

Sensitization induces hypersensitivity in trigeminal nerve

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Abstract

Background and Aims Trigeminal neuralgia is one of the chronic neuropathic pains. The aetiology remains unclear. Chronic inflammation is proposed playing a role in the pathogenesis of trigeminal neuralgia. This study aims to assess the role of allergic inflammation in the pathological status of trigeminal neuralgia.

Methods Allergic inflammation was induced in the infraorbital skin of rats. The expression of 5-HT receptor 3 (R3) in the trigeminal nerve and mastocytosis in the local tissue were observed by immunohistochemistry. The hyperalgesia status of the trigeminal nerve was determined by quantitatively recording the head-withdrawal threshold to mechanical stimulation of the infraorbital nerve territory.

Results After sensitization, the expression of 5-HT R3 in the trigeminal nerve and the frequency of mast cells were markedly increased in the epidermal tissue of the infraorbital area. Applying exogenous 5-HT to the local tissue also increased the expression of 5-HT R3 in the trigeminal nerve. The threshold of mechanical allodynia to stimuli was significantly lower in sensitized rats than control rats, which could be blocked by pre-treatment with either 5-HT R3 antagonists, or neurokinin-1 antagonists, or mast cell stabilizers.

Conclusions Allergic inflammation can induce the overexpression of 5-HT R3 in the trigeminal nerve to induce the hyperalgesia status in the trigeminal nerve. The 5-HT R3 may be a therapeutic target in the treatment of trigeminal neuralgia.

Keywords 5-HT receptor 3, allergy, mast cells, substance P, trigeminal neuralgia

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Introduction

Trigeminal neuralgia is one of the chronic neuropathic disorders, which is defined as sudden, usually unilateral, severe, brief, stabbing, recurrent episodes of pain in the distribution of one or more branches of the trigeminal nerve [1]. The prevalence is about 5–30/100 000 people [2–4]. The treatment is not satisfactory currently, pain control is the mostly used remedy; to remove or destroy the trigeminal peripheral ganglia is performed in some severe cases [5].

The aetiology of trigeminal neuralgia is unknown. Injury may be one of the causes as many animal models of trigeminal neuralgia are established by nerve injury, such as the ligation of the infraorbital nerve [6], or compression of the trigeminal ganglia [7]. The local tissue inflammation is suggested to play a role in the trigeminal neuralgia, which can be ameliorated by administrating the potassium channels blocking agents [8]. Aberrant immune responses are a common cause inducing inflammation in the body. The association

between allergic dermatitis and trigeminal neuralgia has been noted [9]. Allergy is one of the aberrant immune disorders. A conspicuous feature of allergy is the recurrent nature that lasts for many years once initiated. Mastocytosis in local tissue is another feature of allergy. Mast cell activation is one of the major sources of 5-HT in the body [10]. 5-HT and 5-HT receptor 3 (R3) are proposed to play an important role in neuralgia, such as the visceral hyperalgesia [11], in which food allergy is involved [12, 13]. Substance P can be produced from a subset of trigeminal afferents [14]. The trigeminal nerve expresses 5-HT receptors [15, 16]. Cranial nerves V can be activated by 5-HT₃ receptors in the trigeminal tract nucleus [17]. By integrating the above information, we hypothesized that the allergic inflammation may be involved in the pathogenesis of trigeminal neuralgia. As shown by a rat model, the allergic responses in the local tissue induced the overexpression of 5-HT R3 in the trigeminal nerve that conferred the hyperalgesia status in the trigeminal nerve.

Material and methods

Reagents

Antibodies of 5-HT, 5-HT R3A (C-20), isotype IgG and the second antibodies were purchased from Santa Cruz Biotech (Shanghai, China). PGP9.5 (13C4) was purchased from Abcam (Shanghai, China). Mouse anti-RMCP II antibody was purchased from Moredun Scientific (Midlothian, Scotland). 5-HT was purchased from Sigma Aldrich (Shanghai, China). The ELISA kit of 5-HT was purchased from Usen Life Science Inc (Wuhan, China).

Animals

Adult male Sprague–Dawley rats (200–250 g), mast cell-deficient Ws/Ws rats and +/+ rats were obtained from the experimental animal centre of our university. Rats were maintained at a temperature of 20–25°C with a 12-h light-dark cycle; the rats were allowed to freely access to food and water. The procedures of animal experiments were approved by the Animal Experimental Ethic Committee at the Third Military Medical University.

Induction of skin allergic inflammation at infraorbital facial area

The rats were injected with ovalbumin (OVA, 1 mg/rat) mixed with 1 mL alum subcutaneously in the left side infraorbital skin, once a week for four consecutive weeks. The control rats were injected with 1 mL saline.

Detection of 5-HT

The infraorbital skin was excised from the experimental rats and processed for 5-HT assessment using an ELISA kit following the manufacturer's instruction.

Stimulating the skin with 5-HT

The infraorbital skin was shaved and applied with 0.1 mg/mL 5-HT three times daily for five consecutive days. After the rat was killed, the skin was excised and processed for immune staining as described below.

Animal behaviour test

Mechanical allodynia was determined weekly between 5 and 10 weeks after the first sensitization. Mechanical stimuli were applied using eight calibrated Semmes-Weinstein monofilaments (0.41, 0.70, 1.20, 2.00, 3.63, 5.50, 8.50, and 15.10 g) (von Frey hairs; Stoelting, Chicago, IL, USA) following reported procedures [18].

Rats were allowed acclimated in a transparent cage for 10 min; the stimuli were applied to the infraorbital skin (the sensitized skin area) near the centre of the vibrissal pad on hairy skin surrounding the mystacial vibrissae. A clear withdrawal response was recognized as defined by previous studies [19]: (1) rat avoids further contact with the monofilament, (2) rat turns head rapidly away scratching the stimulated area or (3) rat actively attacks the stimulating object. The mechanical threshold of withdrawal response was measured according to the up-down method, and thus the 50% threshold was computed using the formula described by Dixon [6].

Immunohistochemistry

The infraorbital skin was excised and frozen with liquid nitrogen. For nerve staining, the skin piece was flatly laid on the bottom of the sample moulds to reveal the nerve image. For mast cell staining, the orientation of the skin piece was positioned perpendicularly in the moulds. The frozen sections were fixed with cold acetone for 20 min at room temperature and blocked by 1% BSA (bovine serum albumin) for 30 min. For the staining of 5-HT R3 on the trigeminal nerves, the sections were incubated with the goat anti-rat 5-HT R3 (1 : 100) and mouse anti-rat PGP9.5 (1 : 100; a neural marker) at 4°C overnight, and then, incubated with the second antibodies of fluorescein isothiocyanate (FITC)-labelled anti-rabbit and phycoerythrin (PE)-labelled anti-goat for 1 h at room temperature. For mast cell staining, the sections were stained with mouse anti-rat RMCP II antibody (1 : 200) at 4°C overnight, then, incubated with FITC-anti-mouse second antibody at room temperature for 1 h, and stained with propidium iodide (5 µg/mL) for 15 min. The sections were washed with phosphate buffered saline (PBS) for 5 min and three times after each staining step. The sections were mounted with cover slips and observed under a confocal microscope. The observers were not aware of the section type to avoid the observer bias. To obtain data quantitatively, randomly selected 20 pictures were taken from each sample. The positive staining of 5-HT R3 and the trigeminal nerve was processed using image analysis software Photoshop CS5 and expressed as pixels.

Statistics

All experimental data were analysed using a statistical software package, SPSS11.0 (IBM, Armonk, NY, USA). The non-parametric test (Mann–Whitney) was used to analyse NDS and Dunnett's test was used for analysing infarct volumes. $P < 0.05$ was statistically considered different.

Results

Sensitization of the infraorbital skin induces the trigeminal nerve to express 5-HT R3

The 5-HT R3 is proposed to be involved in trigeminal neuralgia [17] and other hyperalgesia syndrome, such as visceral hyperalgesia [12]. To further characterize this phenomenon, the infraorbital skin of the rats was sensitized to a model antigen, OVA, together with the adjuvant alum. The skin was examined by immunohistochemistry. The results showed that positive staining of the 5-HT R3 was detectable in the trigeminal nerve (revealed by PGP9.5 positive staining) in the infraorbital skin of naïve rats, which was significantly increased in rats sensitized to OVA (Fig. 1a and c). Using as a comparison, skin samples were taken from the mast cell-deficient rats (sensitized to OVA) and processed with the above procedures. The staining of 5-HT R3 (Fig. 1a6) was similar to that in naïve rats (Fig. 1a1) and the wild-type littermates $+/+$ rats (data not shown). We also observed mast cells in the local tissue; the confocal images in Fig. 1b and d show that the frequency of mast cells was markedly higher in sensitized rats than naïve rats. As mast cells are one of the major sources of 5-HT in the body [10], we wondered if the

expression of 5-HT R3 was associated with the mast cells in the sensitized tissue. To test the inference, in separate experiments, we applied exogenous 5-HT on the surface of the infraorbital skin daily for 5 days. Indeed, the expression of 5-HT R3 was significantly increased in the trigeminal nerves (Fig. 1a3), but not in the rats received a control Ab (Fig. 1a4). We also assessed the levels of 5-HT in the infraorbital skin. The levels of 5-HT were below the detectable levels in naïve control rats, but significantly increased in sensitized rats (Fig. 1e).

Sensitization-caused alteration in the behaviour of rats

It is proposed that 5-HT and its receptors are involved in the pathogenesis of neuralgia [11]. We next assessed the influence of sensitization on rat trigeminal nerve behaviour. The results showed that, as 5 weeks after the first sensitization lasting to 10 weeks, the head-withdrawal threshold to mechanical stimulation of the infraorbital nerve territory significantly decreased after sensitization of the skin in the infraorbital innervation area (Fig. 2) as compared with control rats. There were also no significant changes in the threshold while stimulus was applied contralateral side (Fig. 2). After challenge with the specific antigen, OVA, on the skin,

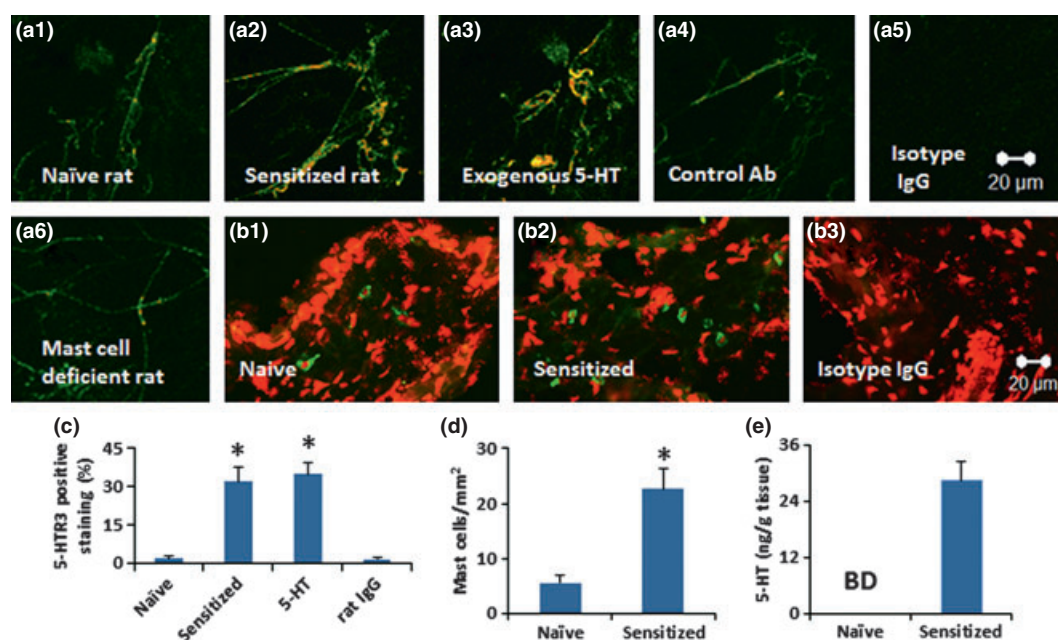


Fig. 1. Trigeminal nerve expresses 5-HTR3 in the infraorbital skin with mastocytosis. Rats were sensitized to antigen, OVA, in the infraorbital skin. The skin was excised and processed for immunohistochemistry. (a) The confocal images show 5-HT R3 (in red) on trigeminal nerve (in green; the yellow colour was merged by red and green colours). (b) The confocal images show the RMCPII⁺ mast cells (in green) in the skin tissue. The nuclei were stained in red. (c) The bars indicate the summarized percentage of 5-HT R3 positive staining against total trigeminal nerve staining (in pixel). (d) The bars indicate the summarized number of mast cells in B. The data in bar graphs were expressed as mean $\pm$ SD [from 20 high power fields ($\times 200$) per rat; six rats per group]. (e) The bars indicate the levels of 5-HT in the infraorbital skin (measured by ELISA). BD: Below detectable levels. *, $P < 0.01$, compared with naïve group. Naïve: Naïve rat. Sensitized: Sensitized rats.

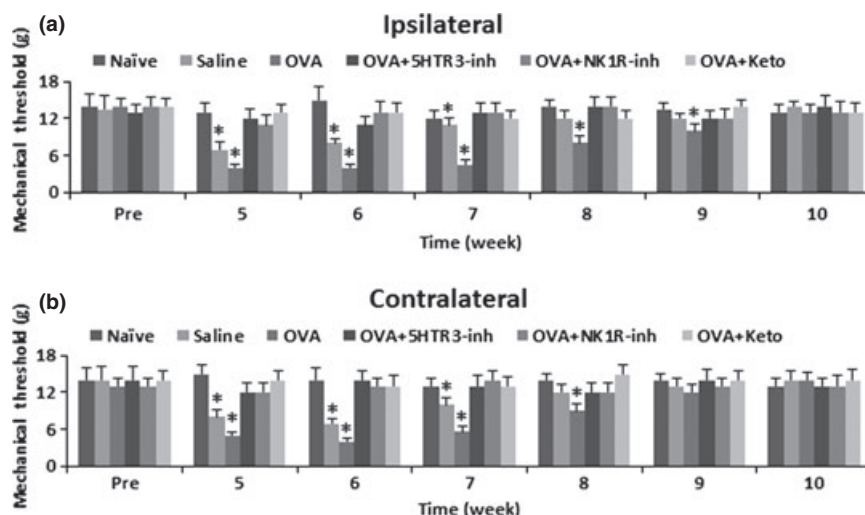


Fig. 2. Mechanical threshold of trigeminal nerve. Naïve rats and sensitized rats were tested the mechanical threshold as described in the text. The bars indicate the mechanical threshold of ipsilateral side (a) and contralateral side (b). The x axes indicate the time points when the mechanical threshold was recorded after the first time of the sensitization. Naïve: Naïve rats. Saline: Sensitized rats treated with saline. OVA: Sensitized rats treated with OVA. 5HT3-inh: Pre-treatment with 5-HT R3 inhibitor, Tropisetron hydrochloride (1 mg/kg i.p.), 30 min before mechanical stimulation. NK-1R-inh: Pre-treatment with NK1 antagonist L-733,060 (10 mg/kg i.p.) 30 min before mechanical stimulation. Keto: Pre-treatment with mast cell membrane stabilizer ketotifen (1 mg/kg i.p.) 30 min before mechanical stimulation.

the head-withdrawal threshold was further decreased, which was abolished by pre-treatment with either NK1 antagonist, or mast cell stabilizer ketotifen, or 5-HT R3 antagonist.

Discussion

The pathogenesis of trigeminal neuralgia is to be further understood. The present data have added novel information to this research area, which indicates that under allergic inflammatory environment, the expression of 5-HT R3 is increased in the trigeminal nerve. The sensitized local tissue has mastocytosis. The threshold of mechanical allodynia to stimuli was significantly lower in sensitized rats than control rats, which can be blocked by either 5-HT R3 antagonists, or mast cell stabilizer, or NK1 inhibitor.

The expression of 5-HT R3 has been observed in the visceral hyperalgesia. Matsumoto et al. [20] reported that 5-HT R3 antagonist attenuated the visceral hyperalgesia. The alterations of 5-HT signalling via 5-HT R3 in mucosa may contribute to the visceral hypersensitivity in colitis mice. Chen et al. [12] indicate that 5-HT R3 mediates the time-dependent vagal afferent modulation of nociception during chronic food allergen-sensitized visceral hyperalgesia in rats. The aberrant nociception activities are proposed as an important factor in neural hypersensitivity. In line with these studies, the present data also observed the overexpression of 5-HT R3 in the trigeminal tissue after the local tissue was sensitized; blocking the 5-HT R3 by its antagonists

could inhibit the allodynia induced by mechanical stimuli. Thus, our data indicate that 5-HT R3 is involved in the pathogenesis of trigeminal hypersensitivity of the rat model.

The initiation factors inducing the overexpression of 5-HT R3 remain unclear. Lomax et al. [21] observed that trinitrobenzene sulphonic acid-treated colon mucosa expressed high levels of 5-HT R3. Burner et al. [22] suggest that mast cells and nerve endings interact to modulate their activities; mediators from mast cells influence neural activities; neural tissue releases peptides in turn modulate mast cell's function. This study revealed that the overexpression of 5-HT R3 was induced in the trigeminal nerve; the mastocytosis was observed in the local tissue. The data imply that the 5-HT released by residential mast cells plays a role in the overexpression of 5-HT R3 in the trigeminal nerve. The deduction is supported by the further experimental results, in which applying extrinsic 5-HT also induced the expression of 5-HT R3 in the trigeminal nerve. Of course, in allergic inflammation, besides mastocytosis, several inflammatory cells, such as eosinophils and Th2 cells, also extravagate into the local tissue; it is possible that those inflammatory cells are also involved in the induction of 5-HT R3 in the trigeminal nerve. However, the data from mast deficient rats have excluded this possibility, no increases in the expression of 5-HT R3 in the trigeminal nerve of mast cell-deficient rats after sensitization.

SP can be produced by a subset of trigeminal afferents upon perceiving stimulation [14]. Mast cells express SP receptor (the NK1 receptor); the interaction

between SP and NK1 induces mast cell activation [23] to release the preformed mediators, such as 5-HT; the latter thus get the opportunity to bind the 5-HT R3 on the trigeminal nerve and induce the aberrant neural activities. Indeed, our data indicate that the response threshold to mechanical stimulation of the experimental rat is significantly lower after sensitization; furthermore, the low threshold can be blocked by NK1 inhibitor or by mast cell membrane stabilizer; the factor implicates that the interaction of SP and NK1 plays an important role in the reduction in the mechanical threshold of the trigeminal nerve.

In summary, the present data indicate that the sensitization-induced mastocytosis in the local tissue induces the overexpression of 5-HT R3 in the trigeminal nerve.

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