

# Superior Laryngeal Nerve

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## 6.1 Introduction

Galenos was the first to describe three pairs of branches from the vagal nerve that innervate the larynx in his historical text entitled “De nervorum dissectione (Anatomy of the nerve).” These branches are considered to correspond to the inferior laryngeal nerve and the internal and external branches of the superior laryngeal nerve. It has been said that the internal branch of the superior laryngeal nerve mainly conveys sensation from the larynx and that the external branch of this nerve innervates the cricothyroid muscle. Our present study, however, provides evidence that the internal and external branches both contain sensory, motor, and autonomic nerve fibers.

The superior laryngeal nerve diverges from the vagal nerve just beneath the nodose ganglion and descends to ramify into the internal and external branches at the level of the middle pharyngeal constrictor muscle. The internal branch of the superior laryngeal nerve runs anteroinferiorly, pierces the thyrohyoid membrane together with the superior laryngeal artery, and ramifies into anterior and posterior fascicles at a level corresponding to the center of the epiglottis. The anterior fascicle mainly covers the glottis, piercing the lateral wall of the larynx through the gap between the internal and lateral muscles and distributing over the glottic mucosa. The posterior fascicle further bifurcates into a branch (middle branch) to the epiglottis, aryepiglottic fold, and arytenoids and another branch that runs toward the caudal side while ramifying into thin branches and distributing over the subglottic mucosa. The last branch of the posterior fascicle merges with the posterior fascicle of the inferior laryngeal nerve, forming Galen’s anastomosis. The presence of an anastomotic branch between the middle branch and the arytenoid muscle branch of the inferior laryngeal nerve has also been reported [1]. The external branch of the superior laryngeal nerve descends along with the inferior pharyngeal constrictor muscle and innervates the cricothyroid muscle. An anastomotic branch is known to be present between the inferior laryngeal nerve and the external branch of the superior laryngeal nerve in humans [2–4]. Displacements for the site of anastomosis reportedly vary among laryngeal nerve branches, such as Galen’s anastomosis [1].

## 6.2 Fiber Composition

It has been reported based on light microscopic studies that the number of myelinated fibers contained in the internal branch of the superior laryngeal nerve is 2,188–2,776 in the cat [5], about 15,000 in humans [6], and 317 and 354 on average on the right and left sides, respectively, in the rat, showing no right-left difference [7]. In recent years, advances in electron microscopic measurement and statistical analysis methods have enabled precise and accurate measurement of nerve fibers. Rosenberg et al. [8] reported the numbers of myelinated and unmyelinated fibers in the adult rat internal branch to be  $335 \pm 40$  and  $325 \pm 62$ ,

respectively. Mortelliti et al. [9] reported the numbers of myelinated and unmyelinated fibers in the adult human internal branch to be  $10,179 \pm 1,969$  and  $10,469$  ( $n=1$ ), respectively. In 1,955, Pressman and Kelemen [10] reviewed prior studies and stated that most of the internal branch consisted of myelinated fibers, with the proportion of unmyelinated fibers being very low. These findings indicate that the ratio of myelinated to unmyelinated fibers is essentially 1:1, at least in humans and dogs. Furthermore, the diameters of the myelinated and unmyelinated nerves in the rat internal branch were reported to be  $2.92 \pm 0.39 \mu\text{m}$  and  $0.453 \pm 0.035 \mu\text{m}$ , respectively [8].

There have been only a few reports on the fiber composition of the external branch of the superior laryngeal nerve. Domeij et al. [7] reported that the mean number of myelinated fibers in the rat external branch was 330 on the right side and 311 on the left side, showing no right-left difference, the same as in the internal branch. A comparison of rat internal and external branches showed that these two branches had nearly the same number of myelinated fibers, showing no bias in the distribution of myelinated and unmyelinated fibers. The diameters of myelinated and unmyelinated fibers were  $0.5\text{--}12 \mu\text{m}$  and  $0.1\text{--}2.3 \mu\text{m}$ , respectively. Thus, the internal and external branches are generally considered to be indistinguishable from each other in terms of the fiber composition alone [7].

The internal and external branches both have several small branches consisting of unmyelinated fibers in the vicinity of the larynx. In the superior laryngeal nerve in the laryngeal area, ganglion cells (paraganglia) are present in the form of clusters of up to 80 cells [11, 12]. (► See Chap. 7 on intralaryngeal ganglion.)

## 6.3 Function of the Superior Laryngeal Nerve

### 6.3.1 Sensory Nerve Fibers in the Superior Laryngeal Nerve

In regard to the sensory innervation of the internal branch, several reports have documented unilateral innervation covering the supraglottic area to the trachea [13] or unilateral innervation in almost all areas except for the subglottic posterior wall, which received bilateral innervation [14]. However, these were electrophysiological studies and failed to clearly identify areas of innervation due to their technical limitations. In 1986, Tanaka et al. [15] conducted a detailed investigation of the course and distribution of sensory nerve fibers in the cat larynx, using the horseradish peroxidase (HRP) method, and reported that the anterior fascicle of the superior laryngeal nerve internal branch was distributed to the laryngeal surface of the epiglottis and the aryepiglottic fold, while the middle branch was distributed to the aryepiglottic fold, arytenoid apex, posterior part, lateral part, laryngeal vestibule, and rostral surface of the vocal cord. They also reported that the posterior fascicle further

divided into four branches which were distributed over the caudal surface of the right and left vocal cords, the mucosa in the subglottic space, the mucosa of the posterior cricoarytenoid muscle, and the hypopharyngeal mucosa, and that the internal branch of the superior laryngeal nerve innervated not only supraglottic but also subglottic areas, in the form of unilateral innervation in the supraglottic area and bilateral innervation with ipsilateral dominance in the subglottic area.

In 1968, Suzuki and Kirchner [16] reported that afferent fibers were contained in the cat external branch, controlling sensory innervation under the anterior commissure. Maranillo et al. [4] who conducted a detailed investigation of the course of the human external branch found that fibers distributed over the subglottic area and thyroarytenoid joint were present in approximately 50 % of individuals and speculated that they might be sensory fibers. Using the HRP method, we previously demonstrated sensory nerve fibers, whose cells of origin lay in the nodose ganglion, to be contained in the dog external branch [17].

### 6.3.2 Motor Nerve Fibers in the Superior Laryngeal Nerve

There has been ongoing controversy since the 1930s as to whether the internal branch contains motor nerves innervating the arytenoid muscle. Lemere [18] and Murtagh and Campbell [19] denied the presence of motor nerves in the internal branch in the dog based on anatomical findings, and Meurmann [20] and Williams [21] also reported that arytenoid muscle contraction in response to stimulation of the internal branch was not observed. In contrast, Vogel [22] identified motor nerve endings in the periphery of the internal branch in human subjects and advocated the theory of double innervation of the arytenoid muscle by the inferior and superior laryngeal nerves. Recently, Sanders and Mu [23] used Sihler's staining technique to study the internal branch of the human superior laryngeal nerve in detail and reported the presence of arytenoid muscle branches. We also previously found, using the nerve tracer technique, that motor nerve fibers were contained in the internal branch in the dog [24].

The external branch consists mainly of motor nerve fibers and controls the motion of the cricothyroid muscle. In the 1950s, Murtagh and Campbell [19] carried out an electrophysiological study and hypothesized that the cricothyroid muscle would be subject to double innervation by the external branch and the inferior laryngeal nerve, but their hypothesis is not currently supported. However, in humans, anastomotic branches between the external branch and the inferior laryngeal nerve (on one side or both sides) have been found at a frequency of 85 % [4]. Therefore, the possibility remains that a few motor nerve fibers are distributed from the inferior laryngeal nerve to the cricothyroid muscle.

### 6.3.3 Autonomic Nerve Fibers in the Superior Laryngeal Nerve

Sympathetic nerve fibers were formerly considered to be derived from the superior cervical ganglion and to run along the superior and inferior laryngeal artery and vein and then enter the larynx [25, 26], but accurate identification was lacking. In 1982, we reported for the first time that numerous noradrenaline (NA) fibers were contained in the inferior laryngeal nerve and the internal and external branches of the superior laryngeal nerve and that these nerve fibers were mainly distributed to the blood vessels in the larynx and laryngeal glands [27, 28].

Starting in the 1980s and extending into the 1990s, studies employing the nerve tracer method revealed that parasympathetic nerve fibers (preganglionic fibers) were contained in the superior laryngeal nerve in the rat [29], dog [30, 31], hamster [32], and guinea pig [33] and that their cell bodies were present in the dorsal motor nucleus of the vagus nerve. (See the section on the dorsal motor nucleus of the vagus nerve.)

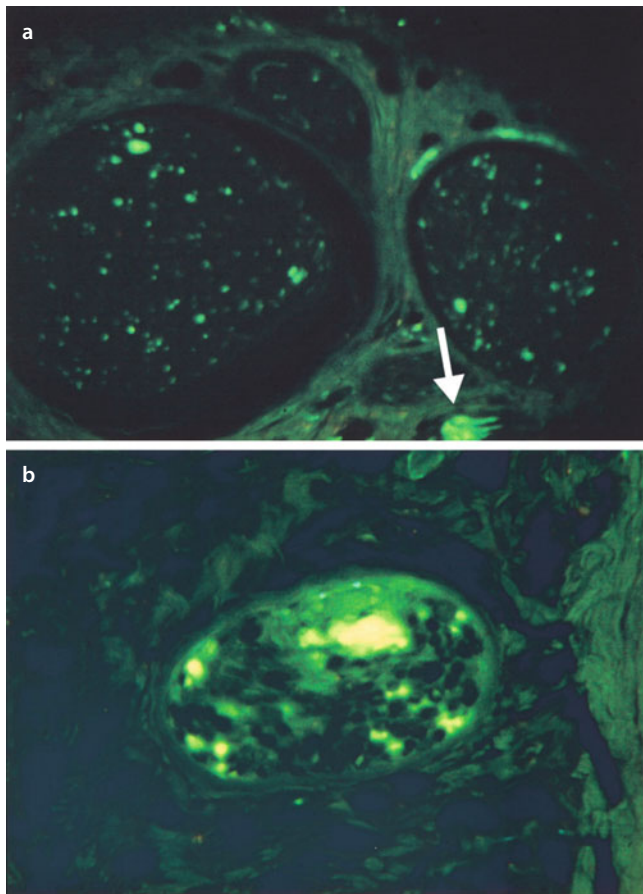
### 6.4 Neuropeptides Contained in the Superior Laryngeal Nerve

Several neuropeptides have been shown to be involved in laryngeal sensory innervation [34–38]. We previously reported on the content of each neuropeptide in sensory nerve fibers contained in the internal branch of the superior laryngeal nerve [38].

### 6.5 Identification of Sympathetic Nerve Fibers in the Superior Laryngeal Nerve [27]

The superior laryngeal nerve contains numerous unmyelinated fibers. However, whether or not they are autonomic nerve fibers has yet to be clarified. In order to identify sympathetic nerve fibers contained in the superior laryngeal nerve, we crushed the internal and external branches of the canine superior laryngeal nerve at the site of laryngeal entry and then visualized NA fibers employing a fluorescent histochemical method (Falck-Hillarp method).

Three or four nerve fascicles were found in the internal branch of the superior laryngeal nerve, and all of these fascicles contained numerous discrete NA fibers (■ Fig. 6.1a). In addition, ramification of small nerve fascicles consisting of NA fibers alone was observed. NA fibers were found in the external branch of the superior laryngeal nerve, just as in the internal branch (■ Fig. 6.1b). From these findings, it was apparent that all branches of the superior laryngeal nerve have sympathetic nerve fibers, and it was inferred that these nerve fibers branched out from the main trunk in the vicinity of the larynx, distributing to the blood vessels and glands in the larynx.

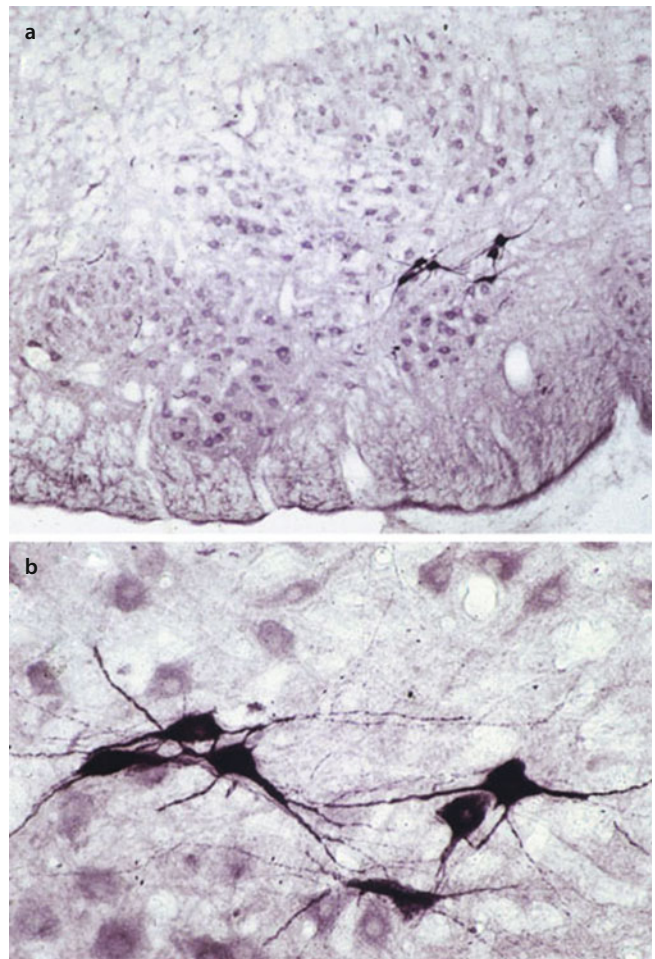


**Fig. 6.1** Superior laryngeal nerve visualized by the Falck-Hillarp method (a internal branch, b external branch) [27]. (a) Nerve fascicles in the internal branch contain many sporadic NA fibers. There is a small nerve fascicle consisting of NA fibers only (→). (b) Numerous NA fibers are also contained in the external branch, just as in the internal branch

## 6.6 Localization of Cells of Origin of Motor Fibers Contained in the Internal Branch of the Superior Laryngeal Nerve [24]

The localization of cells of origin of nerve fibers contained in the internal branch was examined to determine whether motor nerves are contained in the internal branch of the superior laryngeal nerve. The internal branch of the canine superior laryngeal nerve on the right side was cut, and the proximal end was soaked in cholera toxin B (CTB) and subjected to perfusion fixation 2 days later. The brain stem was extirpated, and histological sections were prepared to visualize labeled cells by an immunohistochemical technique for CTB.

Although no labeled cells were found in the nucleus ambiguus, several labeled cells measuring 35–50  $\mu\text{m}$  in diameter were observed in an area 4.1–7.4 mm rostral to the obex on the right ventrolateral side (■ Fig. 6.2a, b). This area corresponds to the region of the facial nucleus, indicating that motor nerve cells of the facial nucleus extend fibers to the internal branch of the superior laryngeal nerve. Further investigations are required to determine



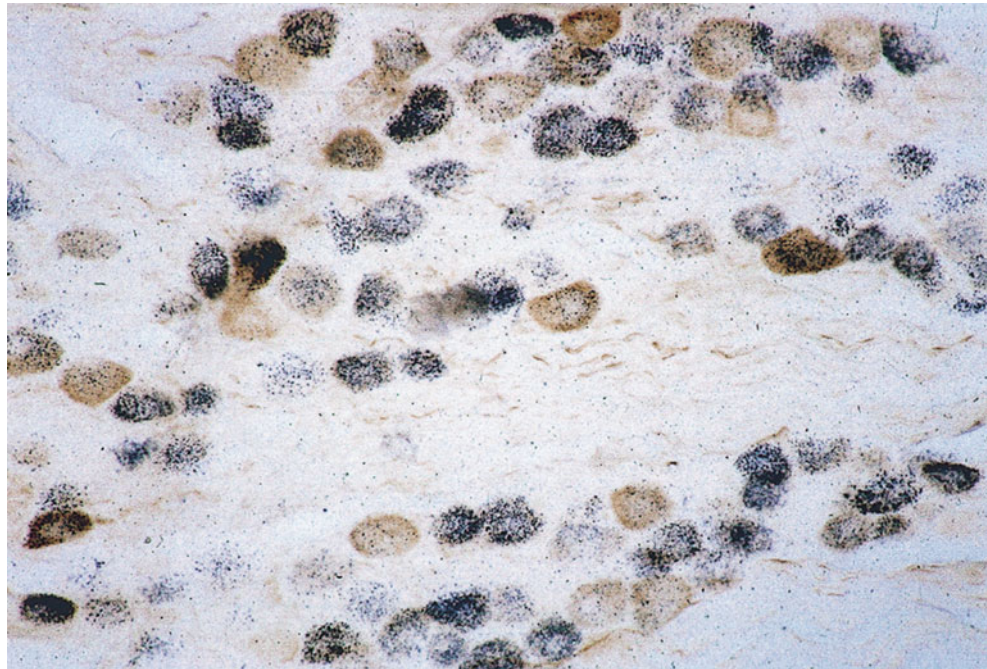
**Fig. 6.2** The proximal end of the internal branch was soaked in CTB, and labeled cells were visualized by immunostaining [24]. (a) Labeled cells were present in the facial nerve nucleus ( $\times 70$ ) (b) The labeled cells were multipolar and measured 35–50  $\mu\text{m}$  in diameter ( $\times 350$ )

whether these cells control the intrinsic muscles of the larynx, such as the transverse arytenoid muscle, or the aryepiglottic muscle.

## 6.7 Involvement of Neuropeptides in Laryngeal Sensory Innervation [38, 39]

We investigated which neuropeptides play important roles in sensory innervation of the larynx. We labeled nodose ganglion cells that supply the internal branch of the superior laryngeal nerve with sensory nerve fibers, by infusing gold-labeled cholera toxin B (CTBG) into the internal branch of the canine superior laryngeal nerve. After colchicine processing followed by perfusion fixation, nodose ganglia were extirpated, and histological sections were prepared. After visualization of labeled cells by silver enhancement, immunohistochemical analyses were carried out using calcitonin gene-related peptide (CGRP), substance P (SP), and leu-enkephalin (ENK), and the positivity

**Fig. 6.3** CTBG was injected into the internal branch, and the SP immunohistochemical method was used to label cells in the nodose ganglion. There were several SP-positive cells among the CTBG-labeled cells [38]



rate of each neuropeptide in CTBG-labeled cells was determined.

Among all labeled cells, the CGRP positivity rate was the highest, 81.5 %, whereas the SP positivity rate was 24.5 %, and the ENK positivity rate was 7.0 % (■ Fig. 6.3).

CGRP and SP were the major neurotransmitters involved in laryngeal sensory innervation, and their distributions in the laryngeal mucosa were similar [34–38]. However, when CGRP and SP were compared in regard to the number of positive fibers, the results were inconsistent; we previously reported that the number of CGRP-positive fibers was higher than that of SP-positive cells [40], whereas others have found no difference between the two [35, 36]. Based on the results of our present study, nerve cells that extend fibers to the internal branch of the superior laryngeal nerve have an approximately threefold greater number of CGRP-positive cells than SP-positive cells. This finding supports the results of our study on the laryngeal mucosa and suggests that CGRP plays the most important role in sensory innervation of the larynx.

As to the neuropeptides contained in the nodose ganglion, cholecystokinin, neurokinin A, vasoactive intestinal polypeptide, and somatostatin, as well as CGRP and SP, have been reported [41, 42]. These observations suggest all of these peptides to play roles in laryngeal sensory innervation. Although this study also revealed the involvement of ENK in laryngeal sensory innervation, its low positivity rate, 7 %, suggests that its involvement may be restricted to the regulation and modification of neural transmission. This issue awaits further clarification.

## References

- Sanudo JR, Maranillo E, Leon X, Mirapeix RM, Orus C, Quer M. An anatomical study of anastomoses between the laryngeal nerves. *Laryngoscope*. 1999;109:983–7.
- Wu BL, Sanders I, Mu L, Biller HF. The human communicating nerve. An extension of the external superior laryngeal nerve that innervates the vocal cord. *Arch Otolaryngol Head Neck Surg*. 1994;120:1321–8.
- Kreyer R, Pomaroli A. Anastomosis between the external branch of the superior laryngeal nerve and the recurrent laryngeal nerve. *Clin Anat*. 2000;13:79–82.
- Maranillo E, Leon X, Quer M, Orus C, Sanudo JR. Is the external laryngeal nerve an exclusively motor nerve? The cricothyroid connection branch. *Laryngoscope*. 2003;113:525–9.
- Dubois F, Foley JO. Experimental studies on the vagus and spinal accessory nerves in the cat. *Anat Rec*. 1936;64:285–307.
- Ogura JH, Lam RL. Anatomical and physiological correlations on stimulating the human superior laryngeal nerve. *Laryngoscope*. 1953;63:947–59.
- Domeij S, Carlsoo B, Dahlqvist A, Hellstrom S, Kourtopoulos H. Motor and sensory fibers of the superior laryngeal nerve in the rat. A light and electron microscopic study. *Acta Otolaryngol*. 1989;108:469–77.
- Rosenberg SI, Malmgren LT, Woo P. Age-related changes in the internal branch of the rat superior laryngeal nerve. *Arch Otolaryngol Head Neck Surg*. 1989;115:78–86.
- Mortelliti AJ, Malmgren LT, Gacek RR. Ultrastructural changes with age in the human superior laryngeal nerve. *Arch Otolaryngol Head Neck Surg*. 1990;116:1062–9.
- Pressman JJ, Kelemen G. Physiology of the larynx. *Physiol Rev*. 1955;35:506–54.
- Domeij S, Carlsoo B, Dahlqvist A, Hellstrom S. Paraganglia of the superior laryngeal nerve of the rat. *Acta Anat (Basel)*. 1987;130:219–23.
- Yoshida Y, Shimazaki T, Tanaka Y, Hirano M. Ganglions and ganglionic neurons in the cat's larynx. *Acta Otolaryngol*. 1993;113:415–20.
- Nagaishi T. Experimental studies about the vocal cord movement. *Pract Otorhinolaryngol*. 1938;33:518–725.
- Suzuki M, Yamamoto W. Function of the recurrent laryngeal nerve. *J Otolaryngol Jpn*. 1971;74:454–5.
- Tanaka Y. Distribution and pathways of peripheral sensory nerve fibers in the larynx and pharynx of cats. *Otologia Fukuoka*. 1986;32:1018–44.
- Suzuki M, Kirchner JA. Afferent nerve fibers in the external branch of the superior laryngeal nerve in the cat. *Ann Otol Rhinol Laryngol*. 1968;77:1059–70.
- Toyoda K. Localization of sensory neurons in the canine nodose ganglion sending fibers to the laryngeal nerves. *J Otolaryngol Jpn*. 1991;94:1888–97.

18. Lemere F. Innervation of the larynx. I. Innervation of the laryngeal muscles. *Am J Anat.* 1932;51:417-37.
19. Murtagh JA, Campbell CJ. Physiology of recurrent laryngeal nerve: report on progress. *J Clin Endocrinol Metab.* 1952;12:1398-401.
20. Meurmann OH. Theories of vocal cord paralysis. *Acta Otolaryngol.* 1950;38:460-72.
21. Williams AF. The recurrent laryngeal nerve and the thyroid gland. *J Laryngol Rhinol Otol.* 1954;68:719-25.
22. Vogel PH. The innervation of the larynx of man and the dog. *Am J Anat.* 1952;90:427-47.
23. Sanders I, Mu L. Anatomy of the human internal superior laryngeal nerve. *Anat Rec.* 1998;252:646-56.
24. Hisa Y, Uno T, Tadaki N, Murakami Y. Sensory, motor and autonomic nerve fibers of the internal branch of the canine superior laryngeal nerve. *Trans Am Laryngol Assoc.* 1992;113:98-103.
25. Ito C. Sympathetic-trophic innervation of the laryngeal muscles. *J Otolaryngol Jpn.* 1929;34:1207-28.
26. Sugano M. Experimental studies about the innervation of the laryngeal nerves. *J Otolaryngol Jpn.* 1930;35:1338-61.
27. Hisa Y. Fluorescence histochemical studies on the noradrenergic innervation of the canine larynx. *Acta Anat (Basel).* 1982;113:15-25.
28. Hisa Y, Matsui T, Fukui K, Ibata Y, Mizukoshi O. Ultrastructural and fluorescence histochemical studies on the sympathetic innervation of the canine laryngeal glands. *Acta Otolaryngol.* 1982;93:119-22.
29. Hinrichsen CF, Ryan AT. Localization of laryngeal motoneurons in the rat: morphologic evidence for dual innervation? *Exp Neurol.* 1981;74:341-55.
30. Wallach JH, Rybicki KJ, Kaufman MP. Anatomical localization of the cells of origin of efferent fibers in the superior laryngeal and recurrent laryngeal nerves of dogs. *Brain Res.* 1983;261:307-11.
31. Uno T. Autonomic neurons sending fibers into the canine laryngeal nerves -using a retrograde tracer technique with cholera toxin-. *J Otolaryngol Jpn.* 1993;96:66-76.
32. Hanamori T, Smith DV. Central projections of the hamster superior laryngeal nerve. *Brain Res Bull.* 1986;16:271-9.
33. Basterra J, Chumbley CC, Dilly PN. The superior laryngeal nerve: its projection to the dorsal motor nucleus of the vagus in the guinea pig. *Laryngoscope.* 1988;98:89-92.
34. Hisa Y, Sato F, Fukui K, Ibata Y, Mizukoshi O. Substance P nerve fibres in the canine larynx by PAP immunohistochemistry. *Acta Otolaryngol.* 1985;100:128-33.
35. Kawasoe M, Shin T, Masuko S. Distribution of neuropeptide-like immunoreactive nerve fibers in the canine larynx. *Otolaryngol Head Neck Surg.* 1990;103:957-62.
36. Tanaka Y, Yoshida Y, Hirano M, Morimoto M, Kanaseki T. Distribution of SP- and CGRP-immunoreactivity in the cat's larynx. *J Laryngol Otol.* 1993;107:522-6.
37. Hisa Y, Tadaki N, Uno T, Okamura H, Taguchi J, Ibata Y. Calcitonin gene-related peptide-like immunoreactive motoneurons innervating the canine inferior pharyngeal constrictor muscle. *Acta Otolaryngol.* 1994;114:560-4.
38. Hisa Y, Tadaki N, Uno T, Okamura H, Taguchi J, Ibata Y. Neuropeptide participation in canine laryngeal sensory innervation. *Immunohistochemistry and retrograde labeling. Ann Otolaryngol Rhinol Laryngol.* 1994;103:767-70.
39. Hisa Y, Koike S, Tadaki N, Bamba H, Shogaki K, Uno T. Neurotransmitters and neuromodulators involved in laryngeal innervation. *Ann Otol Rhinol Laryngol Suppl.* 1999;178:3-14.
40. Hisa Y, Uno T, Tadaki N, Murakami Y, Okamura H, Ibata Y. Distribution of calcitonin gene-related peptide nerve fibers in the canine larynx. *Eur Arch Otorhinolaryngol.* 1992;249:52-5.
41. Helke CJ, Hill KM. Immunohistochemical study of neuropeptides in vagal and glossopharyngeal afferent neurons in the rat. *Neuroscience.* 1988;26:539-51.
42. Helke CJ, Niederer AJ. Studies on the coexistence of substance P with other putative transmitters in the nodose and petrosal ganglia. *Synapse.* 1990;5:144-51.