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Activation of satellite glial cells in the trigeminal ganglion contributes to masseter mechanical allodynia induced by restraint stress in rats

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HIGHLIGHTS

- Chronic restraint stress induces mechanical allodynia in masseter muscle of rats.
- SGCs are activated and substance P expression increases in TG of stressed rats.
- Activated SGCs in TG release IL-1β and up-regulate IL-1RI expression in neurons.
- SGCs inhibition could relieve masseter allodynia induced by restraint stress.

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ABSTRACT

It is commonly accepted that psychological stress contributes to the development of chronic orofacial pain. However, the neural mechanism underlying this process has remained unclear. The present study was performed to determine the involvement of satellite glia cells (SGCs) in the trigeminal ganglion (TG) in stress-induced increases in masseter muscle allodynia in rats. Using a chronic restraint stress model, we found that exposure to a 14-day stress but not a 3-day stress (6 h/day) caused decreased body weight gain, behavioral changes and marked masseter allodynia in rats. SGCs were dramatically activated, and substance P (SP) expression was significantly increased in the TG. A further analysis was undertaken to investigate the contribution of SGCs; the expression of interleukin-1β (IL-1β) in SGCs and interleukin-1 receptor I (IL-1RI) in neurons was significantly increased after chronic restraint stress, whereas injection of L-α-aminoadipate (a SGC inhibitor, LAA) into the TG dramatically inhibited the overexpression of these proteins. In addition, LAA or interleukin-1 receptor antagonist (IL-1ra) administration into the TG could significantly attenuate the mechanical masseter allodynia and overexpression of SP in the TG induced by restraint stress. These results suggest that SGC activation in the TG may play a role in masseter allodynia induced by restraint stress. The over-release of IL-1β and excessive IL1-RI expressions have close relationship with the stress induced masseter allodynia.

1. Introduction

Chronic and persistent masticatory muscle pain is one of the most important symptoms reported by patients with temporomandibular disorders (TMD), a family of conditions involving the temporomandibular joint (TMJ) and masticatory muscles [1]. These chronic muscle pain disorders, often occurring with little or no muscle injury or inflammation, have long been considered linked to central sensitization mechanisms [2]. Psychological factors, such as anxiety and depression, have recently been proven to influence orofacial pain expression [3,4] and appear to have a prominent effect on TMD [5,6]. However, there are few relevant studies addressing the neural basis underlying the integration of psychological stress with orofacial muscle pain.

The sensory information, including pain, of the orofacial area is transmitted to the central nervous system (CNS) via primary afferent neurons which have cell bodies located in the trigeminal ganglion (TG) [7]. In some painful conditions, small-diameter primary afferent neurons of the TG produce and release pain-related neuropeptides, such as substance P (SP), that enhance and maintain the noxious responses of chronic pain [8,9]. Moreover,

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relevant studies have suggested that satellite glial cells (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 [7]. Following trigeminal nerve injury and inflammation (abnormally intense pain elicited by noxious stimuli), increased levels of glial fibrillary acid protein (GFAP), a marker of activated glia cells, can be detected [10,11]. The activated SGCs may directly influence neuronal activity by releasing inflammatory mediators such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), contributing to the development and maintenance of allodynia and hyperalgesia [12,13].

Our group has previously demonstrated that repeated psychological stress could cause structural and metabolic changes [14], as well as oxidative damage [15], in the masseter muscle of rats. Furthermore, repeated stress also increases masseter muscle mechanical sensitivity [4]. Thus, we attempted to clarify the neural mechanisms underlying this process. Due to the important role of the SGCs in the genesis and maintenance of pain, we performed the present study to determine whether SGCs in the TG are activated under chronic stress and whether SGC activation could affect or facilitate neuronal plasticity in the TG and play a role in muscle allodynia induced by stress.

2. Materials and methods

2.1. Experimental animals and restraint stress inducement

Adult male Sprague-Dawley rats (180–200 g) were used in this study. Totally 60 rats contributed to the whole experiments**.** All experiments were performed under the approval from the Animal Use and Care Committee for Research and Education of the Fourth Military Medical University. The animals were repeatedly exposed to daily restraint stress (6 h/day) for 3 days (subacute model) or 14 days (chronic model) ($n = 6$). The restraint stress was performed by keeping the rats in a restrainer consisting of inflexible wire mesh with a sliding door to facilitate restraint. During the procedure, the rats were not allowed to eat, drink or move freely, but their bodies were not constricted [16]. Normally raised rats served as controls ($n = 6$). The rats were weighed every other day. Their body weight gain was calculated according to the following formula: [(Body weight at time point t) − (Baseline body weight)]/(Baseline body weight) [15].

2.2. Behavioral testing

The open-field chamber (RD 1412-OF, Shanghai Mobile datum Corporation) consisted of a 100 cm × 100 cm × 80 cm Plexiglas box illuminated by a single fluorescent light suspended over the chamber. The activities of each rat were automatically monitored for 15 min using a digital video camera [17]. The behaviors of stress rats were measured half an hour after the last restraint stress.

The masseter muscle mechanical sensitivity of the animals was assessed by an electronic von-Frey anesthesiometer (IITC Life Science Instruments). During testing, force was applied with the probe to the masseter muscle belly region, which is located 10 mm inferior to the central point of the line between the orbit and the tragus. The force in grams required to elicit head withdrawal indicative of a nociceptive response was recorded as the head withdrawal threshold (HWT) [18].

2.3. Immunofluorescence staining

Rats were sacrificed using intracardial perfusion after the behavioral evaluation. Then the TGs were removed and cut into 20 μm longitudinal sections with a cryostat (CM1800, Leica). The immunofluorescence staining were performed as our previous report [17]. The sections were visualized under a confocal laser scanning microscope (FV1000, Olympus). For semi-quantification, the number of SP-immunoreactive (SP-IR) neurons or the number of somata of TG neurons encircled with GFAP-IR cells over 2/3 of the soma perimeters of neurons (SensivMeasure) was counted in each rat at the magnification of 200× or 400×. Three sections for each TG were randomly chosen for measurement [10]. The following primary antibodies were used: guinea pig anti-SP (1:200, Abcam), mouse anti-GFAP (1:500, Millipore), rabbit anti-IL-1β (1:300, Endogen), rabbit anti-IL-1RI (1:50, Santa Cruz), mouse anti-NeuN (1:200, Millipore), and rabbit anti-NeuN (1:200, cell signaling).

2.4. Western blot assays

Tissue samples of the TGs were subjected to western blot for IL-1β (rabbit anti-, 1:500, Endogen) and IL-1RI (rabbit anti-, 1:100, Santa Cruz) as before [17]. The concentration of β-actin was also measured using a rabbit anti-β-actin antibody (1:5000, Sigma) as an intra-control.

2.5. Drug administration to the TG

For drug administration, another batch of rats were enrolled in the experiments ($n = 6$). Under anaesthesia, the animal's skull was exposed and a small hole (diameter; 1 mm) was drilled above the location of TG. The guide cannula was extended into the hole 9 mm below the skull surface into the TG (2.8 mm anterior to intra-aural zero and 2.7 mm left of the midline) and was fixed to the skull with screws and dental cement. To define the position of the tip of the cannula, multiunit activities induced by mechanical stimulation of the masseter muscle area were recorded using the trocar as an electrode. L-α-aminoadipate (LAA, 10 nmol, Sigma) or interleukin-1 receptor antagonist (IL-1ra, 20 nmol, Amgen) were injected into the TG (0.5 ml, 10 min) through a 31-gauge injection needle (Heraeus Kulzer) connected to a 10 ml Hamilton syringe. Stressed rats with normal saline (0.9%) was used as the negative control and the unstressed rats with saline (0.9%) was used as the sham group. The rats were allowed to recover for 7 days before experiments were performed [19]. Then the rats were subjected to masseter mechanical sensitivity assessment, after that, the rats were subjected to restraint stress for 14 days. Half an hour after the last restraint stress, masseter mechanical sensitivity of each rat was re-assessed. Then, 10 nmol LAA, 20 nmol IL-1ra or 0.9% saline was given to TG through the cannula according to research design. At 1 h, 2 h, 6 h, 12 h, and 24 h after drug administration, the masseter mechanical sensitivity of each rat was observed. After that, the open-field tests were performed. Then the rats were sacrificed for immunofluorescence staining.

2.6. Statistical analysis

All data are presented as the mean ± SEM. Data from the open field tests, immunohistochemistry and Western blot assays were analyzed using one-way ANOVA followed by the SNK-q test for multiple comparisons. Data from the body weight measurements and von Frey test were evaluated using two-way ANOVA, and the Bonferroni correction was applied to adjust the P value when the ANOVA indicated overall significance. All statistical analyses were performed using SPSS® version 16.0 software (SPSS Inc., USA). $P < 0.05$ was considered statistically significant.