Involvement of TRPV1 and TRPA1 in Incisional Intraoral and Extraoral Pain

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Abstract

Thermal and mechanical hypersensitivity in the injured region is a common complication. Although it is well known clinically that thermal and mechanical sensitivity of the oral mucosa is different from that of the skin, the mechanisms underlying injured pain of the oral mucosa remain poorly understood. The transient receptor potential (TRP) vanilloid 1 (TRPV1) and TRP ankyrin 1 (TRPA1) in primary afferent neurons are known to contribute to pathological pain. Therefore, we investigated whether TRPV1 and/or TRPA1 contribute to thermal and mechanical hypersensitivity following oral mucosa or whisker pad skin incision. Strong heat and mechanical and cold hypersensitivity was caused in the buccal mucosa and whisker pad skin following incisions. On day 3 after the incisions, the number of TRPV1-immunoreactive (IR) and TRPA1-IR trigeminal ganglion (TG) neurons innervating the buccal mucosa and whisker pad skin was significantly increased, and the number of TRPV1/TRPA1-IR TG neurons innervating whisker pad skin, but not the buccal mucosa, was significantly increased. Administration of the TRPV1 antagonist, SB366791, to the incised site produced a significant suppression of heat hyperalgesia in both the buccal mucosa and whisker pad skin, as well as mechanical allodynia in the whisker pad skin. Administration of the TRPA1 antagonist, HC-030031, to the incised site suppressed mechanical allodynia and cold hyperalgesia in both the buccal mucosa and whisker pad skin, as well as heat hyperalgesia in the whisker pad skin. These findings indicate that altered expressions of TRPV1 and TRPA1 in TG neurons are involved in thermal and mechanical hypersensitivity following the buccal mucosa and whisker pad skin incision. Moreover, diverse changes in the number of TRPV1 and TRPA1 coexpressed TG neurons in whisker pad skin-incised rats may contribute to the intracellular interactions of TRPV1 and TRPA1 associated with whisker pad skin incision, whereas TRPV1 and TRPA1 expression in individual TG neurons is involved in buccal mucosa-incised pain.

Keywords: whiskers, mouth mucosa, trigeminal ganglion, mechanical allodynia, transient receptor potential channels, thermal allodynia

Introduction

Thermal and mechanical hypersensitivity occurs due to skin incision, which reflects postinjured pain (Brennan et al. 1996). Various molecular changes are caused in the primary neurons following tissue injury, which cause sensitization of the primary afferent neurons innervating the tissue-injured site (Barabas and Stucky 2013; Mizukoshi et al. 2013). It has been established that thermal and mechanical sensitivity of the oral mucosa is different from that of the facial skin (Green and Gelhard 1987; Komiyama and De Laat 2005). These studies suggest that thermal and mechanical sensory mechanisms are different between intraoral mucosa and facial skin.

The transient receptor potential (TRP) vanilloid 1 (TRPV1) is abundantly expressed in primary neurons (Tominaga and Caterina 2004). TRPV1 is activated by noxious heat (>43 °C), and the functional alterations of TRPV1 contribute heat hyperalgesia after incision (Barabas and Stucky 2013).

Moreover, TRP ankyrin 1 (TRPA1) is a nonselective cation channel expressed in primary neurons and known to be involved in cold and mechanical hypersensitivity after

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A supplemental appendix to this article is published electronically only at <http://jdr.sagepub.com/supplemental>.

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Figure 1. Thermal and mechanical hypersensitivity following oral mucosa or whisker pad skin incision. (A) The precise incised locations of the buccal mucosa and the whisker pad skin incision. Time course of the changes in heat head withdrawal threshold (HHWT) (B), mechanical head withdrawal threshold (MHWT) (C), and cold head withdrawal threshold (CHWT) (D) and each example of bursting electromyography (EMG) activity defined as the threshold ($n = 5$ in each). * $p < 0.05$, ** $p < 0.01$, # $p < 0.05$, ### $p < 0.01$ vs. naive.

incision (Wei et al. 2012; Barabas and Stucky 2013). All TRPA1 positive primary neurons express TRPV1, and 67% of TRPV1 positive primary neurons express TRPA1 (Kobayashi et al. 2005). Intracellular interaction between TRPV1 and TRPA1 contributes to modulation of primary neuronal excitability (Staruschenko et al. 2010; Spahn et al. 2014).

To evaluate the mechanisms underlying thermal and mechanical hypersensitivity in the buccal mucosa or whisker pad skin due to incisions, TRPV1 or TRPA1 expression, TRPV1 and TRPA1 coexpression in trigeminal ganglion (TG) neurons innervating the buccal mucosa or whisker pad skin, and the effect of TRPV1 and TRPA1 antagonists on thermal and mechanical hypersensitivity were studied in the rat model with whisker pad skin or the buccal mucosal incision.

Materials and Methods

Animals

Male Sprague-Dawley rats ($n = 300$; Japan SLC, Shizuoka, Japan) weighing 200 to 300 g were used in this study. The animals were maintained in a temperature-controlled room (23 °C) with a 12/12-h light/dark cycle. Food and water were freely available. This study was approved by the Animal Experimentation Committee at Nihon University School of Dentistry. This study was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the guidelines of the International Association for the Study of Pain

(Zimmermann 1983). The number of rats used in this study was based on the minimum required for statistical analysis.

Buccal Mucosa or Whisker Pad Skin Incision

Rats were deeply anesthetized with an intraperitoneal (i.p.) injection of sodium pentobarbital (50 mg/kg; Schering Plough, Whitehouse Station, NJ, USA). The incision of the left buccal mucosa or whisker pad skin was performed using the scalpel (depth, 2 mm; length, 10 mm) (Fig. 1A). Histologically, the incisions were confined exclusively in buccal mucosa or whisker pad skin. Then, the incised wound was sutured once with 3-0 nylon at the middle of the incision. The deep anesthesia and the placement of device for opening the mouth were performed in control rats, which was identical except for incision and suture.

Thermal and Mechanical Stimulation

Rats were lightly anesthetized with 2% isoflurane as previously described (Shimizu et al. 2006). Every time rats were measured, the heat, cold, and mechanical head withdrawal thresholds (HHWT, CHWT, and MHWT, respectively) on the buccal mucosa or whisker pad skin, bipolar enamel-coated silver-wire electrodes were subcutaneously inserted into the splenius capitis muscle to assess the electromyography (EMG) activity, which was amplified, filtered, digitized, and integrated by Spike 2 software (CED 1401; Cambridge Electronic Design, Cambridge, UK) (Katagiri

et al. 2012). The inserting sites were marked by dye to insert the electrodes into same position. Rats were applied graded heat, cold, or mechanical stimulation after cutting off the supply of 2% isoflurane, once noxious pressure applied to the hindpaw induced an identical weak flexion reflex of the hindlimb to ensure that an adequate level of anesthesia was maintained. A contact heat probe (25 mm²; Intercross, Tokyo, Japan) or an electronic von Frey anesthesiometer (Bioseb, Chaville, France) was used to apply graded heat or cold stimulation (heat: 35 to 60 °C, 1 °C/s [cutoff, 60 °C]; cold: 35 to -10 °C, -1 °C/s [cutoff, -10 °C]) or graded mechanical stimulation (buccal mucosa: 0 to 100 g, 10 g/s [cutoff, 100 g]; whisker pad skin: 0 to 200 g, 10 g/s [cutoff, 200 g]) to measure HHWT, CHWT, and MHWT on the epithelium, which was 1 mm away from the incised wound. The graded mechanical stimulation to buccal mucosa was applied while the head was held with molded silicone rubber to prevent buccal movement due to the stimulation and while the mouth was opened by a mouth opener. An EMG activity exceeding the mean +2 standard deviations of the background activity was defined as the bursting EMG activity. The HHWT was defined as the lowest temperature, the CHWT was defined as the highest temperature, and the MHWT was defined as the lowest pressure required to elicit bursting EMG activities. Three measurements were performed and averaged at each time point for each animal. All behavioral tests were conducted under blind conditions.

Double-Labeling Immunohistochemistry

TG neurons innervating the buccal mucosa or whisker pad skin were identified by means of retrograde labeling with 4% hydroxystilbamidine (FluoroGold [FG]; Fluorochrome, Denver, CO, USA) dissolved in saline or 100 mg/mL (in 100% ethanol) of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine methanesulfonate (DiI) (Thermo Fisher Scientific, Waltham, MA, USA). To label the TG neurons innervating superficial tissues, DiI (5 µL) or FG (10 µL) single subcutaneous injection into the ipsilateral buccal mucosa or whisker pad skin was performed with a 27-gauge needle 4 d before the incision. On day 3 after the incision, rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and transcardially perfused with saline followed by a fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Ipsilateral TGs were dissected out and kept in 0.01 M phosphate-buffered saline (PBS) containing 20% sucrose for 12 h. The specimens were then embedded in TissueTek (Sakura Finetek, Tokyo, Japan) and cut in the horizontal plane along the long axis of the ganglion at a thickness of 15 µm. Every tenth section (5 sections/TG) was collected for analyses. The sections were thaw-mounted onto MAS-coated Superfrost plus microscope slides (Matsunami, Tokyo, Japan). TG sections were incubated with rabbit anti-TRPV1 polyclonal antiserum (1:500, cat. ACC-030;

Alomone, Jerusalem, Israel) (Shinoda et al. 2011), guinea pig anti-TRPV1 polyclonal antiserum (1:1000, cat. AB5566; Millipore, Billerica, MA, USA) (Ohara et al. 2013), or rabbit anti-TRPA1 polyclonal antiserum (1:1000, cat. ab58844; Abcam, Cambridge, UK) (Honda et al. 2014), and then TG sections were reacted with Alexa Fluor 488-conjugated goat anti-rabbit IgG (1:200 in 0.01 M PBS; Thermo Fisher Scientific), Alexa Fluor 568-conjugated goat anti-rabbit IgG (1:200 in 0.01 M PBS; Thermo Fisher Scientific), and/or Alexa Fluor 568-conjugated goat anti-guinea pig IgG (1:200 in 0.01 M PBS; Thermo Fisher Scientific). The sections were coverslipped in mounting medium and examined under a fluorescence microscope. Double-labeled (DiI and TRPV1 or DiI and TRPA1) or triple-labeled (FG, TRPV1, and TRPA1) neurons were identified and analyzed using a BZ-9000 system (Keyence, Tokyo, Japan). Neurons expressing a 2-fold or greater increase in intensity above average background were considered positive. Ratio of the number of TRPV1-immunoreactive (IR), TRPA1-IR, or TRPV1/TRPA1-IR neurons was calculated by the following formula: $100 \times \text{total number of DiI-labeled TRPV1-IR, DiI-labeled TRPA1-IR, or FG-labeled TRPV1/TRPA1-IR neurons in 5 sections of TG} / \text{total number of DiI-labeled or FG-labeled neurons in 5 sections of TG}$. The calculation was performed on FG-labeled TRPV1/TRPA1-IR TG neurons classified according to the area of cells.

Peripheral Administration of TRPV1 or TRPA1 Antagonist

TRPV1 antagonist, N-(3-methoxyphenyl)-4-chlorocinnamide (SB366791; Sigma-Aldrich, St. Louis, MO, USA), or TRPA1 antagonist, 1,2,3,6-Tetrahydro-1,3-dimethyl-N-[4-(1-methylethyl)phenyl]-2,6-dioxo-7H-purine-7-acetamide (HC-030031; Sigma-Aldrich), was dissolved in 50% dimethylsulfoxide in saline. On day 3 after the incision, SB366791 (150 µg) and/or HC-030031 (125 µg) or vehicle was subcutaneously administered once into the incised sites in a volume of 25 µL using a 27-gauge needle. These doses of SB366791 and HC-030031 were determined from previous reports (Kanai et al. 2007; Barabas and Stucky 2013). HHWT, CHWT, or MHWT was defined before and 30, 60, 90, and 120 min after the administration of SB366791, HC-030031, or vehicle as described above. The HHWT, CHWT, or MHWT was also measured in naive rat administered the same volume of SB366791 and/or HC-030031 into the incised sites.

Statistical Analysis

Data were expressed as means ± SEM. Statistical analyses were performed by Student's *t* test or 2-way repeated-measures analysis of variance (ANOVA) followed by Bonferroni's multiple-comparison tests where appropriate. A value of *P* < 0.05 was considered significant.

Results

Changes in Thermal and Mechanical Sensitivity

The HHWT of the buccal mucosa markedly decreased and that of the whisker pad skin significantly decreased (buccal mucosa: 42.9 ± 2.2 °C; whisker pad skin: 50.4 ± 1.0 °C) on day 3 following the incision (Fig. 1B). A significant decrease in heat sensitivity was observed on days 1 to 3 in the buccal mucosa and on days 3 to 5 in the whisker pad skin ($P < 0.05$). The MHWT of the buccal mucosa significantly decreased and that of the whisker pad skin markedly decreased (buccal mucosa: 76.0 ± 5.3 g; whisker pad skin: 105.9 ± 4.3 g) on day 3 following the incision (Fig. 1C). The MHWT was significantly decreased on days 1 to 5 in the buccal mucosa and on days 1 to 11 in the whisker pad skin. The CHWT of the buccal mucosa and whisker pad skin markedly increased (buccal mucosa: -3.4 ± 0.1 °C; whisker pad skin: -2.6 ± 0.7 °C) on day 3 following the incision (Fig. 1D). The CHWT significantly decreased on days 1 to 7 in the buccal mucosa and on days 1 to 5 in the whisker pad skin. No significant changes in the HHWT, CHWT, or MHWT of the buccal mucosa and whisker pad skin in the repeated EMG electrode-inserted rats were observed compared with naive rats (data not shown).

Changes in TRPV1 and/or TRPA1 Expression in TG Neurons

We examined the presence of TRPV1 and/or TRPA1 immunoreactivity in TG neurons innervating the buccal mucosa or whisker pad skin on day 3 following the incision (Fig. 2A, B). The mean percentage of the number of TRPV1-IR or TRPA1-IR neurons innervating the buccal mucosa was significantly increased (TRPV1-IR: $56.6\% \pm 1.2\%$; TRPA1-IR: $60.9\% \pm 4.3\%$) (Fig. 2C). The mean percentage of the number of TRPV1-IR or TRPA1-IR neurons innervating whisker pad skin was also significantly increased (TRPV1-IR: $62.3\% \pm 2.1\%$; TRPA1-IR: $62.9\% \pm 3.1\%$) (Fig. 2D). In advance, it was confirmed that there was no significant difference in the numbers of TRPV1-IR and TRPA1-IR neurons between DiI and FG labeling (data not shown). The cell area analysis revealed that the number of TRPV1/TRPA1-IR TG neurons innervating the whisker pad skin but not the buccal mucosa ipsilateral to the incision increased strikingly in cell groups 200 to $399 \mu\text{m}^2$ in area (incision: $43.0\% \pm 0.9\%$; naive: $31.8\% \pm 4.0\%$) (Fig. 2E, F). The mean percentage of the number of TRPV1/TRPA1-IR TG neurons innervating the buccal mucosa did not change after the incision (naive: $26.0\% \pm 2.0\%$; incised: $30.2\% \pm 2.2\%$), although those of TRPV1-IR TRPA1 nonimmunoreactive (nonIR) and TRPA1-IR TRPV1-nonIR TG neurons innervating the buccal mucosa significantly increased on day 3 following incision. Alternatively, the mean percentage of the number of TRPV1/TRPA1-IR TG neurons innervating whisker pad skin was significantly increased on day

3 following the incision (naive: $22.5\% \pm 4.2\%$; incised: $41.0\% \pm 1.9\%$), although those of TRPV1-IR TRPA1-nonIR and TRPA1-IR TRPV1-nonIR TG neurons innervating the buccal mucosa did not change after incision (Table). The number and size of DiI-labeled TG neurons were virtually the same as that of FG-labeled TG neurons following DiI and FG coinjection in the buccal mucosa ($82.9\% \pm 2.0\%$) and whisker pad skin ($82.3\% \pm 1.6\%$) (Appendix Fig. 1).

Effect of TRPV1 and/or TRPA1 Antagonist on Thermal and Mechanical Hypersensitivity

Effects of SB366791 and/or HC-030031 on thermal and mechanical hypersensitivity of the buccal mucosa or whisker pad skin were tested on day 3 following the incision. SB366791 administration to the incised site completely suppressed heat hyperalgesia in the buccal mucosa and whisker pad skin, and the decreased HHWT was reversed to baseline value. This suppression was peaked at 30 min after the administration (Fig. 3A, B). Although SB366791 administration to the incised site did not suppress the MHWT decrement in the buccal mucosa, the decreased MHWT was reversed in the whisker pad skin (Fig. 3C, D). SB366791 administration had no effect on the CHWT of the buccal mucosa and whisker pad skin (Fig. 3E, F). SB366791 had no effect on the HHWT, MHWT, and CHWT of the buccal mucosa and whisker pad skin in naive rats. HC-030031 administration to the incised site did not recover the decrease of HHWT in the buccal mucosa, but the decrease of HHWT in the whisker pad skin was reversed to baseline value (Fig. 4A, B). Recovery in HHWT peaked at 30 min after HC-030031 administration. The HC-030031 administration to the incised site produced complete inhibition of mechanical allodynia in the buccal mucosa, but the decrease of MHWT in the whisker pad skin was partially reversed (Fig. 4C, D). Moreover, the HC-030031 administration to the incised site produced complete inhibition of cold hypersensitivity in the buccal mucosa or whisker pad skin (Fig. 4E, F). HC-030031 had no effect on the HHWT, MHWT, and CHWT of the buccal mucosa and whisker pad skin in naive rats. SB366791 and HC-030031 coadministration to the incised site produced a significant suppression of heat, mechanical, and cold hypersensitivity in the buccal mucosa. In the whisker pad skin, although the coadministration suppressed heat and cold hypersensitivity, mechanical hypersensitivity was not recovered to the value of naive rats (Appendix Fig. 2). Motor deficits or sedation were not observed during the experimental period (data not shown).

Discussion

Despite the abundance of animal models of pathological pain due to skin incision, there are few data regarding pathological pain associated with oral mucosal incision (Kumar

Figure 2. TRPV1-IR, TRPA1-IR, or TRPV1/TRPA1-IR trigeminal ganglion (TG) neurons innervating the buccal mucosa (A) or whisker pad skin (B) defined by Dil or FluoroGold (FG) on day 3 following the buccal mucosa or whisker pad skin incision. Arrows indicate Dil-labeled TRPV1-IR or Dil-labeled TRPA1-IR or FG-labeled TRPV1/TRPA1-IR TG neurons. Arrowheads indicate FG-labeled TRPA1-IR TG neurons. Double arrows indicate FG-labeled TRPV1-IR TG neurons. Scale bars: 50 μm . Frequency of TRPV1-IR and TRPA1-IR TG neurons innervating the buccal mucosa (C) or whisker pad skin (D) ($n = 5$ in each). Size-frequency histogram illustrating distribution of FG-labeled TRPV1/TRPA1-IR TG neurons on day 3 following the buccal mucosa (E) or whisker pad skin incision (F) ($n = 5$ in each). * $P < 0.05$.

et al. 2013). In this study, heat, mechanical, and cold hypersensitivity occurred in both the buccal mucosa and whisker pad skin following the incisions. The number of TRPV1-IR or TRPA1-IR TG neurons innervating the buccal mucosa or whisker pad skin was significantly increased following the incision. SB366791 administration reversed the heat hyperalgesia in the whisker pad skin and buccal mucosa. Mechanical and cold hypersensitivity was reversed by HC-030031 administration but not by SB366791. In other studies, the loss of TRPV1 completely eliminated incision-induced heat sensitization of C-fiber afferents (Banik and Brennan 2009). TRPA1 was expressed in TG

neurons innervating unmyelinated and small myelinated axons (Kim et al. 2010). Intraplantar injection of TRPA1 antagonist reduced mechanical hypersensitivity following incision (Wei et al. 2012). The number of TRPA1-positive dorsal root ganglion (DRG) neurons innervating the inflamed hindpaw was increased, and cold hyperalgesia occurred in the hindpaw. This cold hyperalgesia was fully prevented by pretreatment with the TRPA1 antagonist (da Costa et al. 2010). Together with these results, our data indicate that the upregulation of TRPV1 and TRPA1 in TG neurons may play an important role in thermal and mechanical hypersensitivity following buccal mucosa and whisker pad

Table. Incidence of TRPV1-IR and TRPA1-IR Trigeminal Ganglion Neurons Innervating Buccal Mucosa or Whisker Pad Skin following Incisions.

		TRPV1-IR TRPA1-nonIR	TRPA1-IR TRPV1-nonIR	TRPV1/TRPA1-IR
Buccal mucosa	Naive	6.8 ± 0.5	12.5 ± 1.6	26.0 ± 2.0
	Incised	14.3 ± 1.5**	19.7 ± 1.5**	30.2 ± 2.2
Whisker pad skin	Naive	8.6 ± 0.8	16.1 ± 1.5	22.5 ± 4.2
	Incised	8.9 ± 1.0	18.9 ± 1.9	41.0 ± 1.9**

Values in mean ± SE (%).

***P* < 0.01 (naive vs. incision). **Figure 3.** Time course of heat head withdrawal threshold (HHWT) (A, B), mechanical head withdrawal threshold (MHWT) (C, D), and cold head withdrawal threshold (CHWT) (E, F) in the buccal mucosa (A, C, E) (*n* = 5 in each group) or whisker pad skin (B, D, F) (*n* = 5 in each group) after subcutaneous administration with SB366791 on day 3 following its incision. **P* < 0.05, ***P* < 0.01 vs. incision/vehicle.

skin incision. In other studies, activation of the phosphatidylinositol 3-kinase (PI3K) signaling in DRG neurons following plantar incision induced peripheral sensitization (Miura et al. 2011). TRPV1 current was strikingly potentiated by phosphorylation through the PI3K pathway (Zhuang et al. 2004). PI3K activation in DRG neurons also induced TRPV1 sensitization through an increase in the number of TRPV1 in the plasma membrane (Stein et al. 2006). In addition to upregulation of TRPV1, incisional pain might also be involved in TRPV1 sensitization in TG due to phosphorylation and/or receptor trafficking.

The TRPV1 antagonist administration to the incised site suppressed mechanical hyperalgesia in the whisker pad skin

but not in the buccal mucosa, although significant mechanical hypersensitivities occurred in both the buccal mucosa and whisker pad skin following incisions. The number of TRPV1/TRPA1-IR small TG neurons innervating the whisker pad skin rather than the buccal mucosa was significantly increased in incised rats. Peripheral TRPA1 sensitization due to skin incision contributes to maintenance of postoperative pain (Wei et al. 2012). TRPV1 activation evokes powerful Ca^{2+} influx into sensory neurons, resulting in an excitation of those neurons (Boillat et al. 2014). TRPA1 excitability is also regulated by intracellular Ca^{2+} via a calmodulin-independent mechanism (Doerner et al. 2007; Zurborg et al. 2007). Our findings suggest that mechanical

Figure 4. Time course of heat head withdrawal threshold (HHWT) (A, B), mechanical head withdrawal threshold (MHWT) (C, D), and cold head withdrawal threshold (CHWT) (E, F) in the buccal mucosa (A, C, E) ($n = 5$ in each group) or whisker pad skin (B, D, F) ($n = 5$ in each group) after subcutaneous administration with HC030031 on day 3 following its incision. * $P < 0.05$, ** $P < 0.01$ vs. incision/vehicle.

hyperalgesia in incised whisker pad skin but not the buccal mucosa may depend on TRPA1 sensitization mediated by activation of TRPV1 in small TG neurons. On the other hand, mechanical hypersensitivity following plantar incision in mice is not mediated by enhanced TRPA1 function (Barabas and Stucky 2013). Although the reason for the discrepancy of TRPA1 function is not known, it might possibly be related to the differences of animal species and/or incision sites.

The administration of TRPA1 antagonist to the incised site suppressed heat hyperalgesia in whisker pad skin but not in the buccal mucosa, although significant heat hypersensitivities were induced in both the buccal mucosa and whisker pad skin following the incisions. The number of TRPA1-IR and TRPV1-IR small TG neurons innervating whisker pad skin was significantly increased, although the number of TRPV1-IR TRPA1-nonIR and TRPA1-IR TRPV1-nonIR neurons innervating the whisker pad skin was not altered following the incisions. TRPA1 activation is involved in sensitization of TRPV1 to heat (Bautista et al. 2006). The effect of heat on TRPV1 currents was significantly enhanced by application of allyl isothiocyanate, which is a TRPA1 agonist (Alpizar et al. 2014). Moreover, TRPV1 was sensitized after TRPA1 stimulation with its ligand in a protein

kinase A (PKA)-dependent manner (Spahn et al. 2014). Therefore, these results suggest that heat hyperalgesia in the incised whisker pad skin but not the buccal mucosa is due to TRPV1 sensitization via PKA signaling mediated by activation of TRPA1 in small TG neurons.

The coadministration of the TRPV1 and TRPA1 antagonist to the incised site suppressed thermal and mechanical hypersensitivity in the buccal mucosa. However, mechanical hypersensitivity in the incised whisker pad skin was not recovered to the value of naive rats following the coadministration. These results suggest that mechanical allodynia in the incised whisker pad skin but not the incised buccal mucosa was also involved in other mechanisms rather than upregulation and sensitization of TRPV1 and TRPA1.

The thermal and mechanical sensitivity of the buccal mucosa or whisker pad skin were assessed under inhalation anesthesia in this study, because sensory stimuli to the intraoral tissue were inapplicable in nonanesthetized rats. Since it was difficult to maintain completely the same level of anesthesia during the measurement of behavioral responses, we could not eliminate the influence of the variation of the anesthetic depth on EMG activity. We should consider the existence of possible limitations due to the use of inhalation anesthesia on analyzing behavioral responses.

In conclusion, we have developed a new rat model of orofacial injured pain by whisker pad skin or the buccal mucosal incision that resulted in thermal and mechanical hypersensitivity. The number of TG neurons coexpressing TRPV1 and TRPA1 was increased following whisker pad skin incision but not the buccal mucosa, whereas the number of TG neurons that expressed either TRPV1 or TRPA1 was increased following the buccal mucosa incision but not the whisker pad skin. TRPV1 antagonism depressed heat hyperalgesia in both whisker pad skin and buccal mucosa, while TRPA1 antagonism depressed heat hyperalgesia in whisker pad skin following the incision. These findings suggest that intracellular interaction between TRPV1 and TRPA1 contributes to postincisional pain due to whisker pad skin injury but not to the oral mucosa.

Author Contributions

K. Urata, M. Shinoda, K. Iwata, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; K. Honda, contributed to design, data analysis, and interpretation, drafted and critically revised the manuscript; J. Lee, contributed to conception, data acquisition, and analysis, drafted and critically revised the manuscript; M. Maruno, contributed to conception, data analysis, and interpretation, drafted and critically revised the manuscript; R. Ito, contributed to conception and data interpretation, drafted and critically revised the manuscript; N. Gionhaku, contributed to design and data analysis, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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