# Scanning electron microscopy of capsaicin-pretreated trachea in the rat during postnatal development

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Summary. The morphological changes of the tracheal surface were studied in neonatally capsaicin-pretreated rats by scanning electron microscopy. One week after neonatal capsaicin treatment, a sparse number of ciliated cells were dispersed among the microvilli-covered goblet cells similar to those of the sham-operated rats. Potential ciliated cells (progenitor cells) possessed a few long cilia and many short cilia. The ciliated cells possessed cilia with a smooth surface and a blunt end similar to that of the sham-operated rats. Two weeks after neonatal capsaicin treatment, numerous ciliated cells frequently in clusters were located among large patches of microvilli-covered goblet cells similar to those of sham-operated rats. Furthermore, the blunt ends of cilia and microvilli contained short star-shaped protrusions extending into the lumen of the trachea. One month after neonatal capsaicin treatment, the star-shaped protrusions became longer, and more irregular than those of sham-operated rats. The short cilia of the potential ciliated cells (progenitor cells) also became blunt and irregular in shape. The star-shaped protrusions of the microvilli of the goblet cells became larger and thicker than those observed at two weeks following capsaicinpretreatment. Two months after neonatal capsaicin treatment, the tracheal surface was lined with a much greater population of the ciliated cells than that at one month. A striking characteristic at this age was that globular mucin-containing secretory products were trapped within the cilia of the ciliated cells. The results of this study suggest that mucus secretion is probably blocked by the capsaicin-pretreatment. Furthermore, the star-shaped protrusions of cilia and microvilli may indicate that the mucociliary clearance mechanism is interfered with by the capsaicin-pretreatment.

**Key words:** Capsaicin, Postnatal development, Scanning electron microscopy, Trachea

## Introduction

The respiratory tract is innervated by the vagal sensory fibres which contain neuropeptides as neurotransmitters or modulators (Springall et al., 1988). The neuropeptides include substance P (Wharton et al., 1979; Ghatei et al., 1982; Sheppard et al., 1983; Lundberg et al., 1984; Luts et al., 1990), and other tachykinins (Hua et al., 1985). These peptides have been found to have strong actions on vascular and airway smooth muscles (Nilsson et al., 1977), glandular secretions (Lundberg et al., 1988), and possibly ciliary motion (Uddman and Sundler, 1986). It is also known that pretreatment of capsaicin, the pungent principle of the red pepper, causes depletion of substance P and then results in the selective degeneration of chemosensitive afferent C-fibres of the vagal nerves (Jancsó et al., 1977). The desensitization of capsaicin to pain stimuli has been reported (Jancsó, 1955; Szolcsányi et al., 1975). Capsaicin is also shown to prevent the neurogenic inflammatory responses (Jancsó et al., 1967). However, in many mammalian species, acute capsaicin injection produces skin irritations, bronchoconstriction, plasma exudation, and goblet cell secretions (Russel and Lai-Fook, 1979; Lundberg and Saria, 1987; Erjefält and Persson, 1989; Kuo et al., 1990). Therefore, capsaicin probably interferes with the synthesis and release of the neurohumor in sensory nerve terminals or secretory cells which are located especially in the epithelium of skin, vascular bed, and airways.

The morphological structure of the epithelium in the airways has been studied by scanning electron microscopy (SEM) (Rhodin and Dalhamn, 1956; Rhodin, 1966; Greenwood and Holland, 1972, 1975; Andrews, 1974). Epithelial cells in the tracheal surface have been identified as ciliated cells and three major types of microvillous cells (including goblet cell, potential ciliated cell, and brush cell) (Andrews, 1980). Although the distributions, patterns and types of the epithelial cells on the tracheal surface have been shown by previous studies, the effect of capsaicin on the surface

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structure of these cells has not been reported. The present study was undertaken to investigate the surface characteristics and morphological changes of the trachea of neonatally capsaicin-treated rats during postnatal development by using SEM.

#### Materials and methods

Sprague-Dawley rats were administered with one subcutaneous injection of 50 mg/kg capsaicin (prepared in a vehicle of 10% ethanol, 10% Tween 80 and 80% isotonic saline) on the second day of life (n=23). Pups from other litter mates injected with the same volume of vehicle, or untreated, served as controls (n=16). At one week (n=11), two weeks (n=11), one month (n=9) and two months (n=8) after capsaicin treatment, the animals were killed. The number within brackets indicates the number of animals in that age group for this study. The trachea were removed and fixed in 3% glutaraldehyde in 0.1M phosphate buffer overnight at 4 °C. The superficial mucus was removed by gently rinsing and shaking with 0.1M phosphate buffer prior to fixation. After fixation the tissues were washed in 0.1M phosphate buffer and then postfixed in 1% osmium tetroxide in 0.1M phosphate buffer for one hour. The trachea were dehydrated in a graded ethanol series to absolute ethanol and transferred to liquid carbon dioxide for critical point drying according to the procedure of Anderson (1951, 1952). The dried specimens were mounted on aluminium or copper studs, coated with gold (30 nm) in a JEOL Ion Sputter JFC-1100 and examined in a JEOL electron microscope (JSM-5300).

## Results

#### Sham treatment

One week after sham treatment, SEM observations revealed that the epithelial surface of the trachea consisted of ciliated cells, potential ciliated cells (progenitor cells), microvilli-covered goblet cells and brush cells. At low magnification, a sparse number of ciliated cells was dispersed among the microvillicovered goblet cells (Fig. 1A). At higher magnifications, scanning micrographs revealed that ciliated cells contained long cilia, and a few short microvilli were pronounced at the base of the cell surface (Fig. 1B,C). Potential ciliated cells (progenitor cells) were characterized by cilia of irregular length intermingling among densely-packed microvilli. Microvilli on potential ciliated cells (progenitor cells) were taller than those on the microvilli-covered goblet cells. Microvillicovered goblet cells contained dense, short microvilli and their surface outlines were polyhedral in shape. The apical surface of a microvilli-covered goblet cell appeared swollen, possibly due to accumulated mucus granules, or possessed the craters resulting from the release of secretory granules. The brush cells were characterized by a dense population of tall microvilli on

their surface. These tall microvilli on the brush cells were more uniformly shaped than those of the potential ciliated cells (Fig. 1B).

Two weeks after sham treatment, the surface morphology of the trachea was characterized by numerous ciliated cells, frequently in clusters, located among large patches of microvilli-covered goblet cells (Fig. 1D). Potential ciliated cells (progenitor cells) containing a few short cilia and many microvilli were localized adjacent to the ciliated cells (Fig. 1E). The length of the cilium of the ciliated cells became longer than that of one week after sham treatment (Fig. 1F).

One month after sham treatment, scanning electron micrographs revealed that the ciliated cells lining the trachea were present in much greater numbers (Fig. 2A). Tracheal ciliated cells were in clusters, interspersed with the microvilli-covered goblet cells, and possessed prominent cilia with a smooth surface and a blunt end (Fig. 2B). The curved shape of the cilia indicated the beating direction of the movement. The apical protrusions of the microvilli-covered goblet cells revealed the accumulation of the mucin. At higher magnification, ciliated cells consisted of long, slender cilia with blunt ends at their free terminals. Domeshaped secretory products covered the apical surface of the microvilli-covered goblet cells. Many microvilli surrounded the periphery of the apical dome-shaped surface. Two months after sham treatment, many craterlike structures possessed surface indentations which had apparently resulted from the apocrine release of microvilli-covered goblet cell secretory product (Fig. 2C). The direction of bending of the cilia indicated its function of mucociliary clearance motion toward the larynx. Occasionally, mucin-containing secretory product just being released was simultaneously aligned with the crater-like structure. This crater-like structure marked the site of recent apocrine secretion of the microvilli-covered goblet cell (Fig. 2D). The mucus secretory product appeared extruding from the craterlike structure.

#### Neonatal capsaicin treatment

One week after neonatal capsaicin treatment, scanning micrographs revealed that topography and morphology of the epithelial surface of the trachea were similar to those of the sham-operated rats (Fig. 3A). Potential ciliated cells (progenitor cells) possessed a few long cilia and many short cilia (Fig. 3B). The ciliated cells possessed cilia with a smooth surface and a blunt end similar to that of the sham-operated rats, but had a tendency to stick together (Fig. 3C).

Two weeks after neonatal capsaicin treatment, the surface morphology of the trachea was similar to that of the sham-operated rats, characterized by ciliated cells, frequently in clusters, located among large patches of microvilli-covered goblet cells (Fig. 3D). The blunt ends of most of cilia, and a few microvilli contained short star-shaped protrusions extending into the lumen of the



Fig. 1. Micrographs of the tracheal surface one week (A-C) and two weeks (D-F) after sham-treatment. A. A sparse number of ciliated cells (arrows) is dispersed among the microvilli-covered goblet cells (arrowheads). x 1,200. Bar= 10  $\mu$ m. B. At higher magnification, ciliated cells (C) contain long cilia (arrows) and short microvilli (arrowheads). A potential ciliated cell (P, progenitor cell) possesses cilia of irregular length (arrows) intermingling among densely packed microvilli (arrowheads). Note the dense short microvilli on the goblet cell (G) and the uniformly tall microvilli on the brush cell (B). x 6,000. Bar= 1  $\mu$ m. C. A ciliated cell (C) is surrounded by the microvilli-covered goblet cells (G). x 7,500. Bar= 1  $\mu$ m. D. Numerous ciliated cells (arrows), frequently in clusters, are located among large patches of microvilli-covered goblet cells (arrowheads). x 1,200. Bar= 10  $\mu$ m. E. At higher magnification, potential ciliated cells (P) containing a few short cilia and many microvilli are localized adjacent to the ciliated cells (C). x 3,750. Bar= 5  $\mu$ m. F. Longer cilium with a smooth surface and a blunt end on the ciliated cell (C). x 7,500. Bar= 1  $\mu$ m.

## SEM of capsaicin-pretreated trachea

trachea (Fig. 3E.F). Some secretory product was located either on the apical surface of the microvilli-covered goblet cells or at the blunt ends of the cilia of the ciliated cells (Fig. 3E).

One month after neonatal capsaicin treatment, the star-shaped protrusions became longer and more irregular than those of the sham-rats (Fig. 4A). The cilia had a tendency to stick together and a sparse number of cilia tapered at their ends (Fig. 4B). The short cilia of the potential ciliated cells (progenitor cells) became blunt and irregular in shape (Fig. 4C). The star-shaped protrusions of the microvilli of the goblet cells became larger and thicker than those of two weeks after neonatal capsaicin treatment (Fig. 4D). These protrusions also possessed a tapered end. At this age the microvilli of the goblet cells appeared to be reduced in number. Two months after neonatal capsaicin treatment, the tracheal

surface was lined with a much greater population of ciliated cells (Fig. 4E). A striking characteristic at this age was that globular mucin-containing secretory products were trapped within the cilia of the ciliated cells (Fig. 4F). The intertwining cilia of the ciliated cells obscured the surface distribution of the microvillicovered goblet cells.

### Discussion

SEM is a useful means for providing a great deal of information on the three-dimensional architecture of various biological specimens. SEM reveals the types and distribution of the surface structure of biological material as well as their relationship to the other regions in the same and different specimens. In addition, SEM can screen a large area of tissues to allow more accurate

Fig. 4. Micrographs of the tracheal surface one month (A, B) and two months (C, D) after sham treatment. A. the clilated cells (arrows) lining the

Fig. 2. Micrographs of the tracheal surface one month (A, B) and two months (C, D) after sham treatment. A. The ciliated cells (arrows) lining the trachea are present in much greater numbers. x 1,200. Bar= 10 μm. B. The apical protrusions of the microvilli-covered goblet cells reveals the accumulation of the mucin (arrows). x 7,500. Bar= 1 μm. C. Many crater-like structures (arrows) possess surface indentations. x 1,200. Bar= 10 μm. D. Mucin-containing secretory products (SP) just being released from the crater-like structures (Cr). x 7,500. Bar= 1 μm.



Fig. 3. Micrographs of the tracheal surface one week (A-C) and two weeks (D-F) after neonatal capsaicin treatment. A. A sparse number of ciliated cells (arrows) is dispersed among the microvilli-covered goblet cells (arrowheads). x 1,200. Bar= 10  $\mu$ m. B. At higher magnification, ciliated cells (C) containing long cilia (arrows) and short microvilli (arrowheads). x 2,600. Bar= 5  $\mu$ m. C. A ciliated cell (C) possesses cilia with a smooth surface and a blunt end. Note the aggregation of cilia (arrows). x 11,000. Bar= 1  $\mu$ m. D. Numerous ciliated cells (arrows), frequently in clusters, are located among large patches of microvilli-covered goblet cells (arrowheads). x 1,200. Bar= 10  $\mu$ m. E. At higher magnification, potential ciliated cells (P) containing a few short cilia and many microvilli are localized adjacent to the ciliated cells (C). Note that secretory products are located either on the apical surface (arrows) of the microvilli-covered goblet cells or at the blunt ends of the ciliated cells. x 2,600. Bar= 5  $\mu$ m. F. Short star-shaped protrusions (arrows) on the blunt ends of cilia of the ciliated cell (C) and on the microvilli of the goblet cell (G). x 7,500. Bar= 1  $\mu$ m.



Fig. 4. Micrographs of the tracheal surface one month (A-D) and two months (E,F) after neonatal capsaicin treatment. A. Irregular star-shaped protrusions (arrows) on the tracheal surface. x 1,200. Bar= 1  $\mu$ m. B. The cilia (arrows) stick together on the ciliated cell (C). Note that the apical star-shaped protrusions (arrowheads) of cilia become longer than those of two weeks after neonatal capsaicin treatment (compare with Fig. 3F). x 7,500. Bar= 1  $\mu$ m. C. Blunt ends (arrows) on the short cilia of the potential ciliated cell. P: progenitor cell. x 7,500. Bar= 1  $\mu$ m. D. Large, thick star-shaped protrusions (arrows) on the microvilli of the goblet cells (G). x 7,500. Bar= 1  $\mu$ m. E. A much greater population of the ciliated cells on the tracheal surface. Note the globular mucin-containing secretory products (arrows) are trapped within the cilia of the ciliated cells. x 1,200. Bar= 10  $\mu$ m. F. At higher magnification, the intertwining cilia of the ciliated cells obscure the surface distribution of the microvilli-covered goblet cells. Note the aggregation of cilia (arrows), the star-shaped protrusions (arrowheads), and the trapped globular mucin-containing secretory products (SP). x 3,750. Bar= 5  $\mu$ m.

information to be elucidated. Respiratory cell types have been characterized in humans (Rhodin, 1966; Greenwood and Holland, 1975), monkey (Castleman et al., 1975), rat (Rhodin and Dalhamn, 1956; Greenwood and Holland, 1972; Andrews, 1974, 1980), and mouse (Hansell and Moretti, 1969; Hama and Nagata, 1970). Previous SEM studies have shown that the tracheal surface is composed of ciliated cells and microvillous (nonciliated) goblet cells (Greenwood and Holland, 1972). The nonciliated goblet cells are mainly mucus secreting in nature (Hansell and Moretti, 1969). Our work confirms these findings in rats.

In addition, the result of our study indicates that a sparse number of the ciliated cells were dispersed among the microvilli-covered goblet cells of the sham-operated trachea in early postnatal development. The length of the cilia and microvilli became longer with increasing age. Furthermore, the morphological structure of the tracheal surface in two-month-old rats was similar to previous reports in adult mice (Greenwood and Holland, 1972).

In the present study numerous ciliated cells often occurred to be in groups interspersing between microvillous cells. This was demonstrated in the shamoperated rats but was not seen in the neonatal capsaicinpretreated rats at one month of age. The great decrease in the ratio of microvilli-covered goblet cells to ciliated cells may be explained by the proliferation of the ciliated cells and/or the differentiation of the goblet cells into ciliated cells (Evans and Shami, 1989). A previous study (Hilding and Hilding, 1966) has proposed the regeneration process of respiratory epithelial cells. The authors were not clear about the developmental relationship of the microvilli to the cilia following mechanical injury to tracheal epithelium. However, their ultrastructural studies revealed that the cilia in tracheal epithelium was formed after the centrioles subdivided to form basal bodies which ascended to the upper cell surfaces (Sorokin, 1962; Hilding and Hilding, 1966). This regeneration process may explain the changes in cilia during postnatal development.

Recently, immunocytochemistry has been widely used. Acetylcholinesterase histochemistry has been used to localize the cholinergic nerve fibres in the respiratory tract (Sheppard et al., 1983, 1984a). Many bioactive peptides have also been found in the mammalian respiratory tract, such as neuropeptide tyrosin (NPY) (Sheppard et al., 1984b) and substance P (Wharton et al., 1979; Ghatei et al., 1982; Sheppard et al., 1983; Springall et al., 1990), etc. Substance P, a tachykinin, is the mediator of the axon reflex in cutaneous tissue (Gamse et al., 1980). Now, it is also known that substance P is a potent bronchoconstrictor, pulmonary vaso-constrictor and airway mucus secretor. However, neonatal capsaicin pretreatment causes degeneration of substance P neurons in the vagus nerve (Gamse et al., 1980) and decreases substance P in regions containing primary sensory neurons (Gamse et al., 1980; Lundberg and Saria, 1982). The results of our study indicate that the goblet cells were reduced in number and obscured by

the intertwining cilia as well as the star-shaped protrusions on the cilia of the ciliated cells, and the microvilli of the goblet cells were noticed at two months after neonatal capsaicin treatment. These star-shaped protrusions were different from the filamentous strands located between adjacent microvilli or cilia as well as between a microvilli and a cilia (Smolich et al., 1976). These strands have been suggested to be the residue of unremoved mucus (Mever et al., 1971) or extensions of the surface glycocalyx (Andrews, 1974; Smolich et al., 1976). Another striking characteristic at this age was that globular mucin-containing secretory products were trapped within the cilia of the ciliated cells. These morphological changes might explain the previous findings of the blockade of mucus secretion (Carstairs and Barnes, 1986; Kuo et al., 1990). The third distinct morphological change was that the cilia had a tendency to stick together, similar to the alteration of the tracheal surface induced by acrolein inhalation (Dahlgren and Dalen, 1972). This morphological change of cilia indicated that the mucociliary clearance mechanism might be interfered with by pretreatment with capsaicin. In summary, results obtained from this study suggest that capsaicin presumably hinders the mucus secretion and interferes with the removal of the inhaled particles in the mucociliary clearance mechanism.

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