

Expression of tyrosine hydroxylase and neuropeptide tyrosine in mouse sympathetic airway-specific neurons under normal situation and allergic airway inflammation

Q. T. Dinh*†, D. A. Groneberg†, C. Witt‡, C. Peiser†, L. B. Cifuentes†, N. Frossard§, B. F. Klapp* and A. Fischer†

*Department of Internal Medicine, †Clinical Research Unit of Allergy and ‡Department of Pneumology, Charité School of Medicine, Humboldt University, Berlin, Germany and §INSERM U 425, Pulmonary Neuroimmunopharmacology, University of Strasbourg, Illkirch, Strasbourg, France

Summary

Background The traditional neurotransmitter catecholamine and the neuropeptide tyrosine in sympathetic airway nerves have been proposed to be involved in the pathogenesis of airway diseases.

Objective The aim of the present study was to investigate the effect of allergic airway inflammation on the expression of catecholamine enzyme tyrosine hydroxylase (TH), neuropeptide tyrosine (NPY) and tachykinins in mouse sympathetic airway ganglia.

Methods Using neuronal tracing in combination with immunohistochemistry, the present study was designed to characterize TH, NPY and tachykinin profiles of superior cervical (SCG) and stellate ganglia after allergen challenge.

Results The vast majority of fast blue-labelled SCG neurons (allergen: $97.5 \pm 1.22\%$ (mean $\pm$ SEM) vs. controls: $94.5 \pm 1.48\%$, $P = 0.18$) and stellate neurons (allergen: $95.3 \pm 1.01\%$ vs. controls: $93.6 \pm 1.33\%$, $P = 0.34$) were immunoreactive for TH. Of the TH immunoreactive and fast blue-labelled SCG neurons, $52.0 \pm 1.01\%$ allergen vs. $51.2 \pm 3.58\%$ controls ($P = 0.83$) and stellate neurons, $57.3\% \pm 0.97$ allergen vs. $56.4 \pm 1.65\%$ controls ($P = 0.64$) were positive for TH only but not NPY, whereas $45.3 \pm 1.05\%$ allergen vs. $43.3 \pm 1.18\%$ controls ($P = 0.47$) of fast blue-labelled SCG neurons and $37.9 \pm 0.86\%$ allergen vs. $37.1 \pm 1.24\%$ controls ($P = 0.62$) of fast blue-labelled stellate neurons were immunoreactive for both TH and NPY immunoreactivities. There was a trend of an increase, but not significant one, in the percentage of TH-/NPY-immunoreactive and fast blue-labelled neurons in allergen-treated animals in comparison with the controls. Tachykinins, however, were not expressed by sympathetic neurons and were also not induced in sympathetic neurons after allergen challenge.

Conclusion The present study indicates that allergic airway inflammation does not alter the expression of noradrenalin and NPY in sympathetic ganglia and also shows that sympathetic neurons do not respond to allergic airway inflammation with tachykinins induction. However, a participation of catecholamine and NPY in the pathogenesis of allergic airway inflammation cannot be excluded in the present study as a higher neurotransmitter output per neuron following allergen challenge could be possible.

Keywords allergic airway inflammation, autonomic nervous system, mouse airway ganglia, neuronal tracing and immunohistochemistry, sympathetic

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Introduction

Sympathetic and parasympathetic airway nerves are of potential interest as factors in the pathogenesis of chronic airway disease since the airway autonomic nervous system is known to have the capacity to influence airway caliber via its effects on airway smooth muscle, bronchial vessels and mucous glands. Therefore, the nerve fibres may contribute to

airway narrowing in asthma or in chronic obstructive pulmonary disease [1, 2].

Parasympathetic cholinergic preganglionic fibres carried by the vagus nerves mediate bronchospasm via activation of cholinergic neurons localized in intrinsic airway ganglion, while sympathetic-adrenergic nerve fibres only play a minor role in directly regulating smooth muscle tone in the human airways [1, 2]. In this respect, the major bronchorelaxant innervation of human, rat and guinea-pig airways is the non-adrenergic and non-cholinergic (NANC) nerves, which can release the bronchodilator neurotransmitter nitric oxide (NO) to mediate bronchodilatation in central and peripheral airways [3–5]. Neuropeptide tyrosine (NPY) is reported to

Correspondence: Q. Thai Dinh, Department of Internal Medicine and Clinical Research Unit of Allergy, Charité, Virchow Campus, Ostring 3, R. 3.0066, Augustenburger Platz 1, D-13353 Berlin, Germany.
E-mail: q-thai.dinh@charite.de

be localized in sympathetic-adrenergic nerves and acts as a vasoconstrictor to control nasal, tracheal and bronchial vascular beds [6–9].

Plasticity has been shown not only for airway sensory nerves by allergic airway inflammation [10–12] but also for sympathetic neurons in superior cervical (SCG) by a variety of mediators [13]. However, sympathetic neurons have not been studied in models of allergic airway inflammation so far. Therefore, using retrogradely neuronal tracing in combination with quantitative double-labelling immunohistochemistry, the present study aimed to characterize not only the expression and distribution of tyrosine hydroxylase (TH), a rate-limiting enzyme of catecholamine synthesis and a marker for noradrenergic neurons and neuropeptide NPY expression, but also tachykinins expression in sympathetic ganglion neurons in allergen-sensitized and control mice. Despite the fact that mouse models of allergic asthma have a number of dissimilarities to human asthma [14], here, the mouse has been chosen as it serves increasingly as a species for animal models to study airway diseases because of the advantages of genetically altered mice with targeted disruption or over-expression of genes [15–17].

Materials and methods

Animals

In total, eight adult female BALB/c mice weighing 15–20 g were used. The animals were kept under standard laboratory conditions. All protocols were performed in accordance with the Animal Welfare Act and the National Institutes of Health 'Guidelines for the care and Use of Laboratory Animals' (NIH publication 85–23, revised 1985) and approved by the state animal committee (0059/01).

Allergen sensitization

The protocol of allergic sensitization and challenge has been reported previously [18]. Four animals were systemically sensitized by means of intraperitoneal injections of 10 µg ovalbumin (OVA, Grad VI, Sigma Chemical, Deisenhofen, Germany) adsorbed to 1.5 mg of aluminium hydroxide $\text{Al}(\text{OH})_3$ (AlumImject, Pierce, Rockford, IL, USA) as adjuvant on days 0, 14 and 21. Before analysis on day 28, animals received two consecutive local aerosol challenges of 1% OVA diluted in phosphate-buffered saline (PBS) delivered by aerosolization of 20 min on days 26 and 27 (2 × challenge) (Fig. 1). Non-immunized control mice received intraperitoneal $\text{Al}(\text{OH})_3$ plus PBS.

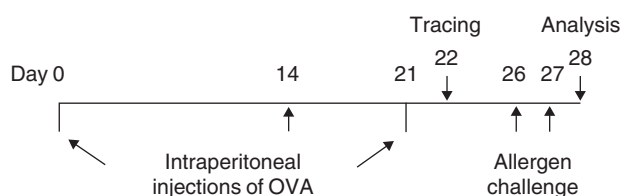


Fig. 1. Allergic sensitization and challenge protocol. The animals were systemically sensitized by means of intraperitoneal injections of ovalbumin (OVA) on days 0, 14 and 21 and were aerosol challenged twice on days 26 and 27. The tracing was carried out after allergen sensitization and before allergen challenge on day 22 and at day 28 the animals were sacrificed.

Determination of antibody titres

Blood was sampled from the lateral caudal veins on day 0 and on the days of the analysis. Serum concentrations of total IgE and allergen-specific IgE antibody titres were determined by ELISA as previously described [18].

Retrogradely neuronal tracing

At day 22 of the sensitization and challenge protocol (Fig. 1), all animals were anaesthetized with intraperitoneal injections of ketamine hydrochloride (Ketanest, Parke-Davies, Freiburg, Germany, 10 mg/kg, body weight) and xylazine hydrochloride (Rompun, Bayer, Leverkusen, Germany, 10 mg/kg). The mid-cervical trachea was exposed and the tip of a 10-µL syringe (Hamilton, Bonaduz, Switzerland) was inserted between the fourth and fifth tracheal cartilage via a glass micropipette, and a 5 µL injection of fast blue tracing dye (2% aqueous solution containing 1% dimethyl sulphoxide, Sigma Chemical) was made into the right main stem bronchus. The cut between the two cartilages was sutured with a 7-0 Vicryl suture and the skin sutures were made with 4-0 Vicryl suture (Ethicon, Norderstedt, Germany). Major bleeding was not observed during and after the operation. After the injection procedure animals were placed with elevated head for about 5 min. With this technique, we have previously demonstrated that the administered tracing substance is confined to the lower trachea and the lung [10, 19]. All animals recovered undisturbed for a post-operative period of 7 days until they were killed by CO_2 asphyxiation. Directly after asphyxiation, the animals were retrogradely perfusion-fixed through the abdominal aorta with freshly prepared Zamboni's solution (2% paraformaldehyde, 15% picric acid, 0.1 M PBS, pH 7.4 for 5 min) as described previously. This routine protocol was performed to ensure optimal conditions for immunohistochemistry and published previously for immunohistochemical analysis of ganglionic neurotransmitter expression [20].

Tissue preparation

Dissection of the Zamboni-fixed sympathetic ganglia (SCG and stellate ganglia) was followed by rinses in 0.1 M phosphate buffer (pH 7.4) and cryoprotection with 18% sucrose in 0.1 M phosphate buffer overnight. Serial sections of the ganglia were cut on a cryostat (HM-500-0, Microm, Walldorf, Germany) to 8 µm and air dried for 30 min.

Histology of the lung

For the assessment of the airway inflammation, serial sections of Zamboni-fixed lung tissues were cut on a cryostat at 8 µm and stained with haematoxylin and eosin.

Immunohistochemistry

For immunohistochemical staining, in total 16 SCG and 16 stellate ganglia were used in the present study. Every eighth section of serial sections of the sympathetic ganglia (to avoid double counting, only neurons with nuclei were analysed) was first incubated for 1 h with PBS containing 10% normal swine serum to block non-specific protein binding sites, and 0.5% Tween 20 (Sigma) to enhance penetration of immunoreagents. Ganglia sections were incubated with a combination of a polyclonal antibody TH from rabbit (ETI, code-no.1012,

Allendale, USA, dilution 1 : 800) and a polyclonal antibody to NPY from sheep (Dr Blessing, Australia, dilution 1 : 1000) overnight. In separate studies, the sections were incubated overnight with a monoclonal antibody from rat raised against substance P/neurokinin A (Boehringer Ingelheim, Heidelberg, Germany, dilution 1 : 400) in combination with a polyclonal antiserum to protein gene product (PGP) 9.5 from rabbit (Biotrend, Köln, Germany, dilution 1 : 1000) to identify neurons. The monoclonal antibody from rat raised against C-terminal of substance P (Boehringer Ingelheim Heidelberg, Germany, dilution 1 : 400) used in the present study was tested by Kummer et al. [21] to have cross-reactivity with NKA and NKB, so that this antibody should be classified as a pan-tachykinin antibody. After rinsing in PBS, a biotinylated anti-sheep/goat IgG from donkey (Amersham Pharmacia Biotech, Freiburg, Germany, dilution 1 : 400) was applied for 1 h. Following several washing steps all sections were incubated with a mixture of a Streptavidin–Texas Red conjugate (Amersham, dilution 1 : 200) and fluorescein-isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Cappel, OH, USA) or with FITC-conjugated goat anti-rabbit IgG and Cy-3-conjugated donkey anti-rat IgG (Jackson Immuno-Research, PA, USA, dilution 1 : 200) for 1 h. Finally, the sections were washed again in PBS and mounted in carbonate-buffered glycerol at pH 8.6 [20, 21].

For immunohistochemical analysis, all neurons with an apparent nucleus were analysed by using an epifluorescence microscopy (DMRA2, Leica, Bensheim, Germany) and appropriate filter combinations. Fast blue was detected with the filter module U1 (excitation filter: band pass, 330–380 nm; barrier filter: long pass, 418 nm), Texas red with the filter G1 module (band pass, 546/10 nm; long pass, 590 nm), Cy3 with the filter module (band pass, 525–560 nm; long-pass, 570–650 nm) and FITC with the B2 module (band pass, 455–490 nm; long pass, 515–560 nm). The sections were visualized using a camera system (Visitron-System, Imagine microscopy, Puchheim, Germany).

For quantification, every eighth section of each ganglion was selected from the serial sections of each ganglion (32 ganglia (four per animal = two SCG and two stellate) of eight animals ($4 \times 8 = 32$) – each ganglion generates 65–100 sections ($8 \mu\text{m}$ per section)). For a mouse, 32–48 sections (8–12 sections/ganglion, per mouse $2 \times \text{SCG}$: 16–24 sections and stellate ganglia: 16–24 sections) were analysed. For immunoreaction specificity testing, pre-absorption studies were carried out and no specific immunohistochemical signalling was detected. Specific immunohistochemical signals also disappeared after replacement of the primary antisera by PBS.

Statistical analysis

The immunohistochemical analysis was carried out in a blinded fashion. The percents of neurons were expressed as mean $\pm$ SEM. The differences between the groups were analysed statistically using Student's *t*-test. *P*-values < 0.05 were considered as significant.

Results

Sensitization of mice to ovalbumin

BALB/c mice treated according to this protocol of allergic sensitization showed very consistent results in the allergic

response and therefore no animal was excluded from the analysis. Animals sensitized to OVA by intraperitoneal injections had high total IgE levels and also developed high anti-OVA IgE titres that provided strong evidence for airway allergic inflammation in these animals (Table 1).

Histology of allergen-sensitized and challenged murine lung

The morphology of the lung tissues of all sensitized and challenged animals showed signs of airway inflammation including infiltration of mononuclear cells in every compartment of the lung as shown by haematoxylin and eosin-stained lung tissue sections from allergen sensitized and challenged mouse. In contrast, sections from the control BALB/c mouse did not contain inflammatory cell aggregates (Fig. 2).

Immunohistochemistry for tyrosine hydroxylase and neuropeptide tyrosine in retrogradely labelled neurons

Fast blue-labelled neurons within the SCG and stellate ganglia were clustered in a particular region of the ganglia, but the exact position of the aggregation of labelled neurons cannot be determined as these ganglia were not topographically oriented before sectioning. Per section of both SCG and stellate ganglia, 30–75 neurons were found to be retrogradely labelled. In total, 9638 fast blue-labelled neurons of the SCG (3589 allergic neurons + 6049 control neurons) and 8594 fast blue-labelled neurons of the stellate ganglia (5242 allergic neurons + 3452 control neurons) were investigated for TH and NPY immunohistochemistry.

All patterns of co-localization of TH and NPY immunoreactivity were found in both SCG and stellate ganglia. The vast majority of fast blue retrogradely labelled SCG neurons, $97.5 \pm 1.22\%$ (mean $\pm$ SEM) allergen vs. $94.5 \pm 1.48\%$ controls ($P = 0.18$) and stellate ganglion neurons, $95.3 \pm 1.01\%$ allergen vs. controls $93.6 \pm 1.33\%$ ($P = 0.34$), were immunoreactive for TH.

Of the TH-immunoreactive and fast blue-labelled SCG neurons, $52.0 \pm 1.01\%$ allergen vs. $51.2 \pm 3.58\%$ controls ($P = 0.83$) and stellate ganglion neurons, $57.3 \pm 0.97\%$ allergen vs. $56.4 \pm 1.65\%$ controls ($P = 0.64$), were immunoreactive for TH only but not for NPY, whereas $45.3 \pm 1.05\%$ allergen vs. $43.3 \pm 1.18\%$ controls ($P = 0.47$) of fast blue-labelled SCG neurons and $37.9 \pm 0.86\%$ allergen vs. $37.1 \pm 1.24\%$ controls ($P = 0.62$) of fast blue-labelled stellate neurons were reactive for both TH and NPY immunoreactivities (Figs 3 and 4).

There was a trend of an increase, but not a significant one, in the percentage of TH-/NPY-immunoreactive and fast blue-labelled neurons in allergen-treated animals in comparison

Table 1. Serum immunoglobulin titres in OVA-sensitized and challenged and PBS-treated BALB/c mice

Immunoglobulin	OVA (intraperitoneal)	Control, PBS
OVA-specific IgE	875 ± 187	–
Total IgE	2586 ± 629	127 ± 25

Serum levels of OVA-specific and total IgE were analysed in mice sensitized and challenged to OVA or PBS by ELISA. Values are presented as means and $\pm$ SEM.

OVA, ovalbumin; PBS, phosphate-buffered saline.

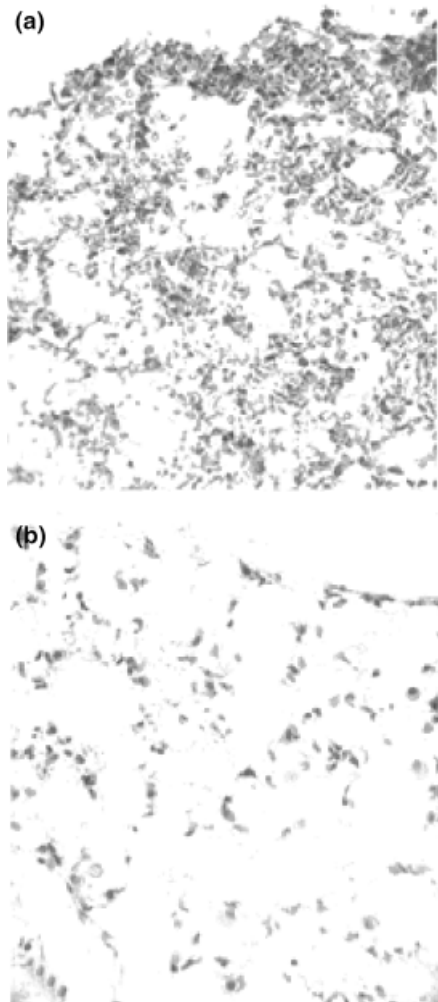


Fig. 2. Lung tissue sections from an allergen-sensitized and challenged mouse were stained with haematoxylin and eosin. (a) Representative field demonstrating mononuclear cell infiltration in the airways of allergen-sensitized and challenged mouse ($\times 240$). (b) Sections from the control BALB/c mouse do not contain inflammatory cell aggregates ($\times 240$).

with the controls. A small number of fast blue-labelled sympathetic neurons showed no immunoreactivity for TH, but for NPY (SCG: 0.4% vs. 0.5%; stellate: 0.5% vs. 0.4%). Fast blue-labelled sympathetic neurons that were found negative for any investigated immunoreactivity were very sparse (SCG: 2.2% vs. 4.9%, stellate: 4.2% vs. 6.0%).

Apart from the findings that TH-/NPY-immunoreactive neurons in the SCG were found to be slightly more numerous when compared with stellate ganglia, other ganglion-specific differences were not observed for both SCG and stellate ganglia.

The counting of the fast blue-labelled and the overall ipsilateral SCG and stellate ganglia neurons that were stained with the pan-neuronal marker PGP 9.5 showed that the majority of neurons of both sympathetic ganglia (SCG: $70.8 \pm 4.6\%$ allergen vs. $70.5 \pm 5.0\%$ controls of the overall SCG neurons and stellate: $68.0 \pm 4.5\%$ allergen vs. $74.5 \pm 5.9\%$ of the overall stellate ganglia neurons) innervated the mouse lower airways. A substantial number of neurons that were not labelled with fast blue were also found in both sympathetic ganglia (Table 2).

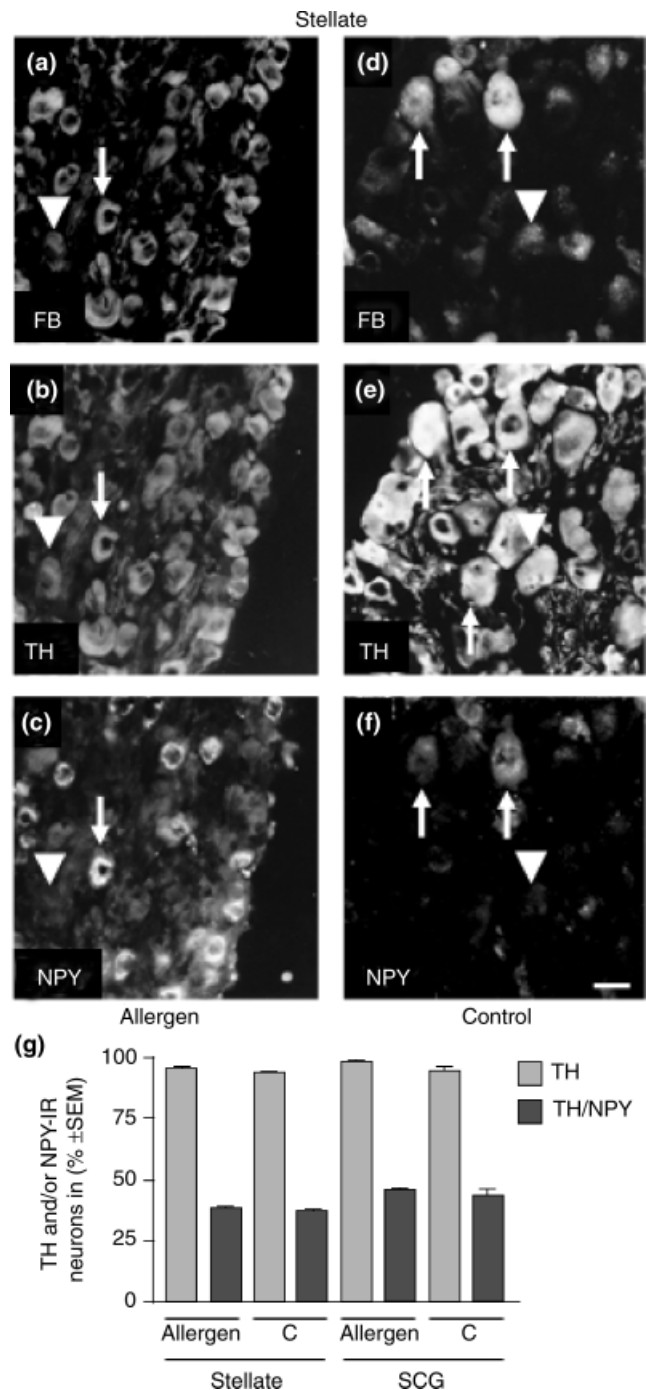


Fig. 3. Tyrosine hydroxylase (TH) and neuropeptide tyrosine (NPY) immunoreactivity (IR) in sympathetic stellate ganglion neurons after allergen challenge. Stellate ganglion neurons are retrogradely labelled with fast blue (FB) dye (allergen: arrows in a and control: d), fast blue-labelled neurons also co-express TH IR (allergen: arrows in b and control: e) NPY IR (allergen: arrow in c and control: f). Other fast blue-labelled neurons (allergen: arrowheads in a and control: d) show immunohistochemical staining for TH (allergen: arrowheads in b and control: e) but not for NPY (allergen: arrowheads in c and control: f). Scale bar: 25 μ m. Histogram showing the percentage of TH- and NPY-immunoreactive neurons innervating the lower airways in SCG and stellate ganglia (e). The percentage of TH- as well as NPY immunoreactive and fast blue-labelled (airway specific) neurons of both sympathetic ganglia is not significantly changed after 24 h allergen challenge, C, control; SCG, superior cervical ganglion.

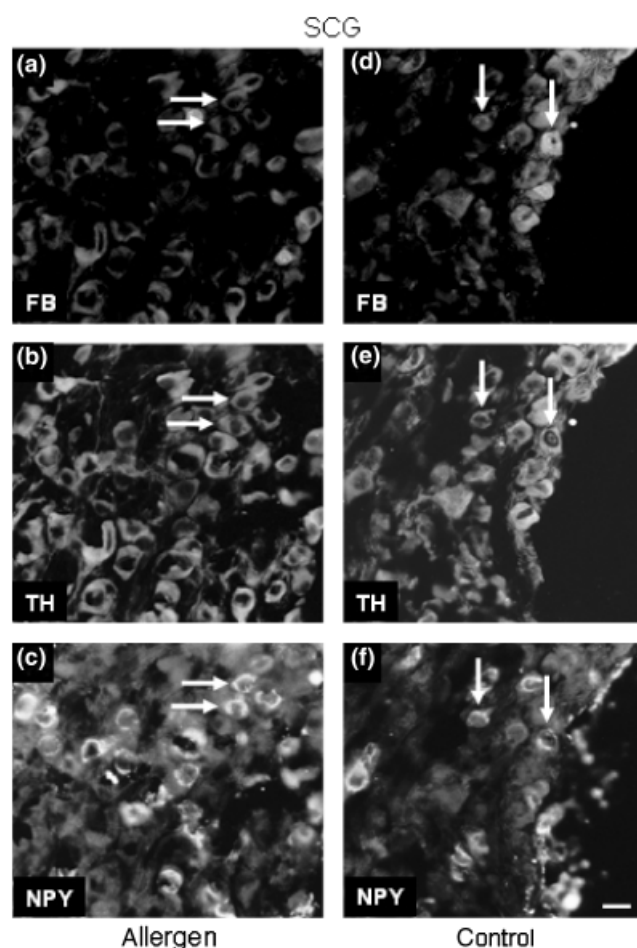


Fig. 4. Tyrosine hydroxylase (TH)- and neuropeptide tyrosine (NPY)-immunoreactivity (IR) in retrogradely labelled neurons of superior cervical ganglion (SCG) after allergen challenge. SCG 24 h after allergen challenge, fast blue (FB)-labelled neurons (a) express TH IR and NPY IR (arrows in b and c). a small number of fast blue-labelled neurons show no immunoreactivity for TH, but for NPY and fast blue-labelled neurons with no immunoreactivity for TH and NPY were very sparse. In control SCG, fast blue-labelled neurons (d) express TH and NPY IR (arrows in e and f). Most of the retrogradely fast blue-labelled neurons in the sympathetic ganglion contain TH or TH plus NPY and only a small number of less than 1% of fast blue-labelled neurons expresses NPY alone, scale bar: 25 µm.

Table 2. The percent of fast blue-labelled neurons in the ipsilateral SCG and stellate ganglia

Non-allergic			
SCG right	70.5 ± 5.0%	Stellate right	74.5 ± 5.9%
Allergic			
SCG right	70.8 ± 4.6%	Stellate right	68.0 ± 4.5%

The number of fast blue-labelled (airway-specific) ipsilateral SCG and stellate ganglia neurons was not increased after allergen sensitization and challenge. The percent of fast blue-labelled neurons in the SCG did not differ from that of the stellate ganglia.
SCG, superior cervical ganglion.

Additionally, immunoreactive staining for tachykinins was not detected in control sympathetic neurons, and was also not observed in SCG and stellate ganglia neurons after allergen challenge, whereas the positive control showed strong immunoreactivity for tachykinins in vagal sensory neurons (Fig. 5).

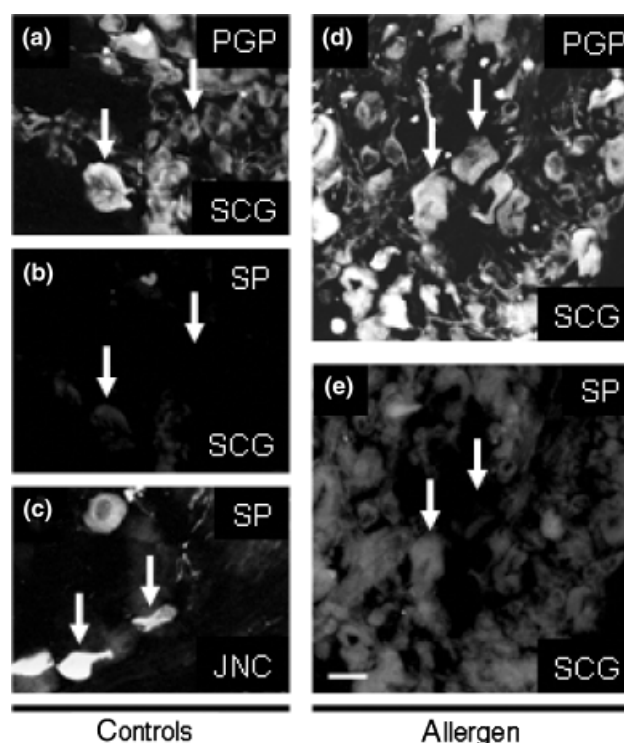


Fig. 5. Substance P (SP) immunoreactivity (IR) in sympathetic neurons before and after allergen challenge. Allergen-sensitized and challenged sympathetic neurons are labelled with a pan-neuronal marker protein gene product (PGP) 9.5 (arrows in a control, d allergen) and these PGP-immunoreactive sympathetic neurons show no immunoreactivity for SP in allergen-challenged ganglia neurons (arrows in e) as well as control neurons (arrows in b control) whereas in the positive controls, SP is strongly expressed by sensory neurons of the jugular-nodose complex (JNC) (arrows in c), scale bar: 15 µm. SCG, superior cervical ganglion.

Discussion

The present study has demonstrated that the majority of airway-innervating sympathetic neurons in both SCG and stellate ganglia of the mouse express TH and a large number of them also express NPY. The mouse airways receive sympathetic supply from both SCG and stellate ganglia as a high number of neurons in these sympathetic ganglia were found to be retrogradely labelled with dye substance from the lower airways [21]. Also, a substantial number of the SCG and stellate ganglia neurons were not labelled with fast blue, indicating another projection of these neurons to other organs such as the heart [22–24].

The high basal expression of TH and NPY in neurons of both sympathetic-adrenergic control ganglia found in the present study suggests that catecholamine and NPY may play an important role in the physiological regulation of airway functions of the mouse. This has previously been suggested for the guinea-pigs [21, 25]. Although there is a high density of NPY- and TH-immunoreactive nerve fibres in small arteries and glands in the nasal mucosa and lower airways of most species, there is little functional evidence for a dense adrenergic innervation of human airway smooth muscles [26]. In this respect, the neural bronchodilator mechanism in human and guinea-pig airways is known to be mediated by inhibitory NANC (i-NANC) nerves that can release NO to mediate bronchodilatation in central and peripheral airways

[3–5]. Catecholamines are known as potent vasoconstrictors and may therefore control pulmonary and bronchial blood flow [7, 27–29]. It is difficult to study the effects of catecholamines on the airways because hormonal and neuronal effects of these classical neurotransmitters cannot be discriminated easily. This difficulty in discriminating hormonal from neuronal effects has therefore contributed to the poor understanding of the functional role of these airway nerves [30]. The high expression of NPY in noradrenergic neurons and nerve fibre varicosities in many mammalian species including humans, cat, guinea-pig and rat suggests that NPY and NA may have similar effects on airway functions [27, 31, 32]. In this respect, NPY is known to be co-localized with NA in sympathetic-adrenergic nerves and is also a potent vasoconstrictor. NPY acts on its receptors that have been cloned and identified as Y1, Y2 and Y5 receptor subtypes and that are present in human and mouse airways [33–37].

Plasticity of the sensory airway innervation has been reported in human and guinea-pig airway diseases such as rhinitis and allergic airway inflammation [11, 12, 19, 38–40]. Also neuronal changes have been shown for the sympathetic innervation in the central and peripheral nervous system [41]. The present study was therefore carried out to investigate the effect of allergic airway inflammation on sympathetic airway nerves. No significant increase in the number of TH- and/or NPY-immunoreactive neurons in sympathetic ganglia was found 24 h after allergen challenge. It is not totally reasonable to investigate TH and NPY expression in sympathetic neurons following airway inflammation as in non-allergic diseases, changes in NPY protein expression were reported for catecholamine neurons after haemorrhage and stress exposition [42, 43] or after axotomy [44]. Not only NPY, but also NPY (Y1) receptor mRNA levels in rat spinal cord and dorsal root ganglia were found to be increased following peripheral tissue inflammation [45].

In rhinitis patients, NPY is also known to have the capacity to reduce major symptoms such as sneezing, itching and secretion, which are caused by allergen, capsaicin or bradykinin challenge, indicating a protective role for this neuropeptide in allergic airway disease [46, 47]. Furthermore, NPY was shown to be able to modulate cholinergic neurotransmission and inhibit tachykinin release from sensory nerves of guinea-pig airways to trigger airway inflammation [48, 49]. NPY plasma levels was found to be increased in asthmatic patients after exacerbation [50], whereas the density of NPY-immunoreactive nerve fibres in biopsies of both asthmatics and patients with chronic bronchitis was significantly decreased in comparison with controls [51].

The two ways in which neurotransmitters may be released in higher amounts after noxious stimulation: neurons may undergo a phenotypic switch and *de novo* neurotransmitter gene expression can be induced. This event was excluded in the present study since we did not find a significant increase in the number of NPY-positive neurons. The second way is the up-regulation of an existing neuropeptide gene expression leading to an increased release of neuropeptides at the terminal's nerve varicosities. This second way cannot be excluded in the present studies.

As not only jugular and nodose sensory neurons [11, 12, 19, 52] but also SCG neurons have been shown to be stimulated

to synthesize tachykinins by a variety of stimuli [13, 53–55], the present study, therefore, also investigated tachykinin expression in control and allergen-treated SCG and stellate ganglia. It was found that sympathetic-adrenergic neurons do not express tachykinins before and also after allergen challenge. In this respect, Jonakait and colleagues have demonstrated that substance P is induced in cultured sympathetic ganglia and substance P induction is mediated by IL-1. The difference between the lack of change in tachykinins in the present study, compared with those observed in the Jonakait studies, may be because of the differences in species and age in the Jonakait studies that have been carried out in rat neonates [55–57].

In conclusion, the present study demonstrated that allergic airway inflammation did not alter the expression of the two main neurotransmitters catecholamine and NPY in sympathetic ganglia and also showed that, unlike vagal sensory neurons, sympathetic neurons did not respond to allergic airway inflammation with an induction of tachykinin gene expression. Although no change in the number of neurons synthesizing neurotransmitters was observed following allergen challenge, a higher neurotransmitter output per neuron following allergen challenge could still be possible. Therefore, the participation of catecholamine and NPY in the pathogenesis of allergic airway inflammation cannot be excluded completely in the present study.

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