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Unilateral cervical vagotomy decreases the magnitude of neurogenic inflammation induced by capsaicin in the ipsilateral bronchial tree of rats

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Abstract. Electrical stimulation of a single cervical vagus nerve produces neurogenic inflammation on the stimulated side of the bronchial tree, including the first (main) to the 4th order bronchi. In the contralateral bronchial tree, in contrast, only the proximal part of the main bronchus exhibits inflammatory changes, suggesting that vagal sensory axons present in the bronchi largely originate from the ipsilateral vagus nerve. Intravenous administration of capsaicin can evoke neurogenic inflammation in bilateral bronchial trees. Sensory axons from various sources are thought to be stimulated by this irritant. The extent to which neurogenic inflammation in both bronchial trees might be reduced by unilateral vagotomy is not known. In the present study, we sought to characterize the effect of unilateral cervical vagotomy on capsaicin-induced changes in plasma extravasation and secretory activity of goblet cells in the bronchial trees of both sides. To quantify the magnitude of neurogenic plasma extravasation, Evans blue was used as a tracer dye to measure spectrophotometrically its amount in the bronchial wall. Another tracer dye, Monastral blue, was used to localize the distribution of leaky blood vessels and to measure morphometrically their area density in the whole mounts. To investigate cell and tissue responses of the mucosa, histological methods were employed. After 2 or 4 postoperative weeks, the rats were intravenously administered with a single dose of capsaicin, 150 μg/kg. This resulted in different magnitudes of Evans blue extravasation in the bronchi of the two sides in vagotomized rats. Extravasation of Evans blue dye in the bronchial tree ipsilateral to vagotomy was one-half to two-thirds of that of the contralateral bronchial tree. For the distal region of the main stem bronchus, the area density of Monastral blue-labeled blood vessels in the operated side was one-half of that in the unoperated side, and for the secondary bronchus, the area density of these blood vessels in the operated side was one-quarter of that in the unoperated side. Histological study indicated that in the bronchial mucosa ipsilateral to vagotomy edematous change was not obvious, and most goblet cells were not responsive to stimulation by capsaicin, in contrast to the contralateral side where edematous change was very prominent and goblet cells were very sensitive to capsaicin. It is concluded that unilateral cervical vagotomy could selectively desensitize the mucosa of the ipsilateral bronchial tree to capsaicin, and therefore, decrease the magnitude of neurogenic inflammation.

Key words: Vagotomy – Capsaicin – Bronchi – Vascular permeability – Goblet cells

Introduction

The bronchial tree receives sensory innervation from several sources, from the right and left vagus nerves (Lundberg et al. 1983a, 1984; Dalsgaard and Lundberg 1984; Cadieux et al. 1986) and from the first three thoracic spinal nerves (Dalsgaard and Lundberg 1984; Lundberg et al. 1984; Saria et al. 1985). The trachea only receives sensory innervation from the paired vagus nerves (Lundberg et al. 1983, 1984; Dalsgaard and Lundberg 1984; Saria et al. 1985; Cadieux et al. 1986). Capsaicin, when administered intravenously, can stimulate the sensory nerve endings to release tachykinins and provoke neurogenic inflammation in the airways of both right and left sides, phenomena such as increased vascular permeability in the tracheobronchial mucosa (Lundberg and Saria 1983), bronchoconstriction (Saria et al. 1985), neutrophil adherence to the tracheal mucosal venules (Umeno et al. 1989, 1990), degranulation of goblet cells (McDonald 1988b; Huang et al. 1989; Kou et al. 1990; Tokuyama et al. 1990) and dilation of intercellular spaces in the surface epithelium of the tracheal mucosa (McDonald 1988b; Huang et al. 1989).

Electrical stimulation of a single vagus nerve produces neurogenic inflammation on both sides of the trachea, but only the stimulated side of the bronchial tree, including the main to 4th order bronchi and the contralateral proximal part of the main bronchus exhibit neurogenic inflammation, as demonstrated by labeling

the venules with Monastral blue pigment (McDonald 1988a; McDonald et al. 1988). These findings suggest that sensory innervation in these airway regions comes from the stimulated vagus nerve. Vagal substance P-immunoreactive sensory axons innervating the bronchial mucosa originate in part from the ipsilateral vagus nerve. Unilateral cervical vagotomy destroys a large number of these axons in the main bronchus of the ipsilateral side, but not in that of the contralateral side (Bałuk et al. 1992). The substance P-immunoreactive sensory axons are located mostly on the bases of mucosal surface epithelial cells (Bałuk et al. 1992).

The aim of the present study was to investigate whether unilateral surgical lesioning of the vagus nerve, on either the right or left side, could change the magnitude of neurogenic inflammation provoked by capsaicin in both ipsilateral and contralateral bronchial trees. The tracer dyes Evans blue and Monastral blue were used to measure the magnitude of vascular permeability. Histological methods were employed to study the inflammatory changes in the lamina propria and the secretory activity of goblet cells.

Materials and methods

The present study was approved by the Animal Care and Use Committee of the National Sun Yat-Sen University, Republic of China.

Animals

Since the respiratory tract of rats of Sprague-Dawley and Long-Evans strains are susceptible to neurogenic inflammation (Lundberg and Saria 1983; Lundberg et al. 1983b; McDonald 1988a, b), they were used in the present study. Sprague-Dawley rats were purchased from the Animal Center in the College of Medicine of National Cheng-Kung University, and Long-Evans rats from the Animal Center of Kaohsiung Medical College. Offspring of these rats were raised in the Animal Center of the Department of Biology, National Sun Yat-Sen University, for the experiments.

For this study, 24 Long-Evans rats and 55 Sprague-Dawley rats of both sexes were used. Most of the rats at the age of 3 months were unilaterally vagotomized, on either the right or left side, at the level of the common carotid artery. The remaining rats were unoperated.

Unilateral cervical vagotomy

To destroy the sensory axons present in the bronchial mucosa that came from the ipsilateral vagus nerve, unilateral cervical vagotomy was performed according to the method of Ling and Wong (1988). Long-Evans rats were anesthetized with an i.p. injection of sodium pentobarbital 40 mg/kg, and Sprague-Dawley rats with an i.p. injection of 30 mg/kg of the same anesthetic. A midventral incision 2 cm long was made on the skin from the location of the sternum to the submandibular glands, and the fascia was cut to expose the pretracheal and sternomastoid muscles. Two glass rods with hooked end were inserted into the space between these muscles to separate the muscle slip and fascia that covered the common carotid artery. The vagus nerve trunk was separated from the common carotid artery and the sympathetic chain by the glass rods. A glass rod was used to hold only the vagus nerve, and then a segment of it, 3–5 mm in length, between the origin and the end of the common carotid artery, was removed. The contralateral vagus nerve was not touched. Aureomycin was applied to the sutured skin. The experiments on neurogenic inflammation were performed following a postoperative period of 2 or 4 weeks.

Neurogenic plasma extravasation in trachea and bronchial trees

Extravasation of Evans blue. To quantify spectrophotometrically the neurogenic plasma extravasation, Evans blue was used as tracer dye. After anesthesia with an i.p. injection of sodium pentobarbital, vagotomized or unoperated rats received an injection of Evans blue dye (30 mg/kg i.v. over 5 s; Merck) dissolved in 0.85% NaCl. About 20 s later, the rats received an injection of capsaicin (150 μg/kg i.v. over 2–3 min; Sigma Chemical Company, St. Louis, Mo.). Ten rats received i.v. injections of Evans blue and vehicle, then 5 min later the rats were perfused for 2 min with 1% paraformaldehyde in 0.05 M citrate buffer (pH 3.5) (Brokaw and McDonald 1988; Huang et al. 1989). To expose in situ the intrapulmonary secondary bronchi, a toothed forceps was used to tear off the accompanying arteries, veins and nerves, as well as the surrounding parenchymal tissue. The rostral-most four cartilaginous rings were removed from each trachea, fixed with 3% glutaraldehyde in 75 mM sodium cacodylate buffer (pH 7.1), and processed for embedding in glycol methacrylate (see histological study below). The bronchial trees and trachea were separated by transecting at their junction. The parenchymal tissue on the intrapulmonary bronchi was further removed by razor blade and forceps under a dissecting microscope (also see the method of Boschetto et al. 1991). Traces of alveolar tissue and bronchioles remained attached to secondary bronchi, since they could not be completely removed. After they had been pressed with forceps to expel any blood remaining in the tissue, washed with citrate buffer and drained of excess buffer solution, the right main bronchus with four secondary bronchi and the left main bronchus with two secondary bronchi together with the right and left halves of the trachea for each rat were blotted between pieces of absorbent paper and weighed. Evans blue dye was extracted by incubating the tracheal or bronchial tissue in 2 ml of formamide at 50° C in the oven for 16–24 h. The optical density of the extracted dye in formamide, measured with a Hitachi H-1200 spectrophotometer at a wavelength of 620 nm, was used to calculate the concentration of dye (Saria and Lundberg 1983).

Bronchial tissue usually had a higher concentration of Evans blue dye than did the trachea. It could not be exluded that this came in part from the tracer dye that remained in the blood vessels within the bronchial wall, which was closely associated with pulmonary blood vessels and their branches.

Extravasation of Monastral blue. To localize and quantify morphometrically the mediator-sensitive venules in the bronchial mucosa, Monastral blue was used as tracer to label these blood vessels. Seven Sprague-Dawley rats, that had been pretreated with left vagotomy 4 weeks before, were anesthetized with an i.p. injection of sodium pentobarbital followed by intravenous injections of Monastral blue (30 mg/kg over 5 s; Sigma Chemical Company) and capsaicin (150 μg/kg over 2–3 min) (for five rats), or its vehicle (for two rats) as in the above experiment. Then, after 5 min, the rats were perfused with the fixative phosphate-buffered paraformaldehyde (McDonald 1988a). The lungs on both sides together with the extrapulmonary bronchi were removed. Extramural tissues surrounding the extra- and intra-pulmonary bronchi were teased off. The left main bronchus with the medial secondary bronchus (the biggest secondary bronchus of the left lung), and the right main bronchus with the third secondary bronchus (the biggest secondary bronchus of the right lung) lateral to the most medial one, were cut open at the ventral surface. The bronchial whole mounts were prepared according to the method for trachea (McDonald 1988a). The following morphometric method was modified from that of Huang et al. (1989). Successive photomicrographs of bronchial mucosal surface were taken along the long axis of bronchi with an

Table 1. Amount of extravasation of Evans blue induced by capsaicin or its vehicle in bronchi and tracheas of Spraque–Dawley rats pretreated with left cervical vagotomy and of controls

Animal group	Injections	Left bronchi	Right bronchi	Left trachea	Right trachea
Intact	Evans blue + vehicle	69 ± 26(10)	70 ± 31(10)	33 ± 9(10)	32 ± 10(10)
Intact	Evans blue + capsaicin	150 ± 22(12)	164 ± 37(12)	105 ± 29(12)	105 ± 24(12)
Vagotomized 2 weeks earlier	Evans blue + capsaicin	127 ± 26(9)[a]	192 ± 24(9)	131 ± 37(9)	133 ± 37(9)
Vagotomized 4 weeks earlier	Evans blue + capsaicin	142 ± 32(10)[b]	226 ± 48(10)	152 ± 27(10)	164 ± 37(10)

The data listed are X ± SD (Evans-blue concentration, ng/mg tissue) and the numbers in parentheses are numbers of animals
[a] Significantly different ($P < 0.001$) from corresponding value for bronchi of right side in the rats vagotomized 2 weeks earlier
[b] Significantly different ($P < 0.001$) from corresponding value for bronchi of right side in the rats vagotomized 4 weeks earlier

Olympus microscope BH 2. Color prints with a magnification of × 40 and area per print of 76 × 127 mm were used for point counting, 3–5 in number for the main bronchus distal region (with 3–5 cartilaginous rings) and 8–10 for the secondary bronchus. The counts were made by superimposing a plastic sheet with a multipurpose test grid (Weibel 1979) on the color prints, d = 10 mm and total number of points = 120. The area density of mucosal surface occupied by Monastral blue-labeled blood vessels was determined by dividing the number of points on the labeled vessels by the total number of points of the test grid.

Histological study of vagus nerves, and bronchial and tracheal mucosa

Four Long-Evans rats and four Sprague-Dawley rats postoperative for either right or left vagotomy for 4 weeks, and another six intact Long-Evans and Sprague-Dawley rats were anesthetized with sodium pentobarbital, then given intravenous injections of Monastral blue followed by a dose of either capsaicin or its vehicle, as in the above experiments. The rats were then perfused with two glutaraldehyde-containing fixatives (McDonald 1988a). The vagus nerves and tracheal and bronchial tissues were removed, osmicated, dehydrated and embedded in Epikote (Merck). Semithin sections, 1 μm in thickness, were cut and stained with toluidine blue.

Tissue blocks were also processed for glycol methacrylate (Merck) embedding. Bronchial and tracheal cross-sections 2 μm in thickness were stained with Alcian blue-periodic acid Schiff (PAS) reagent to study the secretory activity of goblet cells (Lamb and Reid 1969; Bancroft and Stevens 1982; Huang et al. 1989).

Statistical analysis

Average values are expressed as the mean ± standard deviation (SD) of the mean. The significance of differences between groups of data was evaluated by Student's *t*-test. Differences having *P* values less than 0.05 were considered statistically significant.

Results

Decrease of Evans blue extravasation in the bronchial tree ipsilateral to vagotomy

Sprague-Dawley rats. For the intact rats that received intravenous injections of Evans blue and vehicle, the Evans blue concentrations in the left and right parts of the trachea were 33 ± 9 ng/mg tissue and 32 ± 10 ng/mg tissue (mean ± SDM; $n = 10$), respectively. The Evans blue concentrations in the left and right bronchial trees for this rat group were 69 ± 26 ng/mg and 70 ± 31 ng/mg, respectively, i.e., about twice the concentrations in trachea. There was no significant difference between the left and right sides of either trachea or bronchi (Table 1).

The concentrations of Evans blue in the trachea and bronchi of capsaicin-treated intact rats were significantly greater than those of vehicle-treated rats. There was no statistical difference between the two sides of either trachea or bronchi.

In the rats postoperative for unilateral vagotomy for 2 or 4 weeks, capsaicin resulted in statistically different magnitudes of Evans blue extravasation in the bronchial trees ipsilateral and contralateral to vagotomy. The Evans blue concentration in the bronchial tree of the vagotomized side was one-third less than that of the contralateral bronchial tree (Table 1).

Unilateral vagotomy did not result in significant differences in capsaicin-induced extravasation of Evans blue between the two sides of the trachea of any vagotomized animal group, and did not decrease the magnitude of plasma extravasation on either side of the trachea as compared with capsaicin-treated intact rats (Table 1).

Long-Evans rats. For the capsaicin-treated intact rats, the concentration of extravasated Evans blue dye in the bronchi did not show a significant difference between the two sides. In the rats postoperative for vagotomy for 2 weeks that received capsaicin, the Evans blue concentration in the left bronchial tree was 32% less than that of the contralateral bronchial tree (Table 2). In the rats postoperative for vagotomy for 4 weeks that received capsaicin, the extravasated dye in the left bronchi was 49% less than that of the contralateral bronchi. Unilateral vagotomy did not decrease the amount of plasma extravasation in the two sides of the trachea. For all animal groups, the dye concentrations in the left and right sides of the trachea were not significantly different.

Table 2. Amount of extravasation of Evans blue induced by capsaicin in bronchi and tracheas of Long-Evans rats pretreated with left cervical vagotomy and of controls

Animal group	Injections	Left bronchi	Right bronchi	Left trachea	Right trachea
Intact	Evans blue +capsaicin	148 ± 29(6)	140 ± 36(6)	102 ± 29(6)	122 ± 36(6)
Vagotomized 2 weeks earlier	Evans blue +capsaicin	111 ± 31(5)[a]	164 ± 33(5)	110 ± 28(5)	106 ± 23(5)
Vagotomized 4 weeks earlier	Evans blue +capsaicin	95 ± 23(6)[b]	187 ± 70(6)	131 ± 54(6)	141 ± 43(6)

The data listed are X ± SD (Evans-blue concentration, ng/mg tissue) and the numbers in parentheses are numbers of animals
[a] Significantly different ($P < 0.05$) from corresponding value for bronchi of right side in the rats vagotomized 2 weeks earlier
[b] Significantly different ($P < 0.05$) from corresponding value for bronchi of right side in the rats vagotomized 4 weeks earlier

Table 3. Morphometric analysis of capsaicin-induced extravasation of Monastral blue, expressed by area density of Monastral blue-labeled blood vessels in the distal part of main stem bronchi and secondary bronchi of Sprague–Dawley rats pretreated with left cervical vagotomy

	Left main bronchus	Right main bronchus	Left secondary bronchus	Right secondary bronchus
Area density of Monastral blue-labeled blood vessels	15.6 ± 7.1%(5)[a]	34.0 ± 6.1%(5)	10.2 ± 5.2%(5)[b]	37.3 ± 9.7%(5)

The data listed are X ± SD and the numbers in parentheses are numbers of animals
[a] Significantly different ($P < 0.001$) from corresponding value for distal region of right main stem bronchus
[b] Significantly different ($P < 0.001$) from corresponding value for secondary bronchus of right side

Decrease of area density of Monastral blue-labeled blood vessels in whole mounts of bronchi ipsilateral to vagotomy

In the right bronchi of capsaicin-treated Sprague-Dawley rats postoperative for left-side vagotomy for 4 weeks, numerous Monastral blue-labeled blood vessels were present in the mucosa of the right main bronchus to the fourth-order bronchi (Fig. 1). The area density of Monastral blue-labeled blood vessels was 34.0 ± 6.1% ($n = 5$) in the distal region of the main bronchus, and 37.3 ± 9.7% ($n = 5$) in the secondary bronchus of the right-side bronchial tree contralateral to vagotomy (Table 3). In the left bronchial tree, ipsilateral to vagotomy, the number of Monastral blue-labeled blood vessels was markedly decreased in each order of bronchi (Fig. 2). The area density of the distal part of the left main bronchus was 15.6 ± 7.1%, decreased to about one-half of that of the unoperated side ($P < 0.001$), and the area density of the left secondary bronchus was 10.2 ± 5.2%, decreased to a quarter of that of the unoperated side ($P < 0.001$) (Table 3). Vehicle did not cause extravasation of Monastral blue.

Histology of vagus nerves, and bronchial and tracheal mucosa

Vagus nerve. Striking structural differences were observed in the interior of the normal (Fig. 3) and degenerated (Fig. 4) cervical vagus nerves. The normal cervical vagus nerve consisted mainly of numerous myelinated and unmyelinated nerve fibers (Fig. 5), in contrast to the degenerated nerve, which contained numerous Schwann cells (Fig. 6). Myelinated nerve fibers were almost completely lost in the degenerated vagus nerve (Fig. 6).

Bronchial mucosa. In the bronchial mucosa contralateral to vagotomy, neurogenic inflammatory changes caused by capsaicin occurred in most area of the cross-sectional profiles of bronchi. Edema was observed in the connective tissue between mucosal blood vessels and, most prominently, underneath the mucosal surface epithelium (Fig. 7). Many goblet cells in the pseudostratified, high columnar, ciliated epithelium degranulated, and the intercellular spaces were dilated. However, in the bronchial mucosa ipsilateral to vagatomy, most areas were not responsive to capsaicin. In these areas, goblet cells did not degranulate and epithelial intercellular spaces remained unchanged (Fig. 8). Aggregations of lymphoid cells (Fig. 8) could be observed. In the intact rats receiving capsaicin, neurogenic inflammatory changes occurred on both side of the bronchi. Vehicle did not produce these tissue responses.

Tracheal mucosa. Histological changes typical of neurogenic inflammation were observed on both left and right sides of the tracheal mucosa in intact and vagotomized

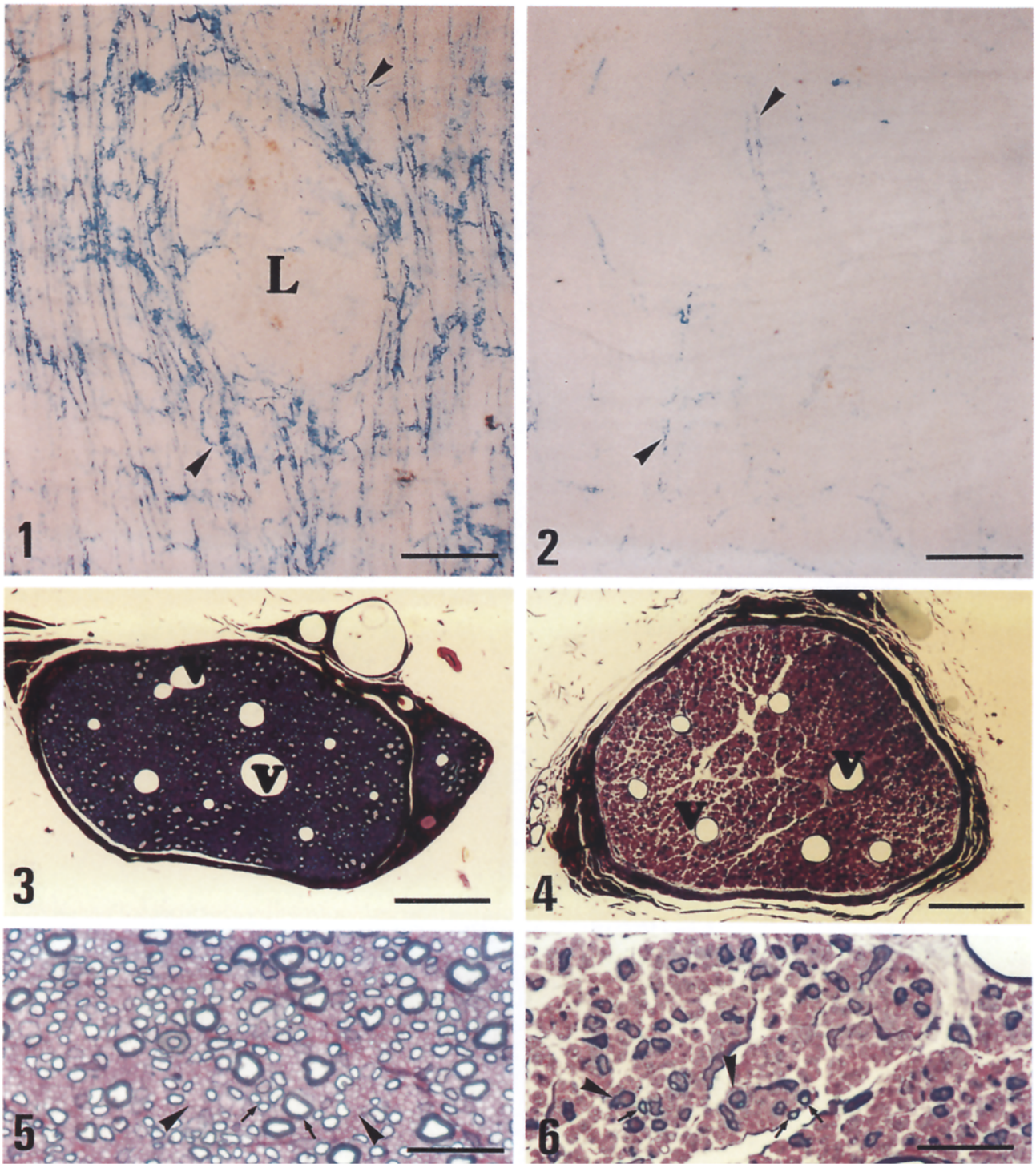

Fig. 1. Whole mount of the third secondary bronchus, lateral to the most medial, of the right bronchial tree, contralateral to vagotomy performed 4 weeks previously, of a Sprague-Dawley rat given intravenous injections of Monastral blue and capsaicin. Numerous Monastral blue-labeled blood vessels *(arrowheads)* can be observed. *L*, lymphoid tissue. × 40, Bar 400 μm

Fig. 2. Whole mount of the medial secondary bronchus of the left bronchial tree, ipsilateral to vagotomy performed 4 weeks previously, of a Sprague-Dawley rat given injections of Monastral blue and capsaicin. Only a few blood vessels *(arrowheads)* labeled with Monastral blue can be observed. × 40. Bar 400 μm

Fig. 3. Light micrograph of toluidine blue-stained epoxy cross-section of a normal cervical vagus nerve from a unilaterally vagotomized rat. Small clear circles in the tissue between blood vessels (*V*) are myelinated nerve fibers. × 200. Bar 80 μm

Fig. 4. Light micrograph of toluidine blue-stained epoxy cross-section of a degenerated vagus nerve distal to surgery site. The degenerated nerve appears loose. Dark dots in the tissue between blood vessels (*V*) are Schwann cells. × 200. Bar 80 μm

Fig. 5. High-power magnification of a part of the normal vagus nerve in Fig. 3, showing numerous myelinated *(arrows)* and unmyelinated *(arrowheads)* nerve fibers. × 800. Bar 20 μm

Fig. 6. High-power magnification of a part of the degenerated vagus nerve in Fig 4, showing numerous Schwann cells *(arrowheads)*. A few myelinated nerve fibers *(arrows)* can also be observed. × 800. Bar 20 μm

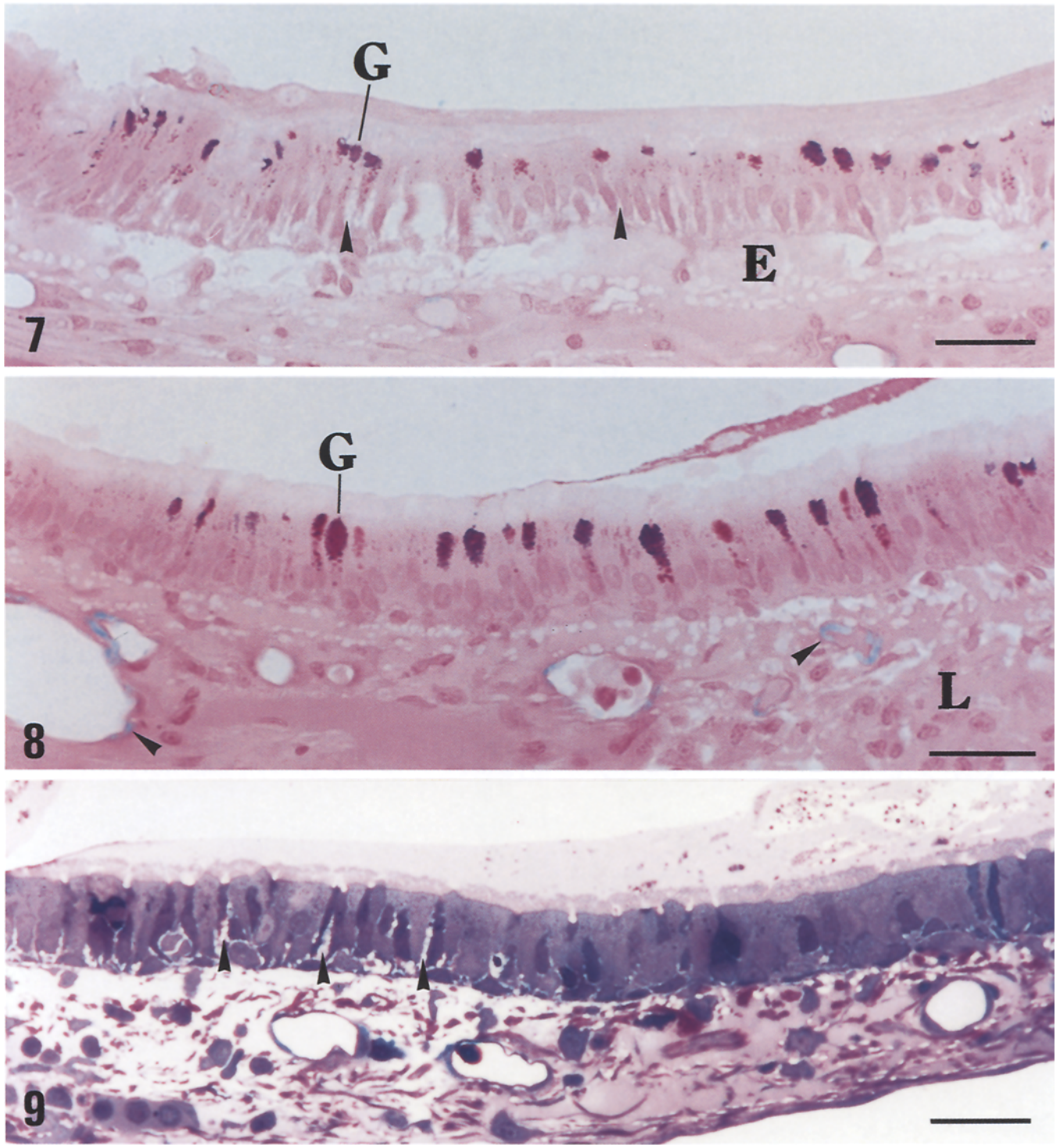

Fig. 7. Alcian blue-PAS staining of methacrylate cross-section of the distal part of the left main bronchus from a Long-Evans rat vagotomized on the right side 4 weeks previously. Neurogenic inflammation is evoked by capsaicin. Edematous change at the subepithelial region (*E*) is prominent. In the mucosal surface epithelium, the goblet cells degranulate (*G*) and the intercellular spaces are dilated *(arrowheads)*. × 560. Bar 30 μm

Fig. 8. Alcian blue-PAS staining of methacrylate cross-section of the distal part of the right main bronchus from a long-Evans rat vagotomized on the right side 4 weeks previously. Capsaicin does not produce structural changes in the mucosal surface epithelium. Goblet cells (*G*) do not degranulate and intercellular spaces are not dilated. Some blood vessels are labeled with Monastral blue *(arrowheads)*. *L*, lymphoid cells. × 560. Bar 30 μm

Fig. 9. Light micrograph of toluidine blue-stained epoxy section from right half of the lower trachea of a Long-Evans rat pretreated with right-side vagotomy 4 weeks previously and injected with Monastral blue and capsaicin. On the left side of the figure can be seen prominent edematous change in the lamina propria and dilated intercellular spaces *(arrowheads)* beside goblet cells in the mucosal surface epithelium. On the right side, these structural changes are not observed. × 560. Bar 30 μm

rats receiving capsaicin. Focal areas of mucosal tissue that were not responsive to capsaicin (Fig. 9) could be observed on both sides of the lower trachea in the vagotomized rats. Lymphatic infiltration was also found in the lamina propria.

Discussion

The results of the present study showed that unilateral cervical vagotomy did not decrease the capsaicin-induced vascular permeability to Evans blue in the right and left regions of the trachea. However, unilateral cervical vagotomy decreased Evans blue extravasation induced by capsaicin in the bronchial tree ipsilateral to the degenerated vagus nerve, leaving the contralateral bronchial tree unchanged. Monastral blue extravasation evoked by capsaicin was also reduced in the bronchi ipsilateral to vagotomy. Histological study indicated that neurogenic inflammation in the bronchial mucosa ipsilateral to vagotomy was almost prevented. Edematous change was not obvious in the lamina propria, especially in the subepithelial region, and most goblet cells were not responsive. It is suggested that unilateral cervical vagotomy could make the ipsilateral bronchi less reactive, or not reactive at all to capsaicin, while the contralateral bronchi remain reactive to it.

Neonatal capsaicin pretreatment, which can induce selective degeneration of chemosensitive primary sensory neurons (Jancso et al. 1977), causes long-lasting desensitization of the mucosa of whole airways to the irritants (Lundberg and Saria 1983). Unilateral cervical vagotomy can abolish a large number of substance P-immunoreactive sensory axons at the base of the mucosal epithelium in the ipsilateral bronchi (Bałuk et al. 1992), and so cause selective desensitization of the mucosa to capsaicin.

In the present study, vascular permeability to Evans blue provoked by capsaicin in the trachea was not decreased following unilateral vagotomy. It is known that each side of the trachea receives sensory nerve fibers from both vagus nerves (Bałuk and Gabella 1989). Although unilateral vagotomy can greatly reduce the number of sensory nerve fibers in the tracheal mucosa (Bałuk et al. 1992), the remaining sensory fibers from the contralateral vagus nerve could probably release a sufficient quantity of tachykinins, caused by capsaicin, to produce neurogenic inflammation in the trachea similar to that of intact rats.

The present study revealed that extravasation of Monastral blue in the bronchial tree, contralateral to vagotomy in capsaicin-treated vagotomized rats, was similar to that ipsilateral to vagal electrical stimulation in the rat (McDonald 1988a). Extravasation of Monastral blue in the bronchial tree ipsilateral to vagotomy was markedly decreased, but Monastral blue-labeled blood vessels were still visible in different parts of bronchial tree, whereas contralateral to vagal electrical stimulation there is no extravasation of tracer beyond the proximal part of the main bronchus (McDonald 1988a). These findings suggest that sensory nerve fibers from both spinal nerves and crossed branches of the contralateral vagus nerve contributed to the occurrence of the lessened vascular permeability in the bronchi of the vagotomized side.

In the tracheal mucosa of the rat and guinea pig, discharge of mucous secretion from the goblet cells is associated with increased vascular permeability by electrical vagal stimulation (McDonald 1988a, b; Tokuyama et al. 1990), or by capsaicin stimulation (McDonald 1988b; Huang et al. 1989, 1990; Kuo et al. 1990). Alcian blue-PAS staining clearly shows release of glycoprotein-containing secretory granules from the supranuclear cytoplasm of goblet cells (Huang et al. 1989). Dilations of intercellular spaces beside the goblet cells are also a prominent feature of neurogenic inflammation (McDonald 1988a; Huang et al. 1989).

After unilateral vagotomy, the ipsilateral recurrent laryngeal nerve and some contralateral nerve bundles degenerated (Dahlqvist et al. 1986; Bałuk and Gabella 1989), leading to focal unresponsiveness of the tracheal epithelium in the previous study (Huang et al. 1990) and in the present study, probably due to loss of sensory nerve endings on the epithelial cells (Bałuk et al. 1992). For the ipsilateral bronchi, unilateral cervical vagotomy effectively inhibited capsaicin-induced discharge of mucous granules from goblet cells and dilation of epithelial intercellular spaces.

The tracheal mucosa of pathogen-free rats without respiratory infections has cuboidal or low columnar, ciliated epithelium in which goblet cells are absent, and has no lymphoid tissue in the lamina propria (Jeffery and Reid 1975; McDonald 1988b; Huang et al. 1989; McDonald et al. 1991). After a few weeks of respiratory infections with *Mycoplasma pulmonis,* Sendai virus and coronavirus, the rats develop a thick, columnar, ciliated epithelium with numerous tall, ciliated cells and hyperplasia of goblet cells. A large amount of lymphoid tissue is present in the thickened lamina propria (McDonald 1988b; Huang et al. 1989). In addition, the infected rats exhibit an abnormally intense neurogenic inflammation in the airway stimulated by capsaicin (McDonald 1988b, Huang et al. 1989). Morphological obervations and the magnitude of neurogenic plasma extravasation suggested that the rats used in the present study had a history of naturally occurring airway infections. It has also been demonstrated that *Mycoplasma pulmonis* infections are responsible for causing morphological alterations of tracheal mucosal tissue (Schoeb et al. 1985; Schoeb and Lindsey 1987; McDonald et al. 1991), and long-lasting potentiation of neurogenic inflammation (McDonald et al. 1991). Pretreatment with dexamethasone, the glucocorticoid, inhibits plasma extravasation in the tracheobronchial mucosa produced by capsaicin (Huang et al. 1989; Piedimonte et al. 1990), substance P (Piedimonte et al. 1990) and platelet-activating factor (Boschetto et al. 1991).

Conclusion

It appears that unilateral lesioning of the vagus nerve can selectively desensitize the mucosa of the ipsilateral bron-

chial tree and make it less reactive to the irritant capsaicin than that of the contralateral bronchial tree. For the contralateral bronchi, capsaicin produced an abnormal increase in the magnitude of vascular permeability, degranulation of goblet cells and dilation of intercellular spaces in the mucosal surface epithelium, and edema formation in the lamina propria. The bronchial tree ipsilateral to vagotomy, in contrast, had a marked decrease in vascular permeability. Numerous goblet cells did not degranulate, and epithelial intercellular spaces did not dilate.

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References

Bałuk P, Gabella G (1989) Innervation of the guinea pig trachea: a quantitative morphological study of intrinsic neurons and extrinsic nerves. J Comp Neurol 285:117–132

Bałuk P, Nadel JA, McDonald DM (1992) Substance P-immunoreactive sensory axons in the respiratory tract: a quantitative study of their distribution and role in neurogenic inflammation. J Comp Neurol 319:586–598

Bancroft JD, Stevens A (1982) Theory and practice of histological techniques. 2nd edn. Churchill Livingstone, Edinburgh

Boschetto P, Rogers DF, Fabbri LM, Barnes PJ (1991) Corticosteroid inhibition of airway microvascular leakage. Am Rev Respir Dis 143:605–609

Brokaw JJ, McDonald DM (1988) Neurally-mediated increase in vascular permeability in the rat trachea: onset, duration, and tachyphylaxis. Exp Lung Res 14:757–767

Cadieux A, Springall DR, Mulderry PK, Rodrigo J, Ghatei MA, Terenghi G, Bloom SR, Polak JM (1986) Occurrence, distribution and ontogeny of CGRP immunoreactivity in the rat lower respiratory tract: effect of capsaicin treatment and surgical denervation. Neuroscience 19:605–627

Dahlqvist A, Carlsoo B, Hellstrom S, Domeij S, Kourtopoulos H (1986) Fiber composition of the recurrent laryngeal nerve after experimental vagotomy and sympathectomy. Acta Anat 125:114–120

Dalsgaard CJ, Lundberg JM (1984) Evidence for a spinal afferent innervation of the guinea-pig lower respiratory tract as studied by the horseradish peroxidase technique. Neurosci Lett 45:117–122

Huang HT, Haskell A, McDonald DM (1989) Changes in epithelial secretory cells and potentiation of neurogenic inflammation in the trachea of rats with respiratory infections. Anat Embryol 180:325–341

Huang HT, Liu KM, Her WY, Chiang CY (1990) Effect of unilateral vagotomy on capsaicin-induced neurogenic inflammation in the trachea of rats. Kaohsiung J Med Sci 6:461–466

Jancso G, Kiraly E, Jancso-Gabor A (1977) Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. Nature 270:741–743

Jeffery PK, Reid L (1975) New observations of rat airway epithelium: a quantitative and electron microscopic study. J Anat 120:295–320

Kou H-P, Rohde JAL, Tokuyama K, Barnes PJ, Rogers DF (1990) Capsaicin and sensory neuropeptide stimulation of goblet cell secretion in guinea-pig trachea. J Physiol (Lond) 431:629–641

Lamb D, Reid L (1969) Histochemical types of acidic glycoprotein produced by mucous cells of the tracheobronchial glands in man. J Pathol 98:213–229

Ling EA, Wong WC (1988) An electron microscopic study of the nodose (inferior vagal) ganglion cells in the monkey. J Neurocytol 17:845–857

Lundberg JM, Saria A (1983) Capsaicin-induced desensitization of airway mucosa to cigarette smoke, mechanical and chemical irritants. Nature 302:251–253

Lundberg JM, Brodin E, Saria A (1983a) Effects and distribution of vagal capsaicin-sensitive substance P neurons with special reference to the trachea and lungs. Acta Physiol Scand 119:243–252

Lundberg JM, Saria A, Martling CR (1983b) Capsaicin pretreatment abolishes cigarette smoke-induced oedema in rat tracheobronchial mucosa. Eur J Pharmacol 86:317–318

Lundberg JM, Hokfelt T, Martling CR, Saria A, Cuello C (1984) Substance P-immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. Cell Tissue Res 235:251–261

McDonald DM (1988a) Neurogenic inflammation in the rat trachea. I. Changes in venules, leukocytes, and epithelial cells. J Neurocytol 17:583–603

McDonald DM (1988b) Respiratory tract infections increase susceptibility to neurogenic inflammation in the rat trachea. Am Rev Respir Dis 137:1432–1440

McDonald DM, Mitchell RA, Gabella G, Haskell A (1988) Neurogenic inflammation in the rat trachea. II. Identity and distribution of nerves mediating the increase in vascular permeability. J Neurocytol 17:605–628

McDonald DM, Schoeb TR, Lindsey JR (1991) *Mycoplasma pulmonis* infections cause long-lasting potentiation of neurogenic inflammation in the respiratory tract of the rat. J Clin Invest 87:787–799

Piedimonte G, McDonald DM, Nadel JA (1990) Glucocorticoids inhibit neurogenic plasma extravasation and prevent virus-potentiated extravasation in the rat trachea. J Clin Invest 86:1409–1415

Saria A, Lundberg JM (1983) Evans blue fluorescence: quantitative and morphological evaluation of vascular permeability in animal tissues. J Neurosci Methods 8:41–49

Saria A, Martling C-R, Dalsgaard C-J, Lundberg JM (1985) Evidence for substance P-immunoreactive spinal afferents that mediate bronchoconstriction. Acta Physiol Scand 125:407–414

Schoeb TR, Lindsey JR (1987) Exacerbation of murine respiratory mycoplasmosis by sialodacryoadenitis virus infection in gnotobiotic F344 rats. Vet Pathol 24:392–399

Schoeb TR, Kervin KC, Lindsey JR (1985) Exacerbation of murine respiratory mycoplasmosis in gnotobiotic F344/N rats by Sendai virus infection. Vet Pathol 22:272–282

Tokuyama K, Kuo H-P, Rohde JAL, Barnes PJ, Rogers DF (1990) Neural control of goblet cell secretion in guinea pig airways. Am J Physiol 259:L108–L115

Umeno E, Nadel JA, Huang HT, McDonald DM (1989) Inhibition of neutral endopeptidase potentiates neurogenic inflammation in the rat trachea. J Appl Physiol 66:2647–2652

Umeno E, Nadel JA, McDonald DM (1990) Neurogenic inflammation of the rat trachea: fate of neutrophils that adhere to venules. J Appl Physiol 69:2131–2136

Weibel E (1979) Stereological Methods, vol 1. Practical Methods for Biological Morphometry. Academic Press, London