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Research Paper

Ascending projections of nociceptive neurons from trigeminal subnucleus caudalis: A population approach

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ABSTRACT

Second-order neurons in trigeminal subnucleus caudalis (Vc) and upper cervical spinal cord (C1) are critical for craniofacial pain processing and project rostrally to terminate in: ventral posteromedial thalamic nucleus (VPM), medial thalamic nuclei (MTN) and parabrachial nuclei (PBN). The contribution of each region to trigeminal nociception was assessed by the number of phosphorylated extracellular signal-regulated kinase-immunoreactive (pERK-IR) neurons co-labeled with fluorogold (FG). The phenotype of pERK-IR neurons was further defined by the expression of neurokinin 1 receptor (NK1).

The retrograde tracer FG was injected into VPM, MTN or PBN of the right hemisphere and after seven days, capsaicin was injected into the left upper lip in male rats. Nearly all pERK-IR neurons were found in superficial laminae of Vc-C1 ipsilateral to the capsaicin injection. Nearly all VPM and MTN FG-labeled neurons in Vc-C1 were found contralateral to the injection site, whereas FG-labeled neurons were found bilaterally after PBN injection. The percentage of FG-pERK-NK1-IR neurons was significantly greater (>10%) for PBN projection neurons than for VPM and MTN projection neurons (<3%). pERK-NK1-IR VPM projection neurons were found mainly in the middle-Vc, while pERK-NK1-immunoreactive MTN or PBN projection neurons were found in the middle-Vc and caudal Vc-C1.

These results suggest that a significant percentage of capsaicin-responsive neurons in superficial laminae of Vc-C1 project directly to PBN, while neurons that project to VPM and MTN are subject to greater modulation by pERK-IR local interneurons. Furthermore, the rostrocaudal distribution differences of FG-pERK-NK1-IR neurons in Vc-C1 may reflect functional differences between these projection areas regarding craniofacial pain.

1. Introduction

Nociceptors that innervate craniofacial tissues terminate mainly on second-order neurons in trigeminal subnucleus caudalis (Vc) and the upper cervical spinal cord (C1) (Sessle, 2000; Bereiter et al., 2009). Electrophysiological and anatomical studies have reported that nociceptive Vc-C1 neurons project to several central nervous system (CNS) areas such as periaqueductal gray (PAG), parabrachial nuclei (PBN), posterior and medial thalamus and hypothalamus (Hirata et al., 2000; Malick et al., 2000; Ikeda et al., 2003; Gauriau and Bernard, 2004; Sugiyo et al., 2005; Chang et al., 2012; Jansen and Giesler, 2015). Although it has long been proposed that the efferent projection targets of second-order neurons in the spinal dorsal horn are more important than the afferent input in determining a role in nociception (Laird and Cervero, 1991), few studies have addressed this issue in the trigeminal system.

Abbreviations: Vc, trigeminal subnucleus caudalis; C1, upper cervical spinal cord; VPM, ventral posteromedial thalamic nucleus; MTN, medial thalamic nuclei; PBN, parabrachial nuclei; pERK, phosphorylated extracellular signal-regulated kinase; IR, immunoreactive; FG, fluorogold; NK1, neurokinin 1 receptor; CNS, central nervous system; PAG, periaqueductal gray matter; MAPK, mitogen-activated protein kinase; TRPV1, transient receptor potentioanl vanilloid 1; TG, trigeminal ganglion; Vi, trigeminal subnucleus interpolaris; mid-Vc, middle Vc (main portion of Vc); NTS, nucleus tractus solitarii; dPa5, dorsal paratrigeminal nucleus; Po, posterior thalamic nuclear group; MD, mediodorsal thalamic nucleus; PF, parafascicular thalamic nucleus; CM, central medial thalamic nucleus; CL, centrolateral thalamic nucleus; KF, Kölliler-Fuse nucleus; Cu, cuneate nucleus; Gr, gracile nucleus; PoT, posterior trianglular area.

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The present study used an anatomical population approach to assess the projections of Vc nociceptive neurons labeled by fluorogold (FG) injection into sensory thalamus, medial thalamus or the PBN and activation of phosphorylated extracellular signal-regulated kinase (pERK). ERK is a member of the mitogen-activated protein kinase (MAPK) family that requires phosphorylation for activation and is an essential signal transduction enzyme for downstream pathways in chronic pain (Ji et al., 2009). ERK phosphorylation occurs rapidly in primary afferent neurons (Dai et al., 2002) and Vc neurons (Noma et al., 2008) following noxious stimulation with a time course that matches well the onset of acute hyperalgesia (Ji et al., 1999; Zhuang et al., 2004). Activation of transient receptor potential vanilloid 1 (TRPV1) selectively activates small myelinated and unmyelinated sensory nerve fibers and mediates cellular responses to a variety of noxious stimuli including capsaicin (Caterina et al., 1997; Tominaga et al., 1998). Approximately 50% of trigeminal ganglion (TG) neurons are gated by capsaicin and likely express TRPV1 (Jordt et al., 2004). Capsaicin-responsive nerves are critical for the development of long-term potentiation and inflammatory hyperalgesia in humans (LaMotte et al., 1992; Henrich et al., 2015). Nociceptive Vc projection neurons were identified on the basis of pERK expression induced by capsaicin injection into the upper lip. The rationale for this approach was based on knowledge that innervation density of the lips is high compared to other regions of facial skin (Nolano et al., 2013) and that chemosensory stimulation of the upper lip is a well-established behavioral test for orofacial pain in animals (Luccarini et al., 2006). ERK-positive neurons were subsequently assessed for substance P receptor expression. Substance P is produced by a high percentage of TG neurons that often express TRPV1 (Price and Flores, 2007). Trigeminal nociceptor activation causes the release of substance P in Vc (Schaible et al., 1997) where it binds to neurokinin-1 receptors (NK1) located mainly on neurons in superficial laminae in the spinal and medullary dorsal horn (Nakaya et al., 1994; Brown et al., 1995). A high percentage of dorsal horn projection neurons in spinal cord (Ikeda et al., 2003; Todd et al., 2000; Lapirot et al., 2009) and Vc-C1 (Li et al., 1996; Li et al., 1998) express NK1. Release of substance P and activation of NK1-IR projection neurons is thought to play a significant role in persistent hyperalgesia (Mantyh et al., 1997; Khasabov et al., 2002; Suzuki et al., 2002; Coste et al., 2008), including the response to intraoral irritants (Simons et al., 2002).

The premise of this study was that Vc-C1 nociceptive neurons that project to ventral posteromedial thalamic nucleus (VPM), medial thalamic nuclei (MTN) and PBN serve different aspects of trigeminal nociception and can be distinguished on the basis of pERK and NK1 expression. Quantitative anatomical approaches were used to test the hypothesis that CNS projection neurons expressing pERK and/or NK1 will differ in distribution within Vc-C1 depending on the efferent projection target.

2. Material and methods

The Animal Experimentation Committee approved the animal protocols at Nihon University, and experimental procedures were performed according to the guidelines of the International Association for the Study of Pain (PHS Low 99-158, revised 2002). A total of 35 adult male Sprague-Dawley rats weighing 200–350 g were used; however, data are presented from 24 rats in which the microinjection was within the targeted regions (capsaicin injection group: VPM n = 5, MTN n = 5, PBN n = 5; vehicle injection group: VPM n = 3, MTN n = 3, PBN n = 3; Japan SLC, Shizuoka, Japan). Rats were housed with free access to food and water. Cages remained in climate and light controlled environment (23 °C, 12:12-h light/dark cycle with the light on at 7:00 AM) for at least 5 days before the experiment. All efforts were made to minimize the number of the animals used for experiments.

2.1. Fluorogold (FG) injection

Rats were anesthetized with intraperitoneal administration of saline solution mixed with 2.5 mg/kg butorphanol (Meiji Seika Pharma, Tokyo, Japan), 0.375 mg/kg medetomidine (ZENOAQ, Fukushima, Japan) and 2.0 mg/kg midazolam (Sandoz, Tokyo, Japan) and placed in a stereotaxic apparatus. The skull was exposed and a small hole (diameter = 3 mm) was drilled over the right hemisphere above the VPM, MTN or PBN. A small opening was cut in the dura and a glass micropipette (tip diameter = 50 μm) filled with 3% FG (Fluorochrome, Denver, CO) dissolved in sterile saline and injected slowly (50 nl/min) from a 1 μl microsyringe. The micropipette was left in the place for 10 min after injection to minimize leakage, and the wound was washed with sterile saline and sutured. After completion of the surgery, penicillin G potassium (20,000 units; Meiji Seika, Tokyo, Japan) was injected intramuscularly to prevent infection. The stereotaxic coordinates (Paxinos and Watson, 1997) were as follows: VPM: 3.5 mm caudal to bregma, 2.5 and 3.0 mm lateral to the midline and depth of 6.0 and 5.7 mm; MTN: 4.0–4.2 mm caudal to bregma, 0.9 mm lateral to the midline and depth of 6.0 mm; PBN: 9.2 mm caudal to bregma, 1.5 mm lateral to the midline and depth of 6.0 mm. The volume of FG was: VPM = 160 nl (80 nl × two sites); MTN = 100 nl and PBN = 100 nl. Multiunit activity was recorded from the VPM, MTN and PBN to confirm that each region was responsive to nociceptive input from the upper lip.

2.2. Capsaicin injection into upper lip

Seven days after FG injection, rats were anesthetized with pentobarbital sodium (80 mg/kg) and received an injection of the small fiber excitant, capsaicin (300 μM, 10 μl, or 0.92 μg, Sigma-Aldrich, St. Louis, MI), via 30-gauge needle into the left upper lip. The vehicle for capsaicin was saline, tween80 (Tokyo Chemical Industry, Tokyo, Japan) and ethanol (Wako, Osaka, Japan). Five minutes after capsaicin or vehicle, the rats were transcardially perfused with 1% paraformaldehyde in 0.1 M phosphate buffer (PB, pH 7.4) followed by cold fixative containing 4% paraformaldehyde in 0.1 M PB. The brain, including brainstem and upper cervical spinal cord, was removed, blocked and postfixed in 4% paraformaldehyde in 0.1 M PB for 24 h at 4 °C.

2.3. FG injection site and brainstem immunohistochemistry

The tissue blocks were immersed in 4% paraformaldehyde for 24 h at 4 °C and then kept in 0.01 M phosphate-buffered saline (PBS) containing 20% sucrose (w/v) for cryoprotection (24 h) at 4 °C. Transverse frozen brain sections (50 μm) were cut at the level of the VPM, MTN, or PBN to identify injection sites. The sections were thaw-mounted on MAS-GP micro slide glass (Matsunami, Osaka, Japan) and coverslipped in mounting medium (Thermo Fisher Scientific, Fremont, CA). The injection sites were confirmed under a fluorescence microscope (BZ-9000 system, Keyence, Osaka, Japan). Transverse frozen brainstem and upper cervical spinal cord sections (40 μm) were cut and transferred serially to multiwell tissue culture plates containing cold 0.01 M PBS. Every fourth section (160 μm intervals) was incubated sequentially in 3% normal goat serum and 0.3% Triton X-100 (Sigma-Aldrich) for 1 h at room temperature, mouse anti-phospho-p44/42 MAPK (ERK1/2) monoclonal antibody (1:300, #9101, lot# 36, Cell Signaling Technology, Beverly, MA) and, rabbit anti-NK1 polyclonal antibody (1:1000, BML-NA4300-0025, lot#11051431, ENZO Life Sciences, Farmingdale, NY) diluted in 0.01 M PBS containing 0.3% Triton X-100 for 72 h at 4 °C. After rinsing with 0.01 M PBS (10 min × 3), sections were incubated in Alexa Fluor 568 anti-mouse IgG (1:200, A11004, lot#1698376, Invitrogen, Paisley, U.K.) and Alexa Fluor 488 anti-rabbit IgG (1:200, A11008, lot#1735088, Invitrogen) diluted in 0.01 M PBS for 2 h at room temperature. After rinsing with 0.01 M PBS (10 min × 3), sections were mounted serially on MAS-GP micro slide glass and coverslipped in mounting medium. The sections were examined under a fluorescence microscope (BZ-9000 system, Keyence, Osaka, Japan). Specific staining was abolished by the omission of primary antibody. pERK-positive neurons appeared as filled nuclei with cytoplasmic staining in 1 or more branches.