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Periodontal CGRP contributes to orofacial pain following experimental tooth movement in rats

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ABSTRACT

Calcitonin-related gene peptide (CGRP) plays an important role in orofacial inflammatory pain. The aim of this study was to determine whether periodontal CGRP contributes to orofacial pain induced by experimental tooth movement in rats. Male Sprague–Dawley rats were used in this study. Closed coil springs were used to deliver forces. Rats were euthanized on 0 d, 1 d, 3 d, 5 d, 7 d, and 14 d following experimental tooth movement. Then, alveolar bones were obtained for immunostaining of periodontal tissues against CGRP. Two hours prior to euthanasia on each day, orofacial pain levels were assessed through rat grimace scale. CGRP and olcegepant (CGRP receptor antagonist) were injected into periodontal tissues to verify the roles of periodontal CGRP in orofacial pain induced by experimental tooth movement. Periodontal CGRP expression levels and orofacial pain levels were elevated on 1 d, 3 d, 5 d, and 7 d following experimental tooth movement. The two indices were significantly correlated with each other and fitted into a dose–response model. Periodontal administration of CGRP could elevate periodontal CGRP expressions and exacerbate orofacial pain. Moreover, olcegepant administration could decrease periodontal CGRP expressions and alleviate orofacial pain. Therefore, periodontal CGRP plays an important role in pain transmission and modulation following experimental tooth movement. We suggest that it may participate in a positive feedback aiming to amplify orofacial pain signals.

1. Introduction

Orofacial pain induced by tooth movement, designated as orthodontic pain in clinical practice, is an inflammatory pain related to tooth movement in the orofacial region (Krishnan, 2007). It has been reported that 87%–100% orthodontic patients experience orthodontic pain during orthodontic treatments (Bergius et al., 2002; Erdinc and Dincer, 2004; Khattab et al., 2013; Long et al., 2013). However, to date, no truly effective approaches have been developed for the relief of orthodontic pain (Angelopoulou et al., 2012; He et al., 2013), justifying the need for further research to elucidate the neurological basis of orthodontic pain.

The painful signals induced by tooth movement are first received by sensory terminals located at periodontal tissues, transmitted to trigeminal neurons located at trigeminal ganglia, relayed to trigeminal nucleus caudalis at medulla oblongata, and at last transmitted to thalamus and perceived by the sensory cortex (Fig. 1) (Crossman and Neary, 2000; Krishnan, 2007). It has been well documented that neuropeptides participate in the sensation and modulation of orofacial pain at the aforementioned sensory stations (*i.e.*, periodontal tissues, trigeminal ganglia, and trigeminal nucleus caudalis) (Deguchi et al., 2003; Liao et al., 2013; Puri et al., 2005; Yang et al., 2009a; Yang et al., 2009b). Therefore, exploring the roles of neuropeptides may help elucidate the neurological mechanisms of orofacial pain (Chichorro et al., 2009; Teodoro et al., 2013).

In particular, CGRP (calcitonin gene-related peptide), a 37 amino acid, has been well-documented to participate in the transmission and modulation of orofacial inflammatory pain (Cady et al., 2011; Capuano et al., 2009; Eberhardt et al., 2009; Kaiser and Russo, 2013; Yisarakun et al., 2015). Moreover, peripheral blockade of CGRP receptor has been revealed to alleviate inflammatory pain in rats (Hirsch et al., 2013). It has been proposed that pronociceptive effects of CGRP are achieved through changing vascular permeability (Yamamoto, 2012) and inducing the expressions of other neuropeptides (Shinoda and Iwata, 2013). However, the exact mechanisms of CGRP whereby it contributes to orofacial pain are still largely unknown. Although previous studies (Kato et al., 1996, Qiao et al., 2012) have determined the expressions of CGRP in periodontal tissues following experimental tooth movement (Kato et al., 1996; Kondo et al., 2013; Norevall et al., 1995; Qiao et al., 2012; Saito et al., 1991), the exact roles of periodontal CGRP in tooth-movement-induced orofacial pain remain poorly understood.

Therefore, in this study, we hypothesized that periodontal CGRP participates in the genesis of orofacial pain following experimental

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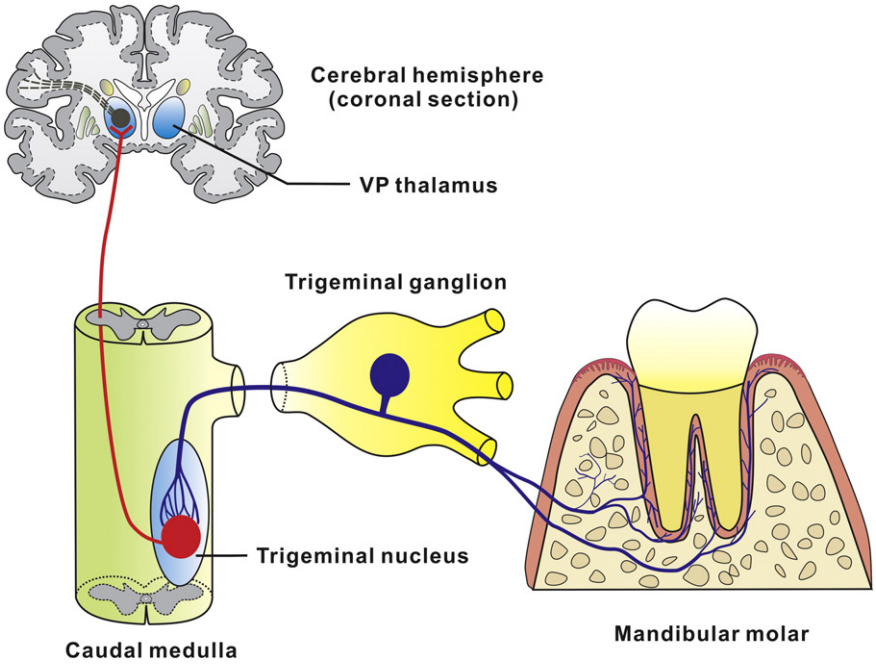

Fig. 1. Sensory pathway for tooth stimuli. A stimulus, once exerted on a tooth, would activate periodontal sensory endings. These sensory fibers would send nociceptive signals to trigeminal neurons located at trigeminal ganglia. Trigeminal neurons would send fibers carrying these painful signals to trigeminal nucleus located at caudal medulla. Then, these noxious signals would be transmitted from trigeminal nucleus to ventral posterior nucleus of thalamus. Lastly, these signals would be replayed to sensory cortex and perceived by organisms.

tooth movement and aimed to examine the roles of periodontal CGRP in orofacial pain induced by experimental tooth movement in rats.

2. Methods

2.1. Animals

In total, 185 male Sprague–Dawley rats (age: 2 months; weight: 200–250 g) offered by the Animal Experimental Center of Sichuan University were used in this study. They were provided with standard rat chow and water *ad libitum* and maintained on a 12/12 day–night cycle.

These 185 rats were divided into force group (n = 37), sham group (n = 37), force + CGRP group (n = 37), force + olcegepant group (n = 37), and force + saline group (n = 37). Closed coil springs were ligated between upper incisor and molar to mimic orthodontic forces in force groups (40 g) while inactivated in the sham group (0 g). They were euthanized through general anesthesia and subsequent cervical dislocation on 0 d, 1 d, 3 d, 5 d, 7 d, and 14 d (5 rats on 0 d, 1 d, 3 d, 5 d, and 7 d in each group and 12 rats on 14 d in each group). Particularly, in all the groups, the rats (n = 12 in each group) euthanized on 14 d were evaluated for orofacial pain levels on 0 d, 1 d, 3 d, 5 d, 7 d, and 14 d. Moreover, the rats euthanized on 0 d did not receive any intervention and were regarded as a baseline control for each group.

As for periodontal injections, CGRP solution (5×10^{-5} mol/L; Chinese Peptide Company, China), olcegepant solution (10^{-3} mol/L; MedChem Express, Priceton, USA), and saline were injected into periodontal tissues around both molars and incisors of the rats in the aforementioned three groups, respectively. Periodontal injections (5 μL) were performed 2 h prior to spring mounting or pain assessment.

2.2. Immunostaining

Following euthanasia, upper alveolar bone containing molars were excised and decalcified in ethylenediaminetetraacetate (EDTA) solutions for at least 1 month. Then, these decalcified hard tissues were immunostained against rabbit-originated monoclonal CGRP (1:200; Abcam). Specifically, following antigen retrieval through microwave oven-citrate buffer, deparaffinized tissue samples were rinsed with phosphate-buffer saline (PBS) for three times. Then, they were blocked with goat serum and incubated with a specific antibody against rabbit originated monoclonal CGRP (1:200; Abcam) at 37 °C for 45 min. The sections were washed with PBS for 10 min and incubated with EnVision™ (K500711; DAKO, Carpinteria, CA) at 37 °C for 45 min. After rinsing again with PBS for 10 min, the sections were developed with 3, 3′-diaminobenzidine (DAB), counterstained with hematoxylin, dehydrated and mounted on coverslips.

Visualizations of immunoreactive tissues were achieved through a light microscope (Model Bx51; Olympus, Tokyo, Japan). For each rat, integrated optical density (IOD)/area for periodontal tissues was calculated in each of 5 randomly consecutive fields (400) in each of 3 slides and the mean value of these 15 fields was employed as the expression level of CGRP for each rat.

2.3. Pain assessment through rat grimace scale (RGS)

Pain levels were assessed through rat grimace scale (RGS) according to Scotocinal et al. (2011) and Liao et al. (2014). In brief, rats were placed individually into a transparent cubicle ($21.0 \times 10.5 \times 9.0$ cm^3) and videotaped for 30 min. Then, for each rat, 10 images of facial expressions were extracted from each videotaping session (30 min) and used for RGS scoring. Specifically, RGS scoring was performed according to the facial expression changes in orbit, nose, ear, and whisker. For each rat, baseline RGS scores were determined before any intervention and RGS scores were assessed on 0 d, 1 d, 3 d, 5 d, 7 d, and 14 d. Then, differences in RGS scores were obtained by subtracting baseline RGS scores from RGS scores at different time points. The differences in RGS scores (simply designated as RGS scores thereafter) were regarded as the surrogate pain levels for each rat at each time point. For each group at each time point, normality of data distributions was confirmed by both Kolmogorov–Smirnov test and Shapiro–Wilk test (all $p > 0.10$). Thus, parametric analysis was used in this study (Norman, 2010).