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# Effect of dural inflammatory soup application on activation and sensitization markers in the caudal trigeminal nucleus of the rat and the modulatory effects of sumatriptan and kynurenic acid

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## Abstract

**Background:** The topical inflammatory soup can model the inflammation of the dura mater causing hypersensitivity and activation of the trigeminal system, a phenomenon present in migraineurs. Calcitonin gene-related peptide, transient receptor potential vanilloid-1 receptor, and neuronal nitric oxide synthase are important in the sensitization process there.

5-HT<sub>1B/1D</sub> receptor agonists, triptans are used as a treatment of migraine. Kynurenic acid an NMDA antagonist can act on structures involved in trigeminal activation.

**Aim:** We investigated the effect of inflammatory soup induced dural inflammation on the calcitonin gene-related peptide, transient receptor potential vanilloid-1 receptor, and neuronal nitric oxide synthase levels in the caudal trigeminal nucleus. We also tested whether pretreatment with a well-known antimigraine drug, such as sumatriptan and kynurenic acid, a compound with a different mechanism of action, can affect these changes and if their modulatory effects are comparable.

**Material and methods:** After subcutaneous sumatriptan or intraperitoneal kynurenic acid the dura mater of adult male Sprague-Dawley rats ( $n = 72$ ) was treated with inflammatory soup or its vehicle (synthetic interstitial fluid). Two and a half or four hours later perfusion was performed and the caudal trigeminal nucleus was removed for immunohistochemistry.

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**Results and conclusion:** Inflammatory soup increased calcitonin gene-related peptide, transient receptor potential vanilloid-1 receptor, and neuronal nitric oxide synthase in the caudal trigeminal nucleus compared to placebo, which was attenuated by sumatriptan and kynurenic acid. This suggests the involvement of 5-HT<sub>1B/1D</sub> and NMDA receptors in neurogenic inflammation development of the dura and thus in migraine attacks.

**Keywords:** Migraine, Trigeminal system, Inflammatory soup, Sumatriptan, Kynurenic acid, CGRP, TRPV1, nNOS

### Article highlights or key findings

Inflammatory soup can cause an increase in the CGRP, TRPV1, and nNOS levels in the TNC.

Sumatriptan was able to mitigate the effect of inflammatory soup.

Kynurenic acid could modulate the effect of inflammatory soup.

### Introduction

Migraine is a common condition affecting up to 15% of the world's population [1]. The pathomechanism of this disorder is not fully understood, but the sterile neurogenic inflammation of the dura mater and the activation and sensitization of the trigeminal system (TS) play a crucial role in the attack [2].

In animal models, stimulation of trigeminal nerves causes a release of neuropeptides, resulting in meningeal blood vessel dilation, plasma extravasation, platelet activation, and mast cell degranulation characteristic of neurogenic inflammation [3]. Calcitonin gene-related peptide (CGRP) is a multifunctional regulatory neuropeptide [4] and a key player in migraine: Serum concentrations of CGRP are elevated during the attack [5] whereas intravenous infusion of CGRP can induce a migraine-like headache in migraineurs [6]. In a rat model of migraine, electrical stimulation of the trigeminal ganglion was able to increase blood flow on the same side of the facial skin which was reduced by intravenous administration of the CGRP antagonist, CGRP 8–37 [7]. Transcranial electrical stimulation in rats was able to cause CGRP release and vasodilation, which was prevented by olcegepant, a CGRP receptor blocker [8]. These results are in line with the clinical data showing that CGRP antagonists are effective in the acute treatment of this disease [9–11]. On the other hand, there are other pathways involved in neurogenic inflammation which are not directly related to CGRP e.g. the appearance of cortical spreading depression might also contribute to this phenomenon [12].

Transient receptor potential vanilloid-1 receptor (TRPV1), a nonselective cation channel, a molecular component of pain detection and modulation [13], is selectively expressed by small- to medium-diameter neurons within the dorsal root ganglion (DRG) and trigeminal ganglia (TG), co-localized with CGRP in

the latter [14]. TRPV1 activation leads to the release of neuropeptides, such as substance P and CGRP. These neuropeptides cause vasodilation and initiate neurogenic inflammation within the meninges under experimental conditions [15].

The synthesis of nitric oxide (NO) is catalyzed by neuronal nitric oxide synthase (nNOS), which can be found in the superficial layers of the dorsal horn of the spinal cord underlining its importance in the trigeminal pain processing [16]. Furthermore, its presence is confirmed in dural mast cells, trigeminal nerve endings, and Gasserian ganglion cells [17]. Systemic administration of nitroglycerin (NTG), a nitric oxide donor can induce an immediate headache and in migraine patients, this is followed by a typical migraine attack without aura [18]. The immediate headache can be explained by the vasodilatory effect of NO, which activates the dural nociceptors, while the delayed headache might be mainly due to an effect of NO on central nociceptors, causing a long-lasting endogenous synthesis of NO by enhancing nNOS resulting in central sensitization process [19].

Triptans are used to relieve migraine attacks, being an agonist on 5-hydroxytryptamine receptors (5-HT<sub>1B/1D</sub>), they can cause the constriction of dilated cranial arteries and the inhibition of CGRP release [20]. They block the depolarization of the trigeminal nerves and inhibit the neurotransmission at the level of interneurons of TNC [21]. In an animal model of migraine, after creating neurogenic inflammation in the dura mater, triptans were able to reduce the plasma protein extravasation, probably by inhibiting nociceptor activation and preventing neuropeptide release [5]. The increase of jugular vein CGRP concentration after the stimulation of the TG can be reduced by sumatriptan [5, 22]. In the rat model of trigeminal neuropathic pain, triptans can selectively inhibit nociceptive [23] and neuropathic pain behavior [24] and evoked activity in trigeminal dorsal horn neurons [21] in response to noxious stimulation of the trigeminal nerve area. In the TG, 5-HT<sub>1B/1D</sub> receptors and glutamate were co-localized in several neurons [25], thus triptans may modulate glutamate release from trigeminal neurons through the 5-HT<sub>1B/1D</sub> receptors and possibly reduce pain [24].

Kynurenic acid (KYNA) is a neuroactive product of the kynurenine pathway of tryptophan metabolism,

which can exert its effect through N-methyl-D-aspartate (NMDA),  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), kainate receptors, and G-protein coupled receptor 35 (GPR35), and these receptors have a relevant role in pain processing and neuroinflammation [26]. A previous study suggests that KYNA has an anti-inflammatory effect on the TS [27]. In rats, KYNA had an analgesic effect in tail-flick test [28]. In an *in vivo* model of acetic acid-induced inflammatory pain, L-kynurenine, which is a precursor of KYNA, caused rise in the KYNA levels in the plasma and the central nervous system (CNS), thereby, was able to elicit antinociceptive effect [29].

Based on the results of clinical trials and animal experiments, trigeminal activation and sensitization occur during the migraine attack. Local administration of inflammatory soup (IS) on the dura mimics this process [30], which might be characterized by the alteration of selected molecular markers. On the other hand, since sumatriptan is effective in the acute treatment it may prevent these alterations and we wanted to compare its possible modulatory effects with a compound with a different pharmacological mechanism of action.

Thus, in our present study, we investigated the effect of IS induced dural inflammation on markers of the sensitization process in the trigemino-cervical complex, namely: CGRP, TRPV1, and nNOS. We also tested whether pretreatment with sumatriptan or KYNA has an effect on the IS induced changes.

## Materials and methods

### Animals

The procedures used in our study were approved by the Committee of the Animal Research of University of Szeged (I-74-49/2017) and the Scientific Ethics Committee for Animal Research of the Protection of Animals Advisory Board (XL/1098/2018) and followed the guidelines the Use of Animals in Research of the International Association for the Study of Pain and the directive of the European Parliament (2010/63/EU).

Seventy-two adult male Sprague-Dawley rats weighing 350–400 g were used. The animals were raised and maintained under standard laboratory conditions with tap water and regular rat chow available *ad libitum* on a 12 h dark-12 h light cycle.

### Drug administration

The animals were divided into two groups of 6 rats ( $n = 6$  per group for 2.5 h and  $n = 6$  per group for 4 h). The animals were deeply anesthetized with an intraperitoneal injection of 4% chloral hydrate (0.4 g/kg body weight, Sigma-Aldrich). The head of the animal was fixed in a stereotaxic frame and lidocaine (10 mg/ml, Egis) infiltration on the skull was used before the

interventions. A handheld drill was used to make a window on the skull. The hole was made posterolaterally (5 mm, 3 mm) to the bregma, on the right side without penetrating the dura mater [31].

The animals in the first group called the placebo group, received synthetic interstitial fluid, (SIF, 135 mM NaCl, 5 mM KCl, 1 mM MgCl<sub>2</sub>, 5 mM CaCl<sub>2</sub>, 10 mM glucose in 10 mM HEPES buffer, pH 7.3). In the second group, we applied inflammatory soup (IS, 1 mM bradykinin, 1 mM serotonin, 1 mM histamine, 0.1 mM prostaglandin in 10 mM HEPES buffer, pH 5.0) on the dural surface. In the third and fourth groups, the animals received subcutaneous sumatriptan (0.6 mg/kg) 10 min before the SIF or IS treatment, while in the fifth and sixth groups received intraperitoneal KYNA (189.17 mg/kg) pretreatment one hour before treatment. Both pretreatment protocols were based on the pharmacological properties of the substances. Sumatriptan has a short half-life, its receptor binding is reversible and the onset of action is 10–15 min after administration. The half-life time of KYNA is about an hour. The dosage we used for both molecules was chosen based on previous reports [32–34]. Two and a half hours or four hours after the SIF or IS administration, the trigemino-cervical complex was processed for immunohistochemistry. Two survival times were used to examine changes over time.

### Immunohistochemistry

Two and a half hours or four hours after the SIF or IS administration, the rats were perfused transcardially with 50 ml phosphate-buffered saline (PBS, 0.1 M, pH 7.4), followed by 200 ml 4% paraformaldehyde in phosphate buffer under chloral hydrate anesthesia, and the trigemino-cervical complex was removed and postfixed overnight for immunohistochemistry in the same fixative. After cryoprotection, 30  $\mu$ m cryostat sections were cut and serially collected in wells containing cold PBS. The free-floating sections were rinsed in PBS and immersed in 0.3% H<sub>2</sub>O<sub>2</sub> in methanol (CGRP staining) or PBS (nNOS and TRPV1 staining) for 30 min. After several rinses in PBS containing 1% Triton X-100, sections were kept overnight at room temperature in anti-CGRP antibody (Sigma, C8198) at a dilution of 1:20000, or TRPV1 antibody (Santa Cruz, s.c.28759) at a dilution of 1:1000, or for two nights at 4 °C in anti-nNOS antibody (EuroProxima, 2263B220–1) at a dilution of 1:5000. The immunohistochemical reaction was visualized by the Vectastain Avidin-Biotin kit of Vector Laboratories (PK6101), and nickel ammonium sulfate-intensified 3,3'-diaminobenzidine. Control experiments included the omission of the primary antisera.

### Data evaluation

All evaluations were performed by an observer blind to the experimental groups. The photomicrographs of the stained sections of trigemino-cervical complex were taken using a Zeiss AxioImager microscope supplied with an AxioCam MRc Rev. 3 camera (Carl Zeiss Microscopy). The area covered by TRPV1-immunoreactive and CGRP-immunoreactive fibers was determined by Image Pro Plus 6.2<sup>®</sup> image analysis software (Media Cybernetics). After image acquisition, the laminae I–II in the dorsal horn were defined manually as areas of interest, and a threshold gray level was validated with the image analysis software. The program calculated the area innervated by the immunoreactive fibers as the number of pixels with densities above the threshold; the data were expressed as area fractions (%) of the corresponding immunolabelled structures. We measured the covered area by the CGRP and TRPV1 immunoreactive fibers and counted the nNOS immunoreactive cells in the area of the dorsal horn innervated by the ophthalmic nerve (V/1 area).

### Statistical analysis

The Shapiro-Wilk test was used to determine the distribution of data. As our data followed a normal distribution in each case, the differences among the groups and sides were examined with a mixed ANOVA model. The pairwise comparisons were performed by paired and independent samples t-tests with Sidak corrections. All statistical analyses were performed using SPSS version 24.0 (IBM Corporation). Values  $p < 0.05$  were considered statistically significant. Our data are reported as means + SEM for all parameters and groups.

## Results

### Inflammatory soup and CGRP

In the dorsal horn, CGRP immunoreactive (IR) axon fibers were distributed in the laminae I and II. IS treatment was able to increase the amount of the area covered by fibers showing CGRP positivity in both time points (Figs. 1, 2, and 3). Sumatriptan and KYNA pretreatments were able to attenuate this effect (Fig. 3). There was no relevant difference between the two time points in the area covered by fibers showing CGRP positivity.

### Inflammatory soup and TRPV1

After 2.5 h there was no significant difference between the IS treated and placebo group (data not shown), but after 4 h we observed a significant increase in the amount of the TRPV1 IR fibers in the IS-treated group, compared to the placebo. Sumatriptan and KYNA pretreatments were able to mitigate the effect of the IS treatment (Fig. 4, Fig. 6).

### Inflammatory soup and nNOS

A significant increase of nNOS was observed only after 4 h in the IS treated group compared to the placebo group in the V/1 area. Sumatriptan and KYNA pretreatments were able to modulate the effect of IS administration (Fig. 5, Fig. 6).

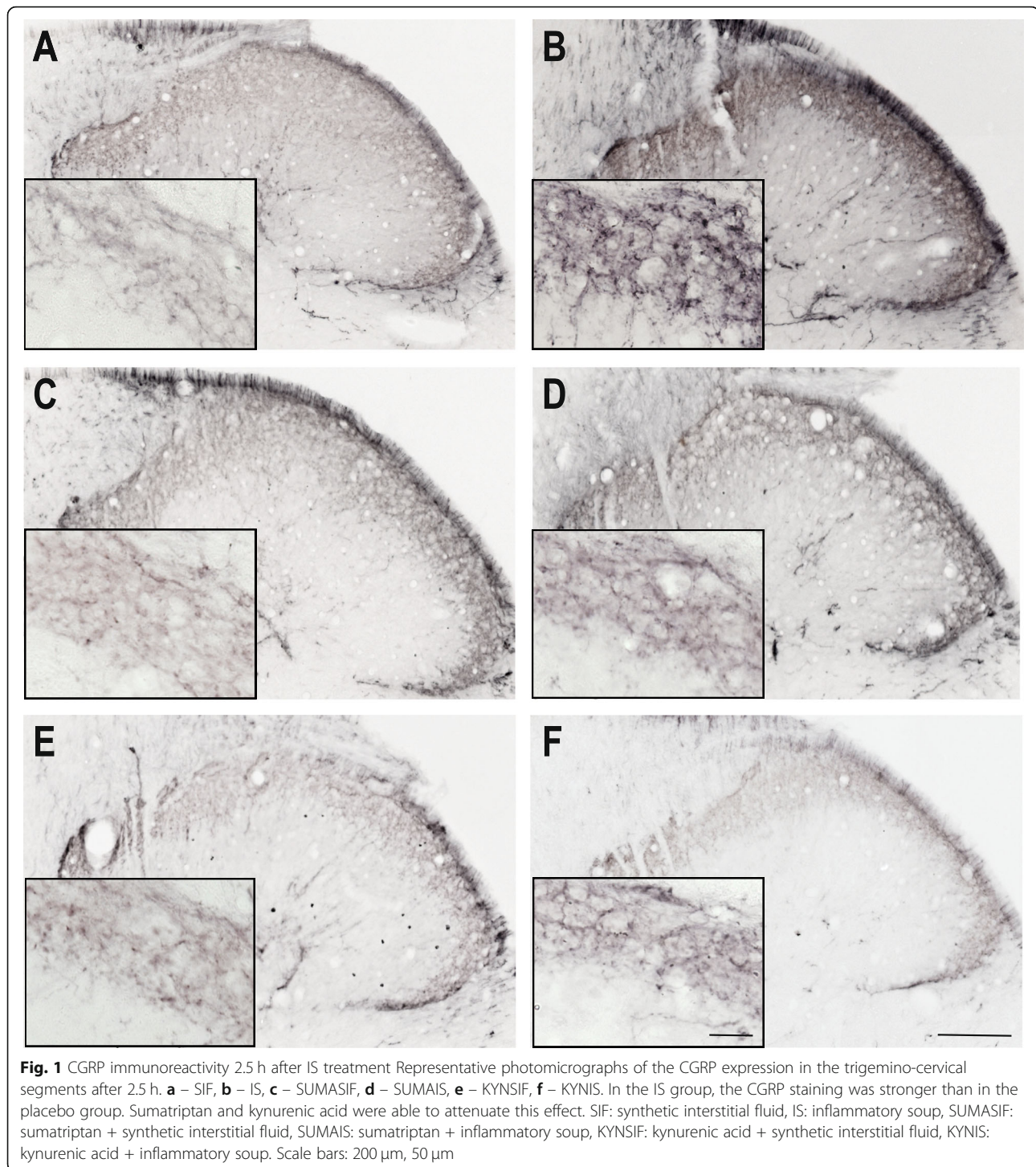
## Discussion

The activation and sensitization of the TS are essential hallmarks of migraine pathomechanism. In our rat model, the topically applied IS activated the trigemino-vascular system [35, 36], and raised the levels of all the three selected markers in the TNC area. Although other pathways, neurotransmitters (e.g. glutamate, 5HT, prostaglandins) and mechanisms might be involved, the release of neuropeptides from the activated peripheral nociceptive terminals may contribute to the development of neurogenic inflammation in this case [3]. These peptides lead to a cascade of inflammatory tissue responses including vasodilation, plasma protein extravasation, and degranulation of mast cells [3], at least in rats.

In our study, as early as two and a half hours after administration, the IS was able to increase the area covered by fibers showing CGRP positivity in the dorsal horn of the cervical spinal cord. The cranial dura mater is densely innervated by CGRP IR fibers [37] thus the increased CGRP level might represent enhanced activation of the primary afferents, which may also be associated with increased CGRP release from the terminals [9] possibly causing a globally higher turnover e.g. intensive synthesis reflected by higher CGRP expression at the terminals [38]. It has been also shown, that intracisternal IS can raise the CGRP concentration in the jugular vein which also reflects release from the nerve endings [39]. This phenomenon might contribute to the activation and sensitization of primary and secondary nociceptors in the TS via the release of numerous pro-inflammatory agents (e.g. cytokines), which can stimulate the nociceptors [40].

In our experiment, IS was able to significantly increase the amount of TRPV1 IR fibers in the dorsal horn after 4 h. Amaya and colleagues described that TRPV1 expression is showing up-regulation in DRG neurons after local inflammation in rats [41]. TRPV1 activation by high temperature or capsaicin allows the entry of  $Ca^{2+}$ , leading to the release of neuropeptides [42, 43], which are able to influence the development of edema and neurogenic inflammation [44, 45]. In another study, after CFA-induced inflammation, increased TRPV1 expression was observed in the digital nerves of the inflamed hindpaw [46]. Pharmacologic studies have also shown that TRPV1 is an essential component of the cellular

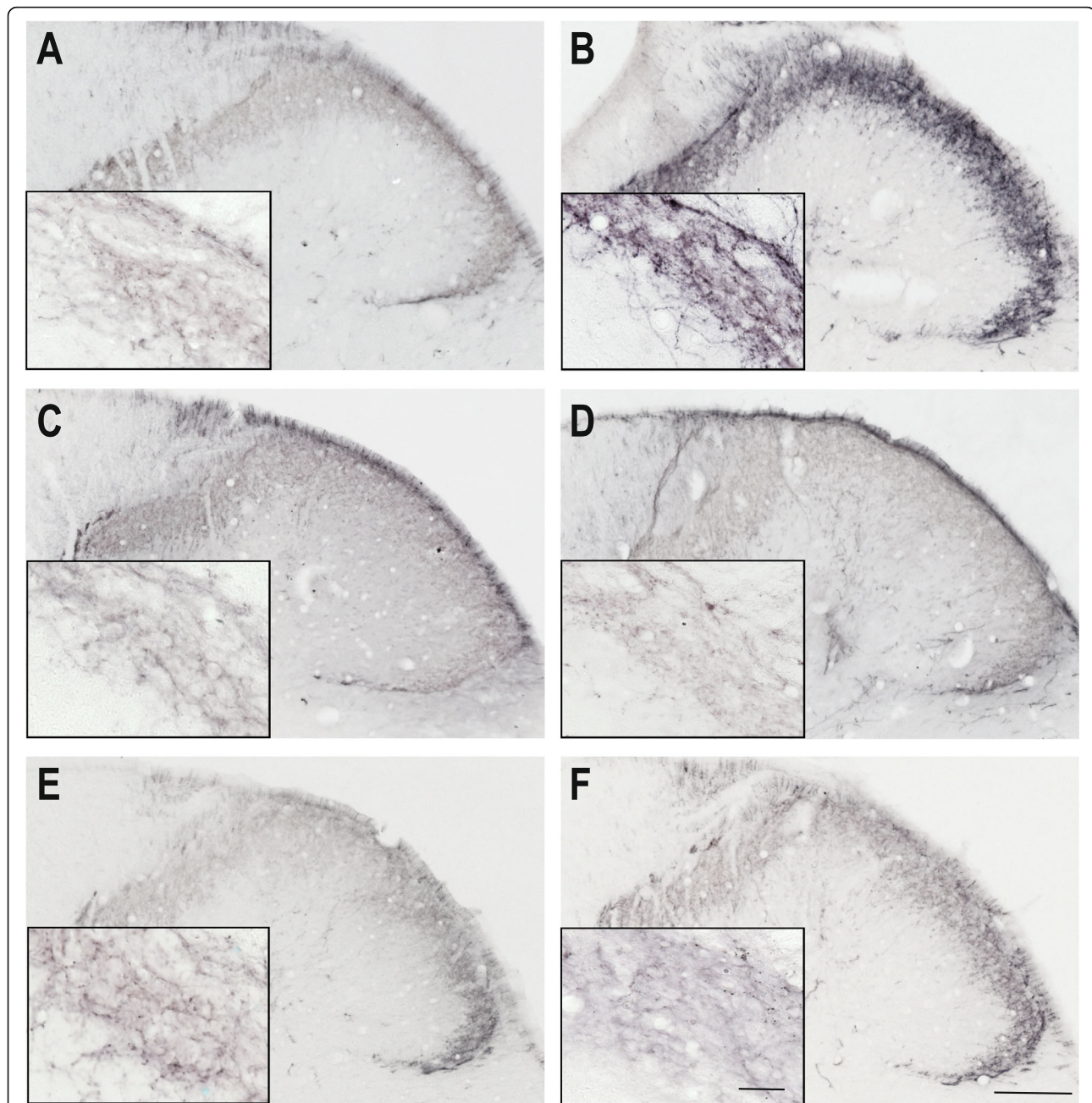




signaling mechanisms through which injury produces thermal hyperalgesia and pain hypersensitivity [47].

TRPV1 is present in the human TG [48] and trigeminal afferents, which innervate the dura mater [49], and these nerve fibers also contain CGRP [50]. TRPV1 expression is also upregulated in painful inflammatory conditions in humans [51]. In chronic migraine patients,

intranasal capsaicin was able to mitigate the migraine pain [52] and TRPV1 agonists might be effective most likely due to desensitization in the acute treatment of migraine [53] as well. Taken together these data point to the fact, that TRPV1 is involved in the trigeminovascular activation and sensitization both in animal models and humans.

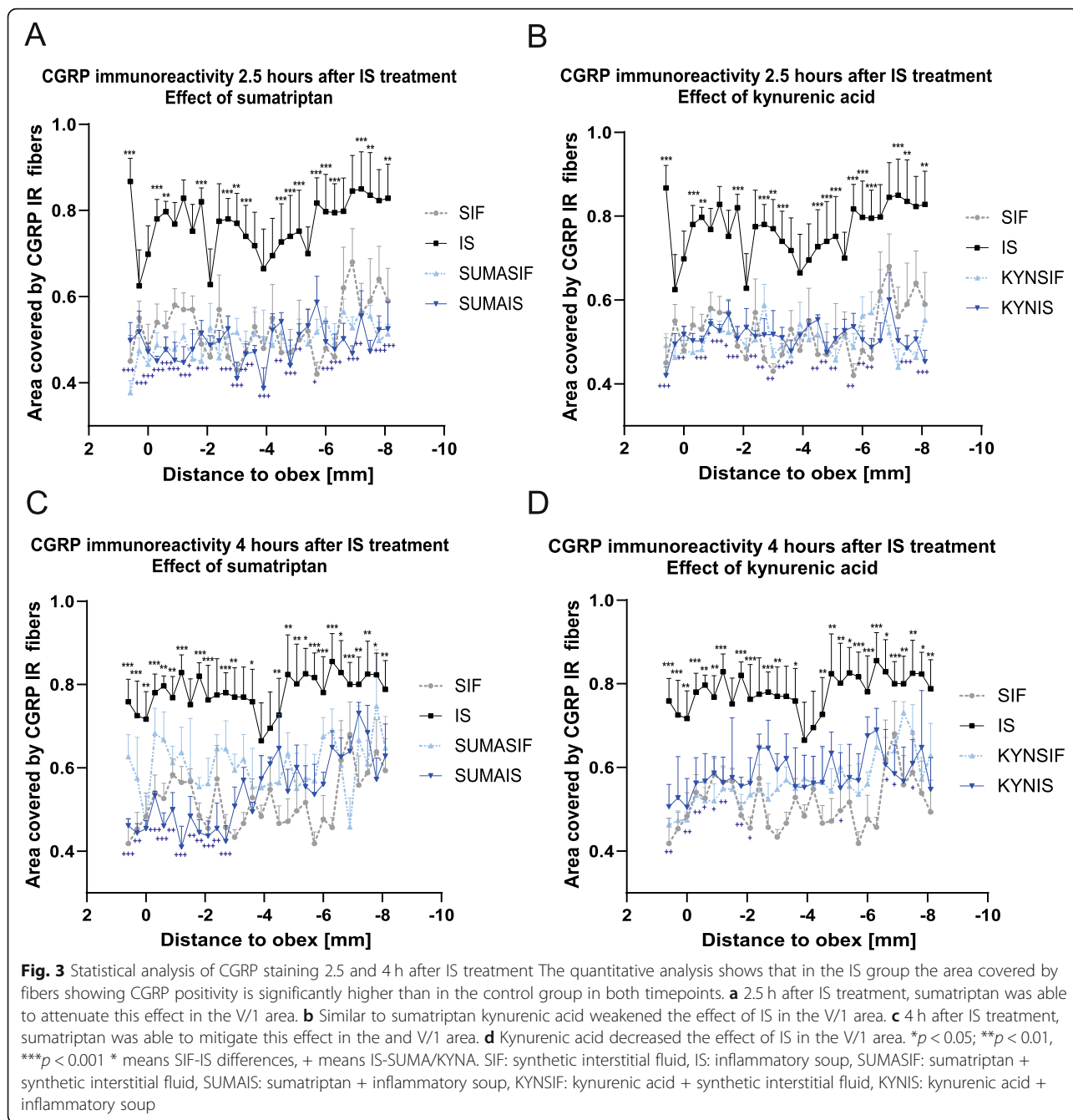


**Fig. 2** CGRP immunoreactivity 4 h after IS treatment Representative photomicrographs of the CGRP expression in the trigemino-cervical segments after 4 h. **a** – SIF, **b** – IS, **c** – SUMASIF, **d** – SUMAIS, **e** – KYNISIF, **f** – KYNIS. In the IS group, the CGRP staining was stronger than in the placebo group. Sumatriptan and kynurenic acid were able to attenuate this effect. SIF: synthetic interstitial fluid, IS: inflammatory soup, SUMASIF: sumatriptan + synthetic interstitial fluid, SUMAIS: sumatriptan + inflammatory soup, KYNISIF: kynurenic acid + synthetic interstitial fluid, KYNIS: kynurenic acid + inflammatory soup. Scale bars: 200  $\mu$ m, 50  $\mu$ m

In our study, IS significantly enhanced the number of nNOS IR cells in the dorsal horn after 4 h due to the activation of primary trigeminal nociceptors conveyed to the second-order neurons [54]. NO donors cause an increase and release of CGRP at the TG and TNC, and NO donors lead to a delayed enhancement of nNOS in the latter [55]. Moreover, bradykinin and histamine

trigger NO release from vascular endothelial cells *in vitro*, suggesting a strong interaction between NO and inflammation [56]. An increase in NO production may contribute to an amplifying process in the meninges, which involves the release of CGRP and possibly prostaglandins and other mediators leading to rapid vasodilatation [19, 57]. The latter can lead to the activation of

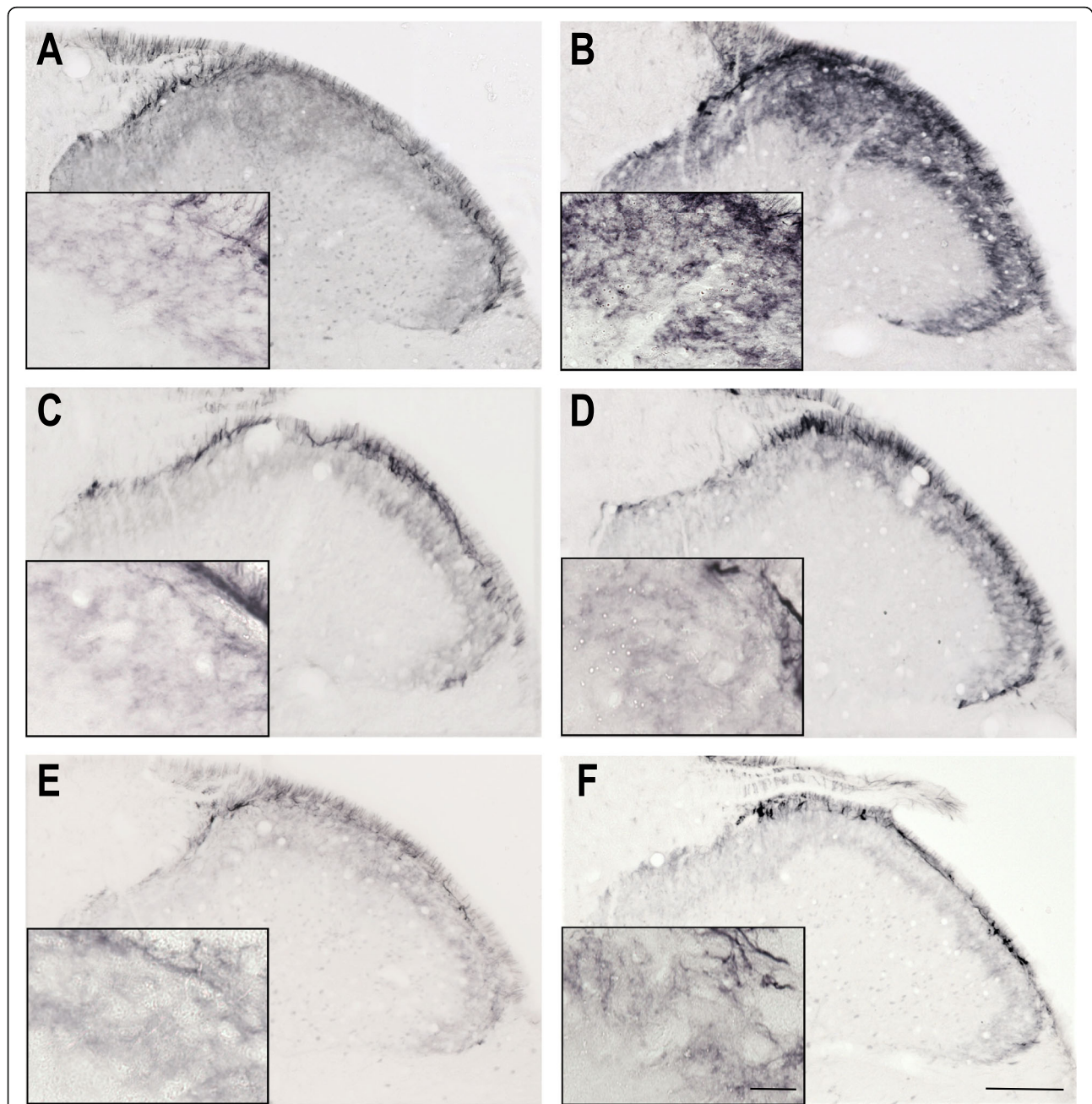




primary afferent neurons and CGRP release, activating satellite glial cells that release NO, which can induce nNOS [58]. In this context nNOS is can be considered as a significant marker of the sensitization process of the TS in animals.

Interestingly, the increase of TRPV1 and nNOS levels are observed later compared to the changes of CGRP reflecting, that the changes of the latter are more likely related to the activation of the primary trigeminal nociceptors whereas TRPV1 and nNOS, which are more likely involved in the sensitization, show a delayed pattern of enhancement.

In our study, sumatriptan was able to modulate the increase of CGRP levels and the TRPV1 activity probably through 5-HT<sub>1B/1D</sub> receptors. This is in line with previous results showing, that CGRP and TRPV1 are co-localized with 5-HT<sub>1B/1D</sub> receptors in trigeminal neurons [59] and sumatriptan presynaptically inhibits the release of nociceptive neuropeptides (e.g. CGRP) from primary afferents [60] and most of the effects of TRPV1 receptors are mediated through CGRP, which is released after TRPV1 activation [61] so 5-HT might have a role in modulation of TRPV1 function too. This is paralleled



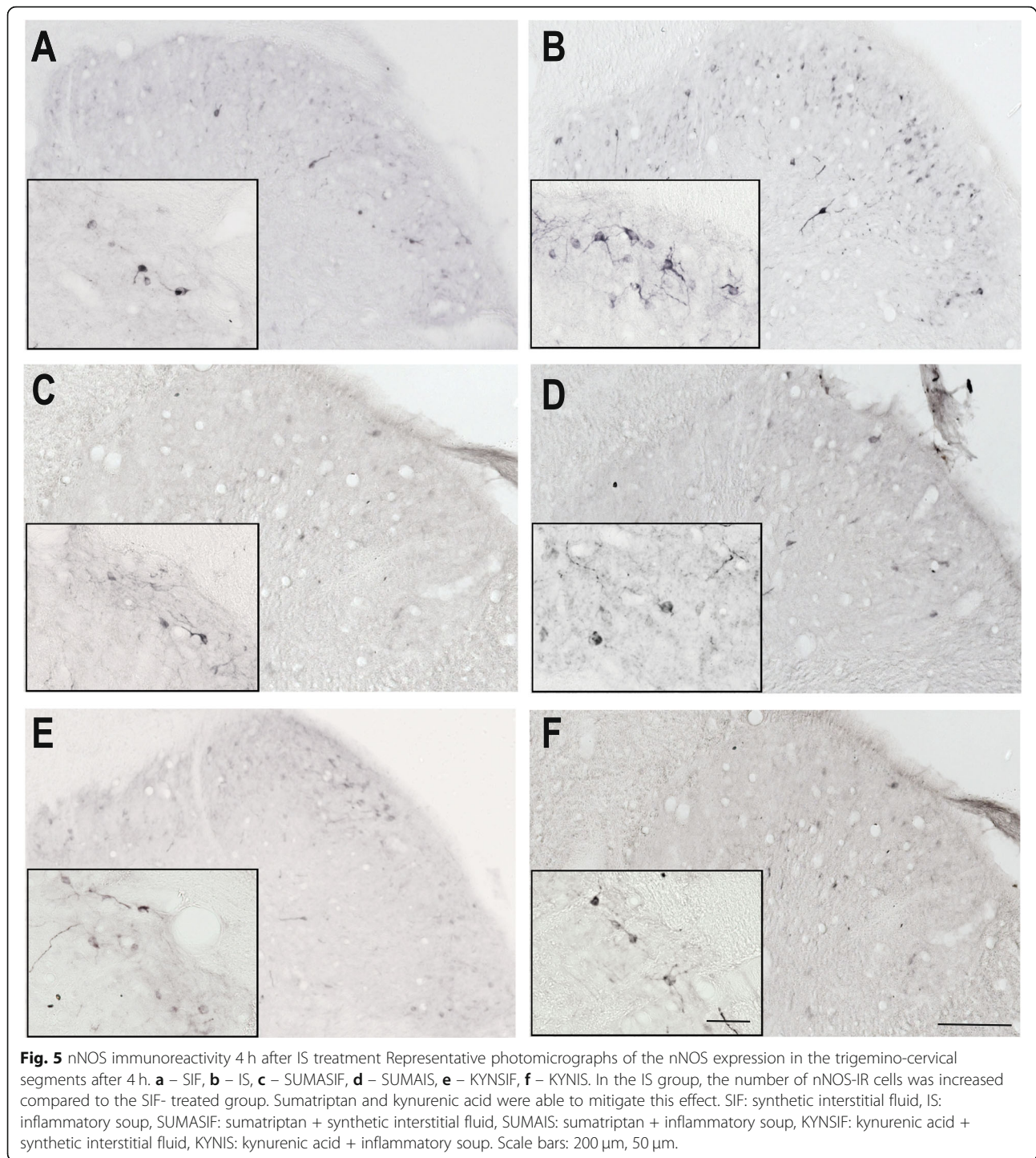
**Fig. 4** TRPV1 immunoreactivity 4 h after IS treatment Representative photomicrographs of the TRPV1 expression in the trigemino-cervical segments after 4 h. **a** – SIF, **b** – IS, **c** – SUMASIF, **d** – SUMAIS, **e** – KYNSIF, **f** – KYNIS. In the IS group, the area covered by TRPV1 was higher than in the placebo group. Sumatriptan and kynurenic acid were able to attenuate this effect. SIF: synthetic interstitial fluid, IS: inflammatory soup, SUMASIF: sumatriptan + synthetic interstitial fluid, SUMAIS: sumatriptan + inflammatory soup, KYNSIF: kynurenic acid + synthetic interstitial fluid, KYNIS: kynurenic acid + inflammatory soup. Scale bars: 200 µm, 50 µm

with the observations, where sumatriptan mitigated the TRPV1 activity after the intracisternal application of IS [62].

In our experiment, sumatriptan also prevented the increase in the number of nNOS IR cells in the rat TNC after 4 h suggesting an important involvement of 5-HT<sub>1B/1D</sub> receptors in the sensitization process in the TS. On the

periphery, sumatriptan inhibits presynaptically the release of vasoactive peptides from primary afferents and impairs the development of neurogenic inflammation [25]. Sumatriptan prevented the increased NOS production in the brainstem after intracisternal carrageenan injection [63]. In humans, NTG-induced headache has been reported to respond to sumatriptan as well



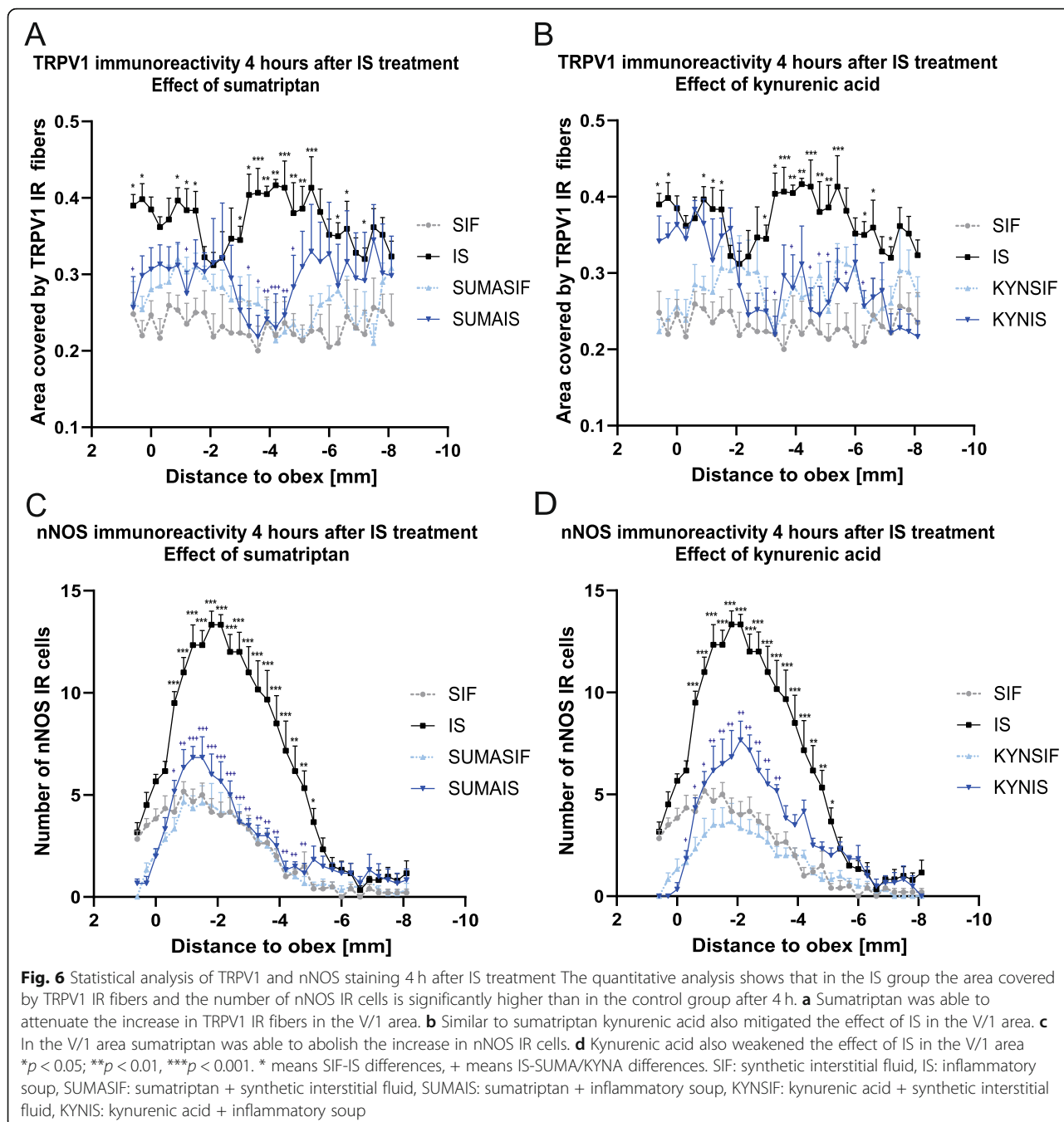


[64]. Taken together, these results suggest that 5-HT<sub>1B/1D</sub> agonism can inhibit IS-induced activation and sensitization present in dural inflammatory process.

Compared to sumatriptan, KYNA had a similar effect on the examined markers in our experimental setting and this phenomenon may be mediated through several different receptors. First KYNA is an endogenous, non-

selective ionotropic glutamate receptor antagonist, which acts on the non-competitive glycine site of NMDA receptors and it is also a GPR35 ligand [26]. Currently, the antagonist effect of KYNA on the  $\alpha$ 7-nicotinic acetylcholine receptor (nAChR) is contested [65].

Three hours after the local IS treatment of the dura, higher glutamate levels can be detected in the TNC [57].



In addition to the NMDA receptors, both AMPA, kainate, and metabotropic receptors are found in the TNC [66] and it has been shown, that the antagonists of non-NMDA glutamate receptors also can inhibit the activation of secondary nociceptive neurons [67]. AMPA receptors can modulate c-fos expression and possibly the neurotransmission in the TS [68] and in a peripheral pain model, activation of the kainate receptors resulted in the appearance of mechanical, thermal hyperalgesia, and allodynia [67]. In the TNC, CGRP can stimulate

glutamate expression and that can be inhibited by 5-HT<sub>1B/1D</sub> receptor agonists [69]. Hence, the relationship between the two systems can be assumed.

In humans, painful stimulation leads to an increase in glutamate concentration in the trigemino-cervical complex [70] and glutamate levels are elevated both ictally and interictally in migraine sufferers [71]. Based on observations in animals and humans, we believe that among others the modulation of glutamatergic neurotransmission is the key event here mitigating CGRP changes.

TRPV1 and NMDAR are co-expressed in the TG [72]. In a mechanical hyperalgesia test of rats, it was found, that NMDAR and TRPV1 functionally interact probably via the calcium/calmodulin-dependent protein kinase type II (CaMKII) and protein kinase C signaling cascades in rat trigeminal sensory neurons and this interaction has a role in the development of mechanical hyperalgesia [72]. GPR35 and TRPV1 co-localize in small- and medium diameter DRG neurons. GPR35 may regulate TRPV1 channel activity by modulating cyclic adenosine monophosphate /protein kinase A pathway [73].

KYNA pretreatment also modulated the IS induced nNOS expression in our animal model. This effect might be related to the anti-glutamatergic effect of KYNA, mainly on the NMDA receptors, which activation is associated with NO production in the spinal trigeminal nucleus [74]. Furthermore, Cosi and colleagues described that elevation of KYNA concentration in the brain could decrease the extracellular glutamate levels in the nervous tissue and reduce inflammatory pain [75]. Another possible explanation for the peripheral effects of KYNA is that it binds to GPR35, which receptor is present in the DRG [76] and KYNA can inhibit adenylate cyclase activity there via G-protein-dependent mechanisms [73] which might interact with nNOS [77, 78]. It has been also reported that abnormalities of the kynurenine pathway are associated with headache disorders e.g. there is evidence that serum kynurenine acid levels decrease during cluster headache and chronic migraine [79–81].

In the present study, sumatriptan and KYNA were similarly effective mitigating the effects of the IS model. They were likely to exert their effects through different receptors/pathways involved in the activation of the trigeminovascular system pointing to different sites of possible pharmacological modulation during this process.

## Conclusion

In our experiment, IS was able to cause sterile neurogenic inflammation in the dura mater. As a consequence of inflammation, changes occur in CGRP, TRPV1, and nNOS levels, which indicates activation and sensitization. Sumatriptan probably acted through the 5-HT<sub>1B/1D</sub> receptors to reduce the expression of the activation and sensitization markers in the TNC by direct (presynaptic) and indirect (lowered dural inflammation) effects on the periphery. In our present study, KYNA possibly acted primarily on peripheral trigeminal nociceptors and secondary sensory neurons and was able to mitigate the activation of these markers in the TNC predominantly through the inhibition of the glutamate system and thereby blocking the sensitization processes, which is important in primary headaches. These findings can help us to understand the pathomechanism processes in migraine.

## Abbreviations

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; CaMKII: calcium/calmodulin-dependent protein kinase type II; CGRP: calcitonin-gene related peptide; CNS: central nervous system; IR: immunoreactive; IS: inflammatory soup; DRG: dorsal root ganglion; KYNA: kynurenic acid; nAChR: α7-nicotinic acetylcholine receptor; nNOS: neuronal nitric oxide synthase; NMDA: N-methyl-D-aspartate; NO: nitric-oxide; NTG: nitroglycerin; PBS: phosphate-buffered saline; PBS-T: PBS containing 1% Triton-X-100; PFA: paraformaldehyde; SIF: synthetic interstitial fluid; TG: trigeminal ganglion; TNC: caudal trigeminal nucleus; TRPV1: transient receptor potential vanilloid-1 receptor; TS: trigeminal system; V/1: ophthalmic nerve; 5-HT: 5-hydroxytryptamine

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## Authors' contributions

ES and KFL carried out the experiments and analyzed the data. ES wrote the manuscript with support from ZSB, GNG, AFSz, KFL, and ÁP. MSz helped to select and perform statistical analysis. ÁP supervised the project. LV participated in the conception and design of the experiments and writing. All authors provided critical feedback and helped shape the research and manuscript. The author(s) read and approved the final manuscript.

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## Availability of data and materials

The data that support the findings of this study are available from the corresponding author upon request.

## Declarations

### Ethics approval and consent to participate

The procedures used in our study were approved by the Committee of the Animal Research of University of Szeged (I-74-49/2017) and the Scientific Ethics Committee for Animal Research of the Protection of Animals Advisory Board (XI/1098/2018) and followed the guidelines the Use of Animals in Research of the International Association for the Study of Pain and the directive of the European Parliament (2010/63/EU).

### Consent for publication

Not applicable.

### Competing interests

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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## References

- Steiner TJ, Stovner LJ, Birbeck GL (2013) Migraine: the seventh disabling. *J Headache Pain*. 14(1):1. <https://doi.org/10.1186/1129-2377-14-1>
- Bartsch T, Goadsby PJ (2003) Increased responses in trigeminocervical nociceptive neurons to cervical input after stimulation of the dura mater. *Brain*. 126(8):1801–1813. <https://doi.org/10.1093/brain/awg190>



3. Kristiansen KA, Edvinsson L (2010) Neurogenic inflammation: a study of rat trigeminal ganglion. *J Headache Pain*. 11(6):485–495. <https://doi.org/10.1007/s10194-010-0260-x>
4. Van Rossum D, Hanisch UK, Quirion R (1997) Neuroanatomical localization, pharmacological characterization and functions of CGRP, related peptides and their receptors. *Neurosci Biobehav Rev* 21(5):649–678. [https://doi.org/10.1016/s0149-7634\(96\)00023-1](https://doi.org/10.1016/s0149-7634(96)00023-1)
5. Goadsby PJ, Edvinsson L (1993) The trigeminovascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. *Ann Neurol* 33(1):48–56. <https://doi.org/10.1002/ana.410330109>
6. Lassen LH, Jacobsen VB, Haderslev PA, Sperling B, Iversen HK, Olesen J, Tfelt-Hansen P (2008) Involvement of calcitonin gene-related peptide in migraine: regional cerebral blood flow and blood flow velocity in migraine patients. *J Headache Pain*. 9(3):151–157. <https://doi.org/10.1007/s10194-008-0036-8>
7. Escott KJ, Beattie DT, Connor HE, Brain SD (1995) Trigeminal ganglion stimulation increases facial skin blood flow in the rat: a major role for calcitonin gene-related peptide. *Brain Res* 669(1):93–99. [https://doi.org/10.1016/0006-8993\(94\)01247-f](https://doi.org/10.1016/0006-8993(94)01247-f)
8. Petersen KA, Birk S, Doods H, Edvinsson L, Olesen J (2004) Inhibitory effect of BIBN4096BS on cephalic vasodilatation induced by CGRP or transcranial electrical stimulation in the rat [published correction appears in *Br J Pharmacol*. 2004 Dec; 143(8):1074–5]. *Br J Pharmacol* 143(6):697–704. <https://doi.org/10.1038/sj.bjp.0705966>
9. Ho TW, Mannix LK, Fan X, Assaid C, Furtek C, Jones CJ, Lines CR, Rapoport AM, On behalf of the MK-0974 Protocol 004 study group (2008) Randomized controlled trial of an oral CGRP receptor antagonist, MK-0974, in acute treatment of migraine. *Neurology*. 70(16):1304–1312. <https://doi.org/10.1212/01.WNL.0000286940.29755.61>
10. Nardin R (2010) Randomized, controlled trial of telcagepant for the acute treatment of migraine. *Neurology*. 74(12):1007–1008. <https://doi.org/10.1212/WNL.0b013e3181d2b6de>
11. Olesen J, Diener HC, Husstedt IW, Goadsby PJ, Hall D, Meier U, Pollentier S, Lesko LM (2004) Calcitonin gene-related peptide receptor antagonist BIBN 4096 BS for the acute treatment of migraine. *N Engl J Med* 350(11):1104–1110. <https://doi.org/10.1056/NEJMoa030505>
12. Schain AJ, Melo-Carrillo A, Stratton J, Strassman AM, Burstein R (2019) CSD-induced arterial dilatation and plasma protein extravasation are unaffected by fremanezumab: implications for CGRP's role in migraine with aura. *J Neurosci* 39(30):6001–6011. <https://doi.org/10.1523/JNEUROSCI.0232-19.2019>
13. Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Traffon J, Petersen-Zeit KR, Koltenburg M, Basbaum AI, Julius D (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science*. 288(5464):306–313. <https://doi.org/10.1126/science.288.5464.306>
14. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature*. 389(6653):816–824. <https://doi.org/10.1038/39807>
15. Lin Q, Li D, Xu X, Zou X, Fang L (2007) Roles of TRPV1 and neuropeptidergic receptors in dorsal root reflex-mediated neurogenic inflammation induced by intradermal injection of capsaicin. *Mol Pain* 3:30. <https://doi.org/10.1186/1744-8069-3-30>
16. Saito S, Kidd GJ, Trapp BD, Dawson TM, Bredt DS, Wilson DA, Traystman RJ, Snyder SH, Hanley DF (1994) Rat spinal cord neurons contain nitric oxide synthase. *Neuroscience*. 59(2):447–456. [https://doi.org/10.1016/0306-4522\(94\)90608-4](https://doi.org/10.1016/0306-4522(94)90608-4)
17. Berger RJ, Zuccarello M, Keller JT (1994) Nitric oxide synthase immunoreactivity in the rat dura mater. *Neuroreport*. 5(4):519–521. <https://doi.org/10.1097/00001756-199401120-00039>
18. Sicuteri F, Del Bene E, Poggioni M, Bonazzi A (1987) Unmasking latent dysnociception in healthy subjects. *Headache*. 27(4):180–185. <https://doi.org/10.1111/j.1526-4610.1987.hed2704180.x>
19. Pardutz A, Krizbai I, Multon S, Vecsei L, Schoenen J (2000) Systemic nitroglycerin increases nNOS levels in rat trigeminal nucleus caudalis. *Neuroreport*. 11(14):3071–3075. <https://doi.org/10.1097/00001756-200009280-00008>
20. Buzzi MG, Carter WB, Shimizu T, Heath H 3rd, Moskowitz MA (1991) Dihydroergotamine and sumatriptan attenuate levels of CGRP in plasma in rat superior sagittal sinus during electrical stimulation of the trigeminal ganglion. *Neuropharmacology*. 30(11):1193–1200. [https://doi.org/10.1016/0028-3908\(91\)90165-8](https://doi.org/10.1016/0028-3908(91)90165-8)
21. Xiao Y, Richter JA, Hurley JH (2008) Release of glutamate and CGRP from trigeminal ganglion neurons: Role of calcium channels and 5-HT1 receptor signaling. *Mol Pain* 4:12. <https://doi.org/10.1186/1744-8069-4-12>
22. Goadsby PJ, Edvinsson L (1994) Human in vivo evidence for trigeminovascular activation in cluster headache. Neuropeptide changes and effects of acute attacks therapies. *Brain*. 117(3):427–434. <https://doi.org/10.1093/brain/117.3.427>
23. Ottani A, Ferraris E, Giuliani D, Mioni C, Bertolini A, Sternieri E, Ferrari A (2004) Effect of sumatriptan in different models of pain in rats. *Eur J Pharmacol* 497(2):181–186. <https://doi.org/10.1016/j.ejphar.2004.06.053>
24. Kayser V, Aubel B, Hamon M, Bourgoin S (2002) The antimigraine 5-HT 1B/1D receptor agonists, sumatriptan, zolmitriptan and dihydroergotamine, attenuate pain-related behaviour in a rat model of trigeminal neuropathic pain. *Br J Pharmacol* 137(8):1287–1297. <https://doi.org/10.1038/sj.bjp.0704979>
25. Ma QP (2001) Co-localization of 5-HT (1B/1D/1F) receptors and glutamate in trigeminal ganglia in rats. *Neuroreport*. 12(8):1589–1591. <https://doi.org/10.1097/00001756-200106130-00015>
26. Vecsei L, Szalárdy L, Fülöp F, Toldi J (2013) Kynurenic acid in the CNS: recent advances and new questions. *Nat Rev Drug Discov* 12(1):64–82. <https://doi.org/10.1038/nrd3793>
27. Csáti A, Edvinsson L, Vecsei L, Toldi J, Fülöp F, Tajti J, Warfvinge K (2015) Kynurenic acid modulates experimentally induced inflammation in the trigeminal ganglion. *J Headache Pain*. 16(1):99. <https://doi.org/10.1186/s10194-015-0581-x>
28. Heyliger SO, Goodman CB, Ngong JM, Soliman KF (1998) The analgesic effects of tryptophan and its metabolites in the rat. *Pharmacol Res* 38(4):243–250. <https://doi.org/10.1006/phrs.1998.0362>
29. Pineda-Farías JB, Pérez-Severiano F, González-Esquivel DF, Barragán-Iglesias P, Bravo-Hernández M, Cervantes-Durán C, Aguilera P, Ríos C, Granados-Soto V (2013) The L-kynurenic-probenecid combination reduces neuropathic pain in rats. *Eur J Pain* 17(9):1365–1373. <https://doi.org/10.1002/j.1532-2149.2013.00305.x>
30. Steen KH, Reeh PW, Anton F, Handwerker HO (1992) Protons selectively induce lasting excitation and sensitization to mechanical stimulation of nociceptors in rat skin, in vitro. *J Neurosci* 12(1):86–95. <https://doi.org/10.1523/JNEUROSCI.12-01-00086.1992>
31. Laborc K, Spekker E, Bohár Z, et al. Trigeminal activation patterns evoked by chemical stimulation of the dura mater in rats. *J Headache Pain*. 2020; 21(1):101. Published 2020 Aug 15. doi:<https://doi.org/10.1186/s10194-020-01169-4>
32. De Felice M, Eyde N, Dodick D et al (2013) Capturing the aversive state of cephalic pain preclinically. *Ann Neurol* 74(2):257–265. <https://doi.org/10.1002/ana.23922>
33. Pardutz A, Szatmári E, Vecsei L, Schoenen J (2004) Nitroglycerin-induced nNOS increase in rat trigeminal nucleus caudalis is inhibited by systemic administration of lysine acetylsalicylate but not of sumatriptan. *Cephalalgia*. 24(6):439–445. <https://doi.org/10.1111/j.1468-2982.2004.00699.x>
34. Körtési T, Tuka B, Tajti J, et al. Kynurenic acid inhibits the electrical stimulation induced elevated pituitary adenylate cyclase-activating polypeptide Expression in the TNC. *Front Neurol*. 2018;8:745. Published 2018 Jan 16. doi:<https://doi.org/10.3389/fneur.2017.00745>
35. Lukács M, Haanes KA, Majláth Z, Tajti J, Vecsei L, Warfvinge K, Edvinsson L (2015) Dural administration of inflammatory soup or complete Freund's adjuvant induces activation and inflammatory response in the rat trigeminal ganglion. *J Headache Pain*. 16(1):564. <https://doi.org/10.1186/s10194-015-0564-y>
36. Strassman AM, Raymond SA, Burstein R (1996) Sensitization of meningeal sensory neurons and the origin of headaches. *Nature*. 384(6609):560–564. <https://doi.org/10.1038/384560a0>
37. Messlinger K, Hanesch U, Baumgärtel M, Trost B, Schmidt RF (1993) Innervation of the dura mater encephali of cat and rat: ultrastructure and calcitonin gene-related peptide-like and substance P-like immunoreactivity. *Anat Embryol (Berl)* 188(3):219–237. <https://doi.org/10.1007/BF00188214>
38. Greco R, Tassorelli C, Sandrini G, Di Bella P, Buscone S, Nappi G (2008) Role of calcitonin gene-related peptide and substance P in different models of pain. *Cephalalgia*. 28(2):114–126. <https://doi.org/10.1111/j.1468-2982.2007.01468.x>
39. Hoffmann J, Wecker S, Neeb L, Dirnagl U, Reuter U (2012) Primary trigeminal afferents are the main source for stimulus-induced CGRP release into jugular vein blood and CSF. *Cephalalgia*. 32(9):659–667. <https://doi.org/10.1177/0333102412447701>
40. Buckley TL, Brain SD, Rampart M, Williams TJ (1991) Time-dependent synergistic interactions between the vasodilator neuropeptide, calcitonin

- gene-related peptide (CGRP) and mediators of inflammation. *Br J Pharmacol* 103(2):1515–1519. <https://doi.org/10.1111/j.1476-5381.1991.tb09819.x>
41. Amaya F, Oh-hashi K, Naruse Y, Iijima N, Ueda M, Shimosato G, Tominaga M, Tanaka Y, Tanaka M (2003) Local inflammation increases vanilloid receptor 1 expression within distinct subgroups of DRG neurons. *Brain Res* 963(1–2): 190–196. [https://doi.org/10.1016/S0006-8993\(02\)03972-0](https://doi.org/10.1016/S0006-8993(02)03972-0)
  42. Nakanishi M, Hata K, Nagayama T, Sakurai T, Nishisho T, Wakabayashi H, Hiraga T, Ebisu S, Yoneda T Acid activation of Trpv1 leads to an up-regulation of calcitonin gene-related peptide expression in dorsal root ganglion neurons via the CaMK-CREB cascade: a potential mechanism of inflammatory pain. *Mol Biol Cell* 2010;21(15):2568–2577. doi:10.1091/mbc.E10-01-0049
  43. Boillat A, Alijevic O, Kellenberger S (2014) Calcium entry via TRPV1 but not ASICs induces neuropeptide release from sensory neurons. *Mol Cell Neurosci* 61:13–22. <https://doi.org/10.1016/j.mcn.2014.04.007>
  44. Brain SD, Williams TJ (1989) Interactions between the tachykinins and calcitonin gene-related peptide lead to the modulation of oedema formation and blood flow in rat skin. *Br J Pharmacol* 97(1):77–82. <https://doi.org/10.1111/j.1476-5381.1989.tb11926.x>
  45. Vincent L, Vang D, Nguyen J, Gupta M, Luk K, Ericson ME, Simone DA, Gupta K (2013) Mast cell activation contributes to sickle cell pathobiology and pain in mice. *Blood*. 122(11):1853–1862. <https://doi.org/10.1182/blood-2013-04-498105>
  46. Carlton SM, Coggeshall RE (2001) Peripheral capsaicin receptors increase in the inflamed rat hindpaw: a possible mechanism for peripheral sensitization. *Neurosci Lett* 310(1):53–56. [https://doi.org/10.1016/S0304-3940\(01\)02093-6](https://doi.org/10.1016/S0304-3940(01)02093-6)
  47. Davis JB, Gray J, Gunthorpe MJ, Hatcher JP, Davey PT, Overend P, Harries MH, Latcham J, Clapham C, Atkinson K, Hughes SA, Rance K, Grau E, Harper AJ, Pugh PL, Rogers DC, Bingham S, Randall A, Sheardown SA (2000) Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. *Nature*. 405(6783):183–187. <https://doi.org/10.1038/35012076>
  48. Hou M, Uddman R, Tajti J, Edvinsson L (2003) Nociceptin immunoreactivity and receptor mRNA in the human trigeminal ganglion. *Brain Res* 964(2): 179–186. [https://doi.org/10.1016/S0006-8993\(02\)03927-6](https://doi.org/10.1016/S0006-8993(02)03927-6)
  49. Quartu M, Serra MP, Boi M, Poddighe L, Picci C, Demontis R, del Fiacco M (2016) TRPV1 receptor in the human trigeminal ganglion and spinal nucleus: immunohistochemical localization and comparison with the neuropeptides CGRP and SP. *J Anat* 229(6):755–767. <https://doi.org/10.1111/joa.12529>
  50. Shimizu T, Toriumi H, Sato H, Shibata M, Nagata E, Gotoh K, Suzuki N (2007) Distribution and origin of TRPV1 receptor-containing nerve fibers in the dura mater of rat. *Brain Res* 1173:84–91. <https://doi.org/10.1016/j.brainres.2007.07.068>
  51. Matthews PJ, Aziz Q, Facer P, Davis JB, Thompson DG, Anand P (2004) Increased capsaicin receptor TRPV1 nerve fibres in the inflamed human oesophagus. *Eur J Gastroenterol Hepatol* 16(9):897–902. <https://doi.org/10.1097/00042737-200409000-00014>
  52. Fusco BM, Barzoi G, Agrò F (2003) Repeated intranasal capsaicin applications to treat chronic migraine. *Br J Anaesth* 90(6):812. <https://doi.org/10.1093/bja/ae572>
  53. Hoffmann J, Suprinsinchai W, Andreou AP, Summ O, Akerman S, Goadsby PJ (2012) Olvanil acts on transient receptor potential vanilloid channel 1 and cannabinoid receptors to modulate neuronal transmission in the trigeminovascular system. *Pain*. 153(11):2226–2232. <https://doi.org/10.1016/j.pain.2012.07.006>
  54. Dubin AE, Patapoutian A (2010) Nociceptors: the sensors of the pain pathway. *J Clin Invest* 120(11):3760–3772. <https://doi.org/10.1172/JCI42843>
  55. Bellamy J, Bowen EJ, Russo AF, Durham PL (2006) Nitric oxide regulation of calcitonin gene-related peptide gene expression in rat trigeminal ganglia neurons. *Eur J Neurosci* 23(8):2057–2066. <https://doi.org/10.1111/j.1460-9568.2006.04742.x>
  56. Palmer RM, Ferrige AG, Moncada S (1987) Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature*. 327(6122):524–526. <https://doi.org/10.1038/327524a0>
  57. Sarker MH, Fraser PA (2002) The role of guanylyl cyclases in the permeability response to inflammatory mediators in pial venular capillaries in the rat. *J Physiol* 540(Pt 1):209–218. <https://doi.org/10.1113/jphysiol.2001.012912>
  58. Capuano A, De Corato A, Lisi L, Tringali G, Navarra P, Dello Russo C. Proinflammatory-activated trigeminal satellite cells promote neuronal sensitization: relevance for migraine pathology. *Mol Pain*. 2009;5:43. Published 2009 Aug 6. doi:<https://doi.org/10.1186/1744-8069-5-43>
  59. Ma QP, Hill R, Sirinathsingji D (2001) Colocalization of CGRP with 5-HT1B/1D receptors and substance P in trigeminal ganglion neurons in rats. *Eur J Neurosci* 13(11):2099–2104. <https://doi.org/10.1046/j.0953-816x.2001.01586.x>
  60. Arvieu L, Mauborgne A, Bourgoin S, Oliver C, Feltz P, Hamon M, Cesselin F (1996) Sumatriptan inhibits the release of CGRP and substance P from the rat spinal cord. *Neuroreport*. 7(12):1973–1976. <https://doi.org/10.1097/00001756-199608120-00023>
  61. Meng J, Ovsepian SV, Wang J, Pickering M, Sasse A, Aoki KR, Lawrence GW, Dolly JO (2009) Activation of TRPV1 mediates calcitonin gene-related peptide release, which excites trigeminal sensory neurons and is attenuated by a retargeted botulinum toxin with anti-nociceptive potential. *J Neurosci* 29(15):4981–4992. <https://doi.org/10.1523/JNEUROSCI.5490-08.2009>
  62. Meents JE, Hoffmann J, Chaplan SR, Neeb L, Schuh-Hofer S, Wickenden A, Reuter U (2015) Two TRPV1 receptor antagonists are effective in two different experimental models of migraine. *J Headache Pain*. 16(1):57. <https://doi.org/10.1186/s10194-015-0539-z>
  63. Demirpence S, Kurul SH, Kiray M, Tugyan K, Yilmaz O, Köse G (2009) The effect of sumatriptan on nitric oxide synthase enzyme production after iatrogenic inflammation in the brain stem of adolescent rats: a randomized, controlled, experimental study. *Curr Ther Res Clin Exp* 70(2):129–135. <https://doi.org/10.1016/j.curtheres.2009.04.003>
  64. Iversen HK, Olesen J (1996) Headache induced by a nitric oxide donor (nitroglycerin) responds to sumatriptan. A human model for development of migraine drugs. *Cephalalgia*. 16(6):412–418. <https://doi.org/10.1046/j.1468-2982.1996.1606412.x>
  65. Stone TW (2020) Does kynurenic acid act on nicotinic receptors? An assessment of the evidence. *J Neurochem* 152(6):627–649. <https://doi.org/10.1111/jnc.14907>
  66. Storer RJ, Goadsby PJ (1999) Trigeminovascular nociceptive transmission involves N-methyl-D-aspartate and non-N-methyl-D-aspartate glutamate receptors. *Neuroscience*. 90(4):1371–1376. [https://doi.org/10.1016/S0306-4522\(98\)00536-3](https://doi.org/10.1016/S0306-4522(98)00536-3)
  67. Dougherty PM, Palecek J, Paleckova V, Sorkin LS, Willis WD (1992) The role of NMDA and non-NMDA excitatory amino acid receptors in the excitation of primate spinothalamic tract neurons by mechanical, chemical, thermal, and electrical stimuli. *J Neurosci* 12(8):3025–3041. <https://doi.org/10.1523/JNEUROSCI.12-08-03025.1992>
  68. Mitsikostas DD (1999) Sanchez del Rio M, Waeber C, Huang Z, Cutrer FM, Moskowitz MA. Non-NMDA glutamate receptors modulate capsaicin induced c-fos expression within trigeminal nucleus caudalis. *Br J Pharmacol* 127(3):623–630. <https://doi.org/10.1038/sj.bjp.0702584>
  69. Choi IS, Cho JH, An CH, Jung JK, Hur YK, Choi JK, Jang IS (2012) 5-HT (1B) receptors inhibit glutamate release from primary afferent terminals in rat medullary dorsal horn neurons. *Br J Pharmacol* 167(2):356–367. <https://doi.org/10.1111/j.1476-5381.2012.01964.x>
  70. Campos F, Sobrino T, Pérez-Mato M, Rodríguez-Osorio X, Leira R, Blanco M, Mirelman D, Castillo J (2013) Glutamate oxaloacetate transaminase: a new key in the dysregulation of glutamate in migraine patients. *Cephalalgia*. 33(14):1148–1154. <https://doi.org/10.1177/0333102413487444>
  71. Li DP, Chen SR, Pan HL (2004) VR1 receptor activation induces glutamate release and postsynaptic firing in the paraventricular nucleus. *J Neurophysiol* 92(3):1807–1816. <https://doi.org/10.1152/jn.00171.2004>
  72. Lee J, Saloman JL, Weiland G, Auh QS, Chung MK, Ro JY (2012) Functional interactions between NMDA receptors and TRPV1 in trigeminal sensory neurons mediate mechanical hyperalgesia in the rat masseter muscle. *Pain*. 153(7):1514–1524. <https://doi.org/10.1016/j.pain.2012.04.015>
  73. Ohshiro H, Tonai-Kachi H, Ichikawa K (2008) GPR35 is a functional receptor in rat dorsal root ganglion neurons. *Biochem Biophys Res Commun* 365(2): 344–348. <https://doi.org/10.1016/j.bbrc.2007.10.197>
  74. Dohm CS, Beitz AJ (1994) NMDA receptor mRNA expression in NOS-containing neurons in the spinal trigeminal nucleus of the rat. *Neurosci Lett* 175(1–2):28–32. [https://doi.org/10.1016/0304-3940\(94\)91070-7](https://doi.org/10.1016/0304-3940(94)91070-7)
  75. McGehee DS, Heath MJ, Gelber S, Devay P, Role LW (1995) Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. *Science*. 269(5231):1692–1696. <https://doi.org/10.1126/science.7569895>
  76. Cosi C, Mannaioni G, Cozzi A, Carlà V, Sili M, Cavone L, Maratea D, Moroni F (2011) G-protein coupled receptor 35 (GPR35) activation and inflammatory pain: studies on the antinociceptive effects of kynurenic acid and zaprinast. *Neuropharmacology*. 60(7–8):1227–1231. <https://doi.org/10.1016/j.neuropharm.2010.11.014>

77. Ohnishi T, Okuda-Ashitaka E, Matsumura S, Katano T, Nishizawa M, Ito S (2008) Characterization of signaling pathway for the translocation of neuronal nitric oxide synthase to the plasma membrane by PACAP. *J Neurochem* 105(6):2271–2285. <https://doi.org/10.1111/j.1471-4159.2008.05325.x>
78. Boissel JP, Bros M, Schröck A, Gödtel-Armbrust U, Förstermann U (2004) Cyclic AMP-mediated upregulation of the expression of neuronal NO synthase in human A673 neuroepithelioma cells results in a decrease in the level of bioactive NO production: analysis of the signaling mechanisms that are involved. *Biochemistry*. 43(22):7197–7206. <https://doi.org/10.1021/bi0302191>
79. Heyes MP, Saito K, Crowley JS et al (1992) Quinolinic acid and kynurenine pathway metabolism in inflammatory and non-inflammatory neurological disease. *Brain*. 115(Pt 5):1249–1273. <https://doi.org/10.1093/brain/115.5.1249>
80. Rejdak K, Bartosik-Psujek H, Dobosz B, Kocki T, Grieb P, Giovannoni G, Turski WA, Stelmasiak Z (2002) Decreased level of kynurenic acid in cerebrospinal fluid of relapsing-onset multiple sclerosis patients. *Neurosci Lett* 331(1):63–65. [https://doi.org/10.1016/s0304-3940\(02\)00710-3](https://doi.org/10.1016/s0304-3940(02)00710-3)
81. Curto M, Lionetto L, Negro A, Capi M, Perugino F, Fazio F, Giamberardino MA, Simmaco M, Nicoletti F, Martelletti P (2015) Altered serum levels of kynurenine metabolites in patients affected by cluster headache. *J Headache Pain* 17(1):27. <https://doi.org/10.1186/s10194-016-0620-2>

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