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Review

Neural communication in the trigeminal ganglion contributes to ectopic orofacial pain

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ABSTRACT

Ectopic orofacial pain develops with local inflammation in remote orofacial structures. However, the mechanism underlying the spreading of pain to remote orofacial areas after local inflammation is still unknown. We investigated the functional significance of neural communication within the trigeminal ganglion (TG) via nerve growth factor (NGF) or calcitonin gene-related peptide (CGRP) in relation to whisker pad skin hyperalgesia following complete Freund's adjuvant (CFA) injection into the lower lip. Heat hyperalgesia and mechanical allodynia were induced in the ipsilateral whisker pad skin following lower lip inflammation, and reversed by local injection of transient receptor potential vanilloid 1 (TRPV1) or $P2X_3$ receptor ($P2X_3R$) antagonist, respectively. The heat hyperalgesia was diminished by neutralizing anti-NGF antibody administration into the lower lip and intraganglionic administration of tyrosine kinase receptor inhibitor. TG neurons that innervate the whisker pad skin and lower lip both expressed labeled NGF, which was administrated into the lower lip. Moreover, NGF and CGRP expression in the TG was increased following lower lip inflammation. The number of TRPV1-positive neurons that innervate the whisker pad skin was increased following lower lip inflammation and this increase was annulled by anti-NGF administration. $P2X_3R$- and CGRP-positive TG neurons innervating whisker pad skin were also increased by lower lip inflammation. The present findings suggest that induced NGF and CGRP following local inflammation in the lower lip increases TRPV1 and $P2X_3R$ in TG neurons, which may result in the development of the whisker pad ectopic pain.

Contents

1. Introduction

Patients who have local inflammation in the orofacial structures often suffer from ectopic orofacial pain. For example, tooth pulp inflammation may cause referred pain in the teeth unilaterally or bilaterally [1,2]. Therefore, trigeminal ectopic pain conditions are difficult to diagnose and treat, and in most cases, their etiology and pathogenesis are unclear. Regarding the mechanisms underlying trigeminal ectopic pain associated with orofacial inflammation, persistent alterations in the excitability of adjacent uninjured neurons due to changes in their membrane properties have been implicated [3,4]. In order to develop effective treatments for atypical orofacial pain, it is important to establish an appropriate model and to investigate the exact mechanisms of ectopic orofacial pain

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induced by local inflammation in the orofacial region. Some studies indicate that injection of complete Freund's adjuvant (CFA) into peripheral tissues induces intense inflammation, heat hyperalgesia, and mechanical allodynia, which persist for several days [5–8].

The transient receptor potential channel vanilloid 1 (TRPV1) is a sensory neuron-specific cation channel sensitive to noxious heat ($>43\,^{\circ}C$), extracellular acidification, various lipids, as well as capsaicin, and is highly expressed in primary sensory neurons. TRPV1 is a critical contributor to normal and pathological pain [9,10]. Recent studies using TRPV1-deficient animals have shown that this channel is required for inflammatory sensitization to noxious thermal stimuli, and its channel functions are modulated during inflammation [11–15].

Adenosine-5′-triphosphate (ATP) is well established as an energy source in cellular biochemical processes and is recognized as an important extracellular neurostimulator [16]. ATP activates cation-permeable ion channels (P2X receptors, P2XR) and G-protein-coupled receptors (P2Y receptors), because $P2X_3R$ is most highly expressed in a subpopulation of small-diameter primary afferent neurons in the pain pathway, including the trigeminal nervous system. It has been assumed that the $P2X_3R$ contained in trigeminal neurons plays a role in orofacial pain transmission [17–20].

Among the factors that affect TRPV1 channel function, nerve growth factor (NGF) possesses a central role [14]. Intravenous application of NGF causes robust, long-lasting thermal hyperalgesia [21,22]. Administration of minute doses of intradermal recombinant human NGF led to a lowering of heat-pain threshold in humans [23,24]. Moreover, NGF rapidly potentiates the activity of TRPV1 channels in dorsal root ganglion (DRG) neurons [25,26]. These results suggest that NGF is a potent activator of nociceptors and an endogenous mediator of heat pain sensation.

Calcitonin gene-related peptide (CGRP) is expressed in small DRG or trigeminal ganglion (TG) neurons, and it has been reported to be the sensory transmitter of nociceptive neurons [27,28]. CGRP is known as a neuropeptide associated with inflammatory pain; for example, total CGRP protein in sensory ganglia increases after CFA-induced inflammation [29]. $P2X_3R$-positive TG neurons are co-expressed with CGRP, and these nerve fibers have been found to be increased in rats with CFA-induced arthritis [30]. These results suggest that CGRP, which increases in sensory ganglia after CFA-induced inflammation, is an endogenous mediator of mechanical pain sensation.

In the present study, we have developed a new mouse model of ectopic orofacial pain induced by injection of CFA into the lower lip resulting in heat hyperalgesia and mechanical allodynia of whisker pad skin. We also have investigated the functional significance of neuron–neuron interaction via NGF or CGRP pathways on ectopic orofacial pain associated with local inflammation.

2. Nocifensive behavior

Among the abundant animal models of inflammatory pain, some models have been developed to clarify the mechanisms underlying orofacial pathological pain. Local injection of CFA [5,31], capsaicin [32], carrageenan [33], or formalin [34] induces local inflammation in the trigeminal region and results in pain-related behavior, including mechanical and heat hypersensitivity at the inflamed site. However, these models have assessed changes in mechanical or heat sensitivity at the inflamed site, and few models inducing ectopic inflammatory pain in the non-inflamed adjacent division of the trigeminal nerve have been developed in the orofacial region. We measured changes in the heat withdrawal latency and head withdrawal threshold to mechanical stimuli following lower lip inflammation induced by CFA injection. Significant heat hyperalgesia and mechanical allodynia were observed in the ipsilateral whisker pad skin following lower lip inflammation. These results indicate that heat hyperalgesia and mechanical allodynia in the whisker pad skin can be induced by local inflammation in the lower lip. Development of useful animal models under this condition will likely increase our knowledge of mechanisms underlying ectopic orofacial inflammatory pain.

3. Changes in properties of trigeminal ganglion neurons

After identification of heat-sensitive TG neurons innervating whisker pad skin, single neuronal activity was examined following lower lip inflammation. Spontaneous activity and evoked responses by heat stimulation of the whisker pad skin significantly increased, indicating that TG neurons innervating whisker pad skin had been sensitized by lower lip inflammation.

We examined the presence of TRPV1 or $P2X_3$ immunoreactivity in TG neurons innervating whisker pad skin using immunofluorescence techniques. TRPV1- or $P2X_3$-positive TG neurons innervating whisker pad skin were significantly increased following lower lip inflammation. Moreover, TRPV1 and $P2X_3R$ antagonist administration produced a marked dose-dependent reversal of heat hyperalgesia and mechanical allodynia, respectively, in the whisker pad skin. These findings suggest that an increase in TRPV1- or $P2X_3R$-positive neurons innervating whisker pad skin is involved in the sensitization of the small-diameter neurons in whisker pad skin following lower lip inflammation.

4. Contribution of NGF to ectopic orofacial heat hyperalgesia

We also aimed to identify the signaling pathways involved in TRPV1-associated mechanisms of pathological ectopic pain following lower lip inflammation. NGF in inflamed peripheral tissues is known to be elevated in several painful inflammatory conditions in humans, including arthritis [35,36], cystitis [37,38], and prostatitis [39]. In the inflammatory state, numerous inflammatory cytokines, such as interleukin (IL)-1, tumor necrosis factor-α, and IL-6, induce NGF production in fibroblasts, endothelial cells, and glial cells in peripheral tissues [40,41]. In animal studies, the concentration of NGF in inflamed tissue increases in response to inflammation produced by injection of some irritants such as capsaicin, endotoxin, turpentine, or trinitrobenzene sulfonic acid [38,42,43]. In inflamed peripheral tissue, NGF receptors TrkA and p75 localized in the distal axons are activated upon NGF binding, the ligand–receptor complex is formed, internalized, and retrogradely transported to the soma of sensory neurons [22]. In the present study, NGF was retrogradely transported to the soma of TG neurons following lower lip inflammation; therefore, its concentration increased not only in the lower lip, but also in the TG. Neurons containing NGF secrete NGF into the extracellular space following neural excitation, resulting in an increase in NGF concentration in the extracellular fluid in vitro [44,45]. Together, these data suggest that NGF produced in the local inflamed site binds to NGF receptors in TG neurons, is transported to the soma of TG neurons and secreted to the extracellular space, resulting in an increase in NGF concentration in the TG. In sensory neurons, the three mitogen-activated protein kinase (MAPK) families of ERK, p38, and c-Jun N-terminal kinase are expressed by NGF signaling [8,14,46]. Activation of downstream transcription factors by these MAPKs contributes to the transcriptional changes in sensory neurons that are associated with heat hyperalgesia [14]. Indeed, retrograde NGF signaling from peripheral terminals in the inflamed tissue to the soma of nociceptive neurons enhances the expression of TRPV1 [47]. NGF signaling also increases the anterograde transport of TRPV1 from the cell body to the peripheral terminals of nociceptors [14]. In cultured TG neurons, chronic application of NGF led to an increase in TRPV1 expression [48]. NGF in DRG neurons activates p38, which in turn increases