

Original article

Modulation of neurotrophin and neurotrophin receptor expression in nasal mucosa after nasal allergen provocation in allergic rhinitis

Background: Patients with allergic rhinitis (AR) feature both allergic airway inflammation and a hyperresponsiveness to nonspecific stimuli which is partly neuronally controlled. Still, it is unclear whether or not neurotrophins are involved in airway pathophysiology of AR and in nasobronchial interaction.

Methods: Nine AR patients with mono-allergy to grass pollen and nine healthy controls underwent nasal allergen provocation (NP). Serum samples, nasal and bronchial biopsies were taken before (T_0) and 24 h after (T_{24}) NP. Pan-neurotrophin receptor p75^{NTR}, tyrosine kinase A (trkA), trkB, nerve growth factor (NGF), and brain-derived neurotrophic factor (BDNF) were assessed with immunohistochemistry, and NGF and BDNF levels with ELISA.

Results: At T_{24} , BDNF and NGF were upregulated in nasal mucosa ($P < 0.05$) and increased in the peripheral blood of AR compared with T_0 . The increase in nasal BDNF expression correlated positively with the maximum increase in total nasal symptom score in AR ($P = 0.02$). p75^{NTR} was expressed on peripheral nerves and epithelial layer, trkA on endothelial cells, and trkB on mast cells. trkB + mast cells significantly decreased after NP in AR ($P < 0.01$). NP did not modulate p75^{NTR} and trkA expression in nasal mucosa and had no effect on the expression of neurotrophins and receptors in bronchial mucosa.

Conclusion: This study shows that neurotrophins and their receptors are expressed in human airways. Allergic rhinitis was characterized by a modulation of BDNF, NGF, and trkB in nasal mucosa after NP and a correlation of nasal BDNF with the maximal increase of total nasal symptom score. Therefore, our data suggest that neurotrophins participate in upper-airway pathophysiology in AR, whereas their role in nasobronchial interaction remains unclear.

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Allergic rhinitis (AR) is the most frequent manifestation of the atopic syndrome affecting the airways. The contribution of genetic, environmental, and immunological factors on the development of AR has been well established (1). In addition, it has been suggested that a neural pathway plays a role in the interaction between upper and lower airways in allergic airways disease (2). So far, no strong evidence has been found to support the allegation of this mechanism after allergen exposure. A key feature of AR is a hyperresponsiveness to nonspecific stimuli possibly due to enhanced neuronal reflex circuits controlling airway function. Changes in the structure and function of the nerves themselves – a phenomenon known as neuronal plasticity – may contribute to the pathophysiology of AR, as well as the response to exogenous

irritants and to endogenous mediators (3). In this regard, possible endogenous mediators, which are able to interact with both sensory nerves and main target effector cells within the inflammatory response, are presented by neurotrophins (4–6).

Neurotrophins were initially described for their neurotrophic activity (7). Meanwhile, it has become evident that neurotrophins modulate the functional activity of a variety of immune cells such as eosinophils and mast cells, which also produce neurotrophins (6, 8, 9).

The effect of neurotrophins is mediated via tyrosine kinase (trk) receptors to which mature neurotrophins bind with high affinity and specificity. Tyrosine kinase A has been identified as the preferred receptor for nerve growth factor (NGF), trkB for brain-derived neurotrophic factor (BDNF) and neurotrophin (NT)-4, and trkC for NT-3 (10). In addition, there is the pan-neurotrophin receptor p75 (p75^{NTR}), a member of the tumor necrosis factor super family, to which mature neurotrophins bind with low affinity and specificity (11).

Abbreviations: AR, allergic rhinitis; BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; NP, nasal provocation; NT, neurotrophin; p75^{NTR}, pan-neurotrophin receptor p75; trk, tyrosine kinase.

Increased serum levels of NGF have been found in patients with AR, allergic asthma, and atopic dermatitis compared with nonatopic subjects (8, 12, 13). Also, nasal and bronchial fluids have been characterized by increased levels of NGF in AR and allergic asthma before and after allergen provocation (14–16). From functional activity, BDNF and NGF contribute to airway hyperreactivity in mouse models for allergic airway inflammation (17, 18). Although it has recently become evident that NGF and NGF receptors are expressed in the nasal mucosa of humans, it is still unclear whether or not there are differences between patients with allergic rhinitis and healthy subjects (19).

On the basis of the pivotal role of NGF and BDNF in atopic diseases, we hypothesized that neurotrophins and their receptors are modulated during nasal allergen provocation both at the nasal and the bronchial level in AR. In addition, we speculated that neurotrophins take part in the nasobronchial interaction in AR. Thus, we determined NGF, BDNF, trkA/B, and p75^{NTR} expression in nasal and bronchial mucosa and NGF and BDNF levels in the serum of patients with AR, compared with nonatopic healthy control subjects before and after NP.

Methods

Subjects

After written informed consent (approved by the medical ethics committee of the Erasmus Medical Centre Rotterdam, the Netherlands), nine patients with mono-allergic rhinitis to grass pollen (five women, four men, age range 22–34 years) and nine healthy

subjects (two women, seven men, age range 19–32 years) were included in the study (Table 1). Patients with AR had at least a 2-year history of isolated grass pollen allergy, confirmed by positive skin prick test reaction to grass pollen extract only and not to a panel of 13 common inhalant allergens (Vivodiagnostik, ALK, Benelux BV, Groningen, the Netherlands). Subjects did not receive any systemic or local anti-inflammatory treatment 4 weeks before the commencement of the study. None of the subjects had an infection of the respiratory tract 4 weeks before allergen challenge. None of the participants had a clinical history of asthma. All participants had a normal forced expiratory volume (FEV₁) and a methacholine PC₂₀ of greater than 8 mg/ml. All subjects were nonsmokers.

Experimental design

Allergen provocation was performed out of pollen season during winter. Collection of blood samples, nasal and bronchial biopsies and assessment of FEV₁ (using a standard spirometry), were performed before (T₀) and 24 h (T₂₄) after NP, as previously described (20). Nasal and bronchial symptoms (visual analog scale scores), peak nasal inspiratory flow (PNIF) and peak expiratory flow (PEF) were recorded at T₀, 30 min after NP, at 2-h intervals after NP (T₂₋₁₂), and 24 h after NP at day 2 (T₂₄).

Nasal biopsy and nasal allergen provocation

All biopsy specimens of nasal mucosa were taken by the same investigator (GJB), as described previously (20). Briefly, local anesthesia was induced by placing a cotton-wool carrier with 50–100 mg of cocaine and three drops of epinephrine (1 : 1000) under the inferior turbinate, without touching the biopsy site. Secondly, a mucosal biopsy sample was obtained from the lower edge of the inferior turbinate about 2 cm posterior to the edge using a Gerritsma forceps with a cup diameter of 2.5 mm. The first nasal biopsy was taken at nine o'clock in the morning to assess baseline

Table 1. Subject characteristics 24 h after NP

Patients	Sex	Age (year)	FEV ₁ (l)	FEV ₁ (%)	IVC (l)	FEV ₁ /VC	BAR* (%)	PC ₂₀ (mg/ml)
Control subjects								
1	M	30	5.30	110	7.49	76	108	32.4
2	F	21	4.34	114	5.41	80	104	32.7
3	M	22	5.75	109	6.64	87	102	33.5
4	F	19	3.79	104	4.79	79	107	40
5	M	22	5.75	118	6.38	90	100	40
6	M	25	4.14	94	4.75	87	101	40
7	M	21	4.68	97	5.55	84	102	40
8	M	24	4.09	86	5.35	76	103	40
9	M	32	4.96	113	6.39	78	102	40
Patients with AR								
10	F	22	3.93	108	4.72	88	103	40
11	M	34	3.20	87	3.65	88	98	40
12	M	26	4.37	95	5.67	77	104	40
13	M	25	4.51	101	4.91	92	105	40
14	F	22	3.82	112	4.06	94	100	40
15	F	26	3.28	93	4.28	77	104	38.4
16	M	22	4.26	90	5.19	82	107	19.6
17	F	26	4.72	126	5.52	86	102	20
18	F	27	3.34	102	3.80	88	105	40

IVC, inspiratory vital capacity; BAR, β -agonist response; FEV, forced expiratory volume; NP, nasal provocation; AR, allergic rhinitis.

* β -agonist response (terbutaline, 1000 μ g) data are presented as percentage improvement compared with initial value; a virtual value of 40 was assigned to subjects who did not reach a PC₃₀ with 38 mg/ml methacholine.

conditions at T_0 . Thereafter, patients and controls underwent NP with 10 000 BU of grass pollen extract (Vivodiagnostic ALK Benelux BV, Groningen, the Netherlands) delivering a fixed dose of 50 μ l into each nostril with a pump spray. After 24 h, at T_{24} , the second nasal biopsy was taken from the contralateral nostril. All biopsy specimens were embedded in Tissue-Teck (Sakura, the Netherlands), immediately snap frozen and stored at -80°C .

Bronchial biopsy specimen

All bronchial biopsy specimens were taken by the same investigator (SEO). After intramuscular premedication with atropine dinitrate (0.5 mg), oropharyngeal anesthesia was accomplished with 1% of topical xylocaine spray. Next, the vocal cords, trachea, and bronchial tree were anesthetized with oxybuprocaine. The fiberoptic bronchoscope (BF, type P20 D; Olympus, Tokyo, Japan) was introduced into the airway via the oral route, and mucosal biopsies were taken from the segmental carinae of the left upper and lower lobes. After 24 h, each subject underwent a repeat bronchoscopy, during which biopsy specimens were taken from lobar segments of the right lung. The bronchial biopsy specimens were embedded in Tissue-Tek II Optimal Cutting Temperature compound (Sakura, the Netherlands), frozen, and stored at -150°C .

Immunohistochemistry and immunofluorescence

Immunohistochemistry was performed on 6- μ m thick sections with avidin-biotin-peroxidase kit (Vectastain ABC-kit, Vector Laboratories Inc. Burlingame, CA, USA), for multiple immunofluorescence labeling with avidin/biotin blocking kit and for multiple immunofluorescence labeling using two mouse monoclonal primary antibody (Ab) with M.O.M. kit (all Vector Laboratories, Inc.) according to the manufacturer's instructions and as previously described (8). Primary Ab or respective isotype control diluted in antibody diluent (DAKO Cytomation, Hamburg, Germany) was added in concentrations of 1–3 μ g/ml and incubated for 24 h at 4°C . For primary Ab, we used p75^{NTR} (anti-human NGF receptor mAb directed against the extracellular domain, clone NGFR5; NeoMarkers, Fremont, CA, USA), trkA and trkB (both mAb directed against the extracellular domain; R&D Systems, Wiesbaden, Germany), BDNF (anti-human BDNF mAb; R&D Systems), a dilution of 1 : 200 NGF (rabbit polyclonal anti-human Ab; Santa Cruz, Biotechnology, Inc., Santa Cruz, CA, USA), a dilution of 1 : 1350 protein-gene product (PGP) 9.5 (rabbit polyclonal Ab; Chemicon, Temecula, CA, USA), and 0.7 μ g/ml mAb to human tryptase (clone G3; Chemicon). For secondary Ab, we used a biotinylated goat anti-rabbit Ab, a biotinylated horse anti-mouse Ab, Fluorescein Avidin DCS, and Texas Red Avidin DCS for immunofluorescence (all Vector Laboratories Inc.). Toluidine-blue staining and naphthol-AS-D chloroacetate esterase reaction was performed to detect mast cells, as previously described (21). Six micrometer samples of human neurinoma was stained with p75^{NTR}, NGF, and PGP 9.5 to ascertain antibody specificity and revealed positively stained sensory nerves (data not shown).

Isotype specific control staining was performed with monoclonal mouse-IgG1 for p75^{NTR}, BDNF and tryptase, mouse IgG2a for trkA, mouse IgG2b for trkB, and rabbit polyclonal IgG for PGP 9.5 and NGF (all R&D Systems).

Microscopic assessment of immunohistochemical staining

For quantification, nasal submucosal areas excluding glands below the basement membrane were analyzed by four independent

subjects in a blinded fashion, as previously described (22, 23). Nerve bundles with a diameter of at least 5 μ m and mast cells were counted in at least five high power fields at 1 : 200 magnification. Counts were expressed as mast cells or nerve bundles per mm². BDNF staining displayed no cell specific staining and was assessed as staining intensity by using a grade from 0 to 4 (absent, weak, moderate, strong, very strong). All slides were microscopically examined and photo-documented (Olympus).

Measurement of neurotrophin protein level

The serum protein levels of BDNF and NGF were assessed with commercial enzyme-linked immunosorbent assays (R&D Systems) according to the manufacturer's instructions and as previously described (8, 12).

Materials

If not otherwise stated, reagents were obtained from Sigma-Aldrich Chemicals (Schnelldorf, Germany).

Statistical analysis

If not otherwise stated results are presented as mean $\pm$ SEM. T tests or Mann-Whitney rank sum test for between-group analysis and Wilcoxon signed rank test for within-group analysis depending on the distribution of the data were applied with the use of a statistical software package SigmaStat for Windows (Jandel Scientific Erkrath Wiesbaden, Germany). A P -value < 0.05 was considered to be statistically significant. To assess the correlation between clinical parameters and neurotrophin/neurotrophin receptor expression, a Person Product Moment Correlation (normality test passed) or a Spearman rank order correlation (normality test failed) was performed; adjusted P -values were obtained via the Bonferroni correction.

Results

Clinical data

At T_0 , control subjects and patients with AR displayed the same clinical parameters. After NP, the patients with AR had an increased total nasal symptom score ($P < 0.001$) and lower PNIF values ($P < 0.0001$) than control subjects [for details see (20)]. FEV₁ did not significantly change after NP (see Table 1). There was no effect of NP on total nasal and bronchial symptom score, PNIF, and PEF in control subjects.

Pan-neurotrophin receptor p75^{NTR} was expressed on basal epithelial layer and sensory nerves in nasal and bronchial mucosa

p75^{NTR} receptor expression was detected on basal epithelial layer and on peripheral nerves in nasal mucosa of both groups studied (Fig. 1). To prove that p75^{NTR} was expressed on nerves, we performed double stainings with PGP 9.5 known as a pan-nerve marker (Fig. 1C–E). At T_0 , p75^{NTR} receptor-positive nerve bundles were lower in AR (0.87 ± 0.15) compared to control subjects (2.87 ± 0.37 ; $P < 0.001$; data not shown). Nasal

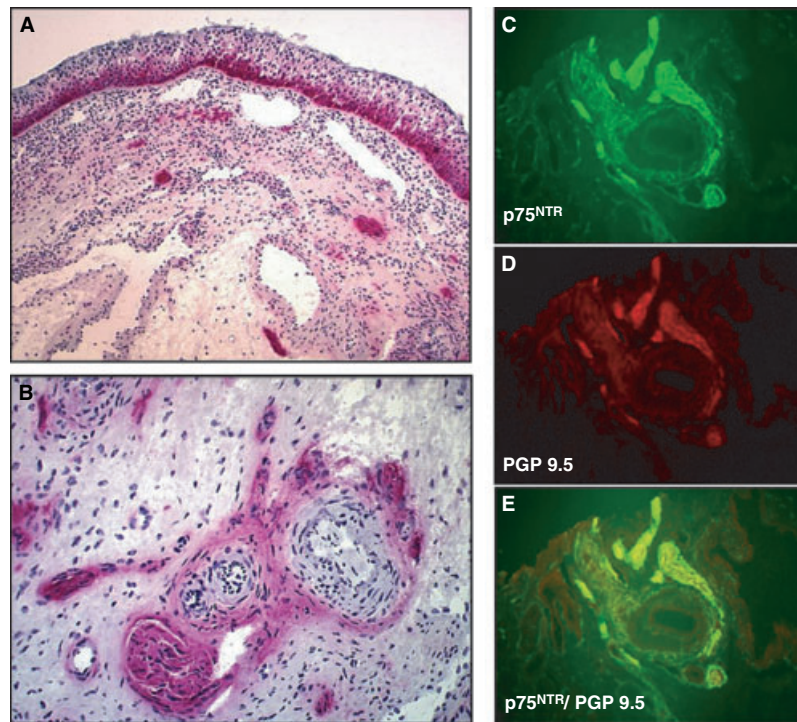


Figure 1. Pan-neurotrophin p 75 (p75^{NTR}) immunohistochemical staining in nasal mucosa sections of allergic rhinitis (AR) (A, magnification $\times 100$) and control subjects (B, magnification $\times 200$) at T₀. p75^{NTR} (fluorescein isothiocyanate) and protein gene-product (PGP) 9.5 (Texas Red) positive nerves surrounding a blood vessel in nasal mucosa of a nonatopic healthy subject assessed with immunofluorescence (C–E).

provocation did not modulate the number of p75^{NTR}-positive nerve bundles in nasal mucosa of AR (1.34 ± 0.29) and control subjects (2.21 ± 0.39 ; data not shown).

In bronchial mucosa, p75^{NTR} was also expressed on peripheral nerves. The level of p75^{NTR} expression was similar in AR and control subjects at T₀. There was no modulation of p75^{NTR} expression on peripheral nerves and no correlation with clinical data in nasal and bronchial mucosa after NP in both groups studied (data not shown).

Tyrosine kinase B was expressed on mast cells and down-regulated after nasal allergen provocation in allergic rhinitis

Tyrosine kinase B receptor was expressed on mast cells as assessed by toluidine blue staining and naphthol-AS-D chloracetate esterase reactivity on consecutive slides (Fig. 2CI,CII) and double staining with tryptase (Fig. 2D). In AR, the number of trkB + mast cells was significantly higher at T₀ (Fig. 2A,E) compared with T₂₄ after NP (Fig. 2B,E; $P < 0.01$). In addition, the number of trkB + positive mast cells was higher in patients with AR compared with control subjects at T₀ ($P < 0.01$, Fig. 2E). There was no effect of NP on trkB receptor expression in control subjects (Fig. 2E). The total number of mast cells was not altered after NP in both groups studied, as assessed with tryptase staining (data not shown).

In nasal mucosa, trkA receptor was expressed on endothelial cells of both groups studied. In bronchial mucosa, trkA and trkB receptor expression was hardly detectable in AR and control subjects. There was no effect of NP on nasal trkA and bronchial trkA/B expression of both groups studied (data not shown).

Increased expression of nerve growth factor and brain-derived neurotrophic factor in nasal mucosa and serum, 24 h after nasal allergen provocation in allergic rhinitis

NGF expression was detected in submucosal glands and nerve bundles in nasal mucosa of both groups studied (Fig. 3A,C). Twenty-four hour after NP, NGF-positive nerve bundles increased significantly in AR compared to T₀ ($P < 0.05$), whereas there was no effect of NP in control subjects (Fig. 3D).

NGF levels in bronchial samples were similar in both groups studied. In AR, NP increased the number of NGF-positive nerve bundles of 2.19 ± 0.3 at T₂₄, however with no significant difference compared to T₀ (1.33 ± 0.45 , $P = 0.19$). There was no effect of NP on NGF expression in bronchial mucosa of control subjects and no correlation with clinical data in both groups studied (data not shown).

BDNF expression was demonstrated in nasal and bronchial mucosa of both groups studied. In comparison with NGF staining, we did not find a cell specific but a

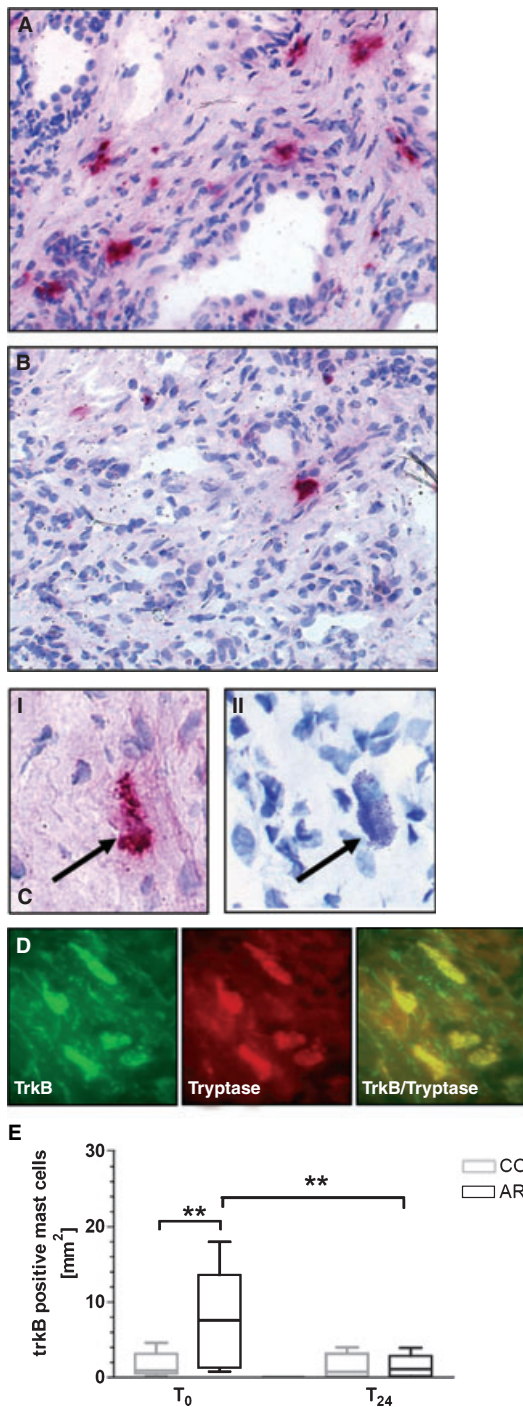


Figure 2. Immunohistochemical staining for tyrosine kinase (trkB) on mast cells in nasal mucosa of allergic rhinitis (AR) at T₀ (A) and T₂₄ (B, magnification $\times 200$). Naphthol-AS-D chloroacetate esterase reactivity (CI) and toluidine-blue staining (CII) on consecutive slides (magnification $\times 400$). TrkB (fluorescein isothiocyanate) and tryptase (Texas Red) positive mast cells assessed with immunofluorescence in AR (D, magnification $\times 400$). Quantitative analysis of trkB + mast cells in nasal mucosa of AR (black line, $n = 9$) and control subjects (grey line, $n = 9$) at T₀ and T₂₄, presented as box blot with median (line within the box) and interquartile range (edge of box; F, $**P < 0.01$).

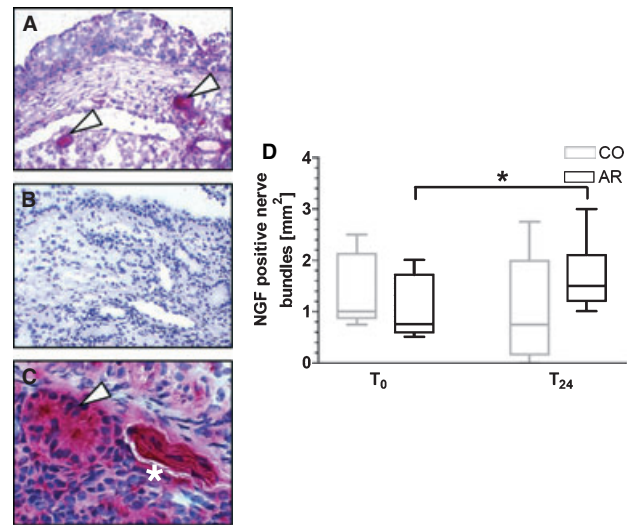


Figure 3. Nerve growth factor (NGF) expression in nasal mucosa of allergic rhinitis (AR) (A, magnification $\times 100$). Negative control (B, magnification $\times 100$). NGF positively stained peripheral nerve (C, magnification $\times 200$, asterisk points to neuronal sheath, arrows point to NGF-positive submucosal glands; A, C). Increased expression of NGF on nerve bundles in AR 24 h after nasal provocation (NP) (T₂₄) compared to T₀ (D). Box blot graphs represent the median (line within the box) and interquartile range (edge of box), grey line represents control subjects (CO, $n = 9$), black line represents AR ($n = 9$; $*P < 0.05$).

positively stained tissue for BDNF. We therefore assessed the staining intensity for BDNF which increased significantly from T₀ to T₂₄ in nasal mucosa of AR ($P < 0.01$, Fig. 4). Interestingly, the increase in BDNF expression (BDNF expression at T₂₄ minus the expression at T₀) correlated positively with the maximal increase in total nasal symptom score after nasal provocation (total nasal symptom score at T₃ minus T₀) in AR (Spearman rank order correlation $r = 0.736$, $P = 0.020$).

In control subjects, BDNF expression was 0.33 ± 0.15 at T₀ and was not modulated after NP (0.38 ± 0.18 at T₂₄). In bronchial mucosa, we found a higher staining intensity for BDNF at T₀ in AR (2.09 ± 0.28) compared with control subjects (1.06 ± 0.31 ; $P < 0.05$, data not shown). However, NP had no effect on bronchial BDNF expression in both groups studied. There was no correlation of bronchial BDNF expression and clinical data.

At T₀, NGF (12.59 ± 4.69 pg/ml) and BDNF (17.46 ± 2.95 ng/ml) serum levels were higher in AR but with no statistical significant difference compared with control subjects (NGF: 8.93 ± 6.66 pg/ml; BDNF: 16.35 ± 2.37 ng/ml). At T₂₄, NGF (23.37 ± 18.51 pg/ml) and BDNF (22.39 ± 0.98 ng/ml) serum levels were increased in AR, however with no significant difference compared to T₀. In control subjects, NGF (5.89 ± 2.78 pg/ml) and BDNF (17.9 ± 2.18 ng/ml)

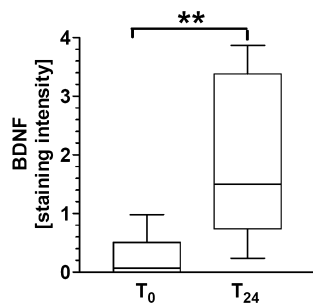


Figure 4. Quantitative analysis of brain-derived neurotrophic factor (BDNF) expression in nasal mucosa of allergic rhinitis (AR) at T₀ and T₂₄ presented as box blot graph with median (line within the box) and interquartile range (edge of box; $n = 9$; $**P < 0.01$).

serum levels were not modulated after NP at T₂₄ compared to T₀. There was no correlation of clinical data with NGF and BDNF serum levels.

Discussion

The results presented in this study demonstrate that BDNF, NGF, p75^{NTR}, and trkA/B are expressed in both upper and lower airways. In addition, this study gives evidence for an allergen induced local down-regulation of trkB and an up-regulation of NGF and BDNF expression in nasal mucosa of AR.

NGF-positive nerves have been shown in lower airways of nonallergic subjects (24). In addition, NGF-positive basal epithelial layer and submucosal glands have been described in human nasal mucosa (19). However, in the latter study, the phenotype of the subjects investigated was not known. Therefore, we wondered if there is a difference of neurotrophin expression in nasal mucosa of patients with allergic rhinitis and nonatopic healthy controls before and after NP.

We confirmed the data of Wu et al. (19) assessing NGF expression on peripheral nerves and submucosal glands in human nasal mucosa. However, we did not find a difference in NGF-positive nerve bundles at T₀ in AR and control subjects. This could be explained by the fact that the AR subjects were sampled out of season. Interestingly, we found an increase in NGF-positive nerve bundles after NP in the nasal mucosa AR which is novel. In a mouse model for allergic airway inflammation, it was demonstrated that mice over-expressing NGF suffer from increased airway hyperreactivity (25). However, in our current study, we could not find evidence for a correlation of nasal or bronchial NGF expression and clinical parameters.

In nasal mucosa of AR, we found an increased expression of BDNF 24 h after allergen provocation. Further, we assessed a positive correlation of nasal BDNF expression and the maximal increase of total nasal symptom score in AR, suggesting, that BDNF has a

Table 2. Summary of staining pattern at T₀ in nasal mucosa and results 24 h after nasal allergen provocation (T₂₄) in AR

	Staining pattern	T ₂₄
BDNF		↑
NGF		↑
trkA		↔
trkB		↓
p75 ^{NTR}		↔

BDNF, brain-derived neurotrophic factor; NGF, nerve growth factor; trk, tyrosine kinase; p75^{NTR}, pan-neurotrophin receptor p75. BDNF was expressed in nasal mucosa, NGF and p75^{NTR} were expressed on peripheral nerves, trkA on endothelial cells, and trkB on mast cells. Twenty-four hour after NP (T₂₄), BDNF and NGF expression were increased whereas the number of trkB + mast cells was down-regulated. There was no modulation of trkA and p75^{NTR} expression after NP at T₂₄.

role in upper airway pathophysiology. Possible sources for increased BDNF levels in nasal tissue are eosinophil granulocytes which are releasers and a source of BDNF and which are increased in peripheral blood and nasal mucosa of AR as we have previously shown (8, 20). In this regard, mast cells, endothelial and epithelial cells also present likely sources of BDNF (26–28).

Interestingly, we found a higher expression of BDNF in bronchial mucosa of AR at T₀ compared to control subjects. What this means for airway pathophysiology is currently not clear. However, this may give evidence for airway remodeling in bronchial mucosa of patients with AR as BDNF is involved in tissue remodeling. Nasal provocation had no effect on BDNF and NGF expression in bronchial mucosa in both groups studied. However, this may relate to the single extra-seasonal nasal allergen provocation in our study being an insufficient stimulus for the bronchial increase of neurotrophins.

A decade ago Bonini et al. revealed significantly higher serum levels of NGF in AR than in healthy subjects (13). We also found higher serum levels of NGF and BDNF in AR compared to control subjects at T₀, although with no statistically significant difference. The NP increased BDNF and NGF serum levels in AR at T₂₄, but without a statistically significant difference compared to T₀. The small sample size of the study could be a limitation in this regard and also the fact that we performed only a single allergen provocation. In addition, this may also be explained by the size of the affected organ, as we did not find an increase in serum or bronchial mucosa neurotrophin levels after NP. In contrast, atopic dermatitis, which affects a larger organ, is characterized by increased levels of NGF and BDNF, as we have recently shown (8, 12).

In the present study, we have demonstrated that trkB receptor is expressed on mast cells. The number of trkB + mast cells was higher in AR than in control

subjects at T₀. Interestingly, NP led to a significant down-regulation of the number of trkB + mast cells in nasal mucosa of AR. It is likely that this accounts for the competitive binding of BDNF, as we found an up-regulation of BDNF expression after NP in AR.

We did not find a modulation of the total number of mast cells after single NP in both groups studied. Also other authors found no difference in the total number of mast cells after NP but an increase in the number of degranulated mast cells in allergic rhinitis (29). Thus, we speculate that trkB receptor down-regulation after single NP may account for the degranulation of mast cells in nasal mucosa of patients with allergic rhinitis.

Our study shows that p75^{NTR} is expressed on the basalepithelial layer and peripheral nerves in bronchial and nasal mucosa in AR and control subjects. This is in agreement with the finding of Ricci et al. who assessed an expression of p75^{NTR} on neurons in human lung (24). Interestingly, we found a lower number of p75^{NTR}-positive nerve bundles in nasal mucosa of AR compared to control subjects at T₀. An explanation for this lower expression could be the internalization of p75^{NTR} and, thereby, the down-regulation of receptor surface expression. In a mouse model, it was shown that p75^{NTR} contributes to airway hyperreactivity and airway inflammation in the lower airways (17). In our present study, we did not find a correlation of p75^{NTR} expression and clinical

data, suggesting that p75^{NTR} has no prominent role in the modulation of airway function in the present model.

NGF and BDNF promote hematopoietic colony growth and participate in wound healing and tissue repair, contributing to airway remodeling in inflammatory conditions. Therefore, our observations of increased BDNF and NGF levels with down-regulation of trkB (Table 2) may have important implications for the pathophysiological role of neurotrophins in mucosal immunity and inflammation in AR, pointing to neuroimmune interactions. In addition, the positive correlation of nasal BDNF with the maximal increase of total nasal symptom score underlines the contribution of BDNF to upper airway pathophysiology. Thus, production of NGF and BDNF may represent a well-integrated mechanism for maintaining and/or restoring homeostatic functions of nasal tissue during or after inflammation in AR. The role of neurotrophins in naso-bronchial interaction could, however, not be confirmed in this allergen provocation model.

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