

EDITORIAL

Nerve growth factor and the vanilloid receptor: partners in crime?

E. van den Worm, F. P. Nijkamp and F. Engels

Neurotrophins and nerve growth factor

Neurotrophins play an essential role in the nervous system. They promote neuronal survival, maintenance and differentiation by binding and activating specific receptors [1]. Neurotrophins also play a role in neurogenic inflammation [2, 3]. Neurogenic inflammation contributes to pathological phenomena in several diseases, including asthma [4], inflamed skin [5] and neuropathic pain [6]. It involves a change in function of sensory neurons due to inflammatory mediators, thereby inducing an enhanced release of tachykinins from these nerves [4]. Nerve growth factor (NGF) was the first neurotrophin to be discovered [7] after an experiment in which the investigators observed a loss of innervating neurons following the removal of peripheral tissue [8]. In the years following its discovery NGF has been a subject of many studies. NGF is a homodimeric molecule [9], and comprises two molecular forms: 7S NGF and 2.5S NGF [10, 11]. NGF can interact with two receptors: either the tyrosine kinase A (TrkA) receptor or p75. TrkA is a receptor with Trk activity and forms a high-affinity binding site for NGF [12]. Upon binding of NGF to the TrkA receptor, the NGF–TrkA complex is internalized and retrogradely transported to the nucleus, where mRNA levels for preprotachykinin, the precursor for tachykinins, are affected [13, 14]. Alternatively, TrkA activation leads, in a Trk-dependent manner, to phosphorylation of proteins at the nerve terminal, which can induce changes in the properties of the nerve ending [15, 16]. NGF displays this effect by inducing a very fast accumulation of second messengers or phosphorylation of pivotal transduction-related proteins or ion channels, thereby sensitizing the peripheral sensory nerve ending [15, 16]. The low-affinity receptor p75 can, besides binding NGF, bind several other neurotrophic factors such as brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3) and NT-4 [17]. The p75 receptor also causes an up-regulation in tachykinin content in sensory neurons.

NGF can be produced by a wide variety of cells and, in turn, can affect many different cells. In addition to neurons, also non-neuronal cells such as mast cells [18], fibroblasts [19], T cells [20, 21], B cells [22], eosinophils [23], lymphocytes [22] and airway epithelial cells [24] can synthesize NGF. Several inflammatory mediators, including IL-1, IL-4, IL-5, TNF- α and IFN- γ , induce the release of NGF [19, 25]. NGF promotes inflammatory mediator release from basophils [26], mast cells [27], T and B cells [21, 28], eosinophils [23] and macrophages [29].

Especially over the past decade, neurotrophic factors have generated much excitement for their potential use as therapy for neurological disorders. In this regard, NGF has generated great interest as a possible target for the treatment of Alzheimer's disease [30–32].

Nerve growth factor and asthma

NGF and other neurotrophins like BDNF, NT-3, and NT-4 are recently discovered players in the field of asthma research [33]. Virchow et al. [34] demonstrated that NGF levels in bronchoalveolar lavage fluid from asthmatic patients were elevated after segmental allergen provocation. Also, circulating NGF levels were increased in patients with allergic diseases and asthma [35, 36]. In allergen-sensitized mice, anti-NGF prevented the development of airway hyper-responsiveness (AHR) [37]. NGF has also been demonstrated to enhance nerve-mediated AHR in mice [37, 38]. We have recently demonstrated that anti-NGF is able to block allergen-induced bronchoconstriction in guinea-pigs [39]. We obtained evidence that NGF acts via the release of substance P (SP), since NGF-induced AHR could be blocked by a tachykinin receptor antagonist, both *in vitro* [40] and *in vivo* [41]. These and other data led us to suggest that NGF sensitizes sensory nerve endings in the airways and evokes the release of neuropeptides, which in turn affect airway function.

In this issue of the journal, Bachar et al. [42] report on the development of an *ex vivo* mouse organ culture assay to investigate the effects of NGF on nerve-mediated airway contractions. Especially since the long-term interaction between inflammatory mediators such as NGF and nerves are not yet fully understood, this study focuses on the effects of prolonged NGF administration on nerve-mediated airway contractions. Bachar et al. found that NGF is able to increase both the contractile responses of the tracheal preparations to electric field stimulation (EFS), and the number of nerve fibres supplying the tracheal segments. Both phenomena were observed exclusively in the proximal part of the trachea, suggesting a causal relationship. The authors also demonstrate a lack of involvement of capsaicin sensitive sensory nerves in the cultured segments in their murine model [42], supposedly ruling out the role of sensory nerve endings. Indeed, Bachar et al. conclude that NGF promotes the preservation or restores cholinergic innervation and that NGF may contribute to airway hyper-reactivity in asthmatic patients by increasing cholinergic neural activity in the peripheral airways. However, they also observed an increase in protein gene product 9.5-positive nerve endings, suggesting an increase of peptide expressing nerves [43]. This leaves open the possibility that sensory nerves, expressing tachykinins, are up-regulated in response to NGF. Indeed, we have observed in a guinea-pig model, that sensory nerves do play a role in NGF-induced AHR [40, 41].

Both Bachar et al. and we recognize that the exact mechanism through which NGF affects nerves supplying the airways is still not fully understood. Based on literature

findings and our own observations, we hypothesize that NGF may have an accomplice in the vanilloid receptor 1 (VR1) when playing a role in allergic asthma.

The vanilloid receptor

VR1, in many aspects, represents an interesting new research target in the field of allergic asthma. It was originally known as the capsaicin receptor [44]. Capsaicin is the pungent ingredient of hot chilli peppers and has long been known to have a unique effect on the pain sensory system [45, 46]. Capsaicin exerts a powerful excitatory effect on peripheral sensory neurons by binding to its receptor in the plasma membrane and activating ligand-gated, non-selective cation channels [33, 47]. The capsaicin receptor has been cloned in 1997 and named VR1 since then [48]. It was shown that it is activated not only by vanilloid ligands like capsaicin, but also by noxious heat and low pH (two conditions frequently found in inflamed tissue), and thus can be viewed as a molecular integrator of noxious stimuli occurring under inflammatory conditions [49]. Since the discovery of VR1, other VRs have been discovered in different types of tissue [50, 51]. VRs are expressed along the entire length of capsaicin-sensitive sensory neurons, from the peripheral terminals to the axons to the somata to the central endings [52], as shown in several species including man. The presence of VRs is not restricted to neuronal cells, since vanilloids have been shown to induce calcium uptake in mast cells [53], to stimulate migration of human polymorphonuclear cells [54], and to release SP from human lymphocytes [55]. Interestingly, capsaicin may exert either a contractile or relaxant effect on isolated human bronchi that might be mediated by VRs on smooth muscle cells [56–58]. Indeed, specific and high-affinity binding of the vanilloid resiniferatoxin (RTX) to human bronchi could be demonstrated [52, 59].

The action of capsaicin and other vanilloids at the neuronal VRs results in opening of the channel pore, leading to calcium influx [60, 61] and the release of pro-inflammatory neuropeptides from the nerve endings [62]. Capsaicin-evoked release of SP has been reported for the guinea-pig respiratory tract [63]. Taken together, there are many indications that VRs, by their virtue of releasing neuropeptides from sensory nerve endings, may play a pivotal role in neurogenic inflammation and the ensuing pulmonary effects like reversible airway obstruction and AHR. This notion is supported by the clinical observations that asthmatic patients have an increased sensitivity to capsaicin [64, 65]. This condition may arise from the inflammatory status of the asthmatic airways. Besides heat and tissue acidosis, a combination of inflammatory mediators, including bradykinin, histamine, serotonin, and prostaglandin E₂, has been shown to activate a membrane conductance also operated by capsaicin [66, 67]. It is conceivable that other factors in the inflamed airways act upon the VRs to negatively affect pulmonary function. So indeed, we hypothesize that the VRs play a role in NGF-induced AHR.

Nerve growth factor and the vanilloid receptor

As mentioned above, NGF is an essential requirement for the survival of the majority of primary sensory neurons during

development [68]. Moreover, in adult life it maintains the nociceptive properties of sensory neurons. Thus, NGF regulates the sensitivity of cultured dorsal root ganglion (DRG) neurons for capsaicin [69, 70]. Very recently, it was shown that NGF treatment of DRG neurons in culture increased both VR-1 mRNA expression and capsaicin evoked neuropeptide release [71]. In an animal model for inflammatory hyperalgesia, i.e. injection of complete Freund's adjuvant into the rat hindpaw, the observed increase of the capsaicin sensitivity of DRG neurons could be inhibited with NGF-neutralizing antibodies [72], again pointing to a regulating role of NGF. It is still not exactly known how NGF maintains the nociceptive properties of sensory neurons. It could act directly on the VR1, lowering their thresholds for activation by heat or low pH. Or possibly, NGF might affect the formation and/or release of other factors that in turn influence VR1. The recent search for endogenous capsaicin-like substances that function as activators of VRs has yielded very exciting findings in this respect. The endogenous cannabinoid anandamide, which can be synthesized by neuronal as well as non-neuronal cells [73, 74], has been shown to activate VRs on sensory nerves [75] as well as recombinant human VR1 [76]. In addition, anandamide induces apoptosis in human neuroblastoma and lymphoma cells via VRs [77]. Alternatively, direct activation of capsaicin receptors in membranes of sensory neurons and cells expressing the cloned VR1 receptor was observed with several products of lipoxygenase enzymes [78]. Apparently, 12- and 15-hydroperoxy-eicosatetraenoic acid (12-/15-HPETE), 5- and 15-hydroxyeicosatetraenoic acid (5-/15-HETE) and leukotriene B₄ may also act as endogenous agonists for VRs. This brings back in mind very early observations from our laboratory, which showed that 15-HPETE is able to induce AHR in guinea-pigs [79]. More recently, we have reported that 13-hydroxyoctadecadienoic acid (13-HODE, a structural analogue of 15-HETE) induced an AHR, which was mediated by sensory neuropeptides [80]. It is tempting to speculate that

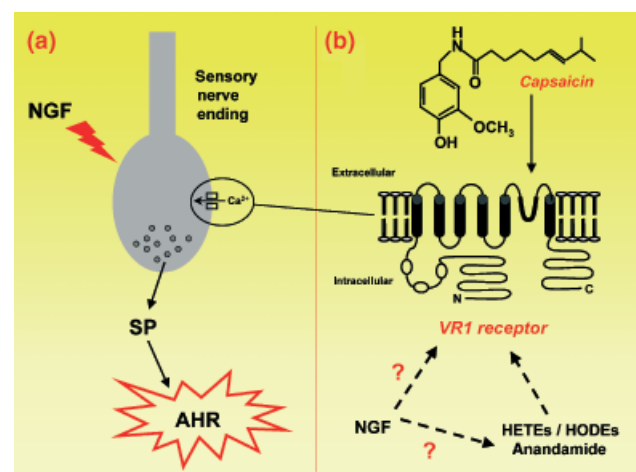


Fig. 1. Figure detailing recent findings on the role of nerve growth factor (NGF) on airway hyper-responsiveness (AHR) through the release of substance P (SP) from sensory nerve endings (panel a), and the proposed mode of action of NGF, i.e. sensitization of the vanilloid receptor (VR1), possibly through the formation of lipoxygenase products (hydroxyeicosatetraenoic acids (HETEs)/hydroxyoctadecadienoic acids (HODEs)) or the endogenous cannabinoid anandamide (panel b).

VRs play a role in these early findings as well. The possible role of VR1 is depicted in Fig. 1. Taking into consideration that NGF obviously is important both in early life as well as in adult life for maintaining sensory nerve function, it is enticing to speculate that NGF may also be at the basis of chronicity of the asthmatic constitution. Alternatively, NGF may be involved in the chronicity of allergic asthma by influencing the number of sensory nerves supplying the airways.

So, ever since its discovery in 1954, NGF has proven to be an intriguing molecule which is still awaiting the clarification of its full potential. Many experiments will undoubtedly be performed to identify the exact role that NGF has in allergic asthma, and future experiments will have to provide proof whether NGF exerts its effects through interaction with the VR1 receptor.

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E. van den Worm, F. P. Nijkamp and F. Engels
Department of Pharmacology & Pathophysiology
Faculty of Pharmaceutical Sciences
Utrecht University
PO Box 80082
3508 TB Utrecht
The Netherlands
E-mail: G.M.H.Engels@pharm.uu.nl