

Antibodies directed against nerve growth factor inhibit the acute bronchoconstriction due to allergen challenge in guinea-pigs

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Summary

Background We have previously demonstrated that the administration of nerve growth factor (NGF) to guinea-pigs results in airway hyper-responsiveness within 1 h.

Objective In the present study we document the involvement of NGF in the acute allergic airway response.

Methods Guinea-pigs that are sensitized to ovalbumin show an acute bronchoconstriction directly after challenge with ovalbumin.

Results Intratracheal application of 10 µg of antibodies directed against NGF (anti-NGF) 1 h before the challenge reduces the acute severe bronchoconstriction to approximately 40% and the sustained bronchoconstriction to approximately 20% of the reaction in controls. This shows a high potency of anti-NGF in diminishing the direct bronchoconstriction. Inhibition of the tyrosine kinases of the tyrosine kinase receptor A, the high-affinity receptor for NGF, has no effect on the bronchoconstriction. Therefore, we postulate that the p75, the low-affinity receptor for neurotrophins, is responsible for the acute bronchoconstriction. Our findings suggest a role for NGF in the induction of the acute asthmatic reaction.

Conclusion These findings offer a new potential therapeutic strategy for the treatment of allergic asthma.

Keywords allergy, asthma, inflammation, lungs, neurotrophins

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Introduction

Nerve growth factor (NGF) is a member of the neurotrophin family of proteins that regulate neuronal development, maintenance, and recovery from injury. NGF is very homologous amongst different species [1,2] and has been extensively studied in relation to neurite outgrowth [3]. However, it now becomes clear that NGF also plays a crucial role in inflammation [4,5].

Inflammation leads to an enhanced production and release of NGF. Inflammatory mediators, including interleukin (IL)-1, IL-4, IL-5, tumour necrosis factor (TNF)- α and interferon (IFN)- γ have been shown to induce the release of NGF [6,7]. In addition to neurones, also non-neuronal cells, such as mast cells [8], fibroblasts [6], T cells [9] and epithelial cells [10], are able to synthesize NGF.

NGF affects immune cell activity, as it promotes inflammatory mediator release from basophils [11], mast cells [12,13], T cells [5,9] and macrophages [14]. NGF is also able to sensitize neurones and induce an enhanced production of substance P and other tachykinins in sensory nerves [4,15]. Tachykinins play a role in neurogenic inflammation and in the development of airway hyper-responsiveness and asthma [16]. Furthermore,

we showed that a neurokinin-1 receptor antagonist blocked NGF-induced airway hyper-responsiveness, indicating a role for the neurokinin-1 receptor ligand substance P [17].

Interestingly, circulating NGF levels are increased in humans with allergic diseases (rhinoconjunctivitis, urticaria-angioedema, asthma [18]). In the study by Bonini and colleagues, NGF serum levels were particularly high in patients with allergic asthma [18]. Furthermore, allergen challenge induces an increase in NGF levels in bronchoalveolar lavage fluid of sensitized mice [19]. Allergen provocation is able to induce an increase in NGF levels in nasal lavage fluid in patients with allergic rhinitis [20].

NGF can interact with two receptors: either the low-affinity receptor p75, which can also bind other neurotrophic factors, such as brain-derived neurotrophic factor and neurotrophin 3, or the tyrosine kinase receptor A (trkA). Of these two receptors, only the trkA is linked to tyrosine kinases for its signal transduction. So far, no data are available on the receptors for NGF and their function in the airways.

In a previous study we have demonstrated that intravenous administration of NGF to guinea-pigs results in airway hyper-responsiveness, indicating a role for NGF in the development of one of the symptoms of the asthmatic disease [17]. These findings prompted us to further investigate the role of NGF in allergic asthma. We tested the effects of antibodies directed against NGF and an inhibitor of the tyrosine kinases of the trkA in the acute ovalbumin-induced bronchoconstriction in guinea-pigs.

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Materials and methods

Ovalbumin sensitization

Male Hartley guinea-pigs (weighing approximately 400 g; Harlan CPB, Zeist, the Netherlands) were sensitized to ovalbumin, grade V (Sigma Chemical Co., St Louis, MO, USA) by injections of a gel containing 20 µg ovalbumin/mL, and 200 mg Al(OH)₃/mL as adjuvant. Six injections were placed: one injection of 0.5 mL intraperitoneally, one of 0.1 mL nuchally, two injections of 0.1 mL axillary, and two of 0.1 mL inguinally. Control animals were not sensitized and received instead a vehicle gel, not containing ovalbumin.

Measurement of acute bronchoconstriction

Fourteen days after the sensitization procedure, the guinea-pigs were individually placed in a box to measure airway function before and after ovalbumin challenge. Each challenge consisted of a 2-s nebulization of 0.01% ovalbumin in sterile saline (Ultra-Neb 2000™). All animals received the ovalbumin challenge.

The airway function of the unrestrained guinea-pigs was measured using whole body plethysmography (Buxco, Troy, NY, USA). As parameter of airway function, the enhanced pause (Penh) was determined. The Penh closely reflects the changes in pulmonary resistance during bronchoconstriction [21,22]. As suggested by Drazen and colleagues [23], we inspected the barometric plethysmograph pressure tracings and assured ourselves that the pressure variations corresponded to those from which the Penh parameter was derived. After 5 min of measuring baseline airway function, the ovalbumin challenge was performed.

Application of antibodies directed against NGF (anti-NGF) and tyrosine kinase inhibitor

Guinea-pigs were treated intratracheally with 10 µg anti-NGF dissolved in 50 µL saline (anti-human β-NGF; R & D Systems, Minneapolis, MN, USA) or 10 µg IgG isotype control antibody (goat IgG, Sigma) 1 h before ovalbumin challenge. The polyclonal antibodies directed against human β-NGF were raised in the goat and purified (IgG fraction) by affinity chromatography. A dose of 0.1 µg/mL anti-NGF neutralizes 5 ng/mL NGF. For treatment with tyrphostin AG879 (Calbiochem Novabiochem, San Diego, CA, USA), 200 µL of 0.2 mm tyrphostin AG879 was applied intratracheally 1 h before ovalbumin challenge. Tyrphostin AG879 is an inhibitor specific for the tyrosine kinases on the trkA [24]. Control animals received the vehicle, saline containing 2% dimethyl sulfoxide (DMSO). In order to apply the solutions intratracheally, the guinea-pigs were anaesthetized using halothane (2% halothane, 0.4 L/min O₂, 2 L/min N₂O, approximately 15–30 s), and placed in a supine position with their heads tilted backwards. Subsequently, the jaws were kept apart by two elastic bands, and a needle with a bulbous tip was inserted to just behind the glottis. Solutions were gently injected in the trachea, the bands were removed and the chest was gently massaged. The animals all regained consciousness within 30 s after this procedure.

The Animal Care Committee of Utrecht University approved all animal studies.

Statistics

Means and standard error of the mean (SEM) were calculated. *P*-values were determined using an unpaired two-tailed

Student's *t*-test for the comparison of two means. Probability values of *P* < 0.05 were considered significantly different.

Results

Ovalbumin challenge in sensitized guinea-pigs resulted in an increase in Penh that started about 3 min after the ovalbumin challenge and resulted in a maximal Penh between 5 and 10 min after the challenge. The maximal Penh rose above values of 20, which is comparable with the bronchoconstriction induced by more than 20 µg/kg body weight histamine intravenously and corresponds to a severe bronchoconstriction.

Anti-NGF prevented the severe and sustained bronchoconstriction induced by the ovalbumin challenge (Fig. 1). Treatment with anti-NGF showed a rapid decline to a low and stable Penh after reaching no peak at all or a very low peak Penh, whereas in the IgG-treated guinea pigs the peak Penh was much higher and decreased more slowly (Fig. 1). The area under the curve was diminished greatly by the anti-NGF treatment. In the typical curves the area under the curve measured from 0 to 20 min after allergen challenge declined from 33.4 to 11.7 arbitrary values. Measurement of the area under the curve for the whole group was not possible, as two of the control animals showed an extreme bronchoconstriction and had to be taken out of the equipment. This indicates that the inhibitory effect of NGF is even more pronounced, as none of the animals treated with anti-NGF showed such a severe bronchoconstriction.

Peak Penh was significantly suppressed by application of anti-NGF (Fig. 2). The peak for non-sensitized animals was 0.34 ± 0.04 (*n* = 4), whereas ovalbumin challenge in ovalbumin-sensitized and control antibody-treated animals led to an increase to 14.60 ± 3.30 (*n* = 4). Anti-NGF reduced the peak to 5.98 ± 1.92 (*n* = 5, *P* = 0.02 anti-NGF vs. control antibody). Treatment with anti-NGF showed a diminished bronchoconstriction compared to IgG treatment, not only when comparing peak bronchoconstriction but also when comparing the sustained bronchoconstriction at 5, 10 and 15 min after the allergen challenge (Fig. 2).

Pre-treatment with the specific tyrosine kinase inhibitor tyrphostin AG879 did not affect the acute bronchoconstriction due to allergen challenge (Fig. 3), making involvement of the trkA receptor in this process rather unlikely.

Discussion

Antibodies directed against NGF inhibited the duration of the severe and sustained bronchoconstriction to allergen challenge (Figs 1 and 2). The Penh value was largely reduced as well at several time points after the ovalbumin challenge (Fig. 2). This inhibitory effect indicates a beneficial effect of anti-NGF on the duration and magnitude of the severe bronchoconstriction.

Locally applied anti-NGF 1 h before allergen challenge markedly reduced the bronchoconstriction; this strongly suggests that a rapid local release of NGF took place. Indeed, increased levels of NGF have been reported in relation to allergic responses several times. In patients suffering from allergic asthma an enhancement in NGF in serum [18], as well as in bronchoalveolar lavage [25,26], is reported. Allergen challenge in patients with allergic rhinitis allegedly leads to an acute increase in NGF in nasal lavage fluid [20]. The NGF increase is confirmed in an animal model: in sensitized mice levels of NGF are enhanced in

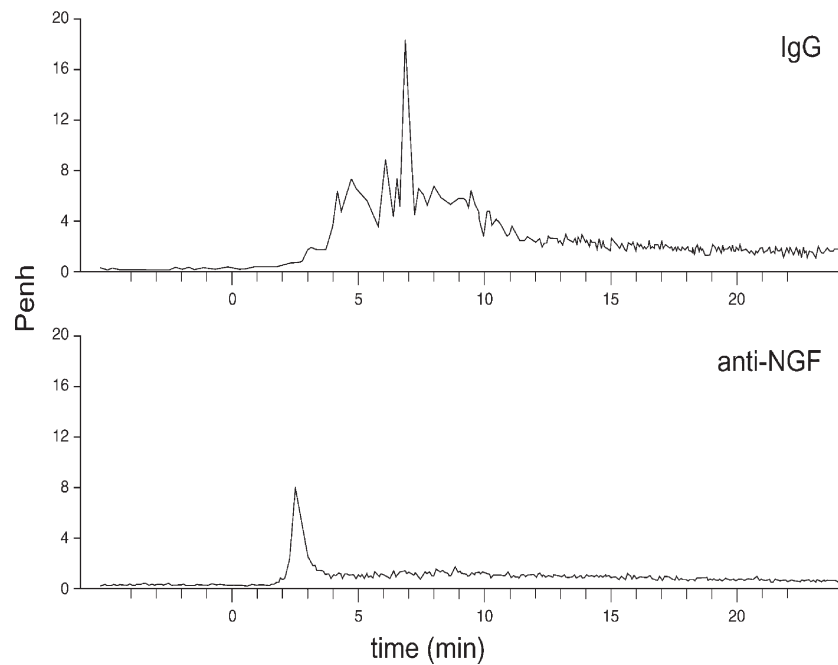


Fig. 1. Typical Penh traces in time after allergen challenge. Guinea-pigs sensitized and challenged with ovalbumin (at time = 0) were treated with IgG control antibody (upper panel) or with anti-NGF (lower panel).

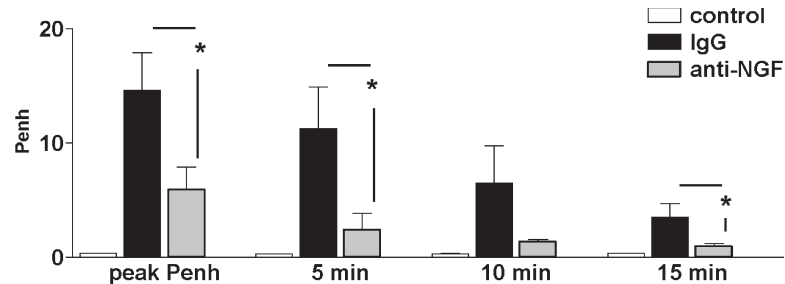


Fig. 2. Treatment with anti-NGF 1 h before ovalbumin challenge reduces the acute bronchoconstriction. Bronchoconstriction is shown at peak, 5 min, 10 min and 15 min after ovalbumin challenge. Controls are the non-sensitized animals (open bars), IgG and anti-NGF are guinea-pigs sensitized to ovalbumin, treated with the control IgG antibody (solid bars) or anti-NGF (grey bars), respectively ($n=4-5$). Bronchoconstriction is expressed as Penh. (* $P < 0.03$, anti-NGF vs. IgG, Student's t -test).

the bronchoalveolar lavage 24 h after allergen challenge [19]. Also, increased amounts of NGF can be found in the lungs of allergen-sensitized and challenged guinea pigs (De Vries and Fischer, personal communication). However, NGF levels in sensitized mice, before challenge, are equal to control animals [19]. This implies that allergen challenge is necessary for NGF up-regulation. The increase in NGF serum levels in patients suffering from allergic asthma could be due to a constant exposure to allergens in their environment. As allergen challenge is presumably necessary for NGF increases [19], we suggest that NGF is released immediately after allergen challenge. Furthermore, a large variety of cells that are able to release NGF, such as eosinophils, epithelial cells, mast cells, basophils, T cells and fibroblasts [6,8-11,27], are present in the airways, and these cells could be the source of NGF.

In our previous study, we showed that intravenously administered NGF induced an airway hyper-responsiveness within 1 h [17]. Our study pointed to a role for substance P in NGF-induced airway hyper-responsiveness, as a neurokinin-1 receptor antagonist was able to prevent airway hyper-responsiveness [17]. Few studies are published on the involvement of tachykinins in acute allergic responses in the airways. Antigen-induced microvascular leakage revealed a role for substance P in the early allergic response [28]. Recently it was shown that toluene diisocyanate induces a rapid increase of substance P; 1 h after inhalation of toluene diisocyanate the number of substance

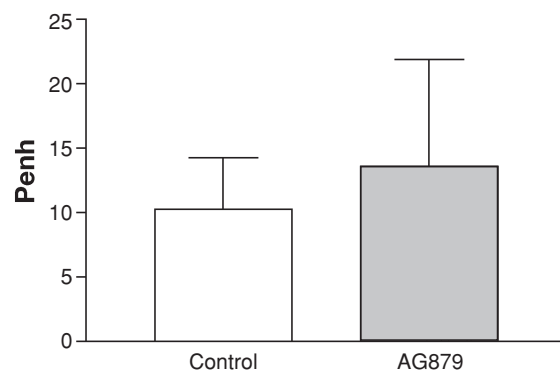


Fig. 3. Treatment with tyrphostin AG879 1 h before ovalbumin challenge does not reduce the acute bronchoconstriction. The peak bronchoconstriction (expressed as Penh) is shown after ovalbumin sensitization and challenge, in animals pre-treated with vehicle (control) or the tyrosine kinase inhibitor (AG879). Peak bronchoconstriction in non-sensitized but challenged animals amounted to 0.32 ± 0.04 ($P < 0.05$ compared with the sensitized control group).

P immuno-reactive nerve fibres was already increased [29]. We postulate that NGF stimulating the sensory neurones could underlie the allergen-induced bronchoconstriction in our present study. Confirming this idea is the acutely enhanced response to capsaicin, inducing tachykinin release from sensory nerve endings, by NGF [30].

We further demonstrated that inhibition of the tyrosine kinases of the *trkA* did not influence the acute bronchoconstriction due to allergen challenge (Fig. 3). NGF can bind to either the *trkA*, the high-affinity receptor for NGF, which is specific for NGF, or to the *p75* with a lower affinity [3]. We hypothesize that the *p75* is responsible for the effects exerted by NGF in the development of the acute allergic response. So far, no studies have reported on *p75* or *trkA* function in the airways. In addition to the acute allergic reaction and airway hyper-responsiveness, allergic asthma is characterized by airway inflammation. In other models, the involvement of *p75* has been revealed in relation to inflammation. Inflammation induced an up-regulation of *p75* in inflamed tissues, suggesting that NGF, or other neurotrophic factors such as brain-derived neurotrophic factor or neurotrophin 3, participate in NGF-induced hyperalgesia [31].

NGF is involved in the development of airway hyper-responsiveness [17], one of the characteristic features of asthma. In our study presented here we show that NGF also plays a crucial role in the cascade of events leading to an acute bronchoconstriction due to allergen challenge, possibly via the *p75*, a receptor for neurotrophic factors. This indicates that NGF is involved in the early events of an acute allergic reaction. Therefore, we hypothesize a regulatory role for NGF in both acute allergic responses as well as in the induction of airway hyper-responsiveness. This offers a new potential therapeutic approach for the treatment of allergic asthma.

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