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Short Communication

ASIC3-immunoreactive neurons in the rat vagal and glossopharyngeal sensory ganglia

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ABSTRACT

ASIC3-immunoreactivity (ir) was examined in the rat vagal and glossopharyngeal sensory ganglia. In the jugular, petrosal and nodose ganglia, 24.8%, 30.8% and 20.6% of sensory neurons, respectively, were immunoreactive for ASIC3. These neurons were observed throughout the ganglia. A double immunofluorescence method demonstrated that many ASIC3-immunoreactive (ir) neurons co-expressed calcitonin gene-related peptide (CGRP)- or vanilloid receptor subtype 1 (VRL-1)-ir in the jugular (CGRP, 77.8%; VRL-1, 28.0%) and petrosal ganglia (CGRP, 61.7%; VRL-1, 21.5%). In the nodose ganglion, however, such neurons were relatively rare (CGRP, 6.3%; VRL-1, 0.4%). ASIC3-ir neurons were mostly devoid of tyrosine hydroxylase in these ganglia. However, some ASIC3-ir neurons co-expressed calbindin D-28k in the petrosal (5.5%) and nodose ganglia (3.8%). These findings may suggest that ASIC3-containing neurons have a wide variety of sensory modalities in the vagal and glossopharyngeal sensory ganglia.

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, visceral sensory neurons of the glossopharyngeal nerve are located in the petrosal ganglion. These neurons innervate the tongue, larynx, pharynx, thorax and abdomen, and convey sensory impulses to the brainstem. Previous immunohistochemical studies have classified primary neurons into several subpopulations on the basis of their chemical markers in the vagal and glossopharyngeal sensory ganglia. Calcitonin gene-related peptide (CGRP) is a marker for small to medium-sized primary sensory neurons in the petrosal, jugular and nodose ganglia (Helke and Hill, 1988; Helke and Niederer, 1990). They supply their peripheral receptive fields with free nerve endings, and are considered to be nociceptors (Silverman and Kruger, 1989). Vanilloid receptor 1-like receptor (VRL-1), a newly cloned capsaicin receptor homologue, is activated by high temperatures with a threshold >52°C (Caterina et al., 1999). VRL-1 is localized to medium-sized to large nociceptors in the vagal and glossopharyngeal sensory ganglia (Ichikawa and Sugimoto, 2002a, 2003). Tyrosine hydroxylase is a rate limiting enzyme of catecholamine synthesis. This enzyme is expressed by small and medium-sized neurons in the petrosal and nodose ganglia (Helke and Hill, 1988; Helke and Niederer, 1990). Because tyrosine hydroxylase-containing petrosal neurons innervate the carotid body, they are thought to be chemoreceptors (Katz and Black, 1986; Ichikawa et al., 1993; Ichikawa, 2002). On the other hand, calbindin D-28k, a member of the calcium-binding protein family, is localized to small to large neurons in the petrosal and nodose ganglia (Ichikawa

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and Helke, 1995; Ichikawa and Sugimoto, 2002a). The carotid body is also innervated by calbindin D-28k-containing chemoreceptors (Ichikawa and Helke, 1995).

ASIC3 belongs to the family of acid-sensing ion channels. The mRNA is expressed by sensory ganglia, brain and many internal tissues including lung and testis (Babinski et al., 1999; Babinski et al., 2000). Previous immunohistochemical studies have demonstrated that ASIC3 is localized to small and large neurons in the dorsal root and trigeminal ganglia (Olson et al., 1998; Ichikawa and Sugimoto, 2002b; Molliver et al., 2005). Subpopulations of ASIC3-containing neurons co-express CGRP in these ganglia. Therefore, this channel is thought to play a role in nociception by functioning as a sensor of tissue acidosis (Olson et al., 1998; Waldmann et al., 1997; Yiangou et al., 2001). In the trigeminal ganglion, large ASIC3-containing neurons co-express parvalbumin, a marker for low-threshold mechanoreceptors (Ichikawa et al., 1994; Ichikawa et al., 1997; Ichikawa and Sugimoto, 1997, 2002b). Thus, ASIC3-containing neurons probably have a wide variety of sensory modalities in the trigeminal ganglion. However, little is known about the distribution of ASIC3 in the vagal and glossopharyngeal sensory ganglia.

In this study, we examine the distribution of ASIC3 and its co-expression with several neurochemical substances to know the phenotype and sensory modality of ASIC3-containing neurons in the vagal and glossopharyngeal sensory ganglia.

The vagal and glossopharyngeal sensory ganglia contained ASIC3-immunoreactive (ir) neurons; 24.8% (134/541), 30.8% (237/769) and 20.6% (311/1509) of jugular, petrosal and nodose neurons, respectively, were immunoreactive for ASIC3 (Fig. 1A). The immunoreactivity (ir) was detected in the cytoplasm but not in the nucleus of these neurons. They were of various sizes in the jugular and petrosal ganglia, and mostly small to medium-sized in the nodose ganglion. ASIC3-ir neurons were numerous throughout these ganglia.

As described previously (Helke and Hill, 1988), CGRP-ir neurons were distributed throughout the jugular ganglion. The rostral portions of the petrosal and nodose ganglia also contained CGRP-ir neurons. The number of ASIC3-ir neurons was fewer than that of CGRP-ir neurons in the jugular and petrosal ganglia. In the nodose ganglion, however, ASIC3-ir neurons were more numerous than CGRP-ir neurons. Our double immunofluorescence method revealed the co-expression of ASIC3 and CGRP in these ganglia (Figs. 1B–G). ASIC3-ir neurons which co-expressed CGRP-ir were abundant in the jugular (77.8%) and petrosal ganglia (61.7%), and relatively rare in the nodose ganglion (6.3%) (Table 1, Figs. 1B–G). Conversely, 36.6% (63/172), 41.6% (129/310) and 22.4% (19/85) of CGRP-ir neurons contained ASIC3-ir in the jugular, petrosal and nodose ganglia, respectively. Sensory neurons which co-expressed these substances were scattered throughout the jugular ganglion. Such neurons were restricted to the rostral portions of the petrosal and nodose ganglion.

VRL-1-ir neurons were distributed throughout the vagal and glossopharyngeal sensory ganglia. Substantial subpopulation of ASIC3-ir neurons co-expressed VRL-1-ir in the jugular (28.0%) and petrosal ganglia (21.5%) but not the nodose ganglion (0.4%) (Table 1, Figs. 1H–M). Conversely, all (33/33) VRL-1-ir jugular neurons and 40.2% (80/199) of VRL-1-ir petrosal neurons exhibited ASIC3-ir (Figs. 1H–K). These neurons were scattered throughout these ganglia. Only less than 1% (1/120) of VRL-1-ir neurons showed ASIC3-ir in the nodose ganglion (Figs. 1L, M).

Tyrosine hydroxylase-ir neurons were detected in the petrosal and nodose ganglia but not the jugular ganglion. Tyrosine hydroxylase-ir neurons were located in the caudal portions of the petrosal and nodose ganglia. The number of ASIC3-ir neurons was more numerous than that of tyrosine hydroxylase-ir neurons in these ganglia. ASIC3-ir neurons which co-expressed tyrosine hydroxylase-ir were very rare in the petrosal and nodose ganglia (Table 1, Figs. 2A–D). Only 4% (2/50) and 3.2% (2/62) of tyrosine hydroxylase-ir neurons showed ASIC3-ir in the petrosal and nodose ganglia, respectively.

Calbindin D-28K-ir neurons were rare in the jugular ganglion and numerous in the petrosal and nodose ganglia. In the petrosal ganglion, calbindin D-28K-ir neurons were detected in the caudal portion. Such neurons were abundant throughout the nodose ganglion. In the nodose ganglion, the number of ASIC3-ir neurons was fewer than that of calbindin D-28K-ir neurons. ASIC3-ir neurons in the jugular ganglion lacked calbindin D-28k-ir (Table 1). However, small but substantial proportions of ASIC3-ir neurons in the petrosal and nodose ganglia co-expressed calbindin D-28K-ir (Table 1, Figs. 2E–G). Eight percent (6/73) and 1.8% (8/458) of calbindin D-28K-ir neurons in the petrosal and nodose ganglia, respectively, showed ASIC3-ir.

The present study demonstrated that the vagal and glossopharyngeal sensory ganglia contained numerous ASIC3-ir neurons. These neurons were of various sizes in the jugular and petrosal ganglia, and small to medium-sized in the nodose ganglion. A previous study has demonstrated that stomach-innervating neurons mostly show ASIC3-ir in the nodose ganglion (Schicho et al., 2004). The proportion of ASIC3-ir neurons among stomach-innervating neurons (75%) is higher than that of ASIC3-ir neurons among entire nodose neurons (21%). This may suggest that the content of ASIC3 in vagal and glossopharyngeal sensory neurons depends on their peripheral receptive fields.

Our double immunofluorescence method also revealed that ASIC3-ir neurons co-expressed CGRP- orVRL-1-ir in the jugular and petrosal ganglia. In the nodose ganglion, such co-expression was relatively rare. Jugular and petrosal neurons innervate the oral, pharyngeal and laryngeal regions, whereas nodose neurons send their peripheral axons to the thoracic and abdominal regions. It is likely that the co-expression of ASIC3 with CGRP or VRL-1 in vagal and glossopharyngeal sensory neurons is dependent upon their peripheral receptive fields. In the dorsal root and trigeminal ganglia, CGRP-ir neurons which co-express VRL-1-ir have myelinated axons (Caterina et al., 1999; Ichikawa and Sugimoto, 2000). Therefore, ASIC3-containing nociceptors with myelinated axons in the jugular and petrosal ganglia probably respond to the thermal stimulus >52 °C.

In the jugular and petrosal ganglia, it is also possible that ASIC3-ir neurons include unmyelinated nociceptors because ASIC3-ir neurons with CGRP-ir were more abundant than those with VRL-1 and because CGRP-ir neurons without VRL-1-ir have unmyelinated axons in the dorsal root and trigeminal ganglia (Caterina et al., 1999; Ichikawa and Sugimoto, 2000). In the nodose ganglion, unmyelinated nociceptors are mostly devoid of CGRP and have the capsaicin receptor VR1