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Research report

Inhibitory effects of tetramethylpyrazine on pain transmission of trigeminal neuralgia in CCI-ION rats

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ABSTRACT

Tetramethylpyrazine (TMP) has anti-inflammatory effects and is used to treat cerebral ischemic injury, but the mechanism of TMP on neural protection is not clear. Trigeminal neuralgia (TN) is a facial pain syndrome that is characterized by paroxysmal, shock-like pain attacks located in the somatosensory distribution of the trigeminal nerve. P2X3 receptor plays a crucial role in facilitating pain transmission. The present study investigates the effects of TMP on trigeminal neuralgia transmission mediated by P2X3 receptor of the trigeminal ganglia (TG). Chronic constriction injury of the infraorbital branch of the trigeminal nerve (CCI-ION) was used as a trigeminal neuralgia model. On day 15 after surgery, there was a significant decline in the mechanical hyperalgesia threshold in the territory of the ligated infraorbital nerve in the TN group, and an increase in expression of P2X3 receptor in the TG of the TN group compared with the Sham group. After treatment with TMP or A-317491, the mechanical hyperalgesia threshold of TN rats was significantly higher, and expression of P2X3 receptor in the TG noticeably declined compared with the TN group. Phosphorylation of p38 and ERK1/2 in the TN group was stronger than in the Sham group. However, the phosphorylation of p38 and ERK1/2 in the TN + TMP group and TN + A-317491 group was much lower than in the TN group. TMP significantly inhibited the ATP activated currents in HEK293 cells transfected with a P2X3 plasmid. Thus, TMP might have inhibitory effects on trigeminal neuralgia by suppressing the expression of P2X3 receptor in the TG and the phosphorylation of p38 and ERK1/2 in the TG.

1. Introduction

Trigeminal neuralgia (TN) is one of the most excruciating pain syndromes afflicting the orofacial region. It is defined as sudden, usually unilateral, severe, brief, stabbing, lancinating, and recurring pain in the distribution of one or more branches of the fifth cranial nerve (Punyani and Jasuja, 2012). TN occurs most often in people over age 50 and affects women more often than men (Liu et al., 2014), clinically, it is generally divided into primary and secondary trigeminal neuralgia. Various drugs and surgical procedures have been utilized for the treatment of TN, but the results are not completely satisfying (Montano et al., 2015). An understanding of its pathological mechanism is necessary to provide useful future directions for the treatment of TN.

Tetramethylpyrazine (TMP) is a biologically active alkaloid isolated from the rhizome of the traditional herbal medicine *Ligusticum walliichi* (Chuanxiong) (Zhang et al., 2014). It has been widely used, especially in the treatment of cerebral ischemic injury, and exhibits an anti-inflammatory effect (Tang et al., 2012), but the mechanism by which TMP effects on the neural protection is not clear and still requires more research (Chen et al., 2015; Lu et al., 2014). In a recent study it was confirmed that P2X3 receptor play a key role in the nociceptive sensory facilitation of TN (Qi et al., 2016; Shinoda et al., 2007; Wirkner et al., 2007). Our experiment will investigate the effects of TMP on the behavioral change of TN rats, and whether the effects are related to the change in the expression of P2X3 receptor in the trigeminal ganglion (TG).

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2. Materials and methods

2.1. Animals

Mature healthy male Sprague-Dawley rats (220–250 g) were provided by the Center of Laboratory Animal Science of Nanchang University. The ethical guidelines of the International Association for the Study of Pain (IASP) for pain research in animals were followed. The trigeminal neuralgia rat model (a chronic constriction injury of the infraorbital branch of the trigeminal nerve, CCI-ION) was established (Vos et al., 1994).

2.2. Experimental design

A chronic constriction injury model of the infraorbital branch of the trigeminal nerve (CCI-ION) was prepared as a trigeminal neuralgia model. Rats were randomly divided into a sham operation group (Sham), a sham operation group treated with TMP (Sham + TMP), and a TN model group (TN); TN rats were treated with TMP (TN + TMP) or with the P2X3 receptor antagonist A-317491 (TN + A). Sham rats received an incision to expose the infraorbital nerve without ligating, but the infraorbital nerve of TN rats was ligated. Then, TMP was administered by intraperitoneal injection in the TN + TMP group (50 mg/kg) after a CCI-ION operation once a day for 2 weeks, and A-317491 was administered by intraperitoneal injection in the TN + A group (0.5 mg/kg).

2.3. Chronic constriction injury of the infraorbital branch of the trigeminal nerve (CCI-ION)

The CCI-ION model was prepared as a trigeminal neuralgia model. Each rat was anesthetized with penthiobarbital sodium (Shanghai Xingya Medical Company, Batch No: 140601) during surgical procedures. After skin preparation and disinfection, we made an arc incision on the brow and achieveda blunt dissection using a glass needle. In this process the skull, frontal bone and nasal bone would be seen gradually until the fossa orbitalis appeared. We pushed the orbital contents aside using the glass needle and exposed the infraorbital nerve located at the bottom of the medial orbital. Two ligatures (5-0 chromic gut) were loosely placed with microsurgical techniques. The interval between the two ligatures was approximately 1 mm. The same investigator created CCI-ION animals to avoid variation. The standard for pressure was that the diameter of the nerve fibers should appear slightly thinner, but not affect neuropotential transmission or unobstructed blood circulation. Finally, we sutured the incision routinely and fed the rats normally after they woke up. In the sham-operated rats the nerve was left untouched.

2.4. Measurement of mechanical withdrawal threshold (MWT)

Noxious-pressure stimulation was used to evaluate mechanical hyperalgesia. Unrestrained rats were placed in a clear plastic chamber (22 × 12 × 22 cm) on a stainless-steel mesh floor and allowed to acclimate. The mechanical hyperalgesia threshold of rats was tested using electric von Frey filaments (BME-404 NO.E5489). We assessed nociception on days 0, 1, 3, 5, 7, 9, 11, 13, and 15 after CCI-ION via the measurement of a withdrawal threshold in the innervations area of the infraorbital nerve. The area of stimulation centered on the rat nasal area and extended to the vibrissa. Stimulations were administered when the rat was in a sniffing/no locomotion state: with four paws placed on the ground, neither moving nor freezing, but exhibiting sniffing behavior. A new stimulus was applied only when the rat resumed this position and at least 30 s after the preceding stimulation.

2.5. Reverse transcription-polymerase chain reaction (RT-PCR)

On day 15 after the operation, the rats in each group were anaesthetized and killed. Ipsilateral TGs on the operation side were removed and transferred immediately into PBS. Then, total RNA was isolated from TGs using a chloroform procedure with TRIzol Reagent Kits (TIANGEN, Beijing, China) according to the respective manufacturer's protocols. cDNA synthesis was performed with a Revert Aid First Strand cDNA Synthesis Kit (Fermentas Co., Shenzhen, China). cDNA was stored at 4 °C after a reaction for 30 min at 42 °C. Then, PCR amplification of the P2X3 receptor and β-actin (control) was performed. The oligonucleotides used for the amplification of P2X3 receptor and β-actin were as follows: for P2X3, sense 5′- ACAGAGTCATGGACGTGTCG −3′ and antisense 5′- TGAGGTTAGGCAGGAGGTTT −3′, and for β-actin, sense 5′-TAAAGACCTCTATGCCAACACAGT-3′ and antisense 5′-CACGATGGAGGGGCCGGACTCATC-3′. The PCR products were amplified using the following cycling parameters: 94 °C for 5 min; followed by 35 cycles of 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 30 s; and finally a single cycle at 72 °C for 10 min.

2.6. Quantitative real-time PCR

On day 15 after the operation, the rats of each group were anaesthetized and killed. Ipsilateral TGs on the operation side were removed and transferred immediately into PBS. Total RNA was isolated with an RNA simple Total RNA Kit (TIANGEN, Beijing, China), according to the manufacturer protocol. The cDNA synthesis was performed with a TransScript First Strand cDNA Synthesis Super Mix (TransGen, Beijing, China). The cDNA was stored at 4 °C after reaction for 30 min at 42 °C. The primers were designed with the Primer Express 3.0 software (Applied Biosystems), and the sequences were as follows: for P2X3, sense 5′- ACAGAGTCATGGACGTGTCG −3′ and antisense 5′-TGAGGTTAGGCAGGAGGTTT −3′, and for β-actin, sense 5′-TAAAGACCTCTATGCCAACACAGT-3′ and antisense 5′-CACGATGGAGGGGCCGGACTCATC-3′. Quantitative PCR was performed with the SYBR® Green MasterMix in an ABI PRISM® 7500 Sequence Detection System (Applied Biosystems, Inc., Foster City, CA, USA). The thermal cycling parameters were 95 °C for 30 s; followed by 40 cycles of amplification at 95 °C for 5 s and 65 °C for 34 s; 95 °C for 15 s, 60 °C for 1 min, and 95 °C for 15 s. The dissolution curve was used to determine the specificity of the amplification, and the results were processed by the software within the ABI7500 PCR instrument.

2.7. Immunohistochemistry (IHC)

Immunohistochemical staining was performed with an SP-9001 kit (Beijing Zhongshan Biotech Co.) according to the manufacturer's instructions. Briefly, trigeminal ganglia (TGs) were isolated and flushed with ice-cold phosphatebuffered saline (PBS). After being fixed with 4% paraformaldehyde (PFA) for 24 h, the TGs were dehydrated in 30% sucrose overnight at 4 °C, and then were cut into 15-μm-thick sections on a cryostat. After washing with PBS three times, the preparations were incubated in 3% H_2O_2 for 10 min to block the endogenous peroxidase activity and then with 10% goat serum for 30 min at room temperature to block non-specific antigen binding. After rinsing and washing in PBS, the blocked sections were incubated with anti- P2X3 (1:100 diluted in PBS; Alomone Labs, Jerusalem, Israel) overnight at 4 °C. After three rinses in PBS, the sections were incubated with a biotinylated goat anti-rabbit secondary antibody (Beijing Zhongshan Biotech Co.) for 1 h at room temperature. The preparations were washed in PBS, and then streptavidin-horseradish peroxidase (Beijing Purinergic Signalling Zhongshan Biotech Co.) was added for 30 min. After development in the diaminobenzidine chromogen for 5 min, the slides were washed with distilled water and cover slipped. After immunohistochemistry, an image scanning analysis system (HMIV-2000, Wuhan) was used to analyze the changes in the levels of integrated optical density (IOD) of P2X3 receptor in the ganglia. The background was determined by averaging the optical density of 10 random areas.

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