

The influence of inhalative corticosteroids on circulating Nerve Growth Factor, Brain-Derived Neurotrophic Factor and Neurotrophin-3 in allergic asthmatics

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Summary

Background The neurotrophins Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF) and Neurotrophin (NT)-3 are produced, stored and released by various immunological cells. The influence of NTs upon the function of these cells is described. Elevated plasma levels were found in inflammatory, autoimmune and allergic diseases with the highest levels in allergic asthma. A connection between bronchial hyper-responsiveness and serum levels has been reported.

Objective Little is known about the influence of treatment with inhaled corticosteroids (ICS) on serum NT levels and their influence on the asthmatic state.

Methods Eighty-seven volunteers were studied. Thirty-eight were stable allergic asthmatics with constant ICS doses, 29 were asthmatics not receiving anti-asthmatic treatment and 20 were age- and sex-matched healthy controls. Demographic and lung function data were evaluated. NT serum levels were determined by ELISA.

Results NGF and BDNF levels were significantly increased in untreated asthmatics compared to the control and the treated group, while NT-3 demonstrated significantly higher levels in treated asthmatics compared to healthy controls. After stabilization of untreated subjects with ICS, the NT levels decreased significantly.

Conclusions These results suggest that NTs participate in allergic inflammation and asthma. Effective treatment leads to a decrease of circulating neurotrophic factors.

Keywords: NGF, BDNF, NT-3, neurotrophins, allergic asthma, inhaled corticosteroids, neurotrophin regulation

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Introduction

The neurotrophins Nerve Growth Factor (NGF), Brain-Derived Neurotrophic Factor (BDNF) and Neurotrophin 3 (NT-3) belong to a chemically well-described family of neurotrophic factors with high homology. NGF was the first described [1,2], and represents the best examined neurotrophin, but BDNF and NT-3 are also well-characterized [3,4]. Various other NTs have been discovered more recently [5,6]. NTs are expressed in the central and

peripheral nervous system, where they play an important role in the development, function and survival of neuronal cells [7] as well as the production of neuropeptides [8]. All actions of these NTs are modulated via their high affinity specific receptors trkA, trkB, trkC and their low affinity unspecific receptor p75 [9].

In recent years numerous studies have described the effects of NTs, especially NGF, on other systems besides the nervous system. In relation to allergic and non-allergic airway diseases it is of great interest that NT receptors have been found on various immunologically active cells, e.g. lymphocytes and mast cells [10]. The crucial role of mast cells in allergic diseases is well-described. They are able to

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produce, store and release NGF [11,12]. *In vitro* stimulation with NGF leads to degranulation with the release of preformed and newly generated mediators such as histamine, leukotrienes and cytokines. Their proliferation and differentiation is also influenced [13]. T and B cells proliferate and can be activated by NGF, which leads to the release of cytokines and the production of immunoglobulins [14]. Basophils can also be stimulated to release their mediators, and their survival is prolonged [15]. Besides neuronal cells and astrocytes, T and B cells, mast cells [11], fibroblasts and basophils are known to produce NGF [16,17].

In view of the influence upon immunologically active cells, the role of NTs in various immune diseases has been discussed, since elevated plasma levels of NGF were described in autoimmune disorders such as lupus erythematosus, psoriasis, Kawasaki syndrome and rheumatoid arthritis [18,19]. Elevated levels have also been discovered in infections [20,21]. However, emotional stress or physical exercise is also able to increase circulating NTs [22].

NTs participate in the pathophysiology of allergic diseases and asthma. Elevated plasma levels were found in patients suffering from asthma, with the highest levels in allergic asthmatics [23,24]. After segmental allergen provocation an increase of the NTs NGF and BDNF in the bronchoalveolar lavage fluid has been described in the late-phase reaction [25]. In mice and rats NGF was able to induce bronchial hyper-responsiveness (BHR). In rats a NK-1 receptor mediated pathway was responsible for this action [26,27]. Mice overexpressing NGF develop a hyperinnervation of the airways which may cause BHR as a consequence of the increased content of NTs in neurons and the liberation of neuropeptides in the airways [28]. NGF is a known regulator of neuropeptide synthesis in sensory nerves [29], which is an important aspect in asthma by triggering neurogenic inflammation.

Various authors have reported on a regulation of neurotrophic factors by stimuli such as hormones, glucocorticosteroids or inflammatory mediators [30–33]. However, it remains unclear if the elevation of NGF is a partial cause of the disease or an effect of inflammatory processes induced by allergens. The origin of circulating NTs in allergic diseases is unknown. Mast cells, migrated blood cells and local pulmonary cells in the inflamed tissue are discussed as important sources. There is little evidence concerning the role of circulating NGF in allergic asthma and even less about BDNF and NT-3. The aim of the presented clinical study was to show if NTs are altered in allergic asthmatics and to demonstrate the influence of inhaled corticosteroids (ICS) on circulating levels.

Methods

Patients

Eighty-seven volunteers were included in the study. Data on age, sex, requirement of ICS and FEV₁ were collected anonymously. All participants gave informed consent. The study was approved by the local ethics committee. Three different groups were examined:

- Thirty-eight patients with stable allergic asthma being treated with ICS in the mean dose of 680 ± 26.1 µg beclomethasone dipropionate (BDP) equivalent for at least one year. Salbutamol metered dose inhaler (MDI) (100 µg per puff) was allowed as rescue medication.
- Twenty-nine patients with allergic asthma – diagnosed for the first time – without any anti-asthmatic treatment. Patients were treated with an individual dose of ICS and salbutamol MDI (100 µg per puff) as rescue medication. Twelve patients requiring permanent ICS were followed over 4–6 weeks until stabilization in weekly intervals. Self-monitored β_2 -agonist consumption was checked after weeks 1 and 4. ICS were applied in a mean dose of 567.3 ± 96.9 µg BDP equivalent.
- Twenty age- and sex-matched healthy controls.

Allergy diagnosis

Allergy was diagnosed on the basis of history and skin prick test with at least one positive reaction (weal greater than the histamine response) to 10 common inhalant allergens including pollen, dust mites and animal dander (Allergopharma, Hamburg, Germany).

Total IgE was detected by the CAP system [34,35] (Pharmacia, Freiburg, Germany).

Lung function

Complete lung function was measured with a bodyplethysmograph (Bodylab, Jaeger, Wuerzburg, Germany). BHR was determined in patients not receiving ICS therapy by the per cent decrease of FEV₁ after inhalation of 20 breaths (20 µg/breath) – total dose 400 µg – acetylcholin (ACh). Administration was performed by using a breath dependent nebulizer (Jaeger).

Serum determination of NGF, BDNF and NT-3

Venous blood was drawn between 8 and 11 AM. Salbutamol was not allowed until 6 h prior to sampling. After clotting, samples were centrifuged (Rotina 35 R, Hettich, Tuttlingen, Germany) at 1500 g. Serum was immediately frozen at -30 °C. Neurotrophic factors were detected in sandwich ELISAs according to the manufacturer's instructions for

NGF (R & D Systems, Minneapolis, MN, USA), and BDNF and NT-3 (Promega, Madison, WI, USA). All assays were performed on F-bottom 96-well plates (Nunc, Wiesbaden, Germany). Tertiary antibodies were conjugated to horseradish peroxidase. Wells were developed with tetramethylbenzidine and measured at 450 nm. NT content was quantified against a standard curve calibrated with known amounts of protein. The detection limits were 4 pg/mL for NGF/BDNF and 8 pg/mL for NT-3. Measurements were performed in duplicate and are expressed as means. Cross-reactivity to related neurotrophic factors were less than 3% for all 3 analysed NTs.

Statistical analysis

Results were calculated as the mean $\pm$ SEM. Values were compared by using the non-parametric test for independent values (Whitney–Mann *U*-test) and dependent values (Wilcoxon signed rank test) with SPSS, Version 8.01 (SPSS Inc., Chicago, IL, USA). *P*-values < 0.05 were regarded as significant. Correlations were calculated using the linear regression and Spearman's rank method.

Results

While BDNF was detected in all subjects, NGF was below the detection limit in 8 of the control group ($n = 20$), 9 in the untreated group ($n = 28$) and 13 cases in the treated group ($n = 38$). NT-3 was undetectable in 8 of the healthy controls, in 6 in the untreated group and in 3 subjects in the treated group.

No influence of age or sex on NT levels was seen [36]. Our results did not show any correlation between serum immunoglobulin (Ig)E, measured lung function parameters or ICS doses and the different serum NT values.

Table 1. Patient data

	Healthy controls <i>n</i> = 20	Untreated asthmatics <i>n</i> = 29	Treated asthmatics <i>n</i> = 38
Male	10	14	20
Female	10	15	18
Age (years)	36.1 $\pm$ 2.4	37.8 $\pm$ 2.5	40.2 $\pm$ 1.9
IgE (kU/L)	22.3 $\pm$ 4.7	412.5 $\pm$ 79.1	224.5 $\pm$ 28.1
FEV ₁ (% of predicted)	99.8 $\pm$ 1.1	76.8 $\pm$ 2.8	66.9 $\pm$ 1.8
ICS (μ g/day)	0	0	680 $\pm$ 26.1
β_2 -usage (puffs/day)	0	0	1.1 $\pm$ 0.1
NGF (pg/mL)	37.6 $\pm$ 17.7	249.2 $\pm$ 100.0	55.5 $\pm$ 21.9
BDNF (ng/mL)	17.3 $\pm$ 1.6	27.0 $\pm$ 2.6	16.8 $\pm$ 1.5
NT-3 (ng/mL)	0.9 $\pm$ 0.3	1.3 $\pm$ 0.3	1.7 $\pm$ 0.2

Serum determination of NGF, BDNF and NT-3

NGF NGF was significantly higher; $P < 0.01$ (249.2 ± 100.0 pg/mL) in the untreated group compared to healthy controls (37.6 ± 17.7 pg/mL). Plasma levels of treated asthmatics were significantly lower (55.5 ± 21.9 pg/mL) compared to untreated asthmatics; $P < 0.01$. No significant difference was found between controls and treated asthmatics. (Table 1; Fig. 1).

BDNF BDNF was significantly higher; $P < 0.01$ (27.0 ± 2.6 ng/mL) in the untreated group compared to healthy controls (17.3 ± 1.6 ng/mL) and the treated group (16.8 ± 1.5). No significant difference was found between

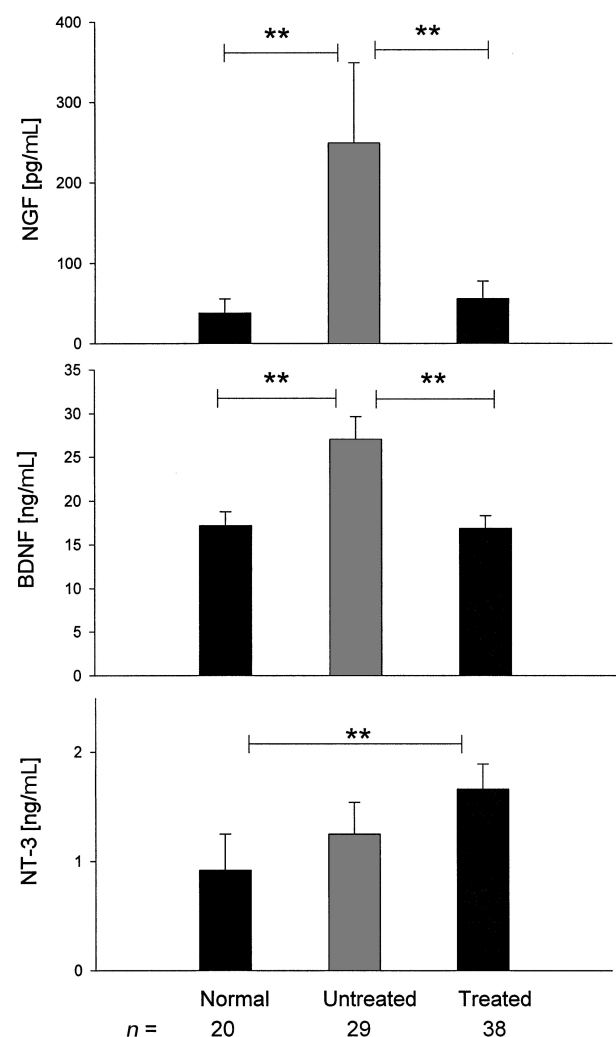


Fig. 1. Serum levels of NGF, BDNF and NT-3 in the control group $n = 20$, untreated group of asthmatics $n = 29$ and the group of treated asthmatics $n = 38$. Values are shown in mean $\pm$ SEM (** $P < 0.01$)

the treated (16.8 ± 1.5 ng/mL) and the control group (17.3 ± 1.6 ng/mL) (Table 1; Fig. 1).

NT-3 NT-3 was significantly higher in the treated group (1.7 ± 0.2 ng/mL) compared to healthy controls (0.9 ± 0.3 ng/mL); $P < 0.05$. No difference was found between the untreated group (1.3 ± 0.3 ng/mL) and the other groups (Table 1; Fig. 1).

Results after stabilization in the untreated group

After clinical stabilization of the untreated group with the individual required dose of ICS, in 12 patients – in which follow-up was possible – serum NT levels as well as lung function parameters were retested.

NGF demonstrated a significant decrease of 55.8%; $P < 0.05$ (458.6 ± 221.4 pg/mL to 202.3 ± 113.5 pg/mL). Mean BDNF levels decreased significantly by 26.9%; $P < 0.05$ (29.4 ± 4.4 ng/mL; 21.5 ± 3.1). In the case of NT-3 mean serum values decreased by 45.3%; $P < 0.05$ (1.15 ± 0.5 ; 0.63 ± 0.4 ng/mL) (Table 2; Fig. 2).

Mean FEV₁ increased from 79.25 to 86.5% of predicted. BHR decreased by 35% from 21.6 to 13.2% of baseline FEV₁ after inhalation of 400 µg ACh. The use of rescue medication (Salbutamol) dropped from 1.3 to 0.3 puffs/day.

Discussion

Neurotrophic factors are a group of mediators influencing neuronal as well as immune cells, representing a connection between the nervous and the immune system. Their

effect on neurons leads to an increased production of neuropeptides which are increased during asthma exacerbations [37]. Their liberation is important for the development of BHR in asthma by neurogenic inflammation [38–40]. This pathway could be triggered by immune cells with the potential to release NTs in allergic inflammation. Vice versa immune cells are widely influenced by NTs. They are able to induce degranulation and differentiation of mast cells, the key cells of the early allergic response [41]. T and B cells as well as granulocytes are activated with the consequence of mediator release and production of

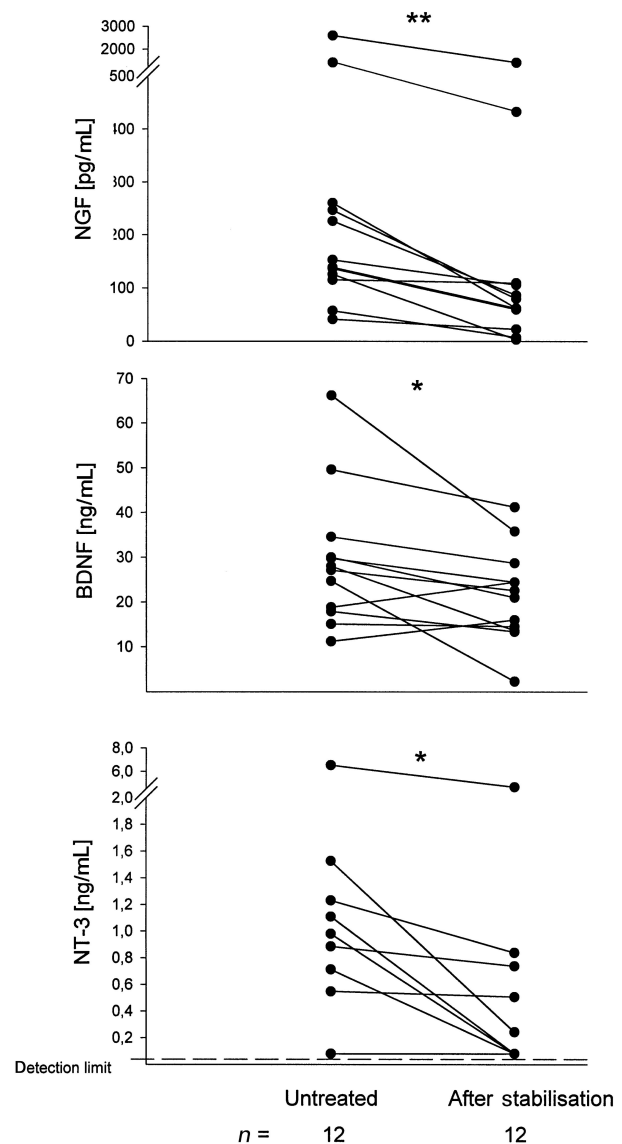


Fig. 2. Serum levels of NGF, BDNF and NT-3 in the untreated group of asthmatics before and with effective treatment with ICS at the required dose. Mean value is shown (* $P < 0.05$; ** $P < 0.01$).

Table 2. Data of re-evaluated patients

	Untreated <i>n</i> = 12	After stabilization <i>n</i> = 12
Male	6	6
Female	6	6
Age (years)	40.0 ± 4.1	40.0 ± 4.1
IgE (kU/L)	416.1 ± 128.6	416.1 ± 128.6
FEV ₁ (% of predicted)	79.25 ± 4.3	86.5 ± 2.8
BHR*	21.26 ± 3.4	13.2 ± 2.4
ICS (µg/day)	0	567.3 ± 96.9
β ₂ -usage (puffs/day)	1.3 ± 0.3 after 1st week	0.3 ± 0.2
NGF (pg/mL)	458.6 ± 221.4	202.3 ± 113.5
BDNF (ng/mL)	29.4 ± 4.4	21.5 ± 3.1
NT-3 (ng/mL)	1.15 ± 0.5	0.63 ± 0.4

*BHR was measured after inhalation of a total dose of 400 mg ACh in percent decrease of FEV₁ from baseline.

immunoglobulins and cytokines. These interactions enable NTs to modulate allergic asthma via influencing neurogenic impulses and immunoactive cells in inflammatory responses of the airways [26,27].

In this study, we could demonstrate that NGF and BDNF levels are elevated in untreated asthmatics. NGF, BDNF and NT-3 were down-regulated by ICS during the clinical stabilization period accompanied by improvement of symptoms and lung function. NT-3 levels in patients treated with ICS for at least 1 years were significantly higher compared to the control group. This indicates that after a primary down-regulation, there must be a counter-regulating mechanism.

Evaluating asthma severity in the 2 groups, both include mild or moderate asthmatics but the majority of the treated group belonged to the moderate state of asthma judged by FEV₁ and steroid dosage necessary for compensation. There was no correlation between the NT concentrations and lung function or the ICS dosage but circulating levels of NTs do not necessarily mirror changes in the airways. Influence of β_2 mimetics on NTs has not been described and the difference in consumption (Table 1 and 2) is unlikely to explain NT regulation considering their half-life period. Additionally, the β_2 mimetics were used as needed with high inter- and intra-individual differences at the time of evaluation. Therefore we conclude that the down-regulation of NTs is a consequence of ICS treatment. The difference in NT-3 levels may be due to divergent asthma history of the patients. The untreated group was recruited from asthmatics diagnosed for the first time with a short history of wheezing and coughing while the treated group had suffered from asthma for a longer period requiring ICS therapy for at least one year. Maybe secondary changes of the remodelled bronchial system might lead to the increase of NT-3 level. This elevation can be a consequence of tissue changes or the release of mediators during the remodelling process. On the other hand, antagonistic functions during chronification with tissue protective effects might be possible as described in neuronal lesions and inflammatory hyperalgesia [42,43].

The functions of different NTs might differ in inflammation. Pro-inflammatory and anti-inflammatory effects are present at the same time mediated by NTs with antagonistic functions in the regulation of inflammation. The time-course of the different NTs remains to be clarified. The liberation of a pro-inflammatory NT might cause a delayed up-regulation of a NT with anti-inflammatory properties. In neuronal cells this effect has been described for BDNF mRNA after NGF stimulation [44]. NT elevation might also be a consequence of pro-inflammatory cytokines such as interferon (IFN) however, NGF and further NTs influence the production and secretion of cytokines as demonstrated for mast cells [45,46].

Down-regulation of NTs after different stimuli including

corticosteroids has been reported in cell culture systems but not *in vivo* [27,47]. Steroids are an effective and established therapy in asthma. Their high anti-inflammatory potential with all the consequences for migration and activation of cells is well-described. Corticosteroids are able to regulate numerous inflammatory mediators by down-regulation of pro-inflammatory or up-regulation of anti-inflammatory mediators [48]. ICSs do not appear in pharmacological relevant doses in the serum. Neither do they influence the hypothalamic pituitary axis in the given dose [49,50]. However, there is no influence on circulating or tissue-connected cells other than pulmonary cells. Since serum levels of NTs – as shown in this study – are modulated by ICS, a high amount of NTs appearing in the serum could be produced by pulmonal cells. Candidates are either immune cells such as leucocytes and mast cells, or various other resident pulmonary cells such as epithelial cells [51], smooth muscle cells [52] endothelial cells, or fibroblasts [53,54] involved in the inflammatory process in asthma.

Regulating neurotrophic factors provides an additional element in the clarification of the outstanding pharmacological potential of corticosteroids.

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