Morphology of intraepithelial corpuscular nerve endings in the nasal respiratory mucosa of the dog

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ABSTRACT

Corpuscular nerve endings in the nasal respiratory mucosa of the dog were investigated by immunohistochemical staining specific for protein gene product 9.5 by light and electron microscopy. In the nasal respiratory mucosa, complex corpuscular endings, which displayed bulbous, laminar and varicose expansions, were distributed on the dorsal elevated part of the nasal septum and on the dorsal nasal concha. The endings were 300–500 µm long and 100–250 µm wide. Some axons gave rise to a single ending while others branched into 2 endings. Cryostat sections revealed that the corpuscular endings were located within the nasal respiratory epithelium. On electron microscopy, immunoreactive nerve terminals that contained organelles, including mitochondria and neurofilaments, were observed within the epithelial layer near the lumen of the nasal cavity. Some terminals contacted the goblet cell. Such terminal regions were covered by the cytoplasmic process of ciliated cells and were never exposed to the lumen of the nasal cavity. These nerve endings are probably activated by pressure changes.

Key words: Sensory nerve endings; mechanoreceptors.

INTRODUCTION

The various sensory receptors found in the respiratory tract are known to play important roles in the reflexes that occur during breathing (Widdicombe et al. 1988). In the nasal respiratory mucosa, physiological studies have indicated that the various trigeminal nerve receptors are activated by different stimuli, such as transmural pressure (pressure receptors), airflow (flow receptors), the motion of nasal muscles (drive receptors) and chemical stimulants (irritant receptors and C-fibre receptors; for review, see Sant’Ambrogio et al. 1995).

On the other hand, morphological studies using silver impregnation and immunohistochemistry for several neuropeptides have demonstrated only non-myelinated intraepithelial free nerve endings in the nasal respiratory mucosa (Retzius, 1892; Cauna et al. 1969; Finger et al. 1990; Grunditz et al. 1994; Lee et al. 1995). The intraepithelial free nerve endings have been regarded as C-afferents activated by noxious chemical stimulants (Tsubone, 1987; Sekizawa & Tsubone, 1994). In the other parts of the respiratory tract, various corpuscular endings, e.g. laminar endings and taste buds in laryngeal mucosa and tree-like endings in the tracheal and bronchial smooth muscles, have been recognised in addition to the intraepithelial free nerve endings (Yamamoto et al. 1994, 1995, 1997a, b, 1998). Some of these endings were suggested to be the mechanosensitive receptors described above, such as the laryngeal pressure receptors and the slowly adapting stretch receptors in the trachea and bronchi.

In the present study, corpuscular endings in the nasal respiratory epithelium were studied by immunohistochemistry for protein gene product 9.5 (PGP 9.5). In particular, distribution and morphological characteristics of such endings were characterised by light and electron microscopy.

MATERIALS AND METHODS

Ten adult mongrel dogs of either sex (7–10 kg) were used. Animals were deeply anaesthetised with pentobarbital sodium (25 mg/kg; intravenous or intra-
peritoneal injection) and killed by exsanguination from the left carotid artery via a polyethylene cannula. The nasal cavity was dissected out and immersed in the same fixative for 3 d for both light and electron microscopy. For whole-mount preparations, vomer and cartilage were removed from samples of 7 dogs, and the nasal respiratory mucosa was separated by fine forceps under a dissecting microscope. Some tissue blocks from the other 3 dogs were decalcified by 10% formic acid with ion-exchanging resin (Amberlite, Organo, Tokyo) for 3 d according to the method described by Hayashida (1995). Decalcified tissues were prepared for thick (75–100 µm) cryostat sections. Some sections were used for immunoelectron microscopy.

The whole-mount preparations and cryostat sections for light microscopy were incubated overnight in phosphate-buffered saline (PBS) containing 0.1% Triton X-100. After washing with PBS, they were treated with PBS containing 0.3% H₂O₂ and washed again with PBS. They were then incubated for 1 h with nonimmune goat serum (diluted 1:100). Next, the specimens were incubated with polyclonal rabbit antibody (IgG) against PGP 9.5 (diluted 1:5000; RA95101; UltraClone, Isle of Wight, UK) for 72 h at 4 °C. After incubation, they were treated for 1 h with biotinylated antibodies that had been raised in goat against rabbit IgG (1:400, Vector Laboratories, Burlingame, CA) and reagents from an ABC kit (Elite ABC kit; Vector Laboratories) for 90 min each at room temperature. Products of immunoreactions were visualised by incubation with Tris-HCl buffer containing 3,3′-diaminobenzidine tetrahydrochloride and H₂O₂. Immunostained specimens were mounted on slides that had been coated with gelatin and chrome alum, air-dried, dehydrated in ethanol, cleared with xylene and sealed with coverslips. The preparations were observed by light microscopy and the size of nerve endings and their parent axons were measured using a micrometer.

For electron microscopy, cryostat sections of nasal mucosa were incubated for 1 h with nonimmune goat serum (diluted 1:100). The specimens were next incubated with polyclonal rabbit IgG against PGP 9.5 for 72 h at 4 °C. After incubation, they were treated for 1 h with biotinylated antibodies that had been raised in goat against rabbit IgG and reagents from an ABC kit (Elite ABC kit; Vector Laboratories) for 90 min each at room temperature. Products of immunoreactions were visualised by incubation with Tris-HCl buffer containing 3,3′-diaminobenzidine tetrahydrochloride and H₂O₂. Portions of sections with immunostained endings were removed under the dissecting microscope, postfixed in 1% osmium tetroxide for 60 min, dehydrated with a graded ethanol series and embedded in Epon 812. Ultrathin sections without contrast were examined in a transmission electron microscope at 75 kV (H-8100; Hitachi, Tokyo).

Results
PGP 9.5-like immunoreactivity was demonstrated in nerve fibres of various thicknesses. Corpuscular nerve endings that were located in the nasal respiratory mucosa were also satisfactorily immunostained. Intraepithelial free nerve endings that arose from thin varicose fibres were also positive for immunostaining.

The corpuscular nerve endings were distributed in the longitudinal mucosal ridge in the dorsal part of the nasal septum (Fig. 1). Such endings were also observed in the nasal dorsal concha. PGP 9.5-like immunoreactive thick nerve fibres gave rise to the corpuscular nerve endings (Figs 2, 3). The parent axons were 7–8 µm in diameter. The corpuscular endings were 300–500 µm long and 100–250 µm wide. Some axons gave rise to a single ending (Figs 2a, 3a), while others were branched and terminated in 2 endings (Figs 2c, 3d). Nerve fibres branched 3–10 times at their ends and terminated at bulbous, laminar, and varicose expansions. Some of the terminals...
Corpuscular nerve endings in whole mount preparation of the mucosa of the nasal septum. Panel a shows a nerve ending in the middle part of the nasal septum. Panel b is a higher magnification view of a. Ring formations (large arrows), laminar expansions (arrowheads) and varicose expansions (small arrows) are visible. Panels c and d are the endings near the nostril and near the olfactory mucosa, respectively. Panel e shows 2 endings from a single parent axon. Bars, 50 µm.

Nerve endings in nasal mucosa

appeared as ring-like formations around goblet cells (Fig. 2b), which were seen as clear circular profiles in whole mount preparations. Neither specialised sheaths nor capsules were observed around the endings. In the mucosa near the atrium of the nasal cavity, most of the terminal expansions in the endings were bulbous in form (Figs 2c, 3b). In the endings in the middle region of the nasal cavity the ring-like formations and laminar expansions were frequently observed (Fig. 2a, b). Some varicose expansions were also seen at this site. In the region near the olfactory mucosa, the endings displayed well-developed varicose expansion and a few ring-like formations (Figs 2d, 3c).

Cryostat sections revealed that the thick nerve fibres intruded into the epithelial layer and extended near the luminal surface (Fig. 4). They were located on the cavernous venous plexus.

On electron microscopy, the parent axons within the epithelial layer were seen to lack a Schwann cell
Fig. 3. Camera lucida drawings of corpuscular nerve endings; a–d correspond to the endings shown in Fig. 2a, c–e.

Fig. 4. Corpuscular nerve ending in a cryostat section. Panel b is a higher magnification view of the area indicated by the arrow in a. Lu, lumen of nasal cavity; v, venous sinuses; Ep, epithelial layer. Bars, a, 500 µm; b, 50 µm.

The morphological characteristics of the corpuscular nerve endings that were observed in the present study, namely a thick parent axon and various terminal expansions, were similar to the nerve endings in the larynx and tracheobronchial tree. Thus bulbous and laminar terminal regions were similar to those in the laminar endings in the larynx (Yamamoto et al. 1997b, 1998) and similar to the tree-like endings in the smooth muscle layer of trachea and bronchus (Yamamoto et al. 1994, 1995). These structures have been regarded as the mechanosensory structures that register the internal pressure of the respiratory cavity. In addition, the nasal corpuscular nerve endings also resemble the mechanoreceptors in other tissues, namely the intraganglionic laminar endings observed in the myenteric ganglia (Berthoud & Powley, 1992; Kuramoto & Kuwano, 1994; Berthoud et al. 1997), the Ruffini endings in the periodontal ligament (Byers, 1985; Maeda et al. 1987, 1989), and the Golgi tendon organ in the muscle tendons (Schoults & Swett 1974). Therefore the corpuscular nerve endings in the respiratory nasal mucosa may belong to one of the mechanoreceptive structures. At the ultrastructural level, a close relationship between the terminals and epithelial cells may be effective for anchoring the

DISCUSSION

were observed. Such terminal regions were covered by the cytoplasmic processes of ciliated cells and were never exposed to the lumen of the nasal cavity. All axon terminals observed in the present study were located beneath the tight junctions between the epithelial cells.
endings within the epithelial layer to receive mucosal tensile changes. In other nerve endings regarded as tension receptors, such as Ruffini endings and the Golgi tendon organ, terminal Schwann cells are intercalated between the terminal portion of the endings and collagen fibrils (Schoults & Swett 1974; Maeda et al. 1989; Kannari, 1990; Munger & Ide, 1988). In addition, it has been suggested that terminal Schwann cells regulate the terminal portion of the nerve endings (Kannari, 1990; Maeda et al. 1991). For the endings observed in the present study, the epithelial ciliated cells may play such roles instead of the terminal Schwann cells.

Electrophysiologically, the presence of pressure-responsive receptors has been demonstrated in the ethmoidal nerve of the rat (Tsubone, 1990) and in the ethmoidal, caudal nasal and infraorbital nerves in the cat (Wallois et al. 1991). Tsubone (1990) reported that the pressure receptors showed a slowly adapting response against maintained negative and positive pressures in the upper airway. The histological location of the corpuscular endings seems to support the concept that such endings possess an ability to respond to pressure, because the cavernous venous sinuses are likely to be the most flexible region within the nasal cavity. Furthermore, the distribution of such endings seems to correspond to the area innervated by the ethmoidal nerve (Seiferle & Böhme, 1992). The nasal corpuscular endings found in the present study may play a role in the defensive reflex when the nostril is occluded.

In conclusion, corpuscular nerve endings that are likely to be pressure receptors exist in the nasal respiratory tract of the dog.
REFERENCES


