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Short communication

Peptide 19 in the rat vagal and glossopharyngeal sensory ganglia

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Abstract

Peptide 19 (PEP 19) is a 7.6-kDa polypeptide which binds to calmodulin and inhibits calcium–calmodulin signaling. In this study, PEP 19-immunoreactivity (PEP 19-IR) was examined in the rat vagal and glossopharyngeal sensory ganglia. Twenty-nine percent, 59%, and 41% of sensory neurons contained PEP 19-IR in the jugular, petrosal, and nodose ganglia, respectively. These neurons were of various sizes (jugular, mean ± SD = 635.8 ± 392.6 μm^2, range = 105.9–1695.9 μm^2; petrosal, mean ± SD = 370.9 ± 228.5 μm^2, range = 57.7–1662.7 μm^2; nodose, mean ± SD = 380.5 ± 157 μm^2, range = 87.5–950.4 μm^2) and scattered throughout these ganglia. Double immunofluorescence method revealed that PEP 19-IR neurons which had parvalbumin-IR were rare in the ganglia (jugular, 4%; petrosal, 10%; nodose, 8%). PEP 19-IR neurons which contained calbindin D-28k were abundant in the petrosal (20%) and nodose (22%) ganglia but not in the jugular ganglion (8%). Retrograde tracing method indicated that many PEP 19-IR neurons projected to the circumvallate papilla and soft palate. In the soft palate, taste buds were innervated by PEP 19-IR nerve fibers. The present study suggests that PEP 19-IR neurons include chemoreceptors in the vagal and glossopharyngeal sensory ganglia.

Theme: Sensory systems
Topic: Somatic and visceral afferents

Keywords: Calbindin D-28k; Jugular ganglion; Nodose ganglion; Parvalbumin; Peptide 19; Petrosal ganglion

Peptide 19 (PEP 19) was first discovered in the developing rat cerebellum [17,19]. The molecule was isolated, sequenced, and found to be a 7.6-kDa polypeptide. Subsequent studies have shown that PEP 19 binds to calmodulin and inhibits calcium–calmodulin signaling [18]. By immunohistochemistry, PEP 19 has been shown to be highly expressed in the central and peripheral nervous systems [1,17,20]. In the dorsal root (DRG) and trigeminal ganglia (TG), PEP 19 is mostly detected in medium sized to large neurons [7,13]. In addition, PEP 19-containing DRG and TG neurons co-express parvalbumin and calbindin D-28k [7,13]. These calcium-binding proteins are localized to large DRG and TG neurons whose peripheral axons innervate muscle spindles and corpuscular endings, respectively [2,3,6,9,11,12]. Therefore, PEP 19 is considered to be expressed by muscular proprioceptors in the DRG and low-threshold mechanoreceptors in the TG.

Somatic sensory neurons of the vagus nerve are located in the jugular ganglion, whereas visceral sensory neurons of the nerve are present in the nodose ganglion. In addition, the somata of visceral sensory neurons of the glossopharyngeal nerve are located in the petrosal ganglion. These ganglia supply various tissues in oral and cervical regions with their peripheral axons. In the tongue and soft palate, taste buds are innervated by the jugular, petrosal, and nodose ganglia [8,10,14,15]. Primary sensory neurons which innervate taste buds transmit chemoreceptive information to the brainstem. The petrosal and nodose ganglion also provide the chemosensory innervation for the carotid body and the aortic bodies, respectively. Thus, the vagal and glossopharyngeal sensory ganglia are considered to contain abundant lingual and palatal chemoreceptors. Besides the vagal and glossopharyngeal ganglia also contain mechanoreceptor neurons.

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Previous studies also have demonstrated that vagal and glossopharyngeal sensory neurons contain parvalbumin and calbindin D-28k [4,5,8]. Calbindin D-28k-containing neurons send their peripheral axons to taste buds in oral and pharyngeal regions [8,16]. Therefore, it has been suggested that calbindin D-28k-containing neurons include chemoreceptors in the vagal and glossopharyngeal sensory ganglia.

To know whether PEP 19 is located in chemoreceptive neurons, we examined the distribution of PEP 19 in the jugular, petrosal, and nodose ganglia, as well as the tongue and soft palate. A double immunofluorescence method was performed to examine co-expression of PEP 19 with parvalbumin and calbindin D-28k. In addition, the peripheral projection of PEP 19-containing neurons was investigated by a retrograde tracing method.

The jugular, petrosal, and nodose ganglia as well as tongues and soft palates were obtained from four male Sprague–Dawley rats (200–300 g). Rats were anesthetized with ether to the level at which respiration was markedly suppressed, and transvascularly perfused with 50 ml of saline followed by 500 ml of 4% formaldehyde in 0.1 M phosphate buffer (pH 7.4). The ganglia, tongue, and soft palate were dissected, frozen sectioned at 12 μm, and thaw-mounted on gelatin-coated glass slides. For evaluation of the distribution and cell size of PEP 19-IR neurons, avidin–biotin–horseradish peroxidase complex (ABC) method was performed. Sections of the sensory ganglia were incubated with rabbit anti-PEP 19 serum [20], followed by biotinylated rabbit anti-rabbit IgG (1:200, Vector Laboratories) and ABC-complex (1:25, Vector Laboratories). Following nickel ammonium sulfate (0.1%)-intensified diaminobenzidine (0.002%) reaction, these sections were dehydrated in a graded series of alcohols, cleared in xylene, and coverslipped with Entellan (Merck). In this study, rostral and caudal halves of the jugular–petrosal complex were regarded as the jugular and petrosal ganglia, respectively. The number and cell size of PEP 19-immunoreactive (IR) and immunonegative neurons was analyzed in every tenth of the serial sections of two ganglia from two animals. In ABC-stained sections, the microscopic image (×215) of the cell bodies was projected over a digitizer tablet using a drawing tube. The cross-sectional area of those cell bodies which contained the nucleolus was recorded. For the peripheral distribution of PEP 19-IR nerve fibers, an indirect immunofluorescence method was used. Sections of the tongue and soft palate were incubated with the primary antiserum (1:100) and lissamine rhodamine B chloride-conjugated donkey anti-rabbit IgG (1:200, Jackson ImmunoResearch Labs, USA).

For co-expression of PEP 19 with calcium-binding proteins, a double-immunofluorescence method was used. Sections of the sensory ganglia were incubated for 24 h at room temperature with a mixture of rabbit anti-PEP 19 serum and either mouse monoclonal anti-parvalbumin antibody (1:1000, Sigma, USA) or mouse monoclonal anti-calbindin D-28k antibody (1:500, Sigma, USA). These sections were then treated with a mixture of lissamine rhodamine B chloride-conjugated donkey anti-rabbit IgG (1:500, Jackson ImmunoResearch Labs) and fluorescein isothiocyanate-conjugated donkey anti-mouse IgG (1:100, Jackson ImmunoResearch Labs) for 1 h at 37 °C.

To study the peripheral projections of PEP 19-IR neurons, four male rats (300–350 g) were used. Under deep anesthesia by ip injection with ethyl carbamate (650 mg/kg) and pentobarbital sodium (20 mg/kg), 0.2 μl of 1% fluorogold (FG, Fluorochrom INC., USA) in distilled water was injected into the circumvallate papilla or soft palate. After 3 days, the animals were reanesthetized with ether and transvascularly perfused with 4% formaldehyde. The right and left sensory ganglia were frozen sectioned at 12 μm, mounted on gelatin-coated glass slides, and processed for PEP 19 immunofluorescence as described above.

The specificities of the primary antisera have been described elsewhere [5,20].

The experiments were carried out under the control of the Animal Research Control Committee in accordance with The Guidelines for Animal Experiments of Okayama University Medical School, Government Animal Protection and Management Law (No. 105), and Japanese Government Notification on Feeding and Safekeeping of Animals (No.6). All efforts were made to minimize the number of animals used and their suffering.

PEP 19-IR neurons were distributed throughout the vagal and glossopharyngeal sensory ganglia (Figs. 1A, C, and E). Twenty-one percent (107/507), 59.3% (275/464), and 41% (252/614) of sensory neurons were immunoreactive for PEP 19 in the jugular, petrosal, and nodose ganglia, respectively (Figs. 1B, D, and F). As shown in Figs. 1A–F, these neurons were of various sizes (jugular, mean ± SD = 635.8 ± 392.6 μm^2, range = 105.9–1695.9 μm^2, n = 507; petrosal, mean ± SD = 370.9 ± 228.5 μm^2, range = 57.7–1662.7 μm^2, n = 464; nodose, mean ± SD = 380.5 ± 157 μm^2, range = 87.5– 950.4 μm^2, n = 614). In the jugular ganglion, 15% of small (<300 μm^2, 26/171) and medium-sized (300–600 μm^2, 32/207) neurons exhibited PEP 19-immunoreactivity (IR). About 40% (36/77) of large neurons had the IR. In the petrosal ganglion, small neurons predominantly contained PEP 19-IR (68.8% or 132/192). Half of medium-sized (107/195) and large (36/77) neurons showed the IR. In the nodose ganglion, half of small (83/188) and medium-sized (143/319) neurons and 24.3% (26/107) of large neurons exhibited PEP 19-IR.

Parvalbumin-IR neurons were common in the nodose ganglion but not in the jugular or petrosal ganglia. These neurons were scattered in these three ganglia. Calbindin D-28K-IR neurons were rare in the jugular ganglion but abundant in the petrosal and nodose ganglia. Such neurons were distributed throughout the jugular and nodose ganglia. In the petrosal ganglion, however, calbindin D-28K-IR neurons were restricted to the caudal portion. The number of calbindin D-28k- or PEP 19-IR neurons was more numerous than that of parvalbumin-IR neurons. Double immunofluor-