Eur Respir J
1991, 4, 673–682

Substance P immunoreactive nerves in airways from asthmatics and nonasthmatics

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Substance P immunoreactive nerves in airways from asthmatics and nonasthmatics. S.L. Ollerenshaw, D. Jarvis, C.E. Sullivan, A.J. Woolcock.
ABSTRACT: Substance P has been localized to nerves supplying smooth muscle, blood vessels and glands in the human lung and may play a major role in the pathophysiology of asthma. We performed a morphological study, using the avidin biotin peroxidase immunostaining technique, to examine sections of airway wall from subjects with and without asthma for the presence of substance P immunoreactive nerve fibres. Airways of 200 μm-12 mm were obtained from autopsy, lobectomy and bronchoscopy. Quantitative morphological analysis was performed on 3 mm diameter airways from three asthmatic and three nonasthmatic subjects collected at autopsy, and on biopsies of 10 mm diameter airways from eight asthmatic and thirteen nonasthmatic subjects. There was an increase in both the number and the length of substance P immunoreactive nerve fibres, in airways from subjects with asthma when compared with airways from subjects without asthma. Fibres were found in the lamina propria and surrounding vessels and glands. The fibres were commonly seen as bundles rather than as single fibres. There was no difference in the number of substance P nerves between normal subjects and subjects with chronic airflow limitation (CAL). The difference in the number, length and morphological characteristics of the substance P immunoreactive nerves between asthmatic and nonasthmatic subjects were striking.
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Keywords: Asthma; Immunocytochemistry, neuropeptide; substance P.

Accepted for publication August 7, 1990.

Work supported by grants from NHMRC Australia and Asthma foundation of NSW, Australia.

We recently reported the absence of vasoactive intestinal peptide (VIP) in airways of subjects with severe asthma [1]. In the present study, the same tissue plus biopsy specimens obtained from less severe asthmatic subjects were studied for the presence of substance P immunoreactive nerve fibres.

The neuropeptide substance P is one of numerous tachykinins present in the human lung [2, 3]. Substance P immunoreactive nerve fibres are found within and beneath the airway epithelium, around blood vessels and, to a lesser extent, in the smooth muscle [4, 5]. The role of these fibres in humans is undetermined. However, animal studies show that substance P is only present in sensory nerves in the respiratory tract [6], and release of substance P occurs following antidromic chemical or mechanical stimulation [7].

In vitro studies show that substance P contracts human airway smooth muscle [8], increases microvascular leakage in animal airways [9] and human skin [10], and stimulates mucus secretion in isolated human airways [11]. Therefore, it may play a role in the pathophysiology of asthma, a disease characterized by bronchoconstriction, oedema and mucus plugging of the small airways. In addition, epithelial desquamation is common, and this may expose sensory nerve fibres. Antidromic stimulation of these afferent nerve fibres by inflammatory mediators may result in the release of tachykinins, such as substance P. This hypothesis, that an axon reflex may be present in the airways, was first put forward by LUNDBERG and SARIA in 1983 [9].

Recent *in vivo* human studies have provided support for the role of the axon reflex in asthma. Both inhaled and infused substance P produce bronchoconstriction [12, 13], and substance P has a capacity to initiate and modulate leucocyte responses. Also, the presence of substance P receptors on inflammatory cells allows speculation that substance P may play a role in asthma [14].

To date there have been no studies describing the distribution of substance P immunoreactive nerve fibres in airways from subjects with asthma. In this morphological study we obtained lung tissue from subjects with and without asthma and stained it for substance P using the avidin biotin peroxidase

immunocytochemical technique. Differences in substance P immunoreactivity in the two groups were quantified using computer image analysis.

Patients and material

Tissue was obtained from 13 subjects with asthma, three at autopsy, two who underwent lobectomy for the treatment of carcinoma of the lung and eight at bronchoscopy. Twenty two nonasthmatic subjects were selected as a control group consisting of eight normal subjects with no respiratory illness, and fourteen subjects with mild chronic airflow limitation (CAL). The control tissue was collected from four normal subjects at autopsy, five subjects with mild CAL at lobectomy, and four normals and nine subjects with CAL at bronchoscopy.

Subjects undergoing lobectomy or bronchoscopy had spirometric function and a bronchial provocation test using methacholine, at least twenty four hours before tissue collection [15]. Clinical histories were obtained from case records, pathology reports, medical practitioners and the patient (where applicable). The clinical details of the autopsy and lobectomy subjects are shown in table 1 and the subjects from whom biopsies were collected in table 2.

Airways ranging from 200 μm–12 mm in diameter were dissected from the seven autopsy and seven lobectomy subjects from areas where no gross pathological changes were present. Autopsy specimens were collected and fixed within 24 h of death and lobectomy specimens within 2 h of surgery.

From each of the 21 subjects undergoing bronchoscopy, four biopsies were taken in the right middle lobe from airways 8–10 mm diameter. All biopsy specimens were obtained from areas where no gross pathological changes were present and were placed in fixative immediately.

Two representative airway diameters, 3 mm and 10 mm, were chosen for computer image analysis to control for the effect of airway diameter on the amount of substance P immunoreactive fibres. The airway diameter was determined as the average distance across the airway lumen measured from the basement membrane.

The 3 mm diameter airways were randomly selected from three subjects with asthma and three subjects without asthma from the 14 autopsy and lobectomy subjects. The six subjects were chosen on the basis that they were nonsmokers and all three asthmatics had died of asthma. Ten sections, which were at least 80 μm apart, were randomly chosen from each of the six subjects and the number of substance P fibres and fibre length (in mm) were measured in the epithelium, basement membrane, lamina propria, bronchial smooth muscle and surrounding vessels and glands.

Computer analysis was also performed on biopsy specimens collected from 10 mm diameter airways from each of the 21 bronchoscopy subjects. Ten sections, at least 80 μm apart, were randomly chosen from each subject and the number of substance P fibres and fibre length (in mm), were measured in the whole biopsy.

Methods

Immunocytochemical techniques

All tissue samples collected at autopsy, lobectomy and bronchoscopy were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer at 4°C for 8 h. After fixation, the specimens were transferred to 25% sucrose for at least 12 h. The specimens were then frozen in liquid nitrogen and sectioned at 20 μm on a Tissue Tek II cryostat.

Table 1. – Clinical characteristics of subjects from whom tissue was collected at autopsy or lobectomy

Subject No.	Disease	Age	Smoker	Autopsy or lobectomy	Mode and cause of death Reason for lobectomy	Asthma medication
1*	Asthma	23	No	Autopsy	Sudden death from asthma	Salbutamol, BDP
2*	Asthma	47	No	Autopsy	Sudden death from asthma	Salbutamol, BDP, oral steroids
3*	Asthma	52	No	Autopsy	Death from asthma after 1 month hospitalization for asthma	Salbutamol, theophylline, BDP, oral steroids
4	Asthma	65	Yes	Lobectomy	Primary adenocarcinoma	Salbutamol, BDP
5	Asthma	67	Yes	Lobectomy	Primary adenocarcinoma	Salbutamol
6	Normal	30	No	Autopsy	Sudden death, car accident	Nil
7*	Normal	15	No	Autopsy	Sudden death, car accident	Nil
8*	Normal	62	No	Autopsy	Sudden death, myocardial infarct	Nil
9	Normal	60	No	Autopsy	Sudden death, ruptured aorta	Nil
10	CAL	66	Yes	Lobectomy	Large cell carcinoma	Nil
11	CAL	64	No	Lobectomy	Secondary adenocarcinoma	Nil
12	CAL	59	Yes	Lobectomy	Primary adenocarcinoma	Nil
13*	CAL	25	No	Lobectomy	Carcinoid tumour	Nil
14	CAL	50	Yes	Lobectomy	Small cell carcinoma	Nil

*: computer image analysis subjects; CAL: chronic airflow limitation; BDP: beclomethasone dipropionate.

Table 2. – Clinical characteristics of subjects from whom tissue was collected at bronchoscopy

Subject No.	Disease	Age	Smoker	PD_{20}	FEV_1 %pred	FVC %pred	Asthma medication
15	Asthma	59	Yes	1.5	57	70	Nil
16	Asthma	30	No	0.65	85	80	Salbutamol, ipratropium bromide, oral steroids, sodium cromoglycate, BDP
17	Asthma	25	No	0.6	85	80	Salbutamol, BDP
18	Asthma	40	Yes	4.0	88	83	Nil
19	Asthma	65	No	4.0	72	65	Salbutamol
20	Asthma	44	No	1.0	110	113	Salbutamol, BDP
21	Asthma	26	No	0.2	75	72	Salbutamol
22	Asthma	27	Yes	0.15	70	73	Salbutamol
23	Normal	55	Yes	0	92	82	Nil
24	Normal	35	No	0	69	75	Nil
25	Normal	47	Yes	0	92	88	Nil
26	Normal	56	No	0	69	74	Nil
27	CAL	60	Yes	0	34	46	Nil
28	CAL	68	Ex	8.0	65	55	Nil
29	CAL	73	Ex	0	60	42	Nil
30	CAL	49	Ex	0	60	70	Nil
31	CAL	61	Ex	0	40	52	Nil
32	CAL	66	Ex	0	77	84	Salbutamol, BDP, theophylline
33	CAL	64	Yes	0	75	70	Nil
34	CAL	58	Ex	0	35	50	Salbutamol
35	CAL	76	Ex	0	60	72	Salbutamol

CAL: chronic airflow limitation; BDP: beclomethasone dipropionate; FEV_1: forced expiratory volume in one second; PD_{20}: provocative dose producing a 20% fall in FEV_1; FVC: forced vital capacity.

The sections were stained either immunocytochemically, for the presence of substance P, or histologically, with haematoxylin and eosin or toluidine blue, to study the pathological features of the airways.

The avidin biotin peroxidase complex technique (ABC), [16] was used for immunostaining tissue samples for substance P. In brief, sections were incubated for 12 hours at 4°C with an anti-substance P antibody (Sera Lab) at a dilution of 1:500 in phosphate buffered saline containing 0.05% Triton X-100 (PBS-Triton). The sections were washed in PBS-Triton and a secondary biotinylated anti-rat immunoglobulin G (IgG) antibody (Vector Laboratories, Burlingame, California) at a dilution of 1:50 in PBS-Triton was applied to the sections for one hour at room temperature. The sections were washed again in PBS-Triton and the avidin biotin peroxidase conjugate was applied for one hour at the concentration recommended by the manufacturer (Vector Laboratories). The sections were rinsed in tris buffer (Sigma, St Louis) containing 0.05% nickel ammonium sulphate and soaked in this buffer for 20 min. The tissue was then allowed to react for 10 min in a solution of tris nickel buffer containing 2 µl of 25% hydrogen peroxide (Boehringer, Ridgefield, Conn) per 10 ml and 5 mg of diaminobenzidine (DAB) (Sigma) per 10 ml for 10 min. To stop the reaction, the sections were rinsed in tris nickel buffer, and finally dehydrated in graded alcohols and cover-slipped from xylene.

To determine if there was any nonspecific immunocytochemical staining to substance P, we used two control techniques. Sections were either incubated in antiserum preabsorbed with excess substance P, or the primary antibody was omitted. The sections were then immunocytochemically stained for the presence of substance P. The two tests were performed alternately on every fourth section from autopsy and lobectomy specimens.

Following immunostaining, every section from each subject was examined by light microscopy. The morphology, amount and location of substance P immunoreactive nerve fibres were noted.

Computer image analysis

Computer image analysis was performed on a total of 270 sections, 10 sections from each subject, randomly chosen from the autopsy, lobectomy and bronchoscopy subjects. Each section was placed under a Zeiss Axioplan light microscope. A clear image was collected *via* a Sony DXC-3000P colour video camera into the Tracor Northern TN-8502 screen which then produced a digitized image. Each section was examined from the epithelium to the submucosa. Substance P immunoreactive nerve fibres were identified and traced to provide a binary image from which the fibre length could be calculated. Images were viewed at high power

(1100 ×) without the use of photomicrographs, and the digitized image was constantly compared with the live image through the attached light microscope. The location of each fibre was recorded.

The mean number of substance P immunoreactive fibres and mean fibre length, (in $mm \cdot mm^{-2}$), were calculated for each subject. On this data a one way analysis of variance test was performed to determine if there was a significant difference in the mean number of fibres and mean fibre length between subjects with asthma, subjects with CAL and normals.

Results

Haematoxylin and eosin stain

In the normal subjects there were no gross abnormalities seen with light microscopy (fig. 1). Airways of all sizes had normal bronchial mucosa with intact ciliated pseudo-stratified columnar epithelium. The airway lumens were clear. There was no oedema in any of the tissue layers but on occasions a moderate lymphocytic infiltration was present in the lamina propria. Goblet cell numbers appeared normal and there was no submucosal gland hypertrophy.

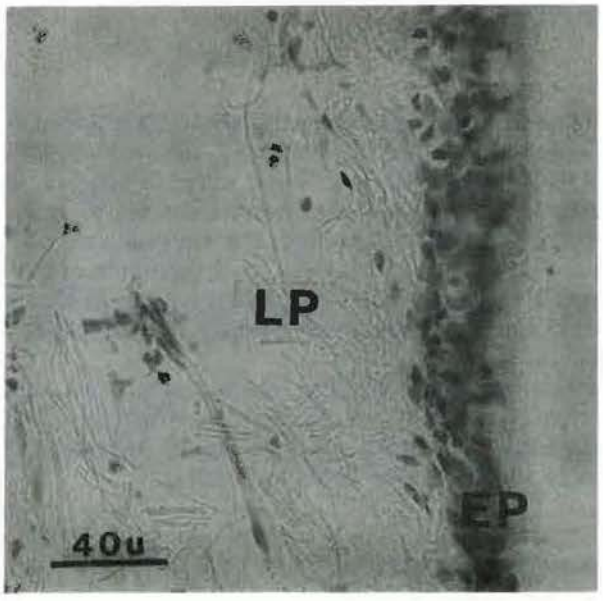


Fig. 1. – Biopsy section of a 10 mm diameter airway from a subject with no respiratory illness. Ciliated columnar epithelium is intact (EP) and the lamina propria is clear (LP). (Haematoxylin and eosin, ×330).

In airways from subjects with CAL there was a moderate loss of cilia and numerous goblet cells in the epithelium. The lamina propria was infiltrated with lymphocytes and on occasions oedematous (fig. 2).

In the subjects with asthma the classical pathological features of asthma were present (fig. 3). Tissue from all of the subjects with asthma showed patchy loss of

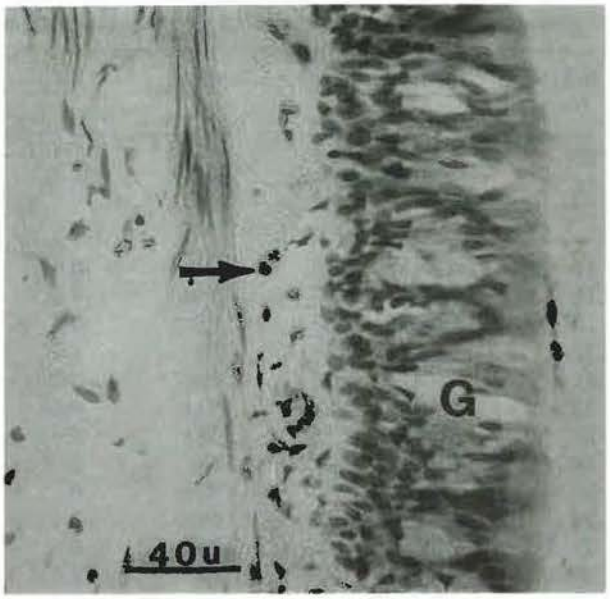


Fig. 2. – Biospy section of a 10 mm diameter airway from a subject with CAL showing lymphocytes (arrow) in the lamina propria and submucosa. Note the numerous goblet cells (G) in the epithelium. (Haematoxylin and eosin, ×330).

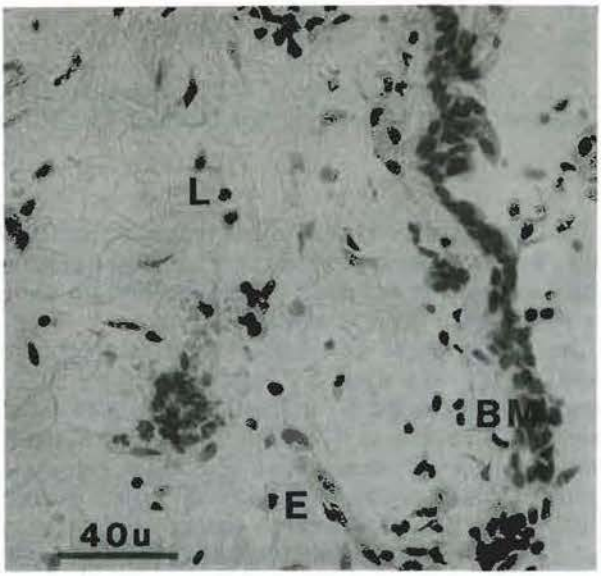


Fig. 3. – Biopsy section of a 10 mm diameter airway from a subject with moderate asthma. Basal cells only line the airway and the basement membrane (BM) is thickened. The lamina propria contains numerous inflammatory cells, lymphocytes (L), eosinophils (E). (Haematoxylin and eosin, ×330).

epithelium which was particularly marked in the subjects who had died of asthma. Even in areas where some epithelium was present it was abnormal with squamous metaplasia or stratified cuboidal cells typical of regenerating epithelium. Frequently the airway lumen was filled with cell debris and mucus plugs. The basement membrane was thick (ranging from 10–15 μm) and the bronchial smooth muscle and submucosal glands were hypertrophied. The lamina propria and smooth muscle were oedematous and contained an inflammatory infiltrate comprised of lymphocytes, eosinophils,

neutrophils and monocytes. Inflammatory cells were also seen in the submucosa and were predominantly lymphocytes.

The overall wall thickness of the asthmatic airways was approximately 1.5 times that of the nonasthmatic airways and most of this increase appeared to be due to oedema.

Immunostaining

In the technique control sections, neither preabsorption of the primary antibody with excess substance P nor omission of the primary antibody produced any immunostaining when reacted with the avidin biotin peroxidase complex technique.

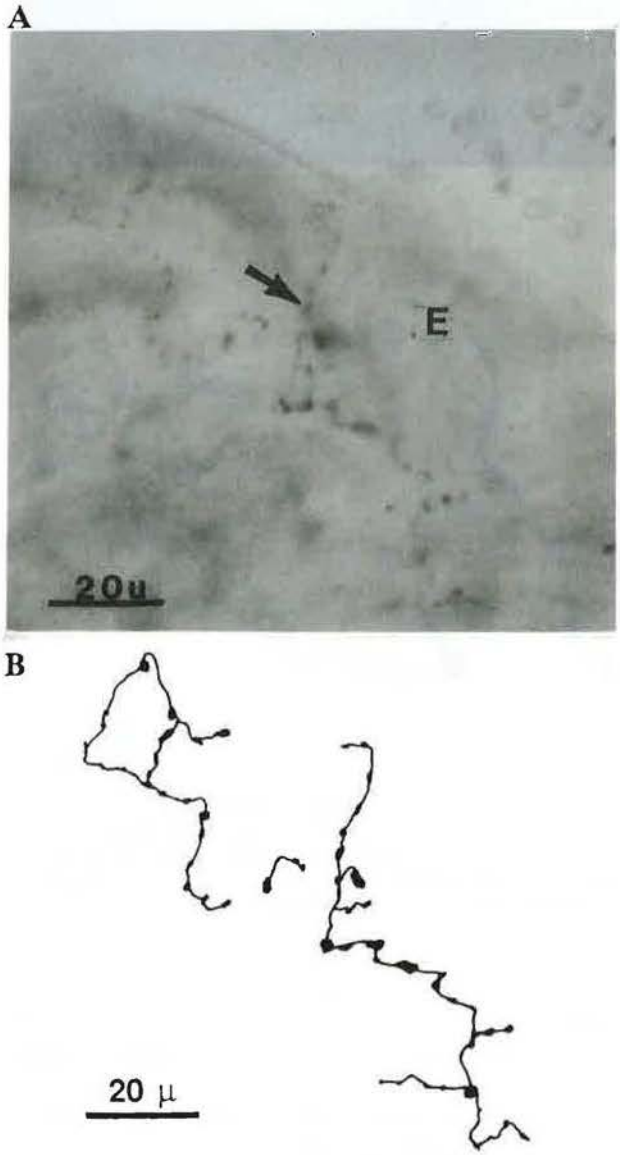


Fig. 4. – A) Nerve fibres immunoreactive to substance P in the basement membrane and epithelium of a subject without asthma. Varicosities can be seen along the fibres (arrow), (E) denotes the epithelium (×660). B) Camera lucida tracing of the substance P nerve fibres seen in figure 4A.

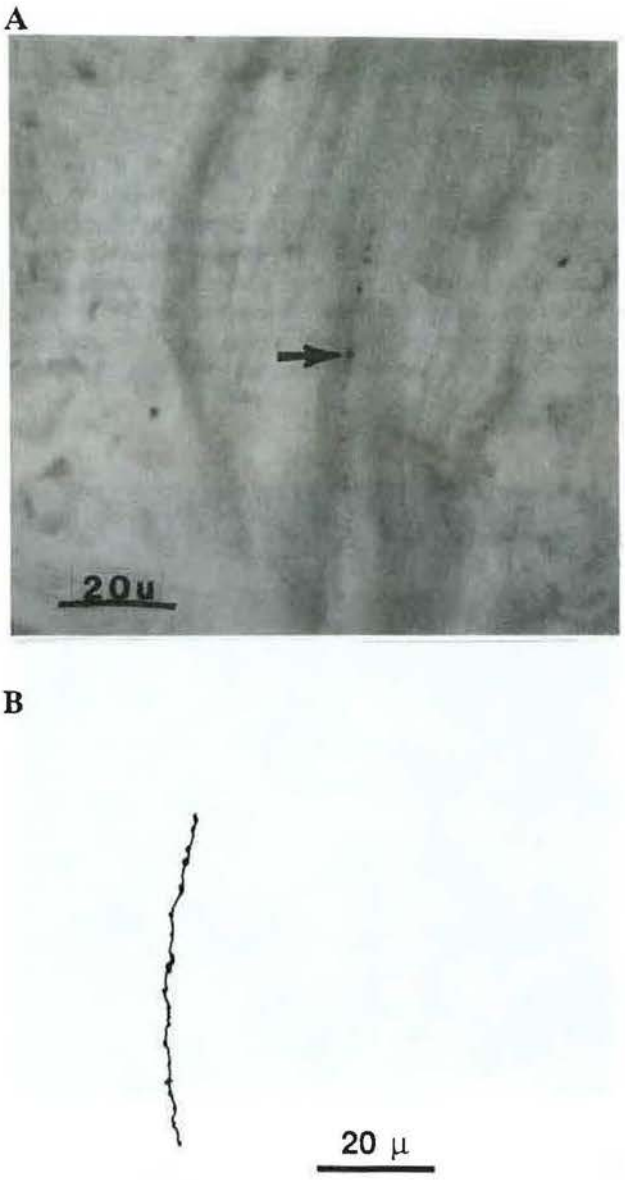
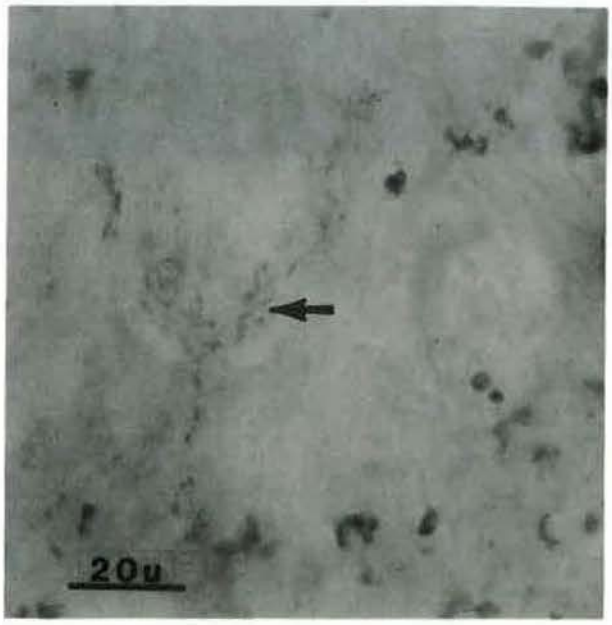


Fig. 5. – A) A single, short, nonbranching nerve fibre immunoreactive to substance P (arrow) in the bronchial smooth muscle of a subject without asthma, (×660). B) Camera lucida tracing of the substance P nerve fibre seen in figure 5A.

In tissue from subjects with mild CAL and normals, substance P immunoreactive nerves were seen as single short fibres in the basement membrane with branches extending into the epithelium (fig. 4). Fibres were also seen in the bronchial smooth muscle (fig. 5) and lamina propria. In the submucosa, single fibres were only occasionally seen in close proximity to the glands and post capillary venules. Fibres in all areas had varicosities along their entire length. There was no difference in the morphology of the substance P nerves between subjects with mild CAL and normals.

In contrast, in tissue from subjects with asthma, substance P immunoreactive nerves were present as branching networks or large fibre bundles and rarely seen as single fibres. There were numerous branching substance P fibres and fibre bundles in the bronchial smooth muscle (fig. 6) and lamina propria (fig. 7) with branches from fibres in the lamina propria extending into the basement membrane. A few fibres were seen in the areas of normal epithelium, but no fibres were found in the abnormal epithelium. Single fibres were seen near the post capillary venules in the submucosa and on occasions large fibre bundles were located between the acini of glands.

A



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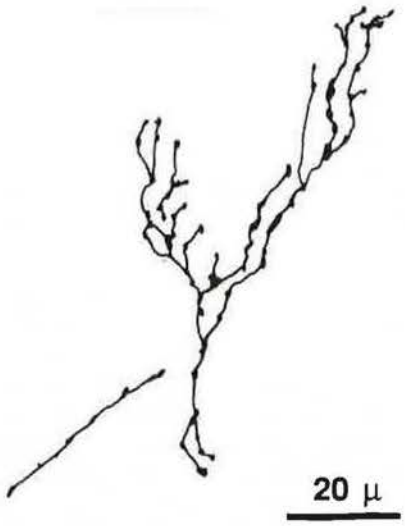
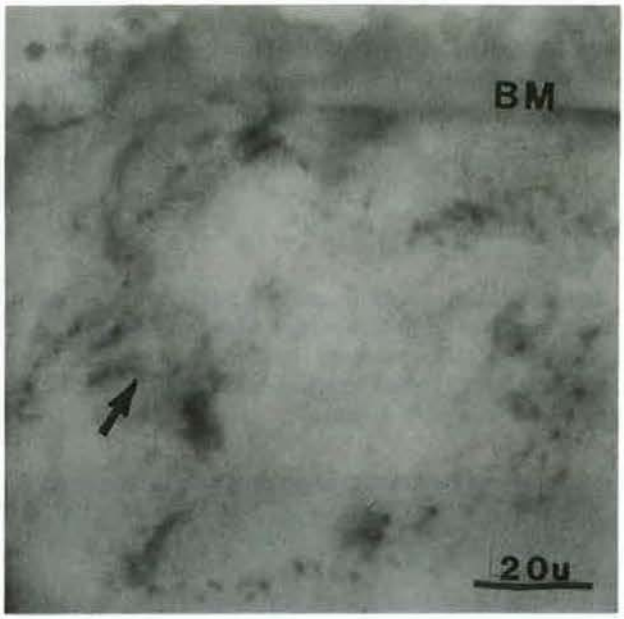


Fig. 6. – A) A bifurcating bundle of nerve fibres immunoreactive to substance P in the bronchial smooth muscle of a subject with asthma (arrow) (×660). B) Camera lucida tracing of the substance P nerve fibres seen in figure 6A.

A



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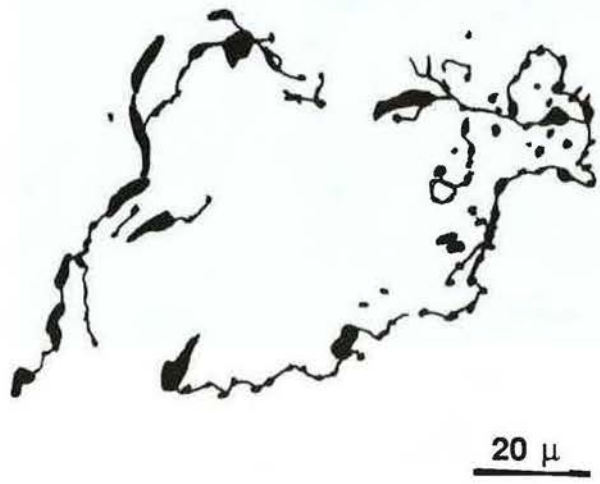

Fig. 7. – A) Numerous branching nerve fibres immunoreactive to substance P (arrow) can be seen in the lamina propria of a subject with asthma. Note the damaged epithelium and the thickened basement membrane (BM), (×660). B) Camera lucida tracing of the substance P nerve fibres seen in figure 7A.

The overall impression was that in the airways from all subjects with asthma there were more substance P immunoreactive nerve fibres in the lamina propria, and in close proximity to vessels and glands. Subjects who had died of asthma had more extensive fibre branching than subjects with mild to moderate asthma. There was not only a difference in quantity, but also a difference in the morphology of these fibres. Branching networks and large fibre bundles dominated and single fibres were uncommon.

Table 3. – Mean number and length of substance P immunoreactive nerve fibres per mm², in 3 mm diameter airways from autopsy and lobectomy subjects

Subject No.	Disease	Mean number fibres·mm^{-2}	Mean fibre length mm·mm^{-2}
1	Asthma	1.45	0.078
2	Asthma	1.42	0.072
3	Asthma	0.73	0.029
7	Normal	0.56	0.025
8	Normal	0.52	0.025
13	CAL	0.66	0.027

CAL: chronic airflow limitation.

Computer image analysis

In 3 mm diameter airways, collected at autopsy or lobectomy both the overall mean number of substance P immunoreactive fibres and fibre length per mm² were increased in subjects with asthma. The results are shown in table 3. The average number of fibres for the three subjects with asthma was 1.2±0.3 compared with 0.58±0.058 in the three subjects without asthma. The average fibre length was 0.0597±0.02 mm in the asthmatic subjects and 0.0257±0.0009 mm in the nonasthmatic subjects.

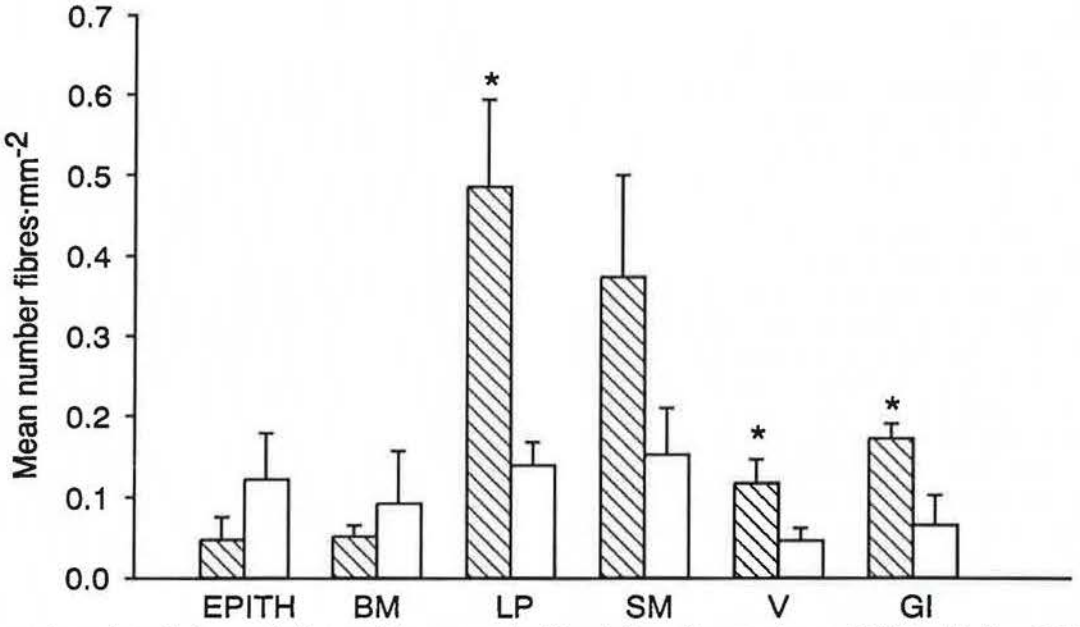

Fig. 8. – Mean number and standard error of substance P immunoreactive fibres in 3 mm diameter airways. EPITH: epithelium; BM: basement membrane; LP: lamina propria; SM: submucosa; V: vessels; GI: glands. ▨ : asthma (n=3); □ : control (n=3); *: p<0.05.

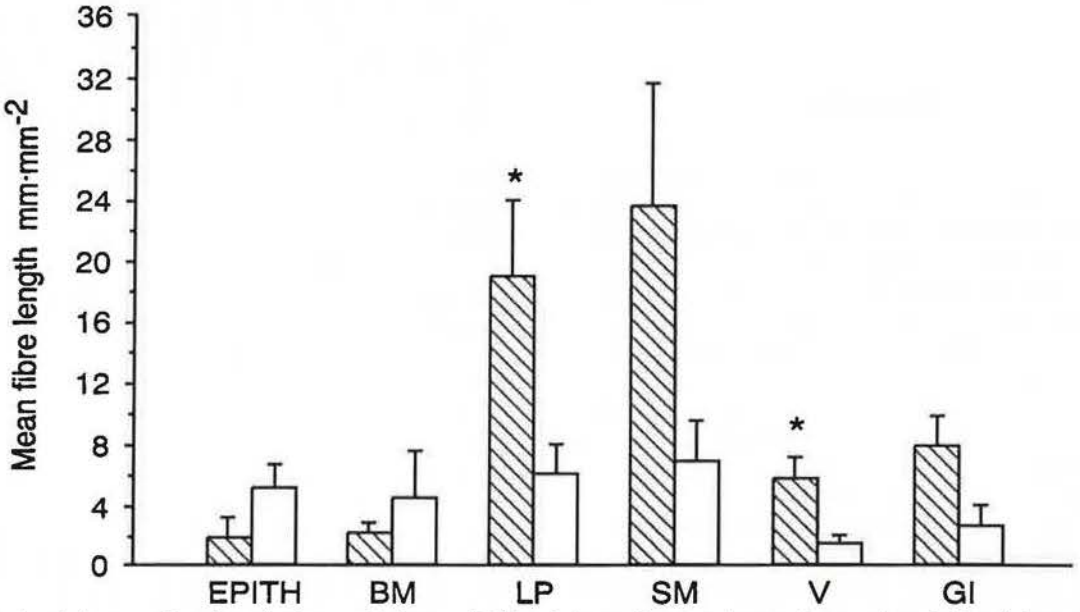

Fig. 9. – Mean length (mm·mm^{-2}) and standard error of substance P fibres in 3 mm diameter airways. For meaning of abbreviations see legend to figure 8. ▨ : asthma (n=3); □: control (n=3); *: p<0.05.

Table 4. – Mean number and length of substance P immunoreactive nerve fibres per mm², in biopsy specimens of 10 mm diameter airways

Subject group	Number of subjects	Number of fibres (mean±SE)	Fibre length (mean±SE)
Asthma	8	2.188±3.36	0.128±0.203
Normal	4	0.299±0.419	0.012±0.022
CAL	9	0.210±0.443	0.013±0.021

CAL: chronic airflow limitation; SE: standard error.

In the airways from subjects with asthma there was a significant increase in the number of substance P immunoreactive fibres in the lamina propria (p=0.015), and surrounding vessels (p=0.046) and glands (p=0.02) when compared to subjects without asthma (fig. 8). There was also a significant increase in the mean fibre length in the lamina propria (p=0.045) and surrounding the vessels (p=0.027) (fig. 9).

In the biopsy sections from airways 10 mm in diameter it was not possible to measure the number and length of substance P immunoreactive fibres in separate tissue layers because of the limited size of the specimen. Therefore, in these sections the number and length of fibres has been determined for the whole section. Table 4 shows the mean values for the number and length of substance P immunoreactive fibres in 10 mm diameter airways from subjects with asthma, subjects with CAL and normal subjects. There was no significant difference in the mean number of fibres (p=0.9) and mean fibre length (p=0.9) per mm² in the biopsy sections from normals and subjects with mild CAL. We then compared subjects with asthma to sub jects without asthma and there was a significant increase in the mean number of fibres (p=0.044) and fibre length (p=0.05) per mm² in the sections from subjects with asthma.

Discussion

This study has shown that 3 mm and 10 mm diameter airways from subjects with asthma contain more substance P immunoreactive nerve fibres than those from subjects without asthma. There is also a clear morphological difference in the presentation of fibres in the asthmatic airways. These changes were most marked in the lamina propria and surrounding-post capillary venules and glands.

The avidin biotin peroxidase technique has been widely used for immunostaining and has been shown to be both reliable and superior to the previously described peroxidase antiperoxidase technique [16].

The primary antibody to substance P is a monoclonal antibody and is directed against the carboxyl terminal fragments of substance P. The antibody reacts with the 5–8 C-terminal fragments as well as intact substance P. In competitive binding studies, somatostatin, B-endorphin and met- and leu-encephalin were not able to displace substance P. A low level of cross reaction was found with the non-mammalian tachykinin eledoisin only.

Both preabsorption of the primary antibody with substance P and omission of the primary antibody eliminated labelling in the lung tissue in both the asthmatic and nonasthmatic subjects. This suggests that the observed reaction product reflects substance P distribution. On occasions inflammatory cells stained black with diaminobenzidine (DAB) and this could be blocked by pretreating the sections with 100% methanol and H_2O_2 without eliminating the staining of nerve fibres. This suggests that the staining of the inflammatory cells was due to a reaction with peroxidase in the cells and not immunoreactive to substance P.

We can think of no systematic differences in the way the tissue was collected, fixed and stained which could affect the results. Tissue was fixed within 24 h of collection to avoid degradation of the neuropeptide and it is unlikely that there was a systematic increase in degradation in the nonasthmatic airways. The enzyme neutral endopeptidase (NEP) is found in airway epithelium and is known to degrade substance P [17, 18]. In this study we report an altered substance P fibre morphology and increased fibre density, however we do not know if there is an increase in the actual amount of substance P. The loss of airway epithelium in asthmatic airways may result in a reduction in the level of NEP and a subsequent increase in substance P. However, there is no evidence that NEP alters the actual fibre morphology and density. Paraformaldehyde fixation used in immunocytochemistry does not greatly reduce the substance P content of myenteric tissue extracts [19]. Thus, it is likely that the differences observed in the present study are representative of the substance P originally contained in the tissue.

Computer image analysis was used to confirm the differences found by microscopic examination. Although this is a time consuming technique, it provides a more accurate measure of fibre length than the digitizing pad or camera lucida techniques [20]. On occasions fibres were visible in two focal planes in the same field of view. By adjusting the focus on the light microscope and redigitizing the image a more accurate measure of fibre length could be made. All sections were 20 μm thick and each fourth section was stained for substance P. The sections analysed were randomly chosen and were at least 80 μm apart and all sections were cut circumferentially to the bronchus. It is therefore unlikely that we were identifying different portions of the same nerve fibre in different sections.

Quantitative analysis of substance P immunoreactivity was performed in 3 mm and 10 mm diameter airways from asthmatics and nonasthmatics for two reasons. Firstly, examination of similar sized airways in the asthmatic and control group made it possible to compare the number of fibres within each cell layer. Secondly, VIP immunoreactive nerves in the human lung are present in decreased numbers in the small airways [21]

and it is possible that the distribution of substance P immunoreactive nerves might also change with airway size.

The pathological changes in the asthmatic airways were so marked that it was not possible to perform the observations completely blind. We decided, therefore, to perform the measurements initially using two observers who were blinded to the detailed clinical characteristics of each subject. There was excellent agreement about what constituted a substance P immunoreactive nerve fibre in both asthmatic and nonasthmatic tissues. For this reason, and because observer mistakes would be likely to occur randomly in both asthmatic and nonasthmatic airways, it is most unlikely that observer bias could explain the significant differences seen in the number and length of substance P immunoreactive nerve fibres and fibre morphology in 3 mm and 10 mm diameter airways.

In the airways from subjects with asthma there was an increase in wall thickness, which was due to oedema, however it is unlikely that this affected the results. Airway wall thickening should cause a decrease in the fibre density, however we report an increase. In addition the fibre morphology was very different in the asthmatic airways.

Asthma produces patchy pathological changes in the airways [22] and in the present study, some areas of the airway wall in asthmatic subjects were only slightly abnormal while others displayed moderate to severe inflammation. However, we were unable to find a relationship between severity of inflammation and the number of substance P immunoreactive nerve fibres in a particular area of airway wall. Similarly we could find no association between the degree of lymphocytic infiltration in the nonasthmatics and the number of substance P immunoreactive nerve fibres.

The cause of the increase in substance P immunoreactive nerve fibres is not known. It has been reported that substance P nerves may diminish with age and with smoking [3]. The asthmatic and nonasthmatic subjects from whom tissue was collected at autopsy or lobectomy were all nonsmokers, however our sample size was too small to draw conclusions about the effects of smoking or of age. It also seems unlikely that the differences were due to the medication being taken by the asthmatic subjects, although this cannot be excluded. It seems more likely that the increase in fibre number and length, and the changes in fibre morphology in the airways from subjects with asthma is caused either from constant stimulation or by lack of inhibition of these nerves and their contained peptides.

We recently reported an absence of VIP immunoreactive nerves in airways of the severe asthmatics, ranging from 200 μm to 12 mm diameter [1]. This observation and the morphological differences reported in this study suggest that substance P and VIP may be involved in the pathophysiology of asthma, but further studies including measurement of amounts of neuropeptides with radioimmunoassay are needed.

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Nerfs immumno-réactifs à la substance P dans les voies aériennes de sujets asthmatiques ou non. S. Ollerenshaw, D. Jarvis, C.E. Sullivan, A.J. Woolcock.

RÉSUMÉ: La substance P a été individualisée au niveau des nerfs qui contrôlent le muscle lisse, les vaisseaux sanguins et les glandes du poumon humain. Elle pourrait jouer un rôle majeur dans la physiopathologie de l'asthme. Nous avons conduit une étude morphologique au moyen de la technique d'immuno-coloration avec l'avidine biotine peroxidase, pour examiner des coupes des parois des voies aériennes de sujets avec ou sans asthme et y rechercher la présence de fibres nerveuses immuno-réactives à l'égard de la substance P. Des voies aériennes de 200 μm à 12 mm ont été prélevées lors d'autopsies, de lobectomies ou de broncoscopies. L'analyse morphologique quantitative a été conduite sur des voies aériennes de 3 mm de diamétre provenant de 3 sujets asthmatiques et de 3 sujets non asthmatiques prélevée à l'autopsie, et sur les biopsies réalisées sur des voies aériennes de 10 mm de diamètre chez 8 asthmatiques et 13 sujets non asthmatiques . L'on a noté, dans les voies aériennes des sujets atteints d'asthme, une augmentation du nombre et de la longueur des fibres nerveuses immuno-réactives à l'égard de la substance P, par comparaison avec celles de sujets sans asthme. Les fibres ont été décelées dans la lamina propria et dans les vaisseaux et les glandes qui l'entourent. Les fibres se présentent sous forme de faisceaux plutôt que comme fibres isolées. L'on n'a pas relevé de différence dans le nombre de nerfs réactifs pour la substance P entre les sujets normaux et ceux atteints de limitation chronique des débits aériens. Les différence dans le nombre, la longueur et les caractéristiques morphologiques des nerfs immuno- réactifs pour la substance P étaient très marquées entre les sujets asthmatiques et non asthmatiques.
Eur Respir J., 1991, 4, 673–682.