. Zhi, S.M. Moochhala, P.K. Moore, M. Bhatia, Endogenous hydrogen sulfide regulates leukocyte trafficking in cecal ligation and puncture-induced sepsis, J. Leukoc. Biol. 82 (2007) 894–905, https://doi.org/10.1189/jlb.0407237.
[53] K. Koga, S. Shimoyama, A. Yamada, T. Furukawa, Y. Nikaido, H. Furue, K. Nakamura, S. Ueno, Chronic inflammatory pain induced GABAergic synaptic plasticity in the adult mouse anterior cingulate cortex, Mol. Pain 14 (2018), https://doi.org/10.1177/1744806918783478.
[54] U. Snekhalatha, M. Anburajan, B. Venkatraman, M. Menaka, Evaluation of complete Freund's adjuvant-induced arthritis in a Wistar rat model, Z. Rheumatol. 72 (2013) 375–382, https://doi.org/10.1007/s00393-012-1083-8.
[55] S.B, Stephen B. McMahon, Wall and Melzack's Textbook of Pain, Elsevier/Saunders, 2013.
[56] A.C. Desiderá, G.C. Nascimento, R.F. Gerlach, C.R.A. Leite-Panissi, Laser therapy reduces gelatinolytic activity in the rat trigeminal ganglion during temporomandibular joint inflammation, Oral Dis. 21 (2015) 652–658, https://doi.org/10.1111/odi.12330.
[57] J.R. Ghilardi, K.T. Freeman, J.M. Jimenez-Andrade, K.A. Coughlin, M.J. Kaczmarska, G. Castaneda-Corral, A.P. Bloom, M.A. Kuskowski, P.W. Mantyh, Neuroplasticity of sensory and sympathetic nerve fibers in a mouse model of a painful arthritic joint, Arthritis Rheum. 64 (2012) 2223–2232, https://doi.org/10.1002/art.34385.
[58] P.R. Kramer, C.A. Kerins, E. Schneiderman, L.L. Bellinger, Measuring persistent temporomandibular joint nociception in rats and two mice strains, Physiol. Behav. 99 (2010) 669–678, https://doi.org/10.1016/j.physbeh.2010.01.037.
[59] G.A. Lemos, R. Rissi, I.L. de Souza Pires, L.P. de Oliveira, A.A. de Aro, E.R. Pimentel, E.T. Palomari, Low-level laser therapy stimulates tissue repair and reduces the extracellular matrix degradation in rats with induced arthritis in the temporomandibular joint, Lasers Med. Sci. 31 (2016) 1051–1059, https://doi.org/10.1007/s10103-016-1946-3.
[60] A.E. Dief, D.K. Mostafa, G.M. Sharara, T.H. Zeitoun, Hydrogen sulfide releasing naproxen offers better anti-inflammatory and chondroprotective effect relative to naproxen in a rat model of zymosan induced arthritis, Eur. Rev. Med. Pharmacol. Sci. 19 (2015) 1537–1546 http://www.ncbi.nlm.nih.gov/pubmed/25967731 , Accessed date: 17 September 2019.
[61] B. Andruski, D.M. McCafferty, T. Ignacy, B. Millen, J.J. McDougall, Leukocyte trafficking and pain behavioral responses to a hydrogen sulfide donor in acute monoarthritis, Am. J. Physiol. Regul. Integr. Comp. Physiol. 295 (2008), https://doi.org/10.1152/ajpregu.90524.2008.
[62] P.G. Winyard, D.A. Willoughby, Inflammation Protocols, Humana Press, 2003, https://doi.org/10.1385/1592593747.
[63] Y.-J. Gao, L. Zhang, O.A. Samad, M.R. Suter, K. Yasuhiko, Z.-Z. Xu, J.-Y. Park, A.-L. Lind, Q. Ma, R.-R. Ji, JNK-induced MCP-1 production in spinal cord astrocytes contributes to central sensitization and neuropathic pain, J. Neurosci. 29 (2009) 4096–4108, https://doi.org/10.1523/JNEUROSCI.3623-08.2009.
[64] P.C. Cordeiro, J.P. Guimaraes, V.A. de Souza, I.M. Dias, J.N. Silva, K.L. Devito, L.L. Bonato, Temporomandibular joint involvement in rheumatoid arthritis patients: association between clinical and tomographic data, Acta Odontol. Latinoam. 29 (2016) 123–129.
[65] R. de Leeuw, Internal derangements of the temporomandibular joint, Oral Maxillofac. Surg. Clin. N. Am. 20 (2008) 159–168, https://doi.org/10.1016/j.coms.2007.12.004.
[66] M. Kobayashi, G.R. Squires, A. Mousa, M. Tanzer, D.J. Zukor, J. Antoniou, U. Feige, A.R. Poole, Role of interleukin-1 and tumor necrosis factor alpha in matrix degradation of human osteoarthritic cartilage, Arthritis Rheum. 52 (2005) 128–135, https://doi.org/10.1002/art.20776.
[67] D.-H. Wang, M.-C. Yang, W.-E. Hsu, M.-L. Hsu, L.-M. Yu, Response of the temporomandibular joint tissue of rats to rheumatoid arthritis induction methods, J. Dent. Sci. 12 (2017) 83–90, https://doi.org/10.1016/J.JDS.2016.12.001.
[68] R. Spears, L.A. Dees, M. Sapozhnikov, L.L. Bellinger, B. Hutchins, Temporal changes in inflammatory mediator concentrations in an adjuvant model of temporomandibular joint inflammation, J. Orofac. Pain 19 (2005) 34–40 http://www.ncbi.nlm.nih.gov/pubmed/15779537 , Accessed date: 17 September 2019.
[69] H.D.C. Francescato, J.R.A. Chierice, E.C.S. Marin, F.Q. Cunha, R.S. Costa, C.G.A. Silva, T.M. Coimbra, Effect of endogenous hydrogen sulfide inhibition on structural and functional renal disturbances induced by gentamicin, Braz. J. Med. Biol. Res. Rev. Bras. Pesqui. Med. Biol. 45 (2012) 244–249, https://doi.org/10.1590/s0100-879x2012007500016.
[70] E. Bałkowiec-Iskra, A. Vermehren-Schmaedick, A. Balkowiec, Tumor necrosis factor-α increases brain-derived neurotrophic factor expression in trigeminal ganglion neurons in an activity-dependent manner, Neuroscience 180 (2011) 322–333, https://doi.org/10.1016/j.neuroscience.2011.02.028.
[71] M. Kandasamy, L. Aigner, Neuroplasticity, limbic neuroblastosis and neuro-regenerative disorders, Neural Regen. Res. 13 (2018) 1322–1326, https://doi.org/10.4103/1673-5374.235214.
[72] R.R. Ji, T. Berta, M. Nedergaard, Glia and pain: is chronic pain a gliopathy? Pain, Elsevier B.V., 2013, , https://doi.org/10.1016/j.pain.2013.06.022.
[73] P.T. Ohara, J.-P. Vit, A. Bhargava, L. Jasmin, Evidence for a role of connexin 43 in trigeminal pain using RNA interference in vivo, J. Neurophysiol. 100 (2008)

3064–3073, https://doi.org/10.1152/jn.90722.2008.
[74] H. Zhang, X. Mei, P. Zhang, C. Ma, F.A. White, D.F. Donnelly, R.H. Lamotte, Altered functional properties of satellite glial cells in compressed spinal ganglia, Glia 57 (2009) 1588–1599, https://doi.org/10.1002/glia.20872.
[75] C.-C. Chen, J.-W. Shen, N.-C. Chung, M.-Y. Min, S.-J. Cheng, I.Y. Liu, Retrieval of context-associated memory is dependent on the Ca(v)3.2 T-type calcium channel, PLoS One 7 (2012) e29384, , https://doi.org/10.1371/journal.pone.0029384.
[76] P. Dublin, M. Hanani, Satellite glial cells in sensory ganglia: their possible contribution to inflammatory pain, Brain Behav. Immun. 21 (2007) 592–598, https://doi.org/10.1016/j.bbi.2006.11.011.
[77] P. Dubový, L. Tučková, R. Jančálek, I. Svíženská, I. Klusáková, Increased invasion of ED-1 positive macrophages in both ipsi- and contralateral dorsal root ganglia following unilateral nerve injuries, Neurosci. Lett. 427 (2007) 88–93, https://doi.org/10.1016/J.NEULET.2007.09.012.
[78] M. Schäfers, C. Geis, D. Brors, T.L. Yaksh, C. Sommer, Anterograde transport of tumor necrosis factor-alpha in the intact and injured rat sciatic nerve, J. Neurosci. 22 (2002) 536–545 http://www.ncbi.nlm.nih.gov/pubmed/11784800 , Accessed date: 17 September 2019.
[79] S. Kadhim, J. McDonald, D.G. Lambert, Opioids, gliosis and central immunomodulation, J. Anesth. 32 (2018) 756–767, https://doi.org/10.1007/s00540-018-2534-4.
[80] E. Hansson, Long-term pain, neuroinflammation and glial activation, Scand. J. Pain 1 (2010) 67–72, https://doi.org/10.1016/j.sjpain.2010.01.002.
[81] G.K. Sheridan, K.J. Murphy, Neuron-glia crosstalk in health and disease: fractalkine and CX3CR1 take centre stage, Open Biol. 3 (2013), https://doi.org/10.1098/rsob.130181 130181–130181.
[82] K. Fried, U. Bongenhielm, F.M. Boissonade, P.P. Robinson, Nerve Injury-!Induced pain in the trigeminal system, Neuroscience 7 (2001) 155–165, https://doi.org/10.1177/107385840100700210.
[83] M. Trevisani, R. Patacchini, P. Nicoletti, R. Gatti, D. Gazzieri, N. Lissi, G. Zagli, C. Creminon, P. Geppetti, S. Harrison, Hydrogen sulfide causes vanilloid receptor 1-mediated neurogenic inflammation in the airways, Br. J. Pharmacol. 145 (2005) 1123–1131, https://doi.org/10.1038/sj.bjp.0706277.