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Immunohistochemical study of TAFII250 in the rat laryngeal nervous system

H. Okano[1,2], H. Bamba[2], Y. Hisa[2], S. Makino[3], S. Ando[3], G. Tamiya[3], S. Goto[4], R. Kaji[5], H. Kimura[1] and I. Tooyama[1]

[1]Molecular Neuroscience Research Center, Shiga University of Medical Science, Setatukinowa-cho, Otsu,
[2]Department of Otolaryngology-Head and Surgery, Kyoto Prefectural University of Medicine, Kawaranachi-Hirokoji, Kyoto,
[3]Department of Molecular Life Science, Tokai University School of Medicine, Kanagawa,
[4]Department of Neurosurgery, Graduate School of Medical Sciences, Kumamoto University, Kumamoto,
[5]Department of Neurology, Graduate School of Medicine, Tokushima University, Tokushima, Japan

Summary. The cause of spasmodic dysphonia, a dystonic disorder of the larynx, remains unclear. Recently, TAFII250, TATA-box binding protein associated factor, was suggested to be involved in dystonia parkinsonism. There is a possibility that TAFII250 is involved in spasmodic dysphonia, but little information is available about the expression of TAFII250 in the laryngeal nervous system. In this study, we investigated the localization of TAFII250 protein in the rat laryngeal nervous system by immunohistochemistry. TAFII250-immunoreactivity was detected in the nodose ganglion and superior cervical ganglion. In these nuclei, TAFII250 was localized in the nucleus of NeuroTrace-positive neurons but not in GFAP-positive glial cells. No positive cells were detected in the motor and parasympathetic nervous system. TAFII250-immunoreactivity was sustained between 3 and 7 days after vagotomy, but at 14 days expression was down-regulated in the distal part of the nodose ganglion. These findings suggest that TAFII250 plays an important role in the laryngeal innervation of the sensory and sympathetic nervous systems.

Key words: TAFII250, Laryngeal nervous system, Nodose ganglion, Supura cervical ganglion, Spasmodic dysphonia

Introduction

Transcription factor IID (TFIID) is a general transcription factor that nucleates the assembly of RNA polymerase II and other initiation factors (TFIIA, TFIIB, TFIIE, TFIIF and TFIIH) at the core promoter of protein-coding genes (Matsui et al., 1980; Buratowski et al., 1989; Orphanides et al., 1996). It is composed of a small TATA-box binding protein (TBP) and other TBP-associated factors (TAFs) ranging in size from about 30 to 250 kDa (Dynlacht et al., 1991; Takada et al., 1992). The largest TAF is a 250-kDa protein designated TAFII p250 (Hisatake et al., 1993; Ruppert et al., 1993), which may specifically function in the activation of genes that mediate cell cycle progression and apoptosis (Hisatake et al., 1993; Ruppert et al., 1993). Recent studies reported that both TAFII130 and TAFII250 are involved in neuronal death. TAFII130 is involved in the pathogenic mechanisms underlying CAG-repeat disease. TAFII130-immunoreactivity was detected in the neuronal intranuclear inclusion in autopsied human brains of Dentatorubral-pallidoluysian atrophy and Machado-Joseph disease (Shimohata et al., 2000). TAFII250 may be involved in X-linked dystonia parkinsonism (Nolte et al., 2002).

In the larynx, spasmodic dysphonia (SD) is a dystonic disorder of the larynx (Blitzer et al., 1988) and characterized by a choked, constrained voice pattern with breaks in vocal flow. The cause of SD are guessed to be focal dystonia of the larynx, but details are unclear. There is a possibility that TAFII250 may be involved in SD. However, the roles of TAFII250 in neurons remain unclear. In particular, there is no report on the localization of the TAFII250 proteins in the peripheral nervous system. In this study, we used immunohistochemistry to investigate the localization of TAFII250 in the rat laryngeal nervous system, that is composed of motor, sensory, sympathetic and parasympathetic nerves that have been well characterized.

Materials and methods

Tissue preparations

This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory

Offprint requests to: Ikuo Tooyama, Professor, Molecular Neuroscience Research Center, Shiga University of Medical Science, Setatukinowa-cho, Otsu 590-2192, Japan. e-mail: kinchan@belle.shiga-med.ac.jp

Animals, the NIH Guide for the Care and Use of Laboratory Animals, and the Animal Welfare Act (7 U.S.C. et seq.). The animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Shiga University of Medical Science. Wistar rats weighing 200-250 g were used in this experiment. Under deep anesthesia with sodium pentobarbital (80 mg/kg body weight), twelve rats were subjected to surgical denervation. For vagotomy, the vagus nerve was cut at a level just above middle cervical ganglion. In all rats, the operations were done on the right side. After a survival period of 3, 7 or 14 days, the animals were deeply anesthetized again and were transcardially perfused with 10 mM phosphate buffered saline followed by an ice-cold fixative of 0.1 M phosphate buffer (PB; pH 7.4) with 4% paraformaldehyde (PFA). Bilateral nodose (NG) and superior cervical ganglion (SCG) as well as the larynx and medulla oblongata were then removed. The specimens were post-fixed for 3 days in the same fixative as used in perfusion at 4°C, and then immersed for 24 hours in 0.1 M PB containing 15% sucrose and 0.1% sodium azide for cryoprotection. The NG and SCG were cut into 12 μm-thick sections, and larynx and medulla oblongata were cut into 20 μm-thick sections using a cryostat. The laryngeal sections were then directly placed on gelatin-chrome-coated glass slides and the other sections were used in a free-floating state. The mirror section technique was used for analyzing TAFII250 localization in the larynx and the medulla oblongata.

Immunohistochemistry

Prior to staining, these sections were kept for at least 4 days in 0.1 M phosphate-buffered saline containing 0.3% Triton X-100 (PBST; pH 7.4) at 4°C. Sections were incubated for 2 days at 4°C with primary antibodies. The primary antibodies used were goat anti-TAFII250 antibody (diluted 1:500; Santa Cruz, California, USA), goat anti-choline acetltransferase (ChAT) antibody (diluted 1:1000; Chemicon Int., California, USA) or rabbit anti-choline acetyltransferase of a peripheral type (pChAT) antibody (diluted 1:10000) (Tooyama and Kimura, 2000). The sections were incubated for 1 hour with biotinylated anti-goat IgG (diluted 1:1000; Vector Laboratories, Burlingame, California, USA) or anti-rabbit IgG (diluted 1:1000; Vector Laboratories) at room temperature, and for 1 hour with avidin-biotinylated peroxidase complex (diluted 1:1000; Vector Laboratories) at room temperature. PBST was used to dilute the antibodies and wash the sections between each step. A purple color was developed with 0.02% 3,3'-diaminobenzidine and 0.3% nickel ammonium sulfate in 50 mM Tris–HCl buffer (pH 7.6). The free-floating sections were mounted on gelatin-chrome-coated glass slides and air-dried.

Some sections mounted on glass-slides were used for double immunostaining. After the first TAFII250 immunostaining with purple color, the sections were treated for 30 min at room temperature with 0.5% hydrogen peroxide in PBST to eliminate the residual horseradish peroxidase activity. Subsequently, the sections were washed in PBST and incubated with rabbit anti-glial fibrillary acidic protein (GFAP) antibody (diluted 1:1000; DAKO, California, USA) for 2 days at room temperature. The sections were incubated for 1 hour with biotinylated anti-rabbit IgG (diluted 1:1000; Vector Laboratories) at room temperature, and for 1 hour with avidin-biotinylated peroxidase complex (diluted 1:1000; Vector Laboratories) at room temperature. They were then incubated with 0.02% 3,3'-diaminobenzidine and in 50 mM Tris–HCl buffer (pH 7.6) to precipitate brown chromogen. These sections were washed in tap water, dehydrated in a graded series of ethanol, cleared in xylene and placed under a cover slip with Entellan (Merck, Germany). Other sections were washed in PBST and incubated with NeuroTrace (diluted 1:200; Molecular Probe, Oregon, USA) at room temperature for 20 min and placed under a cover slip. For immunohistochemical controls, TAFII250 antiserum preadsorbed with an excess amount of the TAFII250 antigenic peptide (20 μg/ ml) was used.

Results

TAFII250 immunostaining was observed in the NG (Fig. 1) and SCG (Fig. 2). This immunoreactivity was specific for TAFII250, since it was abolished by pre-incubation with TAFII250 peptide (Fig. 1B). At high magnification, TAFII250-immunoreactivity was detected in the nuclear region of cells in both NG (Fig. 1C) and SCG (Fig. 2B). Double staining for TAFII250 and NeuroTrace fluorescent Nissl stain or GFAP demonstrated that the TAFII250-positive cells were negative for GFAP (Fig. 1D) and positive for NeuroTrace fluorescent Nissl stain (Fig. 1E,F). The TAFII250-immunoreactive cells are therefore neurons. TAFII250-immunoreactive neurons were distributed throughout the NG (Fig. 1A) and SCG (Fig. 2A) with no specific localization.

The mirror section technique demonstrated that ganglionic cells in the intra-laryngeal ganglia contained pChAT-immunoreactivity (Fig. 3A,B) but not TAFII250-immunoreactivity (Fig. 3C). In the medulla oblongata, nucleus ambiguus (NA), nucleus tractus solitarius (NTS) and dorsal motor nucleus of the vagus (DMNV) displayed ChAT-immunoreactivity ((Fig. 3D) but not TAFII250-immunoreactivity (Fig. 3E).

After axotomy of the vagus nerve, TAFII250-immunoreactivity remained for between 3 and 7 days. At 14 days after vagotomy, TAFII250-immunoreactivity had decreased in the distal part of the NG (Fig. 4A), but was unchanged in the SCG. Double staining for TAFII250 and GFAP in the NG demonstrated that GFAP-immunoreactivity was unchanged (Fig. 4B,C) despite downregulation of TAFII250-immunoreactivity (Fig. 4C).

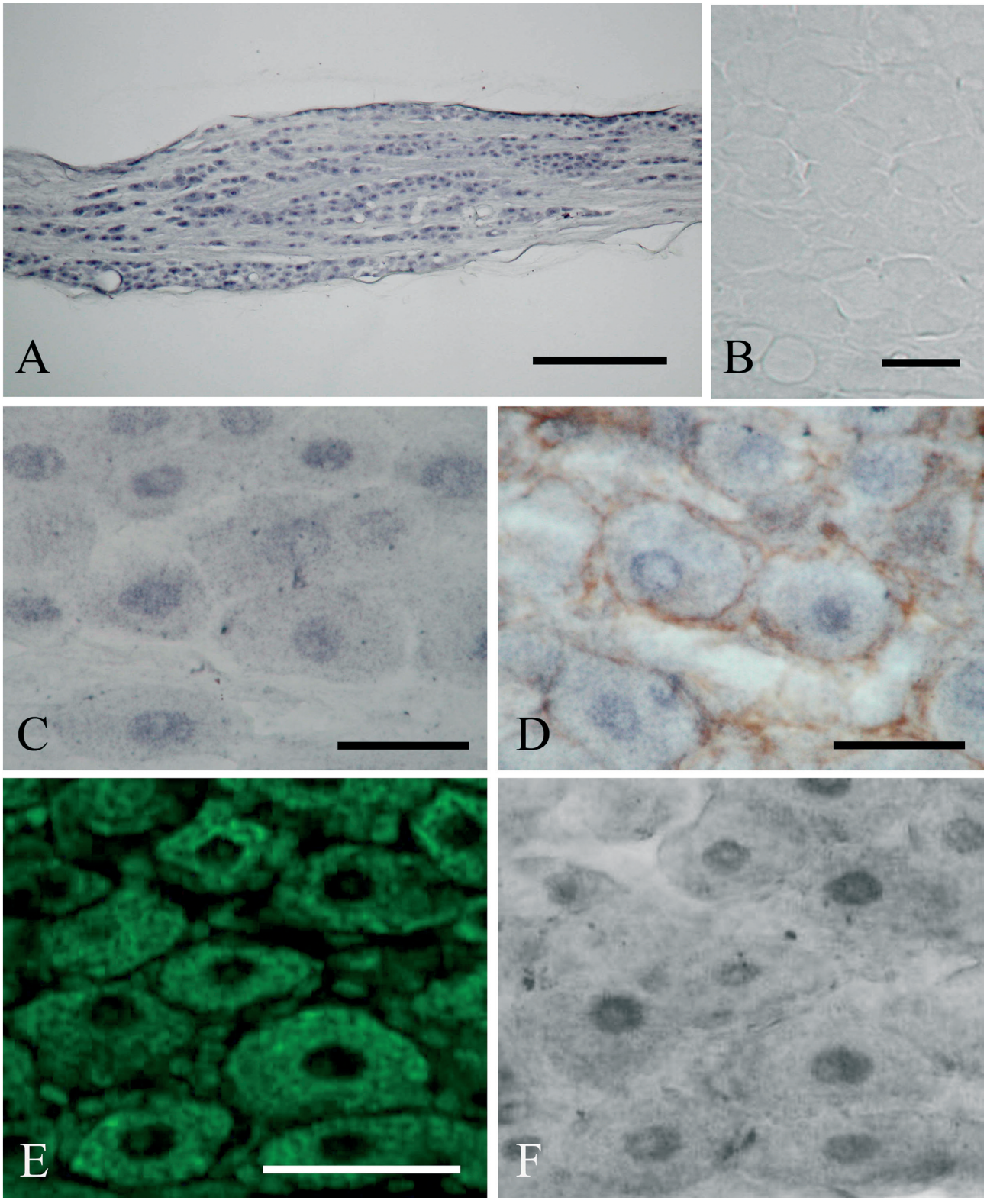

Fig. 1. TAFII250-immunoreactivity in the nodose ganglion. **A.** TAFII250-immunoreactive neurons were distributed throughout the NG with no specific localization. **B.** The staining was abolished by pre-incubation with TAFII250 peptide. **C.** TAFII250-immunoreactivity occurred in the nucleus. **D.** No cells double-stained for TAFII250 (purple) and GFAP (brown). **E.** Neurons positive for the NeuroTrace fluorescent Nissl stain in the NG. **F.** TAFII250-immunoreactive neurons in the NG. Bar: A, 400 μm; D-F, 50 μm.

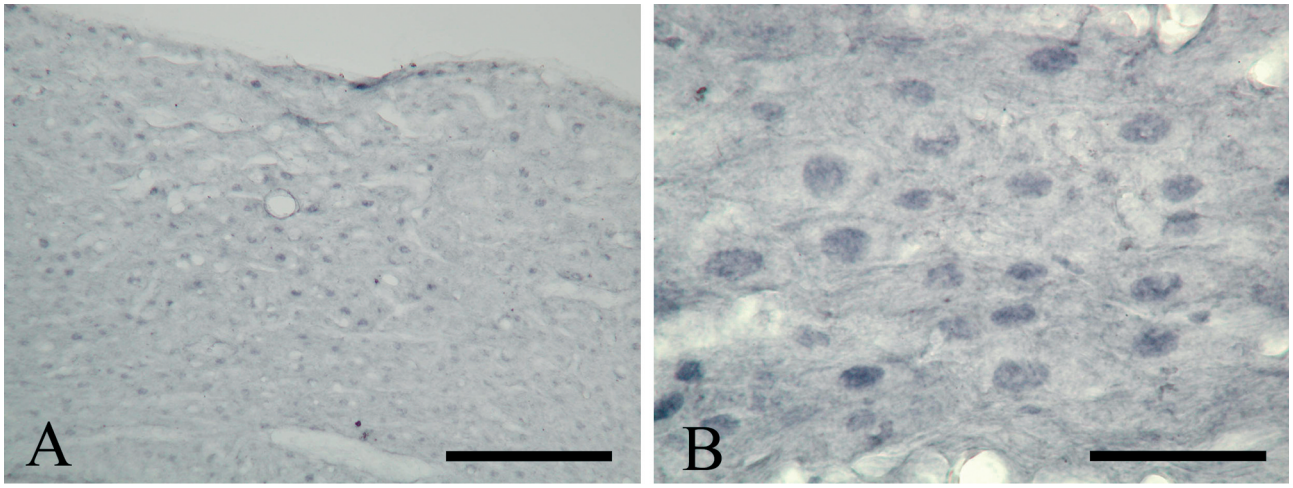

Fig. 2. TAFII250-immunoreactivity in the superior cervical ganglion. **A.** TAFII250-immunoreactive neurons were distributed throughout the SCG with no specific localization. **B.** TAFII250-immunoreactivity in the nucleus. BarA, 200 μm; B, 50 μm.

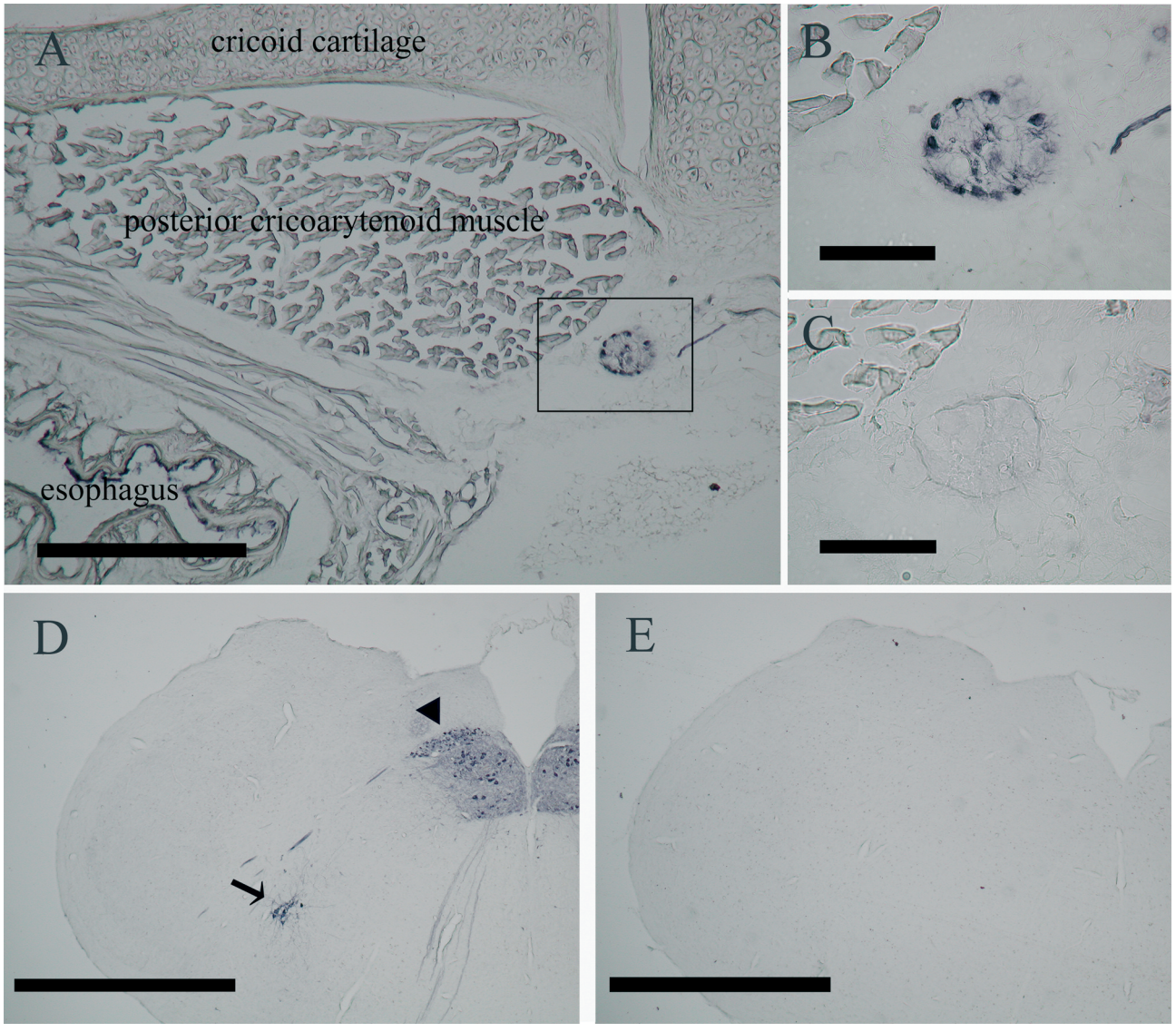

Fig. 3. ChAT, pChAT and TAFII250-immunoreactivity in mirror sections of the intra-laryngeal ganglia and the medulla oblongata. **A.** pChAT-immunoreactive neurons in the intra-laryngeal ganglia. **B.** High magnification of the box cluster in **(A)**. **C.** No TAFII250-immunoreactivity in the intra-laryngeal ganglia. **D.** ChAT-immunoreactive neurons in NA (arrow) and DMNV (arrowhead). **E.** No TAFII250 reactivity was observed in the medulla oblongata including NA, DMNV and NTS. Bar: A, 500 μm; B, C, 100 μm; D, E, 1 μm.

Discussion

The present study is the first demonstration of the localization of TAFII250 protein in the rat laryngeal nervous system (Fig. 5). TAFII250 protein was observed in the nuclear region of cells in the NG and SCG, and double-staining for TAFII250 and NueroTrace or GFAP indicated that these cells were neurons. TAFII250-immunoreactivity in the intra-laryngeal ganglia and medulla oblongata (NTS, NA and DMNV) was below detection level. These results suggest that TAFII250 is relatively highly expressed in the sensory and sympathetic systems, but not in the motor and parasympathetic systems, under physiological conditions.

When the vagus nerve was cut at the level just above middle cervical ganglion to preserve the superior laryngeal nerve (SLN), TAFII250-immunoreactivity

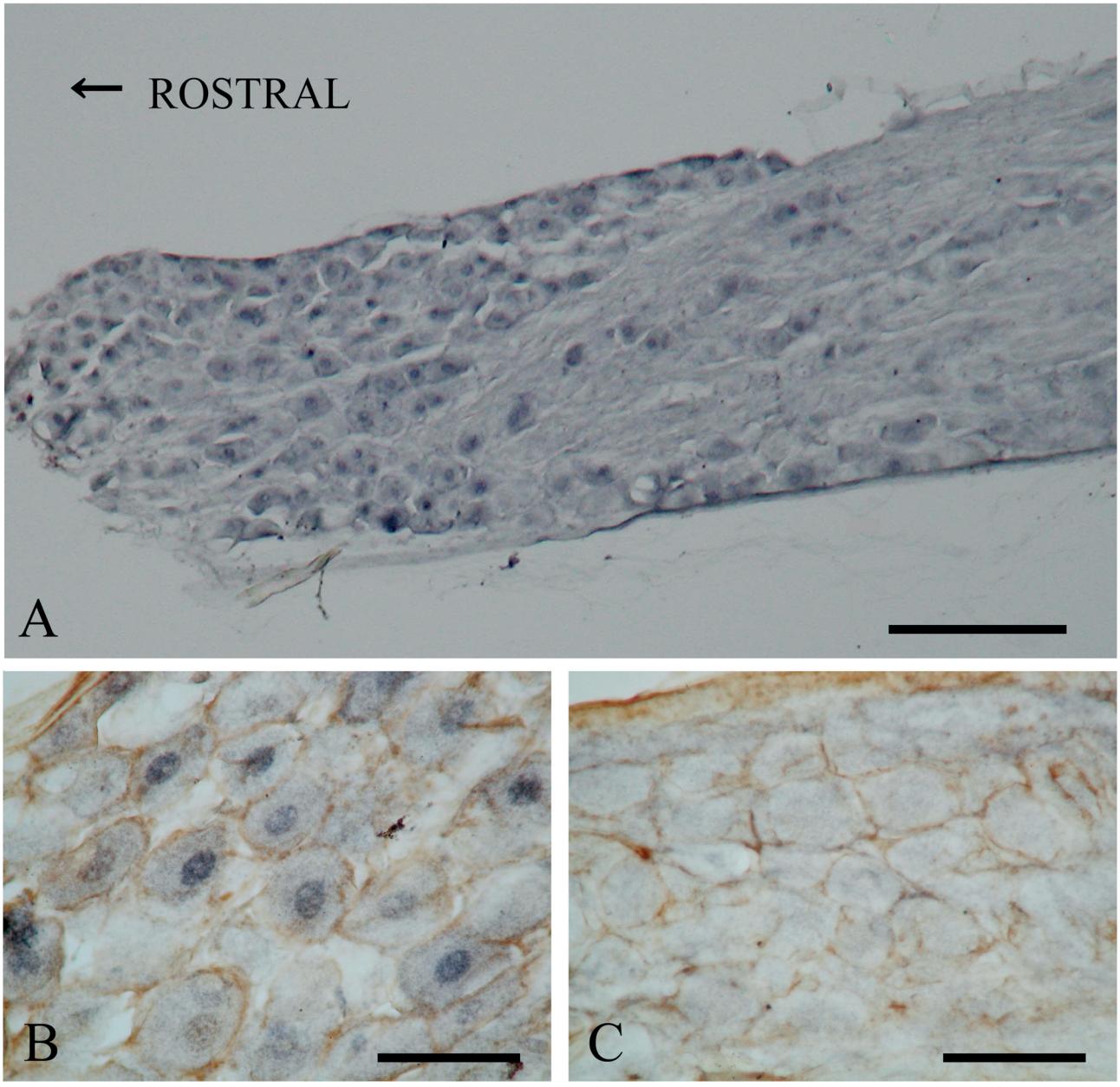

Fig. 4. TAFII250-immunoreactivity in the NG 14 days after vagotomy. **A.**TAFII250-immunoreactive neurons were confined to the rostral region, with TAFII250 expression being downregulated elsewhere. Double staining for TAFII250 (purple) and GFAP (brown): In the rostral region TAFII250-immunoreactivity was unchanged **(B)**; in the distal region, TAFII250-immunoreactivity was down-regulated **(C)**. Bar: A, 200 μm; B, C, 50 μm.

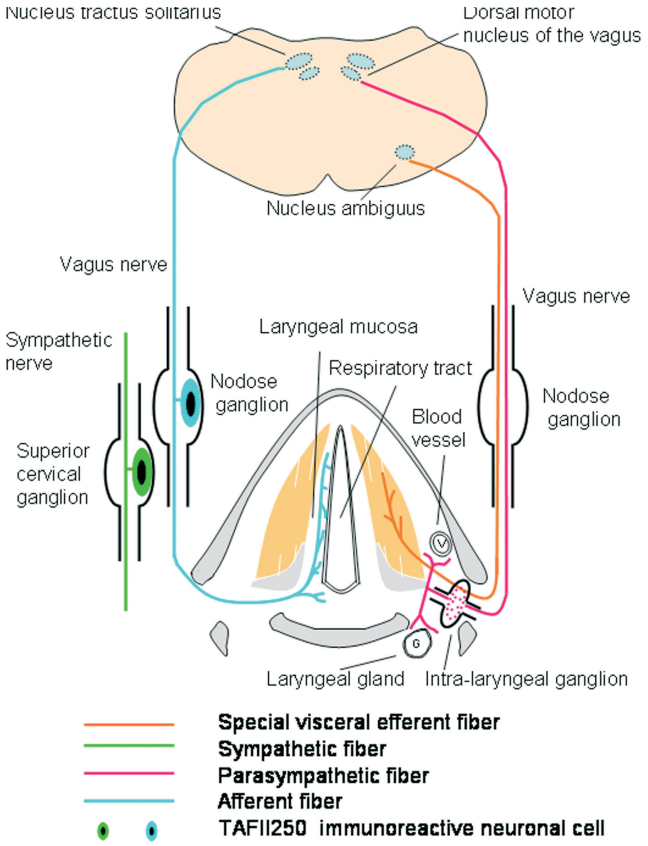

Fig. 5. A schematic drawing of a rat larynx to illustrate the TAFII250-positive structures. TAFII250-immumoreactivity in the NG and SCG. In the intra-laryngeal ganglia, NA, DMNV and NTS, TAFII250-immumoreactivity can not be detected. The efferent (right) and afferent (left) pathways are shown, which is not examined precisely in this study.

remained in neurons in the rostral part of the NG, and decreased in the distal part of the NG. These results confirm our previous study, using horseradish proxidase as a neuronal tracer (Hisa et al., 1991), which showed that NG neurons innervating the larynx through the SLN were mainly localized in the rostral part of the NG. TAFII250 immunoreactive neurons in the rostral part of NG send afferent fibers to the larynx into the SLN and they are the main sensory pathway from the larynx. These results suggest that TAFII250 plays an important role in rat laryngeal sensory innervation.

The SCG is well known to consist of principal postganglionic neurons and paraganglionic cells called small intensely fluorescent (SIF) cells, based on their formaldehyde-induced catecholamine fluorescence (Eranko and Harkonen, 1961). SIF cells are 6-12 μm in diameter. In this study, TAFII250-immunoreactive neurons in the SCG were about 30 μm in diameter, suggesting that they were not SIF cells but principal postganglionic neurons. The results suggest that TAFII250 may also play an important role in the sympathetic innervation of the rat larynx. The lack of TAFII250 reactivity in the intra-laryngeal ganglia and medulla oblongata suggests that other TAFs may function in the motor and parasympathetic nervous systems in the rat larynx. To clarify this issue, further studies will be needed.

In summary, TAFII250-immunoreactivity was detected in the nuclear region of neurons in the NG and SCG, but not in the intra-laryngeal ganglia and medulla oblongata. This specific localization pattern suggests that TAFII250 may play an important role in the sensory and sympathetic nervous system of the rat larynx. Since TAFII250 has been implicated in dystonia (Nolte et al., 2002), both the sensory and sympathetic systems may be involved in this disorder. The recent study suggest that sensory input plays an important role in focal dystonia (Giovanni et al., 2001). SD is a focal dystonia of the laryngeal muscle (Blitzer, 1988). The elucidation of TAFs in the larynx may clarify the disease state of SD. The study of TAFII250 in the larynx has the possibility of elucidation of SD.

Conclusion

The present study is the first to demonstrate the distribution of TAFII250 in the rat laryngeal nervous system using immunohistochemistory. TAFII250-immunoreactivity was detected in the nuclear region of neurons in the NG and SCG but was not seen in the intra-laryngeal ganglia and medulla oblongata. These results indicate that TAFII250 plays an important role in the regulation of transcription in the sensory and sympathetic nervous systems in the rat larynx.

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