Regulation of transient receptor potential vanilloid 1 expression in trigeminal ganglion neurons via methyl-CpG binding protein 2 signaling contributes tongue heat sensitivity and inflammatory hyperalgesia in mice

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Abstract

Background: Pain hypoalgesia has been reported in Rett syndrome patients, a severe neurodevelopmental disorder which can be attributed to mutations in the methyl-CpG binding protein 2 (MeCP2). Here, we examined the role of MeCP2 signaling in tongue heat sensitivity in the normal and inflamed state using Mecp2 heterozygous (Mecp2⁺/-) mice.

Results: Heat hypoalgesia of the tongue occurred in Mecp2⁺/- mice and submucosal injection of complete Freund’s adjuvant into the tongue produced a long-lasting heat hyperalgesia at the inflamed site in wild-type mice but not in Mecp2⁺/- mice. Transient receptor potential vanilloid 1 was expressed in a large number of MeCP2-immunoreactive trigeminal ganglion neurons innervating the tongue in both wild-type and Mecp2⁺/- mice (70.9% in wild type; 72.1% in Mecp2⁺/-). The number of transient receptor potential vanilloid 1-immunoreactive trigeminal ganglion neurons innervating the tongue was smaller in Mecp2⁺/- mice relative to wild-type mice (30.5% in wild type; 20.2% in Mecp2⁺/-). Following complete Freund’s adjuvant injection, the number of transient receptor potential vanilloid 1- and MeCP2-immunoreactive trigeminal ganglion neurons innervating the tongue, as well as MeCP2 protein expression in trigeminal ganglion, was significantly increased in wild-type mice but not in Mecp2⁺/- mice. Additionally, tongue heat hyperalgesia following complete Freund’s adjuvant injection was completely suppressed by the administration of SB366791, a transient receptor potential vanilloid 1 antagonist, in the tongue.

Conclusions: These findings indicate that tongue heat sensitivity and hypersensitivity are dependent on the expression of transient receptor potential vanilloid 1 which is regulated via MeCP2 signaling in trigeminal ganglion neurons innervating the tongue.

Keywords
Trigeminal ganglion, transient receptor potential vanilloid 1, tongue inflammation, Rett syndrome

Background

Mutations in methyl-CpG binding protein 2 (MeCP2) are causally related to Rett syndrome, an X-linked dominant neurodevelopmental disorder characterized by a severe deficit in the peripheral and central nervous system affecting mostly females.¹ The most serious symptoms in Rett syndrome patients are respiratory abnormalities, various motor deficits, absence of speech, and a general growth deficit.² MeCP2 is expressed predominantly in mature neurons and has been shown to be able to change the expression levels of target genes.³,⁴ MeCP2 is considered to be a transcriptional repressor acting...
through its methyl-CpG binding domain and transcriptional repressor domain, and the binding of MeCP2 to DNA acts by altering the chromatin structure.\(^5\) MeCP2 represses transcription by recruiting the nuclear receptor corepressor-silencing mediator of retinoic acid and thyroid hormone receptor corepressor complex. Further, MeCP2 regulates alternative splicing via an interaction with Y-box transcription factor, which modulates microRNA processing.\(^7\) Pain hypoalgesia in skin has also been reported in Rett syndrome patients, suggesting that MeCP2 plays an important role in setting pain sensitivity.\(^10\) It is also reported that heat and mechanical sensitivity of the oral mucosa is different from that of skin.\(^11,12\) Moreover, different interaction between nociceptors in primary afferent neurons contributes difference of characteristic feature between skin pain and oral mucosa pain after the incision.\(^13\) However, it is not known how MeCP2 in the primary afferent neurons is involved in tongue pain sensitivity and the pain modulation under pathological conditions.

Transient receptor potential vanilloid 1 (TRPV1) is mainly expressed in nociceptive primary afferent neurons and is one of the key molecules involved in pain modulation.\(^14,15\) TRPV1 is activated by various noxious stimuli such as high temperature, low pH, and irritant and pungent compounds released during inflammatory processes.\(^16,17\) In previous studies, an increase in the number of TRPV1-positive trigeminal ganglion (TG) neurons and increased TRPV1 expression in primary afferent terminals were induced by injection of turpentine oil into the facial skin,\(^18\) injection of complete Freund’s adjuvant (CFA) into the masseter muscle,\(^19\) or dentinal application of lipopolysaccharide,\(^20\) resulting in consequent orofacial heat hyperalgesia. Taken together, we hypothesized that MeCP2 is involved in regulation of TRPV1 expression in TG neurons, in association with orofacial inflammation. In the present study, we examined the role of MeCP2 in tongue heat sensory transduction and hypersensitivity following tongue inflammation produced by CFA injection into the tongue.

**Methods**

**Animals**

Female Mecp2\(^{+/−}\) mice (20–30 g) and female wild-type littermates (20–30 g) used in this study were obtained by breeding heterozygous females of C57BL/6 background (B6. 129P2(C)-Mecp2\(^{tm1.1Bird}\)) from Jackson Laboratory, Bar Harbor, ME with wild-type C57BL/6 male mice (Jackson Laboratory). The genetic status of the mice was established by polymerase chain reaction for the Mecp2 gene at postnatal day 10 according to Jackson Laboratory protocols. Mice were exposed to a 12h light/dark cycle (lights on at 07:00) and kept in a temperature- and humidity-controlled room (24 ± 1°C, 55 ± 5%) with food and water *ad libitum*. All procedures were conducted in accordance with the animal experimentation committee at Nihon University. The number of mice used was based on the minimum required for statistical analysis.

**Estrous cycle**

To confirm the estrous cycle in each mouse, the electrical impedance of the vagina (EIV) was measured with an impedance reader MK-10B (Muromachi, Tokyo, Japan). The probe was left in the vagina under light anesthesia with 1.5% isoflurane (Mylan). Measuring the EIV allows differentiation between each phase of estrous cycle, with high EIV values (over 3000 omega) occurring only at proestrus and the lowest values (under 3000 omega) at metestrus.\(^21\)

**Induction of inflammation in the tongue**

Under deep anesthesia via intraperitoneal (i.p.) injection of sodium pentobarbital (50 mg/kg, Schering Plough, Whitehouse Station, NJ), 2 µL of CFA or isotonic saline was submucosally injected into the left side of the anterior dorsolateral two thirds of the tongue with a 30-gauge needle attached to a Hamilton syringe (Hamilton, Reno, NV). The location of the needle was limited to the superficial mucosal layer of the tongue without entering the tongue muscle during each injection. Mice were closely monitored for evidence of distress or pain and weight gain following injections.

**Assessment of heat sensitivity**

Under light anesthesia with 1.5% isoflurane (Mylan, Canonsburg, PA), graded heat stimulation (35–60°C, 1°C/s, cutoff: 60°C) was applied to the tongue using a contact heat probe (9 mm²; Intercross, Tokyo, Japan) followed by pulling the tongue forward as previously described.\(^13\) Mice were applied graded heat stimulation after cutting off the supply of 1.5% isoflurane, once noxious pressure applied to the hind paw induced an identical weak flexion reflex of the hind limb to ensure that an adequate level of anesthesia was maintained. The lowest temperature for evoking nocifensive reflex (head withdrawal or avoidance) by heat stimulation of the tongue was defined as heat head-withdrawal reflex threshold (HHWT). The graded heat stimulation was applied at 1 min intervals for each stimulus, and measurements were performed three times and averaged at each time point for each animal. All behavioral tests were performed under blinded conditions.
**Immunohistochemistry**

To label TG neurons innervating the tongue, 5 µL of 5% hydroxystilbamidine (Fluoro-Gold [FG]; fluorochrome, Denver, CO) dissolved in isotonic saline was injected into the superficial mucosal layer of the tongue using a 30-gauge needle 5 days before CFA injection in wild-type and Mecp2<sup>+/−</sup> mice. On day 3, mice were anesthetized with i.p. sodium pentobarbital (50 mg/kg) and transcervically perfused with saline followed by a fixative containing 4% paraformaldehyde in 0.1 M phosphate buffer; pH 7.4). TGs ipsilateral to CFA injection were dissected out, placed in the same fixative overnight at 4°C, and transferred to 0.01 M phosphate-buffered saline (PBS) containing 20% sucrose for 12 h. The TGs were embedded in Tissue Tek (Sakura Finetek, Tokyo, Japan) and cut in the horizontal plane at a thickness of 16 µm. TG sections were thaw mounted onto MAS-coated Superfrost Plus microscope slides (Matsunami, Tokyo, Japan). Every 10th TG section (4 sections/TG) was collected for immunohistochemical analyses. After rinsing with 0.01 M PBS, TG sections were incubated with rabbit anti-MeCP2 polyclonal antibody (1:1000, Millipore; cat. 07–013, Billerica, MA) and guinea pig anti-TRPV1 polyclonal antibody (1:1000, Millipore; cat. AB5566), anti-transient receptor potential melastatin 3 (TRPM3) polyclonal antibody (1:200, Alomone; ACC-050, Jerusalem, Israel), anti-transmembrane protein 16A (TMEM16A) antibody (1:1, Abcam; ab53213, Cambridge, UK), or anti-acetylhistone H3 antibody (1:400, Cell Signaling; #9649, Beverly, MA) diluted in 0.01 M PBS containing 4% normal goat serum and 0.3% Triton X-100 (SigmaAldrich, St. Louis, MO) or Triton X-100 (50 mg/kg, i.p.). TG was removed and homogenized immediately in 50 µL of ice-cold lysis buffer (137 mM NaCl, 20 mM Tris-HCl, pH 8.0, 1% NP-40, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 10 µg/mL aprotinin, 1 g/mL leupeptin, and 0.5 mM sodium vanadate) using a tube pestle (Thermo Fisher Scientific, Waltham, MA). Following centrifugation of samples at 15,000 rpm for 10 min at 4°C, the supernatants were collected and the protein concentrations were determined using a protein assay kit (Bio-Rad, Hercules, CA). The supernatants were heat denatured in Laemmli sample buffer (Bio-Rad, Hercules, CA), and 20 µL of the sample was subjected to electrophoresis on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and electrobotted onto polyvinylidene fluoride membranes (Trans-Blot Turbo Transfer pack, Bio-Rad, Hercules, CA) using Trans-Blot Turbo (Bio-Rad, Hercules, CA). The membrane was rinsed with Tris-buffered saline containing 0.1% Tween 20 (TBST) and incubated with 3% bovine serum albumin (BSA, Bovogen, Essendon, Australia). Then, the membrane was incubated overnight at 4°C with anti-MeCP2 rabbit antibody (1:1000, Millipore) diluted in TBST with 5% BSA. Bound antibody was visualized using a horseradish peroxidase-conjugated donkey anti-rabbit antibody (Cell Signaling, Beverly, MA) and Western Lightning ELC Pro (Perkin Elmer, Waltham, MA). Band intensity was quantified using a ChemiDoc MP system (Bio-Rad, Hercules, CA) and normalized to β-actin immunoreactivity on blots reprobed with anti-β-actin antibody (1:200, Santa Cruz, Dallas, TX) following removal of bound antibody using a stripping reagent (Thermo Fisher Scientific, Waltham, MA).

**Effects of TRPM3 and Anoctamin 1 inhibitors on heat hyperalgesia**

We assessed the involvement of TRPM3 and anoctamin 1 (Ano1) in heat hyperalgesia of the tongue induced by CFA injection. The TRPM3 inhibitor (1 µL, 1 mM; Liquiritigenin, Sigma-Aldrich, St. Louis, MO) and the
Ano1 inhibitor (1 μL, 1 mM; T16Ainh-A01, Tocris Bioscience, Bristol, UK) were dissolved in 50% dimethylsulfoxide in physiological saline. On day 3 after CFA injection into the tongue, HHWTs were measured before and 30, 60, 90 and 120 min after tongue administration of Liquiritigenin or T16Ainh-A01 as described earlier. Before CFA injection into the tongue, prevalue of HHWTs was measured in advance.

**Statistical analysis**

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

**Results**

**Changes in heat sensitivity**

As female mice were used in the present study, we tested whether tongue pain threshold is modulated by the estrous cycle. We did not observe any significant differences in the HHWT of the tongue in female mice at different stage of the estrous cycle (data not shown). Following submucosal CFA injection into the tongue, tongue inflammation was induced accompanied by redness and swelling of the injection site, which lasted for at least 2 weeks (data not shown). In wild-type mice, the HHWT of the tongue significantly decreased on day 1 following CFA injection when compared with that of the saline group (CFA: 39.2 ± 0.5°C; saline: 50.5 ± 0.1°C; Figure 1) and remained significantly lower until day 15 (*p* < 0.05). In contrast, the HHWT in Mecp2+/− (heterozygous) mice was significantly higher than that of wild-type littermates, indicating decreased heat sensitivity in these genetically altered mice. Unlike the wild-type mice, the HHWT in Mecp2+/− mice did not change following CFA injection into the tongue during the experimental period. All mice did not exhibit motor and/or cognitive deficits.

**TRPV1-immunoreactive and MeCP2-IR TG neurons innervating the tongue**

The FG-labeled TRPV1-IR and MeCP2-IR TG neurons innervating the tongue were observed in naive mice and on day 3 after CFA or saline injection in wild-type or Mecp2+/− mice (Figure 2(a)). The FG-labeled acetyl-histone H3-IR TG neurons innervating the tongue were observed in wild-type mice on day 3 after CFA injection (Figure 2(b)). There was no significant difference in the number of FG-labeled neurons on day 3 after CFA or saline injection in wild-type or Mecp2+/− mice (data not shown). TRPV1 was expressed in a large number of MeCP2-IR TG neurons.
innervating the tongue in each group (naive/wild: 63.1%, saline/wild: 70.9%, Saline/Mecp2+/−: 72.1%, CFA/Mecp2+/−: 73.0%). Interestingly, TRPV1 was expressed in almost all of MeCP2-IR TG neurons innervating the tongue on day 3 after CFA injection in wild-type mice (CFA/wild: 97.8%; Figure 3).

**Figure 2.** MeCP2-TRPV1-IR and acetyl-histone H3 TG neurons innervating the tongue. (a) FG-labeled MeCP2-IR and TRPV1-IR TG neurons on day 3 after CFA or saline injection in wild-type or Mecp2+/− mice. Thick arrows indicate FG-labeled MeCP2-IR and TRPV1-IR TG neurons. Arrowheads indicate FG-labeled TRPV1-IR TG neurons. Thin arrows indicate FG-labeled MeCP2-IR TG neurons. Scale bars: 100 μm. (b) FG-labeled acetyl-histone H3-IR TG neurons on day 3 after CFA injection in wild-type mice. Arrowheads indicate acetyl-histone H3-IR TG neurons. Scale bars: 50 μm. MeCP2: methyl-CpG binding protein 2; CFA: complete Freund’s adjuvant; TRPV1-IR: transient receptor potential vanilloid 1-immunoreactive; TG: trigeminal ganglion; FG: Fluoro-Gold.

**Changes in MeCP2 expression in TG neurons**

MeCP2 protein expression in TG was significantly increased on day 3 after CFA injection compared with saline-injected mice (saline, 1.7 ± 0.2; CFA, 2.8 ± 0.3; Figure 4). Subsequently, we examined the presence of MeCP2 immunoreactivity in TG neurons innervating
the tongue on day 3 after CFA or saline injection. In wild-type mice, the number of MeCP2-IR neurons innervating the tongue was significantly increased after CFA injection compared with that of the saline-injected group or naive mice (naive: 29.9 ± 0.4%; saline: 31.0 ± 0.4%; CFA: 57.4 ± 2.5%, respectively; Figure 5(a)). On the other hand, the number of MeCP2-IR neurons innervating the tongue in saline- and CFA-injected Mecp2+/− mice (saline: 20.2 ± 1.2%; CFA: 19.8 ± 0.4%) was significantly less than that of saline-injected wild-type mice (saline: 27.8 ± 1.1%; saline: 30.5 ± 1.2%; CFA: 67.1 ± 2.3%, respectively; Figure 5(c)). On the other hand, the number of TRPV1-IR neurons innervating the tongue in saline-injected and CFA-injected Mecp2+/− mice was significantly increased after CFA injection compared with that of saline-injected wild-type or naive mice (saline: 15.4 ± 1.3%; CFA: 38.5 ± 1.6%; Figure 5(b)). In all cell groups, there was no significant difference in the number of MeCP2-IR neurons in Mecp2+/− mice on day 3 after CFA injection when compared with saline injection. Moreover, in saline- and CFA-injected Mecp2+/− mice, the numbers of MeCP2-IR neurons in cell groups of 201–400 μm², 401–600 μm², and 601–800 μm² in area were significantly less than that of saline-injected wild-type mice (saline: 10.4 ± 0.9%; CFA: 9.1 ± 0.5%; [401–600 μm²] saline: 2.3 ± 0.4%; [601–800 μm²] saline: 0.8 ± 0.2%, CFA: 0.5 ± 0.1%).

**Changes in TRPV1 expression in TG neurons**

We also examined the presence of TRPV1 immunoreactivity in TG neurons innervating the tongue on day 3 after CFA or saline injection. In wild-type mice, the number of TRPV1-IR neurons innervating the tongue was significantly increased after CFA injection compared with that of the saline-injected group or naive mice (naive: 27.8 ± 1.1%; saline: 30.5 ± 1.2%; CFA: 67.1 ± 2.3%, respectively; Figure 5(c)). On the other hand, the number of TRPV1-IR neurons innervating the tongue in saline-injected and CFA-injected Mecp2+/− mice (saline: 20.2 ± 1.2%; CFA: 19.8 ± 0.4%) was significantly less than that of saline-injected wild-type or naive mice. In wild-type mice, the cell area analysis revealed that the number of MeCP2-IR TG neurons innervating the tongue was markedly increased in cell groups of 0–200 μm² and 201–400 μm² in area on day 3 after CFA injection (0–200 μm²) saline: 1.4 ± 0.4%, CFA: 4.9 ± 1.1%; [201–400 μm²] saline: 15.4 ± 1.3%, CFA: 38.5 ± 1.6%; Figure 5(b)). In all cell groups, there was no significant difference in the number of MeCP2-IR neurons in Mecp2+/− mice on day 3 after CFA injection when compared with saline injection. Moreover, in saline- and CFA-injected Mecp2+/− mice,
the number of TRPV1-IR neurons in cell group of 601–800 \( \mu \text{m}^2 \) in area was significantly less than that of saline-injected wild-type mice ([601–800 \( \mu \text{m}^2 \)] saline: 1.3 \( \pm \) 1.0\%, CFA: 1.7 \( \pm \) 0.4\%).

Involvement of TRPM3 and Ano1 in heat hyperalgesia

The TRPM3-IR TG neurons innervating the tongue were observed on day 3 after CFA injection in wild-type mice (Figure 6(a)). The number of TRPM3-IR TG neurons innervating the tongue was significantly increased on day 3 after CFA injection in wild-type but not in Mecp2\(^{+/-}\) mice (Figure 6(b)). The decrease of the HHWT was completely recovered 30 min after Liquiritigenin administration into the tongue and the recovery in HHWT persisted until 90 min (Figure 6(c)). The Ano1-IR TG neurons innervating the tongue were observed on day 3 after CFA injection in wild-type mice (Figure 6(d)). The number of Ano1-IR TG neurons innervating the tongue was significantly decreased on day 3 after CFA injection in wild-type but not in Mecp2\(^{+/-}\) mice (Figure 6(e)). The decrease of the HHWT was completely recovered and tongue heat hypoalgesia was induced 30 and 60 min after T16Ainh-A01 administration into the tongue (Figure 6(f)).

Discussion

Rett syndrome is known to occur predominantly in females and is due to a deficit in MeCP2, which is considered to be a transcriptional factor acting through its methyl CpG binding domain and transcriptional factor domain. Pain hypoalgesia has also been reported in these patients.\(^5,10,22\) In animal studies, mechanical allodynia in hind paw induced by the common peroneal and tibial nerve injury was associated with an increase in MeCP2 in dorsal root ganglion (DRG) neurons, indicating the involvement of MeCP2 in modulation of pain transduction under the pathological condition.\(^23\)

Heat nociception is largely attributed to the TRPV family, which is a critical transducer for noxious heat in primary sensory neurons.\(^16\) In previous studies, the number of TRPV1-IR neurons innervating incised
tissue was significantly increased and TRPV1 antagonism produced a significant suppression of heat hypersensitivity. TRPV1 overexpression in primary afferent neurons induces an inflammatory-mediated heat hyperalgesia. Moreover, mice deficient in TRPV1 had prolonged withdrawal latencies to noxious heat in the hind paw and re-expression of TRPV1 in primary afferent neurons restored normal withdrawal latency. These studies indicate that cutaneous heat sensitivity is highly dependent on the amount of TRPV1 channels in primary neurons innervating the heat-stimulated site. TRPV1 expressed mainly in primary afferent terminals is known to increase in association with peripheral inflammation, and the increased sensitivity in inflamed tissue was attenuated by intrathecal brain-derived neurotrophic factor (BDNF) neutralizing antibody. In the present study, CFA administration into the tongues of wild-type mice enhanced heat sensitivity, while heat hypoalgesia occurred in the inflamed tongues of MeCP2−/− mice. TRPV1 was expressed in a large number of MeCP2-IR TG neurons innervating the tongue in both wild-type and MeCP2−/− mice, and a smaller number of TRPV1-IR TG neurons innervating the tongue were observed in MeCP2−/− mice when compared with the wild type. Together, these results suggest that heat hypoalgesia is induced by inhibition of TRPV1 expression which is highly dependent on MeCP2 signaling.

MeCP2 has an ability to selectively bind the methylated DNA and to interact with HDAC-containing complexes, linking DNA methylation and histone
deacetylation.\(^27\) Further, the MeCP2 signaling is transcriptional repressor that modulates gene expression through the interpretation such as histone deacetylation.\(^28\) Thus, many studies indicate that MeCP2 is moderately expressed under the physiological condition and plays an important role in recruiting transcriptional repressors.\(^27\) However, recent study indicates that MeCP2 may act not only as a transcriptional repressor but also as an activator.\(^29\) We observed that heat sensitivity of the tongue was unchanged in Mecp2\(^{+/−}\) mice following CFA injection into the tongue, whereas heat hyperalgesia was induced in the tongue of wild-type mice. TRPV1 was expressed in almost all MeCP2-IR TG neurons innervating the tongue after CFA injection in wild-type mice. There were no significant changes in the number of MeCP2-IR and TRPV1 neurons innervating the tongue after CFA injection in Mecp2\(^{+/−}\) mice, whereas the number of MeCP2-IR and TRPV1-IR neurons and MeCP2 protein expression in TG were significantly increased after CFA injection in wild-type mice. Furthermore, most TG neurons innervating the tongue expressed acetyl-histone H3 after CFA injection in wild-type mice. Taken together, these results indicates that MeCP2 upregulation associated with histone H3 deacetylation enhances TRPV1 expression in TG neurons, resulting in heat hyperalgesia in the inflamed tongue (Figure 7).

TG neurons were divided into \(<400\,\mu m^2\), \(401–600\,\mu m^2\) and \(>601\,\mu m^2\) in area, predominantly representing neurons that give rise to C-type, Aδ, and Aβ fibers, respectively.\(^30\) Although the number of MeCP2-IR TG neurons innervating the tongue was markedly increased only in small-diameter cell groups (\(<400\,\mu m^2\) in area), TRPV1-IR TG neurons increased in small- and medium-diameter cell groups (\(<400\,\mu m^2\), \(401–600\,\mu m^2\) in area, respectively) following CFA injection. In Mecp2\(^{+/−}\) mice, MeCP2-IR neurons in small-, medium- and large-diameter cell groups (\(<400\,\mu m^2\), \(401–600\,\mu m^2\), and \(>601\,\mu m^2\), respectively) were significantly less than that of the wild-type, though only a small number of TRPV1-IR neurons were observed in large-diameter cell groups. Although further rigorous experiments are needed, our current data suggest that changes in the TRPV1 expression in TG neurons are involved in MeCP2 signaling.

Ano1 is expressed in DRG neurons and activated by heat stimulation over 44°C.\(^31\) Furthermore, TRPV1 have high calcium permeability and TRPV1 activation induced Ca\(^{2+}\) influx.\(^32\) Most Ano1-IR TG neurons expressed TRPV1, and Ano1 activation was also caused by the entry of calcium through TRPV1.\(^33,34\) Inflammatory pain-related behaviors were reduced in Ano1 conditional-knockout mice, suggesting that changes in Ano1 reactivity are involved in the inflammatory pain.\(^35\) TRPM3 which is Ca\(^{2+}\) permeable nonselective cation channel is expressed in a large subset of DRG and TG neurons.\(^36\) TRPM3-knockout mice exhibit loss of the response to noxious heat stimuli and failed to develop inflammatory heat hyperalgesia.\(^37\) In this study, the number of TRPM3-IR TG neurons innervating the tongue was significantly increased and TRPM3 inhibition completely depressed heat hyperalgesia under inflammatory states. The number of Ano1-IR TG neurons innervating the tongue was significantly decreased and Ano1 inhibition induced heat hypoalgesia. The changes in the number of TRPM3-IR and Ano1-IR TG neurons did not occur in Mecp2\(^{+/−}\) mice. These findings suggest that the enhancements of TRPM3 and Ano1 activities in TG neurons via MeCP2 signaling are also involved in the tongue heat hyperalgesia in inflammatory states. However, the interaction between the decrease of

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**Figure 7.** Schematic illustration of potential interactions between MeCP2 signaling and TRPV1 expression following CFA injection into the tongue. A complex formed by MeCP2, HDAC, and DNMT binds mCpG and histone deacetylation is accelerated. The histone deacetylation may link with activation of TRPV1 gene transcription. MeCP2: methyl-CpG binding protein 2; TRPV1: transient receptor potential vanilloid 1; CFA: complete Freund’s adjuvant; AC: histone acetylation; DNMT: DNA methyltransferase; HDAC: histone deacetylase; mCpG: methyl-CpG.
Ano1 expression and the enhancement of Ano1 activity via MeCP2 signaling is unclear, further studies are needed.

Finally, tongue has multimodal innervations and the main function is the taste detection. In cultured DRG neurons, MeCP2 regulates BDNF expression and down-regulation of MeCP2 by microRNAs decreases BDNF expression. Further, a subpopulation of taste cells in adulthood persistently expresses BDNF, which suggests that BDNF has a role in mature taste cells. Indeed, BDNF presumably assists to modulate the innervation in a specific subpopulation of taste bud cells. These reports including the present study presume that MeCP2 signaling plays a pivotal role in not only changes in heat sensitivity via the alteration of TRPV1 expression but also the taste detection in the tongue.

Conclusion

Heat hypoalgesia of the tongue occurred in Mecp2+/− mice and inflammatory heat hyperalgesia induced in wild-type but not in Mecp2+/− mice. The number of TRPV1- and MeCP2-IR TG neurons innervating the tongue was significantly increased in wild-type but not in Mecp2+/− mice following CFA injection. These findings indicate that tongue heat sensitivity and hypersensitivity are dependent on the expression of TRPV1 which is regulated via MeCP2 signaling in TG neurons innervating the tongue. Further elucidation of MeCP2-mediated pain modulation may shed new light on the pathogenesis of Rett syndrome and provide new therapeutic strategies for pain hypoalgesia in Rett syndrome patients.

Author Contributions

AS, MS, KH, KI: contributed to conception, design, data acquisition, analysis, and interpretation, drafted and wrote the manuscript; KH, KI: contributed to design, data analysis, and interpretation; TS: contributed to design and data analysis and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

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