Neuroscience Letters 664 (2018) 84–90

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet

Research paper

Role of KCNQ2 channels in orofacial cold sensitivity: KCNQ2 upregulation in trigeminal ganglion neurons after infraorbital nerve chronic constrictive injury

Jennifer Ling, Ferhat Erol, Jianguo G. Gu*

Department of Anesthesiology and Perioperative Medicine, University of Alabama at Birmingham, Birmingham, AL, 35294, United States

ARTICLE INFO

Keywords:
Trigeminal ganglion neurons
Neuropathic pain
Cold allodynia
Cold hyperalgesia
KCNQ2 channels
Retigabine

ABSTRACT

Sensitivity to cooling temperatures often becomes heightened in orofacial regions leading to orofacial cold allodynia/hyperalgesia after chronic trigeminal nerve injury. KCNQ2 channels are involved in controlling excitability of primary afferent neurons and thereby regulate sensory functions under both physiological and pathological conditions. In the present study, we sought to determine whether KCNQ2 channels in trigeminal nerves are involved in regulating orofacial operant behavioral responses to cooling stimulation. We also sought to examine whether chronic trigeminal nerve injury may alter KCNQ2 channel expression in trigeminal ganglions. Using the orofacial operant tests, animals show cold allodynia/hyperalgesia in orofacial regions following infraorbital nerve chronic constrictive injury (ION-CCI), which could be alleviated by subcutaneous administration of retigabine, a KCNQ2 activator. In contrast, subcutaneous administration of the KCNQ2 inhibitor XE991 directly elicits cold allodynia/hyperalgesia in sham animals. Using immunostaining, we show that KCNQ2 channels are primarily expressed in small-sized TG neurons. Interestingly, KCNQ2 channel expression becomes significantly upregulated in TG neurons following the ION-CCI. Our results suggest that KCNQ2 channels are involved in regulating orofacial cold sensitivity. Upregulation of KCNQ2 channels may be a compensatory change in attempting to limit injury-induced trigeminal hyperexcitability.

1. Introduction

Trigeminal neuropathic pain is a significant clinical issue due to its severity, location, and resistance to conventional treatment [1]. Similar to neuropathic pain in other parts of the body, trigeminal neuropathic pain is often manifested with exaggerated painful sensations triggered by innocuous mechanical stimuli (mechanical allodynia) and also by innocuous or mild noxious cooling temperatures (cold allodynia/hyperalgesia). Cold allodynia/hyperalgesia is more difficult to manage in areas such as orofacial regions that are innervated by the trigeminal nervous system. This is because these regions normally directly expose to ambient temperatures, and covering these regions with clothing is not practical to avoid cold allodynia/hyperalgesia. A peripheral sensitization to cooling stimuli at primary afferent endings of the trigeminal nerves is believed to be an underlying mechanism of orofacial cold allodynia/hyperalgesia. Cooling stimuli at primary afferent endings are known to be primarily transduced by the cooling temperature sensor TRPM8 channels [2,3]. However, ion channels that are involved in regulating membrane excitability of the trigeminal nerves may also have a profound impact on the sense of cooling temperatures.

KCNQ2 channels are voltage-gated K^+ channels expressed on the membranes of neurons in the CNS and the peripheral nervous system (PNS) [4]. In the PNS, functional KCNQ channels in dorsal root ganglion (DRG) neurons are thought to be mainly formed by KCNQ2 and KCNQ3 subunits in a heteromeric form (KCNQ2/3 channels), and these KCNQ2/3 channels are believed to underlie the M-type outward K^+ currents (M-currents) recorded in DRG neurons [5–7]. KCNQ2/3 channels have been shown to play a key role in regulating excitability of nociceptive DRG neurons [4]. Previous studies have shown that KCNQ2 and KCNQ3 subunits are down-regulated in nociceptive DRG neurons after nerve injury and in an animal model of a bone cancer [6,8,7]. These studies have further shown that KCNQ channel down-regulation is associated with the increase of the excitability of nociceptive DRG neurons [8,7]. In TG neurons, we have recently shown that KCNQ2 channels are expressed in nociceptive TG neurons including those that sense cooling temperatures, and we have also found that pharmacological inhibition of these channels leads to TG neuron hyperexcitability [9]. More recently, we have found that KCNQ2 channels

* Corresponding author at: Department of Anesthesiology and Perioperative Medicine, University of Alabama at Birmingham, 901 19TH Street South, BMR II 210, Birmingham, AL, 35294, United States.
E-mail address: jianguogu@uabmc.edu (J.G. Gu).

https://doi.org/10.1016/j.neulet.2017.11.026
Received 20 October 2017; Received in revised form 8 November 2017; Accepted 9 November 2017
Available online 10 November 2017
0304-3940/

become down-regulated in rat TG neurons that innervate orofacial regions (the V2 branch of trigeminal nerves, or V2 TG) following oxaliplatin-induced trigeminal neuropathy [10]. Furthermore, we have shown that systemic administration of retigabine can alleviate cold allodynia/hyperalgesia in animals following chronic trigeminal nerve injury or in animals treated with oxaliplatin [9]. In the present study, we sought to determine whether KCNQ2 channels at peripheral endings of the trigeminal nerve play a role in regulating behavioral sensitivity to cooling temperatures. We also sought to determine whether there was a significant change in KCNQ2 channel expression in TG neurons following infraorbital nerve chronic constrictive injury.

2. Materials and methods

Male adult Sprague Dawley rats at the age of 6–10 weeks were used. Animal care and use conformed to National Institutes of Health guidelines for care and use of experimental animals. Experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham. After 2 weeks of the pre-surgical adaptation training for orofacial operant behavioral test (see below), the rats were divided into sham and infraorbital nerve chronic constrictive injury (ION-CCI) groups. ION-CCI was created by unilateral ligation of the infraorbital nerves as described in our previous studies [11,12]. In brief, under anesthesia with ketamine/xylazine, a 2-cm curvilinear incision was made superior to the right orbital cavity. The infraorbital nerve was freed from the surrounding connective tissues and two ligatures were made approximately 5 mm apart with a 5–0 absorbable chromic gut suture Superion®. The incision was then closed with suture. The sham group had a similar surgery, but without any ligatures. After a 2-week healing period, the rats underwent a 2-week period of post-surgical adaption training of orofacial operant tests.

Orofacial operant tests were conducted on sham and ION-CCI animals as described in our previous studies [11]. In brief, animals initially underwent 4–6 sessions of adaptation training in two weeks using the Ugo Basile Orofacial Stimulation Test System® (Comerio VA, Italy). For each training session, animals were first fasted for a 12-h period. Each rat was then placed in a cage of Orofacial Stimulation Test System. The Orofacial Stimulation Test System had a drinking window for the rat head to enter and acquire a reward (30% sweetened condensed milk, Nestle Carnation®). The Orofacial Stimulation Test System also consisted of a thermal module with its temperature being set at 24 °C for training sessions and at 17, 12, or 5 °C for cold stimulation. An infrared beam was built in the drinking window to automatically detect and record the head accessing the milk. A training session was started by placing a rat in the cage. After the rat was given 10 min to familiarize itself with its environment, the drinking window was opened and the testing rat was subsequently timed for 10 min to allow drinking. After 2 weeks of training, procedures were performed on the animals to create ION-CCI group and sham group as described above. Subsequent orofacial operant tests were performed at different days for up to 6 weeks in the same manner as the adaptation training. In a set of experiments with sham animals the KCNQ2 blocker XE991 was subcutaneously injected to both sides of orofacial regions at the dose of 300 μg (30 μl) each side 28–42 days following sham surgery. In another set of experiments with ION-CCI animals, the KCNQ2 activator retigabine was administered subcutaneously to ipsilateral sides of orofacial regions at the doses of 300 μg (μl) 28–35 days following ION-CCI procedure. Orofacial operant tests were performed 60 min after XE991 or retigabine administration. The events of head assessing the milk were detected by the infrared beam. Orofacial operant behavioral parameters were the total contact time (measured by total time the infrared beam breaks) and total contact number (measured by total number of beam breaks) in a 10-min experimental session.

For immunostaining, TGs were harvested from bilateral ION-CCI and sham groups 28–30 days after the surgery procedures. We used bilateral procedures in this set of experiments to maximize the numbers of TGs that we could work with for immunostaining experiments. Immunostaining was performed in the same manner as described in our previous study [10]. In brief, animals were fixed with 4% paraformaldehyde (PFA), and TGs were removed and cryoprotected in 30% sucrose for two nights. The TGs were then cut into 10 μm sections on a cryostat. The sections of TGs were thaw-mounted onto slides. For each slide, three sections from sham animals (control) and three sections from ION-CCI animals were mounted on the same slide. These sections were then encircled together with PAP Pen so that the two groups were paired in immunostaining. Immunostaining was conducted with a polyclonal rabbit anti-KCNQ2 antibody (1:2000 diluted with 5% normal goat serum in PBS; Abcam, Cambridge, MA, USA, incubated for 2 nights at 4 °C) and a secondary antibody of goat anti-rabbit IgG conjugated with Alexa-594 (1:1000 in 5% normal goat serum in PBS, ThermoFisher Scientific, Waltham, MA USA). Slices after immunostaining were viewed under an upright fluorescent microscope and images under a 20× objective were captured using a CCD camera. KCNQ2-ir positive and total neurons in TG sections were visually identified and counted from the acquired fluorescent images.

Data were presented as Mean ± SEM, analysed by the unpaired Student's *t* test or one-way AMOVA with the Tukey post hoc test, *P < 0.05, **P < 0.01, and ***P < 0.001.

3. Results

Orofacial operant tests performed at cooling temperatures showed differences in orofacial cold sensitivity between ION-CCI group and sham group (Fig. 1A–D). In sham group, the total contact time in a 10-min testing session was 312 ± 13 s (n = 16) at 24 °C, 246 ± 19 s (n = 15) at 17 °C, 215 ± 17 s (n = 16) s at 12 °C, and 171 ± 15 s (n = 12) s at 5 °C. The total contact time in ION-CCI group was 154 ± 12 s (n = 11, P < 0.05) at 12 °C and 70 ± 12 s (n = 15, P < 0.001) at 5 °C, significantly shorter than that of sham group at the same cooling temperatures (Fig. 1A–C). ION-CCI animals showed significantly higher total contact numbers at 24 °C in comparison with sham group (Fig. 1D). However, at cooling temperatures tested, there were no significant differences in total contact numbers between ION-CCI group and sham group.

XE991, a highly selective KCNQ2 channel blocker, was subcutaneously administered to orofacial regions of sham animals. This allowed us to determine whether KCNQ2 channels at peripheral trigeminal nerve endings were involved in controlling cold sensitivity of the skin in orofacial areas (Fig. 2A–D). As tested at 12 °C, the subcutaneous XE991 administration resulted in cold hyper-sensitivity as indicated by significantly shortening total contact time (Fig. 2C). The total contact time was 222 ± 20 s (n = 7) in vehicle-injected group and was shortened to 136 ± 22 s (n = 7, P < 0.05) in XE991-injected group (Fig. 2C). The total contact numbers were not significantly different between the two groups (Fig. 2D).

To further demonstrate that KCNQ2 channels at peripheral trigeminal nerve endings were involved in controlling cold sensitivity of the skin in orofacial areas, we subcutaneously administered either vehicle or the KCNQ2 channel activator retigabine into orofacial regions of ION-CCI animals and performed orofacial operant tests at 5 °C. In ION-CCI group injected with vehicle, the total contact time was 62 ± 16 s (n = 6, Fig. 3A & C). In contrast, in ION-CCI group injected with retigabine, the total contact time was 147 ± 16 s (n = 6, Fig. 3B & C), significantly (P < 0.01) longer than that of vehicle-injected ION-CCI animals. We also examined effects of retigabine in sham group. The total contact time in sham group injected with retigabine was 185 ± 16 s (n = 7), and was not significantly different from vehicle-injected sham group (190 ± 17 s, n = 7). We examined total contact numbers in ION-CCI group following subcutaneous administration of either vehicle or retigabin. The total contact numbers were 39 ± 4 (n = 6) in retigabine-injected ION-CCI group, significantly more than that of vehicle-injected ION-CCI group (21 ± 3, n = 6). In sham