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Increased expression of interleukin-18 in the trigeminal spinal subnucleus caudalis after inferior alveolar nerve injury in the rat

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HIGHLIGHTS

- A trigeminal neuropathic pain model was used with inferior alveolar nerve transection.
- IL-18 upregulation occurred in hyperactive microglia in the trigeminal spinal subnucleus caudalis.
- IL-18 was increased in the Vc around obex area from 3 d until 14 d after nerve injury.
- IL-18 induction coexisted with phosphorylated p38 MAPK.
- The findings suggest a role of IL-18 in orofacial neuropathic pain mechanism.

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ABSTRACT

Interleukin-18 (IL-18) is an important regulator of innate and immune responses, and is known to be expressed in various types of cells and upregulated in pathological conditions including tissue injury and inflammation, suggesting it has both proinflammatory and compensatory roles. Here we show that IL-18 was increased in microglia in the trigeminal spinal subnucleus caudalis (Vc) after peripheral nerve injury. We used a trigeminal neuropathic pain model in which the withdrawal threshold of maxillary whisker pad skin was significantly decreased after inferior alveolar nerve transection, and observed a striking increase in IL-18 expression in the Vc around the obex area from 3 d and continued until 14 d after nerve injury. The IL-18 labeled cells were largely colocalized with Iba1, suggesting this upregulation occurred in hyperactive microglia. We also found that the IL-18 induction coexisted with phosphorylated p38 MAPK, indicating a possible role of p38 in the regulation of IL-18. Our findings are the first report that injury of trigeminal nerve induced IL-18 upregulation in activated microglia in the Vc, suggesting a possible role of IL-18 in orofacial neuropathic pain.

1. Introduction

A number of previous reports have shown that hyperactivity of non-neuronal (glial) cells is involved in neuroplastic changes, which reflect the hyperexcitability of nociceptive pathways in both medullary (trigeminal spinal subnucleus caudalis; Vc) and spinal dorsal horns (DH) [see review, 2]. While glial cells are known to have important functions in nourishing and supporting neurons, recent studies have reported that hyperactive glial cells have important roles in modulating the Vc and spinal DH neuronal activity after peripheral nerve injury or inflammation [19,25,28,29]. Following peripheral nerve injury, glial cells in the Vc and spinal DH are hyperactive and may change their morphological features and manifest large somata with many thick processes [10,30]. This Vc and spinal glial activation is likely involved in the production and release of pro-inflammatory cytokines, such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α), which may augment the nociceptive signals in the spinal cord and Vc [21,22].

Interleukin-18 is a multifunctional cytokine affecting both innate and adaptive immune responses [5,16,18]. IL-18 is a member of the IL-1 family and IL-18 is produced as an inactive precursor (pro IL-18), which is activated by proteolytic cleavage, primarily by cysteine protease caspase-1 [1,16,18]. IL-18 is upregulated in several human autoimmune and

Abbreviations: IL-18, interleukin-18; Vc, trigeminal spinal subnucleus caudalis; IANT, inferior alveolar nerve transection; DH, dorsal horn; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α; IAN, inferior alveolar nerve; Iba1, ionized calcium-binding adapter molecule 1; GFAP, glial fibrillary acidic protein; p-p38, phosphorylated-p38; IR, immunoreactivity.

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inflammatory diseases and therefore might represent a novel therapeutic target [1,20,23]. It is known that IL-1β is upregulated in astrocytes in the Vc following tissue inflammation and nerve injury [6,22]. In contrast to IL-1β, there has been no study examining IL-18 signaling pathways in the Vc after trigeminal nerve injury.

In this work, we investigated whether IL-18 is induced in the Vc in neuropathic pain models with inferior alveolar nerve transection (IANT) [7,19,24]. Because a number of reports have indicated that the p38 MAPK in microglia has a critical role in neuropathic pain, we also examined the colocalization of IL-18 and phosphorylated p38. We now show that nerve injury induces an increase in IL-18 expression in microglia in the Vc, and these data suggest a role of microglia mediated IL-18 in the development and maintenance of orofacial tactile allodynia through glial cell-specific signal transduction cascades.

2. Materials and methods

2.1. Animals and surgical procedures

Male Sprague–Dawley rats weighing 150–250 g were used. All procedures were approved by the Hyogo College of Medicine Committee on Animal Research and were performed in accordance with the National Institutes of Health Guidelines on Animal Care. All procedures were performed with the rats under pentobarbital anesthesia (50 mg/kg, i.p.). In all animals, no surgery was performed on the right side. Special care was taken to prevent infection and to minimize the influence of inflammation. To produce an IANT [7,19], a small skin incision was made on the surface of the facial skin over the masseter muscle, and the surface of the alveolar bone covering the inferior alveolar nerve (IAN) was removed to expose the IAN. The IAN was transected at two points of the nerve trunk at just above the angle of the mandible and at a 1 mm distal from the angle of the mandibular bone and then the amputated nerve stump was removed. Animals were allowed to survive for 1, 3, 5, 7, 14 and 28 d after surgery ($n = 4$ for immunohistochemistry for each time point). The nerve was exposed without transaction in sham-operated animals, and the naïve animals were also used for immunohistochemistry.

2.2. Behavioral testing

The measure of allodynia following IANT has been previously described [7,19,24]. After 1 week of training, rats became capable of receiving the mechanical stimuli during protrusion of their perioral regions. The criterion performance was when the rats could keep their nose protruding for 5–10 min without escape from high intensity mechanical stimulation applied by the von Frey filament to the maxillary whisker pad skin. Each von Frey filament was applied five times to the same region at approximately 5 s intervals on sides ipsilateral and contralateral to the nerve injury. Head withdrawal, touching or scratching the facial regions upon von Frey filament applications was considered as positive pain response. The response threshold was defined as the lowest force of the filaments that produced at least three positive responses in five trials. The threshold for escape behavior in response to mechanical stimulation was measured in the two groups of rats (IANT rats and sham-operated rats). The behavioral testing was performed 1 d before surgery and 1, 3, 5, 7, 14 and 28 d after surgery.

2.3. Immunohistochemistry

The rats were perfused as previously reported [12] and the medulla and upper cervical cord were dissected out and postfixed in the 4% paraformaldehyde for 2 d at 4 °C, then replaced with 30% sucrose for several days ($n = 4$ at each time point). Transverse medulla and upper cervical cord sections (10 μm and free floating, 20 μm) were cut and processed for IL-18, ionized calcium-binding adapter molecule 1 (Iba1), glial fibrillary acidic protein (GFAP) and phosphorylated-p38 (p-p38) for immunohistochemistry [12]. The polyclonal primary antibodies were used in the following dilutions: IL-18 (1:200; R&D Systems), Iba1 (1:2000; Wako), GFAP (1:2500; Millipore Bioscience Research Reagents), p-p38 (1:500; Cell Signaling Technology). Double immunofluorescent staining was performed with IL-18 and Iba1, GFAP, p-p38 at 3 d after surgery. The specificity of the IL-18 antibody was confirmed by a preabsorption control experiment with the IL-18 protein, and the Western blots with other antibodies and IL-18 antibody were used to show their specificity [12,15].

To quantify the signal of IL-18-immunoreactivity (IR), five adjacent sections in the superficial layers of the Vc and cervical DH per each rat (each time point $n = 4$) were randomly selected and an image in a square (316 μm × 236 μm) on each section was captured. The signal on each image was measured with a computer-assisted imaging analysis system (NIH Image Version 1.63). Upper and lower thresholds of gray level density were set such that only positive immunostained profiles were accurately discriminated from the background in the squares and read by the computer pixel-by-pixel. Subsequently, the area of discriminated pixels was measured and divided by the area of the outlined square, giving a relative intensity of IL-18-IR. All results are expressed as mean ± SEM. Differences in changes of values over time of each group were tested using one-way ANOVA, followed by individual post hoc comparisons (Fisher's exact test).

3. Results

3.1. Nocifensive behavior

The withdrawal threshold to mechanical stimulation of the whisker pad was measured to assess control threshold 1 d before IANT. Fig. 1 illustrates the behavioral responses upon mechanical stimulation of the whisker pad in IANT rats and sham-operated rats. The head withdrawal thresholds upon mechanical stimulation of the whisker pad ipsilateral and contralateral to the IANT and sham-operated rats were significantly reduced at 1 d after IANT. Although the head withdrawal threshold returned to preoperative level at 3 d in sham-operated rats, the threshold was reduced significantly on

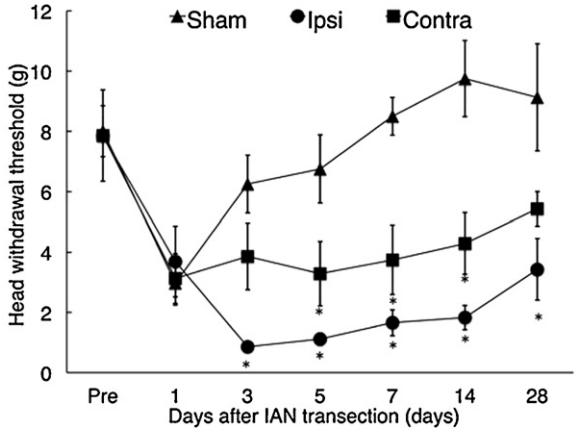

Fig. 1. Changes in head escape thresholds to mechanical stimulation in the maxillary whisker pad skin of the sham-operated and IANT rats. Ipsilateral and contralateral sides of IANT rats were measured. The response threshold was defined as the lowest force of the filaments that produced at least three positive responses in five trials. Sham, sham-operated rats; Ipsi, ipsilateral side to IANT; Contra, contralateral side to IANT.