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Distribution of hemokinin-1 in the rat trigeminal ganglion and trigeminal sensory nuclear complex

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ABSTRACT

Objective: A new mammalian tachykinin peptide encoded in a TAC4 gene was identified and designated as hemokinin-1 (HK-1). A representative of the tachykinin peptide family is substance P (SP), and the function of SP has been well characterized as a pain transmitter or modulator, while it is possible that HK-1 is involved in pruriceptive processing, but, as yet, the distribution of HK-1 peptide in the trigeminal sensory system is still unknown. Thus, the aim of the present study was to elucidate the distribution of HK-1, while comparing the expression of SP, in the trigeminal ganglion and trigeminal sensory nuclear complex.

Design: The trigeminal ganglion and the brain stem of male SD rats were used in the immunohistochemical study. Since the amino acid sequence in the carboxyl-terminal regions of HK-1 and SP is common, polyclonal antibodies of HK-1 and SP derived from 6 amino acids consisting of amino-terminal regions of these peptides were produced in guinea pig and rabbit, respectively. The immunohistochemical staining of HK-1 and SP was conducted using frozen sections of the trigeminal ganglion and brain stem in rats.

Results: Immunohistochemical studies revealed the expression of HK-1 in small- and medium-sized trigeminal ganglion neurons, in the paratrigeminal nucleus, and in lamina I of the trigeminal nucleus caudalis, while there was no immunoreactivity of HK-1 in the trigeminal nucleus principalis, trigeminal nucleus oralis, and trigeminal nucleus interpolaris.

Conclusion: These findings indicate that HK-1 is a target molecule for treatment of itch in the orofaicial regions.

1. Introduction

A new mammalian tachykinin peptide encoded in a preprotachykinin gene, TAC4, was identified in mouse bone marrow cells and designated as hemokinin-1 (HK-1) (Zhang, Lu, Furlonger, Wu, & Paige, 2000). HK-1 exhibits structural homology with known members of the tachykinin family, of which peptides share a carboxyl-terminal F-X-G-L-M-amide motif and more varied amino-terminals (Page, 2004; Page, 2005; Page, 2006).

A representative of the tachykinin peptide family is substance P (SP), and its function has been well characterized. SP and its proper receptor, neurokinin 1 (NK1) receptor, are mainly expressed in the superficial layer of the spinal dorsal horn, in which nociceptive primary afferents terminate (Brown et al., 1995; Greco et al., 2007; Hökfelt, Kellerth, Nilsson, & Pernow, 1975; Naono-Nakayama, Sunakawa, Ikeda, & Nishimori, 2011). SP is found in and released from primary afferents, and is believed to function as a pain transmitter or modulator (De Koninck & Henry, 1991; Henry, 1976). Meanwhile, the function of HK-1 remains poorly understood, but recent investigations have suggested its involvement in processing of pruritus (Funahashi et al., 2014; Naono-Nakayama et al., 2014) or neuropathic pain (Matsumura et al., 2008; Sakai, Takasu, Sawada, & Suzuki, 2012).

Additionally, there is currently no information available about the distribution of HK-1 peptide in either the spinal cord or the trigeminal sensory system, although the expression of mRNA derived from TAC4 gene encoding HK-1 has been reported in several regions, including the brain, spinal cord, dorsal root ganglia, brain stem, and trigeminal ganglion (Duffy et al., 2003;

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Kurtz et al., 2002; Matsumura et al., 2008). These findings suggest the expression of HK-1 in the trigeminal sensory system, as well as in the spinal cord. In the trigeminal sensory system, peripheral axons of trigeminal ganglion neurons are distributed in the orofacial regions, and central axons terminate in the trigeminal sensory nuclear complex that is structurally and functionally divided into the trigeminal sensory nucleus principalis (Vp) and three subnuclei of the spinal trigeminal nucleus: trigeminal nucleus oralis (Vo), trigeminal nucleus interpolaris (Vi), and trigeminal nucleus caudalis (Vc) (Olszewski, 1950).

Scratching is a widely-used behavior marker for evaluating pruritus, and administration of a pruritic stimulus to the cheek, an orofacial region, as well as the nape of the neck, elicits scratching behavior and neuronal activity in trigeminal nucleus caudalis neurons (Akiyama, Carstens, & Carstens, 2010; Akiyama, Curtis, Nguyen, Carstens, & Carstens, 2016; Klein, Carstens, & Carstens, 2011; LaMotte, Shimada, & Sikand, 2011; Shimada & LaMotte, 2008). These findings suggest that transmitters or modulators used in the spinal cord may also be involved in pruritus signaling in the trigeminal sensory system. Recently, pharmacological studies have demonstrated the involvement of HK-1 in pruriceptive processing in the spinal cord (Funahashi et al., 2014; Naono-Nakayama et al., 2014). Thus, it is possible that HK-1 may similarly contribute to pruriceptive processing in the trigeminal sensory system. Undoubtedly, the clarification of HK-1 distribution in this system provides a clue to elucidate the underlying mechanisms of pruriceptive processing in the trigeminal sensory system.

Therefore, the aim of the present study was to elucidate the expression of HK-1 in the trigeminal ganglion and trigeminal sensory nuclear complex. SP and HK-1 are peptides belonging to the same tachykinin family, and share a common sequence of amino acids in the C-terminal, but not the N-terminal regions. Thus, to avoid the cross-reactivity, two different antibodies derived from the N-terminal regions of SP and HK-1 were produced from two different species, rabbit and guinea pig, respectively. The immunoreactivity of these two peptides was investigated in the trigeminal ganglion and trigeminal sensory nuclear complex, and the their coexpression was examined in trigeminal ganglion neurons.

2. Materials and methods

2.1. Experimental design

The experimental procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 85-23, revised 1996). All efforts were made to minimize the number of animals used (Zimmermann, 1983).

Male Sprague-Dawley rats, weighing 200–250 g, were acclimated for a period of at least one week at the Experimental Animal Center of the University of Miyazaki, and maintained under a 12/12 h light/dark cycle with food and water available.

2.2. Antibodies against HK-1 and SP

The amino acid sequences of HK-1 (1–11) and SP (1–11) are Arg-Ser-Arg-Thr-Arg-Gln-Phe-Tyr-Gly-Leu-Met-amide and Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-amide, respectively. The amino acid sequence of HK-1 proposed by Zhang et al. (2000) was adopted in this study, since it remains to be determined whether arginine exists at the N-terminus of HK-1 (Page, 2004). Since the C-terminal regions of HK-1 and SP share an amino acid sequence common to the tachykinin peptide family, Phe-X-Gly-Leu-Met, antibodies against HK-1 or SP were produced using peptides derived from the N-terminal regions of each of these two peptides for reducing the possibility of cross-reactivity of antibodies. The 1st to 6th consecutive amino acid sequences of the N-terminal regions of HK-1 (1–6): Arg-Ser-Arg-Thr-Arg-Gln, and SP (1–6): Arg-Pro-Lys-Pro-Gln-Gln, were synthesized, and, HK-1 (1–6) and SP (1–6) peptides, were injected into guinea pig and rabbit, respectively, for producing polyclonal antibodies against these peptides. IgG isolated from their sera was purified, and the final concentration of IgG was adjusted to 2 mg/ml (TAKARA BIO INC, Kusatsu, Shiga, Japan). Antibodies derived from each terminal fragment of HK-1 and SP were designated as HK-1 (1–6) antibody and SP (1–6) antibody, respectively.

2.3. Immunohistochemistry of HK-1 and SP

Animals were anesthetized with an overdose of sodium pentobarbital, and perfused intracardially with saline (200 ml), followed by 4% cold paraformaldehyde in 0.1 M phosphate buffer (PB) (500 ml) for 30 min. The brain stem and trigeminal ganglion were removed, postfixed for 1 h in the same fixative, and cryoprotected in 10% sucrose in PB for 1 h and then in 30% sucrose in PB overnight. Frozen serial sections of the brain stem and trigeminal ganglion, 50 μm in thickness, were prepared, collected in phosphate-buffered saline (PBS; pH 7.4), and processed as free-floating sections for immunohistochemical staining against HK-1 and SP.

All sections were incubated in hydrogen peroxide and Triton X-100, and then in normal goat serum. Part of sections were immersed in a solution of polyclonal guinea pig anti-HK-1 (1–6) antibody (1:15,000 or 1:5000) overnight at 4 °C, and then reacted with biotinylated goat anti-guinea pig IgG and avidin-conjugated horseradish peroxidase (VECSTAIN ABC kits, Burlingame, CA, USA). The remaining sections were incubated in polyclonal rabbit anti-SP (1–6) antibody (1:15,000 or 1:5000) or monoclonal rabbit anti-NeuN antibody (1:10,000, Abcam, Cambridge, MA, USA) overnight at 4 °C, and then reacted with biotinylated goat anti-rabbit IgG and avidin-conjugated horseradish peroxidase (SAB kit; Nichirei Biosciences Inc., Tokyo, Japan). All sections with an avidin-biotin complex were visualized using diaminobenzidine tetrahydrochloride (DAB; Sigma Chemical Co., St. Louis, Mo, USA) and hydrogen peroxide. The sections placed on Frontier-coated glass slides (Matsunami Glass, Kishiwada, Japan) were mounted using NEW M·X mounting agent (Matsunami Glass, Kishiwada, Japan) and coverslips, after dehydration by ethyl alcohol and penetration by xylene. Preabsorption of HK-1 (1–6) and SP (1–6) antibodies with HK-1 (1–11) and SP (1–11) peptides (Genenet Co., Fukuoka, Japan), respectively, or omission of the antibody from the protocol, eliminated positive staining.

Trigeminal ganglion cells were stained with various degree of density. Then, cells labeled heavily and contained a clearly defined nucleus were regarded as cells positive to HK-1, SP and NeuN, and their sizes were measured for generating cell-size histograms of HK-1-positive cells, SP-positive cells and NeuN-positive cells in the trigeminal ganglion. Trigeminal sensory nuclear complex was divided into Vp, Vo, Vi and Vc. The dorsal horn of Vc was further divided into lamina I, lamina II, lamina III, lamina IV and lamina V, and especially lamina II was subdivided into lamina IIo consisting of the outer one-third of this lamina and lamina IIi consisting of the inner two-thirds of this lamina.

In addition, some sections were incubated in hydrogen peroxide and Triton-X, and reacted with polyclonal guinea pig anti-HK-1 (1–6) antibody (1:10,000 or 1:5000) and then goat anti-guinea pig IgG conjugated with Alexa Fluor 488 (1:10,000, Abcam, Cambridge, MA, USA), polyclonal rabbit anti-SP (1–6) antibody (1:10,000 or 1:5000) and then donkey anti-rabbit IgG conjugated with Alexa Fluor 594 (1:10,000, Abcam, Cambridge, MA, USA), or monoclonal rabbit anti-NeuN antibody (1:10,000, Abcam, Cambridge, MA, USA)