Ultrastructural organisation of intraepithelial nerves in the human airway tract

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ABSTRACT   Intraepithelial nerves of human airway tract were studied by electron microscopy after conventional glutaraldehyde fixation. Specimens were obtained from five patients at three different airway levels—(1) the trachea, (2) the right upper lobe bronchus, and (3) segmental bronchus. Intraepithelial axon profiles were located either near the basement membrane or close to the lumen but were rare in the intermediate area of the epithelium. Axon profiles close to the lumen were seen only in the central airways (levels 1 and 2), while profiles close to the basement membrane were seen in all three levels.

Nerve fibres have been found in the bronchial airway epithelium of several species, including man, by light microscopy with various staining techniques, such as methylene blue and silver methods.[1-5]

The presence of intraepithelial axons both in animals and in man has been confirmed at the ultrastructural level by the use of electron microscopy.[6] In man, however, only studies presenting illustrations of a single nerve fibre have been published.[7-9] Systematic studies giving detailed information of the concentration of axons at different airway levels have been carried out in several animal species[10-12] but none report the findings in human airway epithelium.

Understanding of the functional significance of intraepithelial nerves may be aided by detailed study of their distribution, organisation, and frequency in the human airway. The present study reports the results obtained from a systematic examination of bronchial biopsy specimens from human patients.

Methods

The specimens were obtained from five patients who were admitted to hospital and subjected to direct bronchoscopy because of a pulmonary infiltrate evident on the chest radiograph. All patients (one woman aged 40 years and four men aged 44–77 years) were found to be otherwise healthy on

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routine clinical examination. Their blood gas tensions at rest were normal. All patients had smoked for more than 15 years.

The specimens for this study were additional to those required for diagnostic purposes and permission to take them was obtained from the patients before bronchoscopy. Specimens were taken from three standard airway levels: (1) the middle of the trachea, (2) inside the right upper lobe bronchus, and (3) the right lower lobe from a subsegmental carina of a first order segmental bronchus. The specimens were fixed in 3% glutaraldehyde in 0.1 mol/l phosphate buffer at pH 7.4 for four hours at 4°C. After being rinsed in phosphate buffer they were postfixed with 1% OsO_4 for one hour. The postfixed tissue pieces were dehydrated in a graded series of ethyl alcohol and embedded in Epon.

For light microscopy 1 μm sections were stained with toluidine blue. Light microscopy was used to find epithelium in the block. Ultrathin sections were cut with a LKB Ultrotome I ultramicrotome and stained with uranyl acetate and lead citrate.[13] Coated copper slot grids 2 × 1 mm were used for quantitation, because with ordinary 200–300 mesh grids the whole section cannot be viewed. The sections were studied with a Jeol JEM-100 CX Temscan electron microscope operated at 60 kV.

For measuring the length of the epithelium, one whole section was first photographed with the electron microscope at a low magnification (×680). To calculate the number of the nerves, the section was then scanned at a high magnification (×5000). Later a photomontage was made from the neighbouring
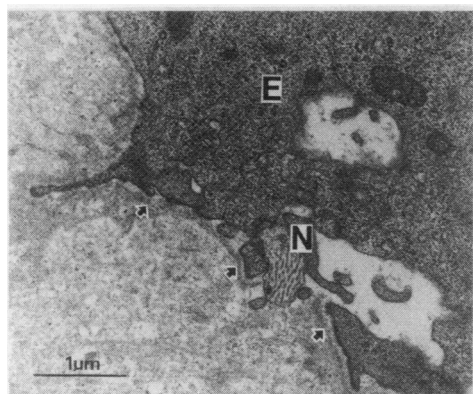
Fig 1   *Patient 5 (airway level 1): Nerve fibre (N) containing longitudinally cut neurotubuli penetrating the epithelial basement membrane (arrows). E—epithelium. (×12 300.)*

electronmicrographs with the low magnification and the length of the epithelial basement membrane measured with a map measurer. In the sections the measured lengths of the basement membrane varied from 300 to 554 μm.

Results

ORGANISATION OF INTRAEPITHELIAL NERVES
The photomontages gave a good general display of the epithelia. Neither light nor electron microscopic examination of the specimens showed pathological changes. The epithelia had a firm structure with ciliated, goblet, and basal cells. Cell junctions and intercellular spaces were normal, and all cellular organelles were well preserved.
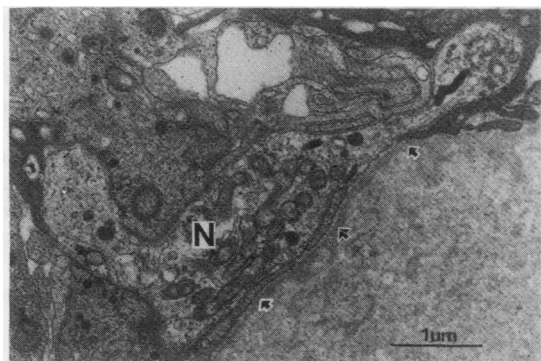

Fig 2   *Patient 1 (airway level 2): Intraepithelial nerve fibre (N) containing neurotubuli and mitochondria running parallel to the epithelial basement membrane (arrows). (×12 300.)*
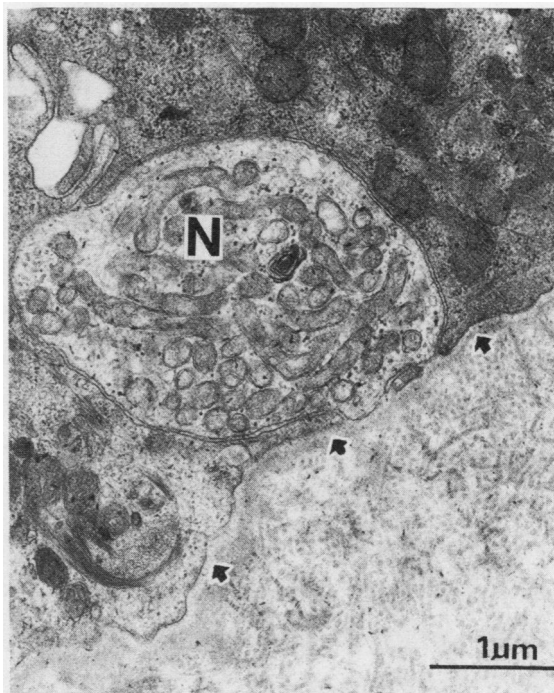

Fig 3   *Patient 1 (airway level 2): Axon profile (N) rich in mitochondria seen at the base of the epithelium close to the basement membrane (arrows). (×19 000.)*

In the lamina propria axons formed nerve bundles surrounded by Schwann cells and some of these axons were myelinated. The myelinated axons did not come into close contact with the epithelium, the closest distance observed between myelinated axon and the epithelial basement membrane being 30 μm. Axons penetrating the basement membrane (fig 1) and running in the epithelium and axonal profiles in the epithelium were all devoid of Schwann cells. Only a few axons could be seen to penetrate the epithelial basement membrane.

In the epithelium the axons were seen to run parallel to the basement membrane (fig 2) or to have a course towards the lumen between the columnar epithelial cells. The axonal profiles, containing vesicles, neurotubuli, and mitochondria, were predominantly located either near the basement membrane (fig 3) or close to the airway lumen beneath the tight junctions (fig 4). Axonal profiles close to the airway lumen were 0.4–3.5 μm from the luminal border (the luminal zone). At the base of the epithelium the greatest distance of axonal profiles from the basement membrane was 8 μm (the basal zone); but 90% of the profiles at the base were situated within a distance of 2.1 μm from the basement
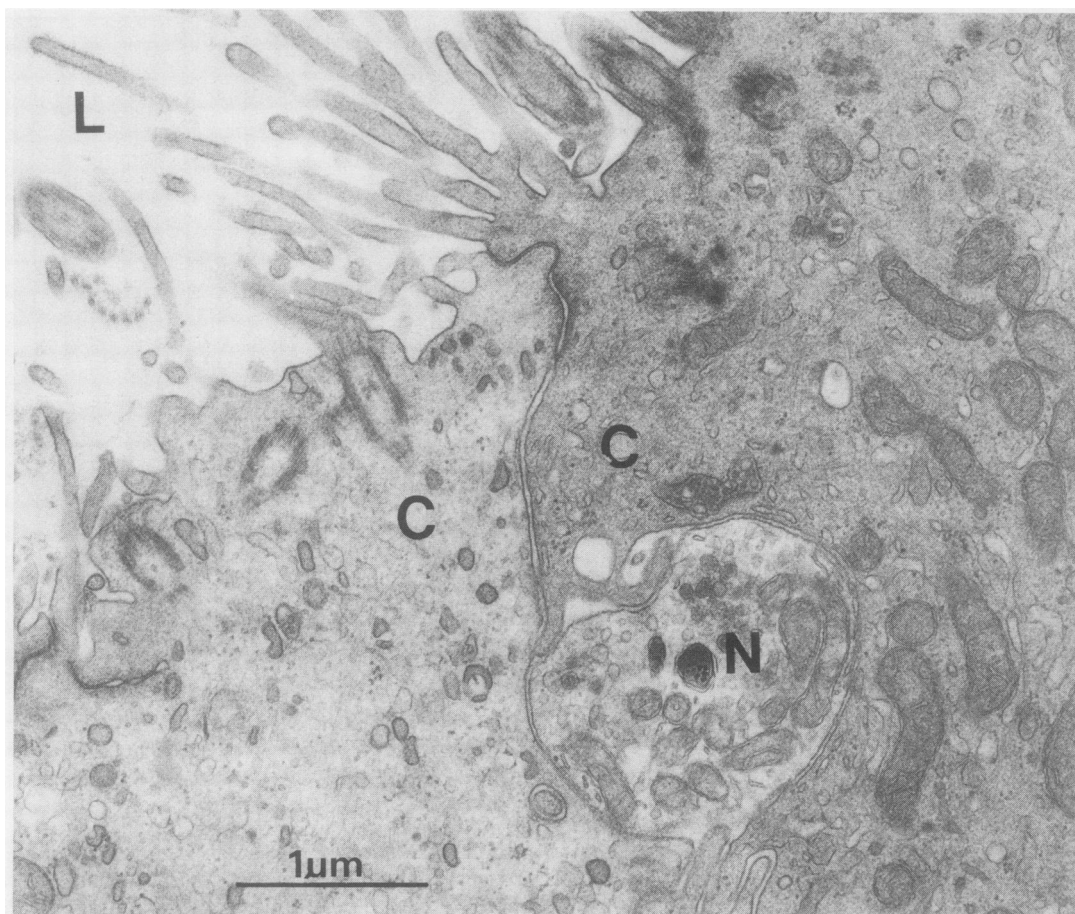
Fig 4 *Patient 4 (airway level 1): Axon profile (N) with mitochondria and vesicles located close to the airway lumen (L) between two ciliated cells (C). (×24 600.)*

membrane. Only two axon profiles were found in the epithelium between the luminal and the basal zones (the intermediate zone). In the intermediate zone only a few axons containing neurotubuli and filaments were observed to run between epithelial cells towards the lumen.

DISTRIBUTION AND FREQUENCY OF AXONS IN DIFFERENT AIRWAY LEVELS

The distribution and numbers of intraepithelial axon profiles are shown in the table. In the specimens from the trachea and right upper lobe bronchus (airway levels 1 and 2) the axon profiles were seen both near the lumen and near the basement membrane. In the specimens from the segmental bronchi (airway level 3) axon profiles were seen only close to the basement membrane and none were found near the lumen. In the basal zone the frequency of axon profiles was highest in the right upper lobe bronchus

(one axon profile per 131 μm), whereas in the middle of the trachea and in the segmental bronchus they were seen almost as often (one axon profile per 239 μm and 248 μm respectively). In the luminal zone most axon profiles (83%) were found in the samples from the middle of the trachea, and the rest (17%) were located in the samples from the upper lobe bronchus. In the luminal zone of the trachea one axon profile was found per 215 μm, while in the upper lobe bronchus the mean distance between axon profiles was 1118 μm.

Discussion

The present study is the first to establish the concentration of intraepithelial nerves at various airway levels in man. In the epithelium axon profiles seemed to have two predominant locations: they were situated either close to the airway lumen or at

Distribution and number of intraepithelial axon profiles

Patient No	Length of basement membrane in one section (µm)	No of axon profiles	
		Luminal zone†	Basal zone‡
Airway level 1*			
1	498	1	3
2	554	1	0
3	305	2	0
4	430	3	3
5	368	3	3
	2155	10	9
Airway level 2			
1	419	2	6
2	402	0	4
3	373	0	2
4	520	0	5
5	522	0	0
	2236	2	17
Airway level 3			
1	300	0	2
2	—	—	—
3	311	0	0
4	303	0	2
5	328	0	1
	1242	0	5
Totals	5633	12	31

*In addition to these 43 axon profiles two were found in airway level 1 intraepithelially between the luminal and the basal zone. Airway level 1—middle of the trachea; 2—right upper lobe bronchus; 3—segmental bronchus.
†0.4–3.5 µm from lumen.
‡Within 8 µm from basement membrane.

the base of the epithelium close to the basement membrane. Nerve fibres penetrating the basement membrane and traversing the epithelium from the base to the luminal side were also present. Furthermore, I could show the site at which the epithelial basement membrane was pierced by subepithelial nerves, which neither Jeffery and Reid[10] nor Das et al[11] were able to see in the rat and the cat respectively. Since only a few nerves are seen to penetrate the basement membrane and enter the epithelium probably the nerve fibres entering the airway epithelium divide further and form several axon profiles.

The present results show that there is a difference between different airway levels in respect of the location of intraepithelial nerves. Nerves near the lumen were mostly found in the larger airways—for example, the trachea—and relatively few were observed in the smaller airways. This is in accordance with the findings of previous animal studies.[10–12 14] On the other hand, in the base of the epithelium, although some differences between airway levels were seen, axon profiles were constantly found at every level. Ultrastructural studies in animals have rarely shown nerves in the intrapulmonary airways.[6]

The location of the intraepithelial nerves in different airway levels may have an important bearing on the production of bronchoconstriction and bronchodilatation. For instance, histamine, in addition to its direct local effects, is known to stimulate receptors in the airways, which results in vagally mediated bronchoconstriction and possible magnification of the changes in airway resistance.[15] In animals atropine or vagotomy significantly decreases the effect of histamine on airflow resistance, but does not alter the effect of histamine on lung compliance.[16 17] It has also been shown that histamine given by rapid intravenous injection causes a significant decrease in static lung compliance in man.[18] Irritating particles are mainly deposited at levels 1 and 2, the central airways, depending on their size. So the nerves close to the lumen could act as "primary" sensory receptors, which by causing cough or acute bronchoconstriction could act as a first clearing mechanism in the airways. In dogs it has been shown that the major conducting airways are the principal location of rapidly adapting "irritant receptors."[19] When there is a defect in permeability[20] or damage to the epithelium[9] the nerves close to the basement membrane could act as "secondary" receptors, which might cause more general bronchoconstriction (including also level 3).

Physiological studies have confirmed the presence of sensory receptors in mammalian airways and have indicated their close association with the airway epithelium.[21–23] Sensory input from the airways has also been recorded in man by physiological measurement.[24 25] It has been suggested that vagally mediated airway hyperreactivity occurs as a result of irritant receptor sensitisation.[26] At the ultrastructural level, however, the afferent site of these reflexes has not yet been found. Because the epithelium with its tight junctions, cells, and basement membrane forms a very firm barrier to luminal irritants, nerves associated with afferent activity might reasonably be expected to be situated close to the airway lumen. This study shows that in the human airway epithelium there are axon profiles that by ultrastructural criteria[12] resemble sensory nerve endings from which afferent stimuli may originate. The present results are supported by the findings of Lauweryns et al, who have described intraepithelial nerves in human airways in close contact with neuroepithelial bodies.[27] Their animal studies with the selective nerve degeneration method indicate that most of the nerves close to the neuroepithelial bodies are afferent.[28]

Morphological studies alone cannot establish whether the intraepithelial nerves are afferent or

motor; but our finding of intraepithelial nerves in man give a morphological basis for the transmission of afferent stimuli from the airway.

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