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Involvement of trigeminal astrocyte activation in masseter hyperalgesia under stress

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HIGHLIGHTS

- Chronic restraint stress causes rodent behavioral change and masseter hyperalgesia.
- There is a potential link between astrocyte activation and hyperalgesia by stress.
- Astrocyte toxin relieves both hyperalgesia and behavioral change of stressed rats.
- Astrocyte-IL-1β-NMDAR-neuron pathway may underlie masseter hyperalgesia by stress.

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ABSTRACT

It is commonly accepted that psychological stress contributes to the development of temporomandibular joint disorders, in which chronic orofacial pain is the main symptom. However, the central mechanism underlying the development of these disorders has remained unclear. The current study was performed to determine the involvement of the glia in the trigeminal spinal subnucleus caudalis in stress-induced increases in masseter muscle hyperalgesia in rats. After being subjected to chronic restraint stress, the animals showed decreased body weight gain, behavioral changes and marked masseter allodynia. We also found that astrocytes, but not microglia, in the trigeminal subnucleus caudalis (Vc) were dramatically activated. A further analysis was undertaken to investigate the contribution of the glia; we intrathecally injected L-α-aminoadipate (astrocyte-specific inhibitor) and/or minocycline (microglia-specific inhibitor) into the stressed rats. Our results showed that L-α-aminoadipate (LAA), but not minocycline, could significantly attenuate the mechanical masseter allodynia and behavioral changes induced by restraint stress. In addition, the expression of interleukin-1β (IL-1β) and phosphorylated N-methyl-D-aspartic acid receptor 1 (p-NR1) in the Vc was significantly increased after chronic restraint stress, whereas LAA dramatically inhibited the overexpression of IL-1β and p-NR1. Taken together, these results suggest that activated astrocytes in the Vc may be one of the most important factors in the pathophysiology of masseter hyperalgesia induced by restraint stress and the following overexpression of IL-1β and excessive NMDAR phosphorylation may ultimately contribute to masseter hyperalgesia. Thus, inhibiting spinal astrocytic activation may represent a novel therapeutic strategy for the treatment of orofacial pain induced by stress.

1. Introduction

Temporomandibular joint disorders (TMDs) form a group of conditions associated with pain in the temporomandibular joint (TMJ), masticatory muscles, and related head and neck musculoskeletal structures. Chronic or persistent pain of the masticatory muscles is one of the most important symptoms reported by TMD patients [1,2]. However, these muscle pain disorders, similar to other functional pain syndromes such as fibromyalgia, chronic headache and arthritis, are often described as fluctuating and non-progressive, rather than occurring with muscle injury or inflammation [1]. These facts suggest that the mechanisms underlying the orofacial muscle pain of TMD patients may involve central sensitization. In recent years, numerous studies have demonstrated that psychological stress, such as anxiety and depression, may contribute to the occurrence and development of TMD [3–6]. Additionally, a number of animal studies have indicated that experimental exposure to chronic stress could induce hyperalgesia or enhance nociception in

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the masticatory muscles of the orofacial region [7–10]. Nevertheless, there are few relevant studies addressing the neural mechanisms of psychological stress and orofacial muscle pain.

In general, the medullary dorsal horn (trigeminal spinal subnucleus caudalis; Vc) is considered to integrate the nociceptive information coming from the mandibular area of the orofacial region. Many reports have demonstrated that chronic masticatory muscle pain is usually accompanied by detectable hyperactivated sensory neurons in the Vc [11–14]. Previous research has indicated that activation of the N-methyl-D-aspartate receptor (NMDAR), a glutamate receptor localized on neurons, plays an important role in the occurrence of pain [15–17]. However, recent literature has suggested that the central glia (including astrocytes and microglia), which had been considered to be supporting and nourishing cells, play a crucial role in the development and maintenance of various types of pain conditions. When nerve injury or inflammation occurs, the central glia can be activated, releasing a host of proinflammatory cytokines, such as interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and subsequently modulating neuronal activity, resulting in pain facilitation [18–21]. Previous studies have clarified that microglia, for which ionized calcium-binding adapter molecule 1 (Iba-1) or complement receptor type 3 (OX-42) is used as an activation marker, usually play a role in the early establishment of acute neuropathic pain, whereas the activation of astrocytes, detected by increased glial fibrillary acid protein (GFAP) expression, more frequently contributes to the maintenance of chronic pain [22,23]. It has also been shown that the activation of astrocytes in the Vc and the subsequent triggering of IL-1β signaling may facilitate NMDAR to enhance neuronal activity, resulting in allodynia in the orofacial area in experimental inflammatory or neuropathic pain models [24–26].

It has been established that experimentally induced chronic stress can alter the neural properties of the Vc and cause decreased orofacial nociceptive thresholds [7,9]. Unfortunately, the mechanisms underlying this process are poorly understood. Due to the important role of the central glia in the genesis of chronic pain, we hypothesized that trigeminal glial activation could affect or facilitate neuronal plasticity by interacting with neuronal glutamate receptors and could play a role in the masticatory muscle hyperalgesia induced by psychological stress. In the present study, we chose to examine the masseter muscle because it is the most superficial and one of the strongest muscles involved in mastication. In addition, we investigated whether either astrocytes or microglia, or both, were activated in the development of mechanical allodynia of the masseter muscle induced by restraint stress.

2. Materials and methods

2.1. Animals

A total of 62 adult male Sprague–Dawley rats (180–200 g, from the Laboratory Animal Center of the Fourth Military Medical University, Xi'an, China) were used in this study. The rats were caged in a room with controlled temperature (22 ± 1 °C), humidity (60 ± 5%), and light–dark cycles (light on from 8:00 to 20:00 h). The animals were given access to food and water ad libitum. This study was performed in strict accordance with the recommendations in the ethical guidelines for investigations of experimental pain in conscious animals [27]. All experimental procedures received prior approval from the Animal Use and Care Committee for Research and Education of the Fourth Military Medical University (Xi'an, China), and every effort was made to avoid animal suffering at each stage of the experiment.

2.2. Restraint stress

The restraint procedure started between 8:00 and 10:00 am every day. The rats were kept in a restrainer consisting of inflexible wire mesh with a sliding door. During the procedure, the rats were not allowed to eat, drink, or move freely, but their bodies were not constricted [28]. Normally raised rats served as controls (n = 8). During restrain procedures, time points of 3, 7, 14 days or longer (21 days) have been typically chosen in studies on the effects of restraint stress on glial or neuronal activity in the central neural system [28–30]. In our previous study, the masseter mechanical sensitivity after stress was found to be significantly changed from 3 days to 14 days. Thus, the rats in the present study were subjected to restraint stress (6 h/day) for 3, 7 or 14 consecutive days (n = 8).

2.3. Body weight measurements

Initially, the body weights of the animals were measured and recorded as the baseline level. After the experiment began, 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) [31].

2.4. Behavioral testing

2.4.1. Open-field test

The open-field chamber (RD 1412-OF, Shanghai Mobile Datum Corporation, Shanghai, China) 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. After each test, the box was thoroughly cleaned with 20% alcohol. The distance traveled in center, the total distance traveled and the velocity were calculated [32,33].

2.4.2. Assessment of mechanical sensitivity

According to previous studies [34,35], animals were first habituated to standing on their hind paws and against the tester's gloved hand. The habituation lasted approximately half an hour. An electronic von-Frey anesthesiometer (IITC Life Science Instruments, Woodland Hills, CA, USA) was used to assess muscle mechanical sensitivity. The unit was supplied with a rigid plastic tip capable of transmitting pressure onto the masticatory muscles. In the present study, the masseter muscle belly region, a site located 10 mm inferior to the central point of the line between the orbit and the tragus, was tested. At this location, obvious muscular contraction can be palpated during mastication. When testing, force was applied with the probe oriented perpendicular to the sagittal plane. The force in grams required to elicit head withdrawal indicative of a nociceptive response was recorded five times for each animal at 1-min intervals. The average of these five values was used as the withdrawal threshold. For this study, we defined mechanical hyperalgesia as a statistically significant decreased withdrawal threshold following stress compared with the pre-stress baseline. The rats were tested half an hour after the application of stress every other day. For all of the data reported here, the observer was blinded to the grouping situation of the rats.

2.5. Immunofluorescence staining

After being deeply anesthetized with pentobarbital (60 mg/kg), the rats were perfused through the ascending aorta with 100 ml of 0.9% saline, followed by 500 ml of 0.1 M phosphate buffer (PB, pH 7.3) containing 4% paraformaldehyde and 2% picric acid. After perfusion, the medulla and upper cervical spinal cord were removed and post-fixed in the same fixative for 4 h and then transferred to 30% sucrose in 0.1 M PB for cryoprotection. Transverse frozen spinal sections (30 μm thick) of the caudal medulla and upper cervical spinal cord were cut with a cryostat (Leica CM1800; Heidelberg, Germany) and collected. The sections were rinsed in 0.01 M phosphate-buffered saline (PBS, pH 7.3) three times (10 min each), then blocked with 2% goat serum in 0.01 M PBS containing 0.3% Triton X-100 for 1 h at room temperature and subjected to immunofluorescent staining. The sections were incubated overnight at room temperature with the primary antibodies mouse anti-GFAP and goat anti-Iba-1 (1:500; Chemicon, Temecula,