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The transient receptor potential cation channel subfamily V members 1 and 2, P2X purinoceptor 3 and calcitonin gene-related peptide in sensory neurons of the rat trigeminal ganglion, innervating the periosteum, masseter muscle and facial skin

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ABSTRACT

Objective: Distribution of the transient receptor potential cation channel subfamily V members 1 (TRPV1) and 2 (TRPV2), and P2X purinoceptor 3 (P2 × 3) was investigated in rat trigeminal ganglion neurons innervating the periosteum, masseter muscle and facial skin.

Design: Double immunofluorescence method for TRPV1 and TRPV2 ion channels or ATP receptor P2 × 3 with calcitonin gene-related peptide (CGRP) was performed on trigeminal ganglion neurons retrogradely labeled from the mandibular periosteum, masseter muscle, or facial skin in 15 male Wistar rats.

Results: The cell size of periosteum neurons (mean ± S.D. = 810.7 ± 36.1 μm^2) was smaller than that of masseter muscle neurons (927.0 ± 75.6 μm^2), and larger than that of facial skin neurons (661.3 ± 82.2 μm^2). Periosteum neurons contained TRPV1- (26.7%), TRPV2- (47.1%) and P2 × 3-immunoreactivity (50.1%). Expression of TRPV2-immunoreactivity was more abundant among periosteum neurons than among facial skin neurons (16.1%). Regarding to TRPV1 and P2 × 3 expression, however, there was no significant difference between periosteum neurons and, masseter muscle and facial skin neurons. TRPV1- immunoreactive trigeminal ganglion neurons which innervated the periosteum, masseter muscle and facial skin mostly had small and medium-sized cell bodies, whereas TRPV2- and P2 × 3-immunoreactive trigeminal ganglion neurons innervating those tissues were of various cell body sizes. Approximately 20% of periosteum (19.2%), masseter muscle (19.2%) and facial skin (21.5%) neurons contained both TRPV1- and CGRP-immunoreactivity. Some periosteum neurons also co-expressed CGRP-immunoreactivity with TRPV2- (10.9%) or P2 × 3- immunoreactivity (11.1%). Distributions of perivascular and free nerve fibers containing CGRP and either TRPV1, TRPV2, or P2 × 3 were occasionally very similar in the mandibular periosteum.

Conclusions: The present study indicated that trigeminal ganglion nociceptors innervating the periosteum as well as those innervating the masseter muscle and facial skin have vanilloid, acidic, thermal, mechanical and ATP sensors. In some periosteum neurons, CGRP may act as inflammatory mediator through activation of TRPV1, TRPV2 and P2 × 3.

1. Introduction

Primary nociceptive neurons respond to mechanical, chemical, and thermal stimuli, and convey the information to the brainstem and spinal cord. The transient receptor potential cation channel subfamily V member 1 (TRPV1) responds to vanilloids, acids and temperatures > 43 °C (Caterina, Rosen, Tominaga, Brake, & Julius, 1999). The receptor channel is expressed by small and medium-sized neurons in the sensory ganglion (Caterina et al., 1999; Guo, Vulchanova, Wang, Li, & Elde, 1999; Ichikawa & Sugimoto, 2001, 2004; Hironaka et al., 2014; Kim, Kim, Lee, Ko, & Bae, 2018). Such neurons have unmyelinated axons and send free nerve endings to their peripheral receptive fields (Lawson, McIlwrath, Woodbury, Davis, & Koerber, 2008; Yeo et al., 2010). TRPV2 is activated by temperatures > 52 °C and mechanical stimulus (Caterina et al., 1997; McGahon et al., 2016; O'Neil & Heller, 2005). TRPV2-containing sensory neurons are medium-

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Table 1
List of antibodies.

Antibody	Species	Source	catalog No.	Dilution
Primary antiserum				
TRPV1	rabbit	Neuromics, USA	RA10110	1:400
TRPV1	goat	R&D systems Inc., USA	AF3066	1:300
TRPV2	rabbit	EMD Millipore, USA	AB5398	1:2000
P2 × 3	rabbit	Neuromics, USA	RA14139	1:2000
CGRP	guinea pig	Peninsula Laboratories, USA	T-5053	1:1000
Secondary antibody				
Lissamine rhodamine red™-X-conjugated anti-rabbit IgG for ion channels	donkey	Jackson ImmunoResearch Labs., USA	711-296-152	1:300
Lissamine rhodamine red™-X-conjugated anti-goat IgG for TRPV1	donkey	Jackson ImmunoResearch Labs., USA	705-295-147	1:300
Fluorescein isothiocyanate-conjugated anti-guinea pig IgG for CGRP	donkey	Jackson ImmunoResearch Labs., USA	706-096-148	1:100

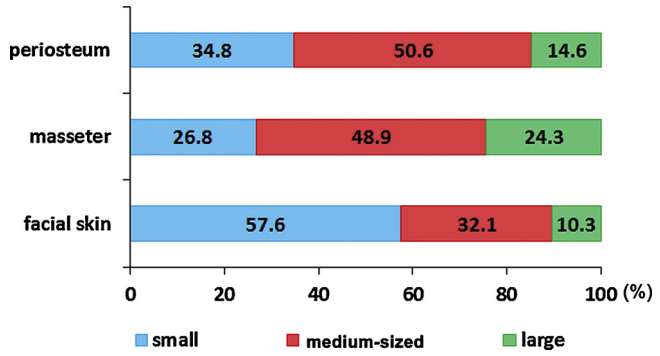

Fig. 1. The proportion of small, medium-sized and large trigeminal ganglion neurons which innervate the periosteum, masseter and facial skin. The data were obtained from design of a specific tissue labeled per rat, with n = 5 rats for each tissue. A total of 1580 periosteum trigeminal ganglion neurons, 912 masseter trigeminal ganglion neurons and1413 facial skin trigeminal ganglion neurons were analyzed.

sized to large, and have myelinated axons (Caterina et al., 1997; Hironaka et al., 2014; Ichikawa & Sugimoto, 2000; Ichikawa et al., 2005; Lawson et al., 2008; Sato et al., 2017). P2X purinoceptor 3 (P2 × 3) is sensitive to extracellular ATP (Saloman, Chung, & Ro, 2013; Yasuda et al., 2016). P2 × 3-containing sensory neurons with small to medium-sized cell bodies have TRPV1, and those with medium-sized to large cell bodies contain TRPV2 (Guo et al., 1999; Ichikawa & Sugimoto, 2004). In addition, small to medium-sized sensory neurons co-express TRPV1 and TRPV2 ion channels or ATP receptor P2 × 3 with calcitonin gene-related peptide (CGRP), a marker for unmyelinated and finely myelinated nociceptors (Ichikawa & Sugimoto, 2000, 2001; Yeo et al., 2010; Bae, Kim, Cho, Mah, & Bae, 2015; Quartu et al., 2016).

Sensory neurons in the trigeminal ganglion innervate facial, oral and cranial structures. In the masseter muscle and facial skin, nociceptive afferents contain TRPV1 and TRPV2 ion channels, ATP receptor P2 × 3 and neuropeptides. Many sensory neurons which innervate the masseter muscle and facial skin contain TRPV1, P2 × 3 and CGRP in the trigeminal ganglion (Ichikawa & Sugimoto, 2000, 2004; Carleson, Lundeberg, & Appelgren, 2004; Ambalavanar, Yallampalli, Yallampalli, & Dessem, 2007; Qi et al., 2016; Wong, Hossain, & Cairns, 2017). However, TRPV2 expression is relatively infrequent in the facial skin (Ichikawa & Sugimoto, 2001). We have a hypothesis that the morphology and function of nociceptive afferents may depend on their peripheral targets in the trigeminal system.

The periosteum consists of dense connective tissue, and covers the outer surface of bones. This tissue is exclusively innervated by nociceptive afferents. In previous studies, CGRP-containing trigeminal ganglion neurons innervate blood vessels and make nerve plexuses in the periosteum (Hill & Elde, 1988, 1991). Because neonatal capsaicin treatment decreases the CGRP-containing nerve fibers (Hill & Elde, 1991), occurrence of TRPV1 has been also predicted in the periosteum. To our knowledge, however, distribution of TRPVs or ATP receptors in periosteum afferents has not been reported. And, little is known regarding to function of TRPV1 and TRPV2 ion channels, and ATP receptor P2 × 3 in the periosteum.

In this study, we investigated and compared the distribution of TRPVs, P2 × 3 and CGRP in trigeminal ganglion neurons which innervated the periosteum, masseter muscle and facial skin to facilitate our understanding the morphology and function of nociceptive afferents in the periosteum.

2. Materials and methods

2.1. Specimens

To know expression of TRPV1, TRPV2, P2 × 3 and CGRP in periosteum, masseter muscle and facial skin neurons in the trigeminal ganglion, 15 male Wistar rats (200–250 g) were used. They were anesthetized by intraperitoneal injection of ethyl carbamate (650 mg/kg) and pentobarbital sodium (20 mg/kg). One μl of 1% fluorogold (Fluorochrom, USA) in distilled water was injected into either the mandibular periosteum, the masseter muscle, or the mandibular skin with Hamilton syringe. To demonstrate trigeminal ganglion neurons innervating the periosteum, the tissue was exposed by incision of the facial skin on the right side and the right masseter muscle. Fluorogold was injected into the periosteum in the middle portion of the mandibular ramus on the right side. The excessive fluorogold was wiped with dry cotton and paraffin wax was applied over the injection site to prevent leakage of the tracer into the muscle and skin. To show masseter muscle neurons, the facial skin on the right side was incised, the right masseter muscle was exposed and fluorogold was put into the central portion of the right masseter muscle. The incised masseter muscle and/or facial skin were stitched with silk sutures. To label facial skin neurons, the tracer was injected into the skin over the middle portion of the mandibular ramus on the right side. Three days later, the animals were anesthetized with isoflurane and perfused with Zamboni fixative (Stefanini, De Martino, & Zamboni, 1967). After checking with a fluorescence microscope (ECLIPSE 80i, Nikon, Japan) that there is no spreading of fluorogold to the surrounding different tissues, the ipsilateral trigeminal ganglion was dissected.

Additional 2 Wistar rats (180–250 g) were used to show distribution of TRPV1 and TRPV2 ion channels, ATP receptor P2 × 3 and CGRP in the periosteum. They were anesthetized, and perfused with Zamboni fixative. Mandibles were dissected, and decalcified with 10% ethylenediaminetetraacetic acid (pH 7.4). Eight μm thickness sections of the trigeminal ganglion and mandibles were processed for expression of TRPV1 and TRPV2 ion channels or ATP receptor P2 × 3, and their co-expression with CGRP.

2.2. Immunohistochemistry

Sections were applied with a mixture of antibodies against either