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Silencing of TRPV4-expressing sensory neurons attenuates temporomandibular disorders pain

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Abstract

Identification of potential therapeutic targets is needed for temporomandibular disorders (TMD) pain, the most common form of orofacial pain, because current treatments lack efficacy. Considering TMD pain is critically mediated by the trigeminal ganglion (TG) sensory neurons, functional blockade of nociceptive neurons in the TG may provide an effective approach for mitigating pain associated with TMD. We have previously shown that TRPV4, a polymodally-activated ion channel, is expressed in TG nociceptive neurons. Yet, it remains unexplored whether functional silencing of TRPV4-expressing TG neurons attenuates TMD pain. In this study, we demonstrated that co-application of a positively charged, membrane-impermeable lidocaine derivative QX-314 with the TRPV4 selective agonist GSK101 suppressed the excitability of TG neurons. Moreover, co-administration of QX-314 and GSK101 into the TG significantly attenuated pain in mouse models of temporomandibular joint (TMJ) inflammation and masseter muscle injury. Collectively, these results suggest TRPV4-expressing TG neurons represent a potential target for TMD pain.

Keywords

TRPV4, trigeminal ganglion sensory neurons, temporomandibular joint disorders, pain, QX-314

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Introduction

Temporomandibular disorders (TMD) are a group of painful conditions that involve the temporomandibular joint (TMJ), masseter muscles, and surrounding connective tissues.[1–3] For the majority of patients, one of the cardinal symptoms of TMD is pain in the joint and/or chewing muscles.[3–5] A wealth of evidence demonstrates that TMD patients have significantly lower bite strength compared with healthy controls, which can be regarded as functional masticatory pain in clinics.[6–10] Detailed understanding of the mechanisms underlying TMD pain is still absent.

TMD pain transmission critically relies on trigeminal ganglion (TG) sensory neurons,[11,12] which innervate the TMJ, masseter muscles, and surrounding connective tissues and provide the substrate for pain arising from such tissues. Hence, nociceptive neurons (nociceptors) residing in the TG represent an attractive therapeutic target for TMD pain. One potential strategy to functionally inhibit these neurons is to deliver QX-314 (N-ethyl-lidocaine), a cell impermeant and permanently charged sodium channel blocker, by entry through large-pore ion channels expressed in nociceptors.[13,14]

We have previously shown that TRPV4, a polymodally-activated ion channel, is expressed in TG nociceptive neurons innervating the TMJ and masseter muscle.[15,16] Here, in this

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micro report, we sought to determine whether: (1) QX-314 can suppress the excitability of TG neurons in the presence of the TRPV4 selective agonist GSK1016790A (GSK101);[17] (2) co-administration of QX-314 and GSK101 into the TG ameliorates pain in mouse models of TMD-induced by TMJ inflammation or masseter muscle injury.

Methods

Animals

Male WT mice (C57bl/6j) were used at 2.5–3 months of age for behavioral tests and immunostaining analysis. Animals were housed in climate-controlled rooms on a 12/12 h light/dark cycle with water and standardized rodent diet available *ad libitum*. Animal protocol was approved by the Duke University-Institutional Animal Care and Use Committee (IACUC) in compliance with NIH guidelines.

Mouse models of TMD: TMJ inflammation and masseter muscle injury

Whereas TMD has multifactorial etiologies,[1,18] a significant subgroup of patients suffers joint inflammation and/or masseter muscle injury.[1,19,20] Following previous studies,[15,16,21] we induced TMJ inflammation and masseter muscle injury to mimic these conditions in mice. For TMJ inflammation, mice were injected with 10 µL of complete Freund's adjuvant (CFA, 5 mg/mL; Chondrex) into the joint. Controls received incomplete Freund's adjuvant (IFA). For masseter muscle injury, ligation of the tendon of the anterior superficial part of masseter muscle (TASM) with two 6.0-chromic gut ligatures was conducted. Control mice received IFA or sham procedure of TASM.

Pain behavioral test

Bite force test was used to measure masticatory pain as we and others previously described.[15,16,22] When the bite transducer was slowly moved towards the mouse, a bite was invariably elicited. The voltage output during each bite was recorded using Labview 8.0 (National Instruments). The voltage of each bite was determined and converted into force (newton) based on the regression equation derived from calibration. Each animal was tested 3–5 times per time point and the values were averaged. The interval between two trials was >1 min. The experimenter was blinded to the treatment conditions.

TG sensory neurons culture

Following our previous method,[23,24] TGs from male WT mice (1–2 months old) were dissected and digested with 1 mg/mL collagenase (Worthington Biochemical Co.) and 5 mg/mL dispase (Invitrogen) for 1 h, then triturated. Neurons were cultured in DH10 medium (1:1 DMEM:Ham F12, Invitrogen) on coverslips coated with poly-D-lysine and laminin (Invitrogen), and incubated with 5% CO_2 at 37°C overnight for electrophysiological experiments.

Voltage-gated sodium current recording

Following our previous study,[25] whole-cell patch-clamp was used to record voltage-gated Na^+ currents at room temperature. Data was acquired by an Axopatch-200B amplifier with a Digidata 1440A by using pClamp10 software (Axon Instruments). The pipette solution contained: 130 mM CsCl, 9 mM NaCl, 1 mM $MgCl_2$, 10 mM EGTA, and 10 mM HEPES, adjusted to pH 7.3 with CsOH. The external solution contained: 131 mM NaCl, 10 mM tetraethylammonium chloride, 10 mM CsCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 0.3 mM $CdCl_2$, 3 mM 4-aminopyridine, 10 mM HEPES, and 10 mM glucose, adjusted to pH 7.4 with NaOH. Data were sampled at 10 kHz and low-pass-filtered at 2 kHz. In voltage-clamp experiments, Na^+ currents were evoked by a test pulse (40 ms) to 0 mV from the holding potential (−70 mV). The baseline of the Na^+ currents was recorded and followed with perfusion of vehicle (2% DMSO), GSK101 (1 µM, Sigma), QX-314 (5 mM, Tocris) or GSK101+QX-314 solution. Small-medium sized TG neurons, where TRPV4 is mostly expressed,[16] were selected for recording.

Intraganglionic injection

To assess the contribution of functional inhibition of TRPV4-expressing TG neurons to TMD-like pain behaviors, GSK101 and QX-314 were intraganglionic (i.g.) co-administered into the TG. I.g. injection is a simplified and well-established method for targeted delivery of pharmacological agents into mouse TGs and it has been widely used by many groups.[26–28] Mice were briefly anesthetized with 2% isoflurane and bilaterally injected using a 30G needle on a 5 µL Hamilton syringe. The tip of the needle was terminated at the medial part of the TG, and 2 µL of solution containing 1 µM of GSK101 and 2% (~7.6 mM) of QX-314 was slowly delivered within 10 s.

Neural tracing

To track TMJ or masseter muscle innervation by the TG neurons, mice were injected with 2 µL of neuraltracerfast blue (FB, 2% aqueous solution; Polysciences) into the TMJ or masseter muscle 15 min before CFA/IFA and TASM/sham.

Immunohistochemistry and quantitative analysis

Mouse TG sections at 12 µm were blocked with 5% normal donkey serum (Jackson-ImmunoResearch) and incubated overnight with primary antibody: rabbit anti-TRPV4 (1:2500, Novus Biologicals). Immunodetection was accomplished with secondary antibodies (AlexaFluor-594, 1:600;

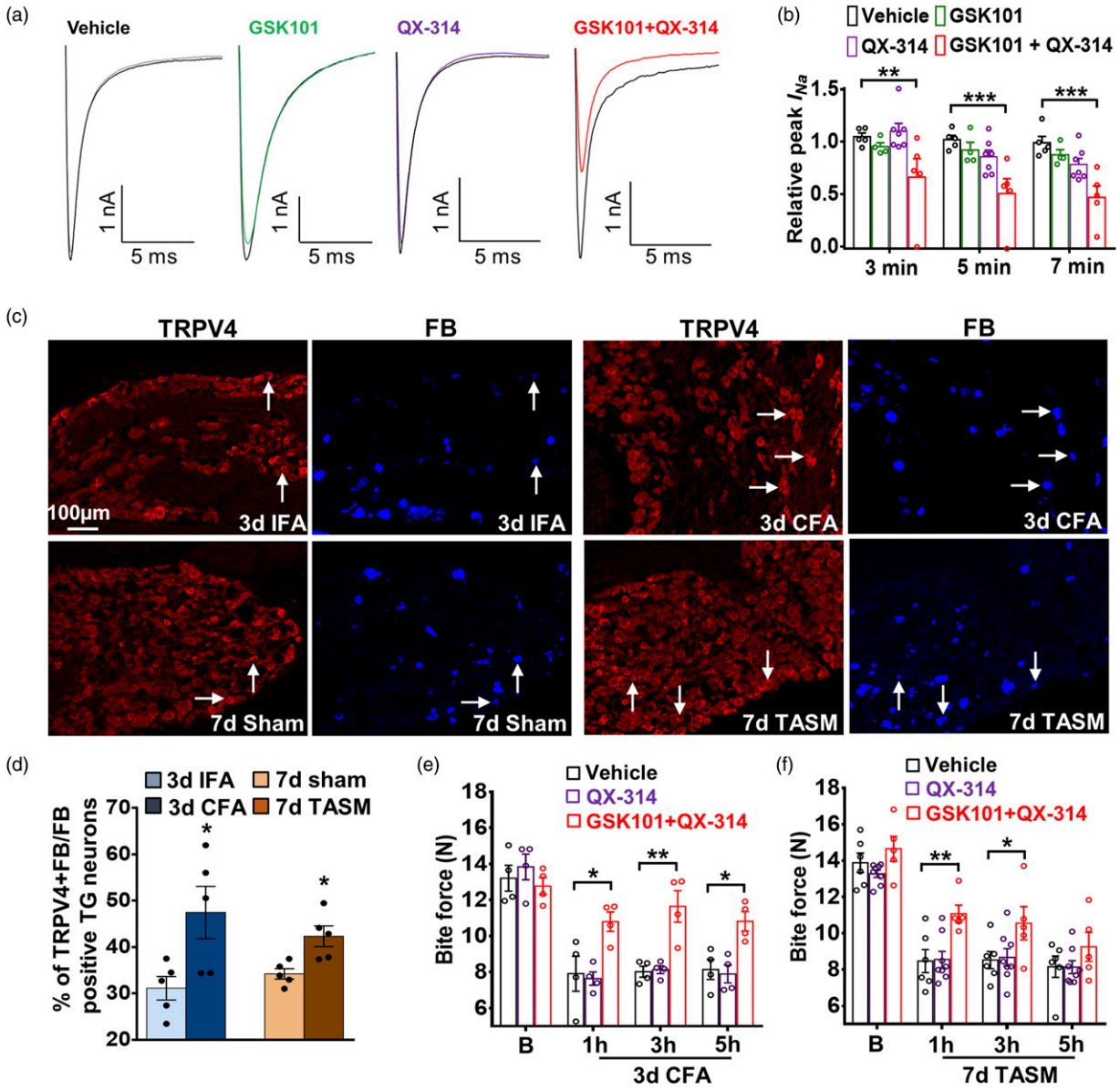

Figure 1. Co-application of QX-314 and GSK101 suppresses sodium currents in TG neurons and attenuates TMD-like pain. (a) Traces and (b) quantification of sodium currents in responses to vehicle (2% DMSO), GSK101(1 μM), QX-314 (5 mM) and GSK101 (1 μM) + QX-314 (5 mM) treatments. $^{**}p < .01$ and $^{***}p < .01$ vs. vehicle, two-way ANOVA followed by Dunnett's post hoc test. $N = 4–7$ neurons from four mice were recorded for each group; (c) representative images and (d) quantitative analysis show an increase of TRPV4-expressing neurons which innervate the TMJ and masseter muscle (% of TRPV4 + FB/FB) after CFA or TASM, respectively. $^{*}p < .05$ vs. 3 days IFA or 7 days sham, unpaired t test. $N = 5$ mice/group; (e) and (f) i.g. coinjection of GSK101 (1 μM) and QX314 (2%) significantly reduced CFA- or TASM-induced attenuation of bite force. $^{*}p < .05$ and $^{**}p < .01$ vs. vehicle, two-way ANOVA followed by Bonferroni post hoc test; $N = 5–8$ mice/group.

Invitrogen). Images were acquired using Keyence Microscope (Keyence Co.). 4–6 sections/TG were analyzed. % of TRPV4-expressing TG neurons innervating the TMJ or masseter muscle (TRPV4 + FB/FB) was analyzed.

Statistical analysis

Data were expressed as mean ± SEM. Unpaired t test and two-way ANOVA followed by Dunnett's or Bonferroni *post-hoc* test were used for groups comparison. $p < .05$ was considered statistically significant.

Results

Our whole-cell patch-clamp recordings in cultured TG neurons demonstrated that extracellular co-application of QX-314 and GSK101 produced a substantial inhibition of voltage-gated sodium currents. In contrast, QX-314 or

GSK101 alone had a marginal, non-significant effect on the current (Figure 1(a) and (b)). These data indicate that TRPV4 can be employed in a strategy to functionally inhibit sensory neurons by the channel-mediated uptake of QX-314.

We have previously shown that TRPV4 is expressed in TG neurons that innervate the TMJ and masseter muscle.[15,16] Here, using a retrograde neural tracer-FB combined with TRPV4 immunostaining, we extended this finding and found that the TMJ and masseter muscle innervation by TRPV4-expessing neurons increases after TMJ inflammation and masseter muscle injury (Figure 1(c) and (d)), suggesting TRPV4-containing TG neurons might be a potential cellular site contributing to TMD pain. Neural tracing analysis revealed that the percentage of FB-labeled neurons/all TG neurons remained unchanged after TMJ inflammation or masseter muscle injury: 14.9 vs 14.3% (IFA vs CFA) and 14.5 vs 13.8% (sham vs TASM). These data suggest that the increased TMJ and masseter muscle innervation by $TRPV4^{+}$ afferents after CFA or TASM might be attributable to the increased TRPV4 expression in TG neurons as we recently reported.[15]

Considering our observations demonstrated activation of TRPV4 permeates QX-314 into the channel-expressing TG neurons and lead to electrical silencing of cells, we next leveraged this method to examine the effects of silencing of TRPV4-expressing TG neurons on pain 1day after TMJ inflammation and 7 days after masseter muscle injury, when pain is established and most prominent as shown in our previous studies.[15,16] Bite force measurement, as a clinically relevant read-out for assessing masticatory pain of TMD,[15,16] showed that i.g. co-injection of QX-314 and GSK101 produced a significant attenuation of bite force reduction-evoked by TMJ inflammation or masseter muscle injury (Figure 1(e) and (f)).

Discussion

Previous studies have documented that activation of certain types of ion channels can change their membrane permeability that permits large molecules (≤900 Da) to enter cells via their pore dilation.[29] For instance, it was reported that TRPV1 allows QX-314 (~260 Da), a membrane impermeable sodium channel blocker, to enter into sensory neurons when the channel is opened, leading to functional silencing of cells and producing analgesic effects under pathophysiological conditions.[13,30,31] QX-314 can also permeate through TRPA1 and TRPM8 when the channels are activated.[32–34] Further in vivo behavioral assays showed that co-injection of QX-314 with TRPA1 or TRPM8 agonist blocked cold-evoked allodynia and hyperalgesia.[32–34] Interestingly, TRPV4 can permeate an organic cation Yo-Pro (629 Da) in huTRPV4-transfected Chinese hamster ovary (CHO) cells,[35] suggesting a possibility that TRPV4, similar to TRPV1, TRPA1, and TRPM8 ion channels, allows large molecules to enter cells. Here, we demonstrated that treatment of QX-314 led to robust attenuation of sodium currents of TG neurons when co-applying with GSK101. In contrast, extracellular application of QX-314 or GSK101 alone to neurons did not significantly affect the sodium current. Based on our best knowledge, this is the first time to show that functional blockade of TRPV4-expressing cells can be achieved by co-application of QX-314 and the TRPV4 selective agonist. Previous studies reported that TRPV4 is expressed in TG nociceptive neurons,[16] In this study, we were able to observe that these neurons contribute to TMD pain: delivery of QX-314 and GSK101 simultaneously into the TG produced a long-lasting (>3–5 h) attenuation of TMD-like pain-induced by TMJ inflammation or masseter muscle injury. One limitation of our study is that it remains elusive whether co-injection of QX-314 and GSK101 into the ganglion can also reduce the excitability of satellite glial cells, where TRPV4 is also expressed.[36] However, our data on TRPV4-expressing TG neurons' excitability and innervation of TMJ and masseter muscle clearly inform us that TRPV4-containing TG neurons contribute to TMD pain. Together, our findings suggest that silencing of TRPV4 neurons in TG may provide a potential approach for mitigating TMD pain. Considering TRPV4 is also involved in dental pain, neuropathic pain, pancreatitis pain, visceral pain, and migraine,[37,38] future studies are warranted to investigate whether functional silencing of TRPV4 sensory neurons can also attenuate these types of pain.

Author contributions

YC designed the study. FCD, ZLW, GS and YC performed the experiments and analyzed the data. YC wrote the manuscript with input from all the authors. All authors read and approved the final manuscript.

Declaration of conflicting interests

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