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Excitatory non-adrenergic–non-cholinergic neuropeptides: key players in asthma

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Abstract

Professor David de Wied first introduced the term 'neuropeptides' at the end of 1971. Later peptide hormones and their fragments, endogenous opioid (morphine-like) peptides and a large number of other biogenic peptides became classified as neuropeptides. All of these peptides are united by a number of common features including their origin (nervous system and peptide-secreting cells found in various organs such as skin, gut, lungs), biosynthesis, secretion, metabolism, and enormous effectiveness. Neuropeptides are biologically active at extremely low concentrations. The past decade, neuropeptide research has revealed that neuropeptides also participate strongly in immune reactions. The neuro-immune concept has opened up a whole new research area. In the last 20 years, significant advances have been made in investigations of the interaction between immune and nervous systems in chronic inflammatory diseases such as asthma. The goal of this review is to bring together the functional relevance of excitatory non-adrenergic–non-cholinergic (NANC) nerves and the interaction with the immune system in asthma.

Keywords: Asthma; NANC (non-adrenergic–non-cholinergic) nerve, excitatory; Neuropeptide; Animal model; (Human)

1. Introduction

Bronchoconstriction, cough, mucus production and airway hyperreactivity to bronchoconstrictor mediators are the main clinical manifestations of asthma and these features correlate well with the severity of the disease (O'Byrne, 1988). The exact mechanisms responsible for these effects are not fully elucidated. Airway inflammation and alterations in neuronal function are believed to play a role. Asthmatic patients are exquisitely sensitive to a number of bronchoconstrictor agents. For example, bradykinin (Simonsson et al., 1973; Fuller et al., 1987; Polosa and Holgate, 1990), sulfur dioxide (Tan et al., 1982; Sheppard et al., 1980), distilled water (e.g. fog) (Anderson et al., 1983), adenosine (Cushley et al., 1983, 1984) and capsaicin (Fuller et al., 1985) cause bronchoconstriction in asthmatic subjects, while modest or no effects have been found on respiratory function in healthy individuals. A common feature of these stimuli is that they can cause changes in lung function as a result of (indirect) stimulation of airway nerves.

Human airways are innervated by efferent and afferent autonomic nerves which regulate many aspects of airway function (Barnes, 1986a,b; Richardson, 1982). Neuronal control of airways may be abnormal in asthmatic patients and neurogenic mechanisms contribute to the pathogenesis of asthma. The parasympathetic nervous system is the dominant neuronal pathway of airway smooth muscle tone. Stimulation of cholinergic nerves causes bronchoconstriction, mucus secretion, and bronchial vasodilatation (White, 1995; Lammers et al., 1989; Zaagsma et al., 1997; Barnes, 1992; Minette and Barnes, 1988). Recently, the evidence for cholinergic dysfunction in asthmatic patients is not convincing (Van der Velden and Hulsmann, 1999). Sympathetic nerves control bronchial blood vessels, but no innervation of human smooth muscle has been demon-

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strated (Daniel et al., 1986; Davis et al., 1982; Richardson, 1979; Doidge and Satchell, 1982). β-Adrenoceptor are abundantly expressed on airway smooth muscle and result in bronchodilatation when activated (Carstairs et al., 1985; Emorine et al., 1989; Engels et al., 1989; Gothert and Hentrich, 1985). The physiological role of β-adrenoceptor seems unclear and their function appears to be normal in asthmatic patients (De Jongste et al., 1987; Bai et al., 1992; Van der Velden and Hulsmann, 1999). A third neural network in the lung is collectively termed the non-adrenergic–non-cholinergic (NANC) nervous system (Barnes, 1986a,b; Barnes et al., 1991a,b). Inhibitory NANC nerves contain vasoactive intestinal peptide (VIP) and nitric oxide (NO) and may be the only neural bronchodilator pathway in human airways (Fisher et al., 1996a,b; Hauser-Kronberger et al., 1996; Bai and Bramley, 1993; Belvisi et al., 1992, 1995). VIP and NO seem to counteract the bronchoconstriction and thus may function as a braking mechanism for cholinergic nerves. VIP is a potent relaxant of human airways (Wulff et al., 1997)). Although dysfunction of inhibitory NANC nerves has been proposed in asthma, no differences in inhibitory NANC responses have been found between asthmatics and healthy subjects (Van der Velden and Hulsmann, 1999).

In addition to inhibitory NANC efferent system in the airways, there is also a NANC afferent nervous system that protects the airways against inhaled irritants, chemical particles, and stretch (Barnes et al., 1991a,b, Lundberg et al., 1983; Solway and Leff, 1991). These excitatory NANC nerves play an important regulatory role in airways in that their activation provides input of peripheral information to the central nervous system resulting in maintenance of pulmonary homeostasis via reflex pathways such as bronchoconstriction, mucus secretion, and cough (Barnes, 1986a,b). Excitatory NANC nerves or so-called sensory nerves have been extensively studied in animal airways and have also been detected in human airways. Unmyelinated C-fibres, that are characterised by their sensitivity to the neurotoxin capsaicin, are localised between and beneath the airway epithelium, around blood vessels and submucosal glands, within the smooth muscle layer and around local tracheobronchial ganglion cells (Coleridge and Coleridge, 1994; Lalloo et al., 1995; Karlsson, 1993; Lundberg et al., 1984; Joos et al., 1994). When excitatory NANC nerves are stimulated, neuropeptides are released via axon reflex mechanisms. These neuropeptides contract airways smooth muscle, dilate bronchial arteries, increase vascular permeability, increase mucus production and modulate ganglionic transmission, thereby affecting airway function in a way that resembles features found in the pathogenesis of asthma (Barnes, 1986a,b; Barnes et al., 1991a,b; Joos et al., 1994). In addition, these neuropeptides may influence the recruitment, proliferation and activation of leukocytes and tissue resident inflammatory cells such as mast cells (Barnes, 1986a,b). On the other hand, inflammatory cells can modulate the neuronal function and phenotype (Lammers et al., 1988; Patterson and Nawa, 1993). The goal of this review is to bring together the functional relevance of excitatory NANC nerves and the interaction with the immune system in asthma.

2. Excitatory NANC neuropeptides and receptors in airways

The majority of vagal excitatory NANC nerve fibres are non-myelinated excitatory NANC C-fibre afferents that have cell bodies in the nodose, jugular and thoracic dorsal root ganglion. The lung receives C-fibres that are of vagal and spinal origin and the trachea is innervated with C-fibres of vagal origin (Kummer et al., 1992). The majority of C-fibres originates within the airway epithelium and contains vesicles filled with bioactive neuropeptides. These unmyelinated C-fibres are characterised by their sensitivity to the neurotoxin capsaicin (extract of hot pepper). At high doses, capsaicin effectively depletes neuropeptides from pulmonary C-fibres, while at low doses it mimics endogenous neuropeptide release and has thus been used extensively as an investigational tool (Barnes et al., 1991).

The main neuropeptides localised in excitatory NANC nerves include tachykinins (substance P, neurokinin A, and neurokinin B) and calcitonin gene-related peptide (CGRP) (Coleridge and Coleridge, 1994; Lalloo et al., 1995; Karlsson, 1993). Upon activation, afferent central reflex effects such as bronchoconstriction, mucus secretion, and cough take place as do efferent effects induced by the local release of neuropeptides. Local efferent effects include contraction of airway smooth muscle, vasodilatation, and increased vascular permeability (also referred to as neurogenic inflammation) (Barnes, 1986a,b; Barnes et al., 1991a,b).

A number of studies have demonstrated the presence of substance P, neurokinin A, and CGRP in human airways. Nerve fibres containing substance P-like immunoreactivity have been described in the larynx, trachea, in and around bronchi, bronchioles, the more distal airways, and occasionally extending into the alveoli. The nerve fibres are found beneath and within the airway epithelium, around blood vessels and submucosal glands, within the bronchial smooth muscle layer and around local tracheobronchial ganglion cells. (Lundberg et al., 1984; Luts et al., 1993; Baluk and McDonald, 1998). Numerous neurokinin A-like immunoreactive nerves are present around intrinsic neurones of local bronchial ganglia and within the bronchial smooth muscle layer (Sheldrick et al., 1995). As measured by radioimmunoassay, the substance P content in segmental human bronchi was reported to be 3 pmol/g tissue and the content of neurokinin A was reported to be lower at 0.3 pmol/g tissue (Lundberg et al., 1984; Martling et al., 1987). No immunoreactivity corresponding to neurokinin B was found (Martling et al., 1987). CGRP has been

localised using immunocytochemistry in airway neuroendocrine cells, tracheal serous cells and in terminals of C-afferent NANC fibres (Carstairs, 1987; Palmer et al., 1987).

Three types of tachykinin receptors have been characterised: neurokinin 1 (NK_1), NK_2, and NK_3 (Joos et al., 1994; Regoli et al., 1994). These receptors are preferentially activated by substance P, neurokinin A, and neurokinin B, respectively. Animal studies have shown that tachykinin NK_2 receptors and to a lesser extent tachykinin NK_1 receptors are involved in bronchoconstriction, whereas tachykinin NK_1 receptors are involved in vasodilatation, increase of vascular permeability, mucus secretion and the effects on inflammatory cells (Maggi, 1993; Frossard and Advenier, 1991). The latter effect can also be non-receptor mediated.

Tachykinin NK_1 and NK_2 receptors are present in human airways (Mapp et al., 2000). In man, substance P and neurokinin A contract large bronchi (Lundberg et al., 1983; Naline et al., 1989). Tachykinin NK_2 specific receptor agonists [Nle^{10}] neurokinin A-(4-10) or [β-Ala^8] neurokinin A-(4-10), but not tachykinin NK_1 receptor agonists ([Sar^9, $Met(O_2)^{11}$]SP) induce constriction of human isolated bronchus (Naline et al., 1989). Neurokinin B has no contractile effect on human airways (Naline et al., 1989). Studies using specific tachykinin NK_2 receptor antagonists SR48968 and GR159897, demonstrated antagonism for the contraction induced by tachykinin NK_2 receptor agonist [Nle^{10}] neurokinin A-(4-10) or substance P (Advenier et al., 1992a,b; Ellis et al., 1997). Since a specific tachykinin NK_1 receptor antagonist CP96345 has no effects, it can be concluded that tachykinin-induced contractions in large, normal human airways are mediated through tachykinin NK_2 receptor stimulation (Advenier et al., 1992a,b). Neurokinin A and substance P contract smaller airways to a larger extent and at lower concentrations compared to large airways (Frossard and Barnes, 1991). The effects of substance P are mediated via the release of thromboxane A_2 in addition to the activation of tachykinin NK_1 receptors (Naline et al., 1996). The tachykinin NK_1 receptor-mediated contraction is small, transient and subject to tachyphylaxis, probably due to desensitisation and internalisation of the tachykinin NK_1 receptor (Naline et al., 1996; Baluk and McDonald, 1998). A number of potent and selective NK receptor antagonists have become available and are represented in Table 1.

Table 1
Excitatory NANC neuropeptide receptor antagonists

Receptor type	Code	References
NK_1 selective	CP 96,345	Snider et al., 1991
	RP 67,580	Garret et al., 1991
	FK 888	Fujii et al., 1992
	SR 140333	Emonds-Alt et al., 1993
	LY 303870	Gitter et al., 1995
	GR 203040	Beattie et al., 1995
NK_2 selective	MEN 10,376	Maggi et al., 1991
	SR 48968	Emonds-Alt et al., 1992
		Advenier et al., 1992a,b
	MEN 10,627	Maggi et al., 1994
	GR 159897	Beresford et al., 1995
	TAC-363	Higashide et al., 1997
	MEN 11,420	Catalioto et al., 1998
NK_3 selective	SR 142801	Emonds-Alt et al., 1995
NK_1/NK_2	FK224	Murai et al., 1992
	S. 16474	Robineau et al., 1995
	MDL 105,212A	Kudlacz et al., 1996
CGRP	BIBN4096BS	Doods et al., 2000
	CGRP(8-37)	Poyner, 1992

Modified from Advenier et al., 1997.

3. Excitatory NANC nerves and asthma

The role of excitatory NANC nerves and their neuropeptides has been extensively studied in many animal models for asthma. Several studies have reported that exposure of guinea pigs to an aerosol of either capsaicin (thus causing the release of endogenous NANC neuropeptides) or substance P, elicited airway hyperresponsiveness to bronchoconstrictor agents (Hsiue et al., 1992; Ladenius et al., 1995; Boichot et al., 1993; Matsuse et al., 1991). The substance P-induced hyperresponsiveness was not associated with changes in eosinophil number or activation status (Kraneveld et al., 1997). In addition, we have demonstrated that in guinea pigs, interleukin-5-induced hyperresponsiveness but not eosinophilia was completely blocked by pretreatment with a tachykinin NK_2 receptor antagonist (Kraneveld et al., 1997). In a guinea pig model for allergic asthma, it was demonstrated that a three- to four-fold increase of substance P, neurokinin A, and CGRP was found 24 h after challenge (Fischer et al., 1996). Quantitative immunohistochemistry showed that at the same time point after challenge, the number of tachykinin-immunoreactive nodose ganglion neurones had increased by 25% (Fischer et al., 1996). Moreover, capsaicin-induced neuropeptide depletion or tachykinin NK_2 receptor blockade profoundly inhibited the antigen-induced hyperreactivity (Ladenius and Nijkamp, 1993; Ladenius et al., 1995; Boichot et al., 1995). In viral infection models (Ladenius et al., 1995), and in other types of experimental airway hyperresponsiveness induced by platelet activation factor (Perretti and Manzini, 1993), toluene diisocyanate (Thompson et al., 1987; Scheerens et al., 1996) and dinitrobenzene-induced (Buckley and Nijkamp, 1994) delayed type hyperreactivity reactions, capsaicin-induced neuropeptide depletion also resulted in a marked inhibition of airway hyperresponsiveness. Recently, it has been demonstrated in guinea pigs that tachykinin NK_1 and NK_2 receptors are differentially involved in the development of allergen-induced airway obstruction, airway hyperrespon-

siveness, and infiltration of inflammatory cells. Both tachykinin NK_1 and NK_2 receptor antagonists had no effects on the severity of early airway obstruction, while the tachykinin NK_2 receptor antagonist caused a significant inhibition of the late asthmatic response (Schuiling et al., 1999a). Tachykinin NK_1 receptor antagonist reduced allergen induced airway hyperresponsiveness both after early and late asthmatic response (Schuiling et al., 1999b), while tachykinin NK_2 receptor antagonist significantly attenuated hyperresponsiveness found after late asthmatic response (Schuiling et al., 1999a). The tachykinin NK_1 as well as the tachykinin NK_2 receptor antagonist inhibited cellular infiltration and accumulation of ciliated epithelial cells in the airway lumen (Schuiling et al., 1999c).

Immunohistochemical studies of neuronal tachykinins in airways of asthmatics have yielded conflicting results. While in some studies in asthmatics an increase in both the number and length of tachykinin-immunoreactive nerve fibres was found in the airways compared with non-asthmatics (Ollerenshaw et al., 1991; Nieber et al., 1992), others have detected significantly less substance P like immunoreactivity in lung tissue from asthmatic than from non-asthmatic patients (Howarth et al., 1991; Lilly et al., 1995). However, this latter finding may reflect augmented release of substance P followed by degradation (Lilly et al., 1995). These findings are in accordance with a report of increased plasma levels for substance P in patients with acute asthma (4.6 ± 0.4 pmol/l in 25 asthmatics compared to 2.2 ± 0.2 pmol/l in 21 healthy controls) (Cardell et al., 1994). Substance P and neurokinin A have been measured in bronchoalveolar lavage fluid (Hazbun et al., 1993; Nieber et al., 1992; Heaney et al., 1998). An increased amount of substance P was measured in healthy subjects after ozone exposure (Hazbun et al., 1993). Moreover, a significant larger amount of substance P was found in atopic subjects with grass pollen allergy compared with non-allergic controls (Nieber et al., 1992). After intrasegmental provocation with allergen, a significant increase in bronchoalveolar lavage fluid substance P was observed in the allergic subjects. Substance P has been measured in sputum induced by inhalation of hypertonic saline. Patients with asthma and with chronic bronchitis demonstrated a significantly higher concentration of sputum substance P (17.7 ± 2.4 fmol/l and 25.6 ± 5.5 fmol/l, respectively) when compared to normal subjects (1.1 ± 0.4 fmol/l) (Tomaki et al., 1995). The concentration of substance P in sputum correlated with the index of airway obstruction. In addition, asthmatic patients with chronic cough have abnormal density of intra-epithelial airway nerves containing increased quantities of CGRP (O'Connell et al., 1995).

Several groups have reported in vivo bronchoconstrictor effects of substance P and neurokinin A in asthmatic patients (Advenier et al., 1999). Neurokinin A was found to been a more potent bronchoconstrictor than substance P and asthmatics were found to be hyperresponsive to substance P and neurokinin A (Joos et al., 1994). Inhalation of substance P and neurokinin A by six healthy subjects did not cause a change in specific airway conductance (Joos et al., 1987; Joos, 1989). After inhalation of neurokinin A by asthmatics, a concentration-dependent bronchoconstriction was observed as measured by increases in airway conductance. Crimi et al. (1988) demonstrated that inhaled substance P was also able to cause bronchoconstriction in patients with asthma. Cheung et al. (1992, 1993) found that neurokinin A caused bronchoconstriction not only in asthmatics, but also in normal persons with the asthmatics being more sensitive that the normal subjects. Aerosolised substance P was also shown to cause bronchoconstriction in children with asthma, an effect that was dependent on the severity of disease (Nakai et al., 1991). In mild asthmatics, nedocromil sodium prevented the bronchoconstriction caused by neurokinin A and substance P (Joos et al., 1989; Crimi et al., 1988).

Effects of tachykinins on bronchial responsiveness in man are less well studies. Inhalation of substance P was found to enhance the maximal airway narrowing to metacholine in patients with asthma (Cheung et al., 1994). This effect was observed 24 h after inhalation of substance P. Some studies have documented possible changes in tachykinin receptor expression in asthma, and an increase in mRNA transcripts for both tachykinin NK_1 and NK_2 receptors has been found (Adcock et al., 1993; Bai et al., 1995).

4. Immunomodulatory role for excitatory NANC nerves in asthma

NANC neuropeptides do not only play a role in asthma via a direct action on bronchial smooth muscle or vasculature, it has recently become evident that neuropeptides are modulators of immune cells such as mast cells, eosinophils, macrophages, neutrophils and lymphocytes. All these immune cells have been shown to be involved in the pathogenesis of asthma. The relationship between NANC nerves and immune cells seems to be bi-directional since neuropeptides influence the activity of these cells and mediators released from mast cells, macrophages, eosinophils and lymphocytes (such as histamine, serotonin, nerve growth factor (NGF), eosinophils cationic proteins and cytokines) are able to activate excitatory NANC nerves leading to the release of neuropeptides.

5. Mast cells and excitatory NANC nerves

Histological studies reveal an intimate association between mast cells and neurones in the peripheral and central nervous system (Dimitriadou et al., 1990; Stead et al., 1989). Mast cells fulfill a critical role in immediate hyper-

sensitivity and allergic reactions when activated through immunoglobulin E (IgE) by specific antigens. Mast cells thus form both an anatomical and a functional link between the immune and nervous systems, and may be involved in neurogenic-inflammation. MacQueen et al. (1989) elegantly demonstrated "Pavlovian" conditioning of rat mucosal mast cells to secrete rat mast cell protease II. In their experiments, antigen (egg albumin) injections were paired with an audiovisual cue. Animals re-exposed to only the audiovisual cue released a quantity of protease similar to that from animals re-exposed to both the cue and the antigen. In addition, both these groups released significantly more protease than animals that had received the cue and antigen in a non-contingent manner. Recent data suggest that neural mechanisms are involved in mast cell activation, and that mast cells act as principal transducers of information between peripheral nerves and local inflammatory events (Williams et al., 1995). Peripheral nerves are highly populated with mast cells (Torcia et al., 1996), and manipulation of these peripheral nerves causes changes in mast cell densities (Church et al., 1989). Mast cells and macrophages lining the mucosal layer of the respiratory tract have also been found frequently in the vicinity of substance P and CGRP-immunoreactive nerves (Ganguly et al., 1978; Nilsson et al., 1990). Mediator release from mast cells can be induced by neurotrophic factors such as NGF (Pearce and Thompson, 1986; Horigome et al., 1993) and neuropeptides (e.g. substance P, CGRP, neurotensin and neuropeptide Y) (Piotrowski et al., 1984; Devillier et al., 1986). Recently, Forsythe et al. (2000) have demonstrated that substance P, neurokinin A, and CGRP induced histamine release from airway mast cells obtained by bronchoalveolar lavage from non-atopic controls and from patients with cough variant asthma. Mast cells from patients appear to have an increased responsiveness to CGRP compared with controls.

Janiszewski et al. (1994) reported that mast cells respond electrophysiologically to very low concentrations (5 pM) of substance P without degranulation, but that degranulation occurred after repeated doses. These data indicate that substance P may act as a priming substance rather than a substance causing direct degranulation. On the other hand, we have shown that when murine mast cells are "primed" with cytokines, substance P causes direct degranulation (Karimi et al., 1999,2000). Micromolar concentrations of substance P causes degranulation of rat peritoneal mast cells through direct activation of pertussis toxin-sensitive G proteins in the inner surface of the plasma membrane (Mousli et al., 1990). The activation is mediated by the N-terminal domain (arginine and lysine) of substance P, and does not appear to involve one of the known NK-receptor subtypes. Other literature also suggests that neurokinin receptors on mast cells do not fall into the known categories (Church et al., 1989), however, by performing binding studies and examining serotonin release, Cooke et al. (1998), recently demonstrated the presence of functional tachykinin NK_1 receptors in RBL–2H3 cells, a mucosal-like mast cell line. Furthermore, histamine release from the murine MC/9 mast cell line is mediated by tachykinin NK_2 receptors (Krumins and Broomfield, 1993).

We have developed several murine models for non-atopic asthma. Pulmonary delayed-type hypersensitivity (type 1V hypersensitivity) reactions are induced by skin sensitisation followed by an intra-airway application of the low molecular weight molecule dinitrofluorobenzene (Buckley and Nijkamp, 1994). Airway hyperresponsiveness as well as pulmonary inflammation in this model could be inhibited by depletion of excitatory NANC neuropeptides using capsaicin or by pretreatment with tachykinin NK_1 receptor antagonists (Buckley and Nijkamp, 1994). Interestingly, in the same animal model, we have also demonstrated that in two strains of mast cell deficient mice (W/W^v and Sl/Sl^d mutant), tracheal hyperreactivity was inhibited (Kraneveld et al., 1998). These data point to a mutual role for excitatory NANC nerves and mast cells in the induction of airway hyperresponsiveness. Similar findings have been found by us and other investigators using viral infection models as well as in animal models for allergic asthma in which mast cells are involved (Saban et al., 1987; Folkerts et al., 1993; Ladenius et al., 1995).

The interaction between mast cells and excitatory NANC nerves is bi-directional. Many studies have shown that mast cell-derived mediators (such as histamine, serotonin, prostaglandin, leukotrienes and cytokines) modulate NANC neurotransmission (Martin et al., 1988; Michaelis et al., 1998; Hua and Yaksh, 1993). NANC nerve endings express receptors for both histamine (H_1 and H_3 receptors) and serotonin ($5\text{-}HT_{2A}$ receptors) (Sekizawa et al., 1998; Imamura et al., 1996; Ohkubo et al., 1995; Carlton and Coggeshall, 1997; Grubb et al., 1988). Under inflammatory-like conditions, it has been shown that primary NANC nerves show an upregulation of histamine H_1 receptor expression (Kashiba et al., 1999). Histamine, serotonin, prostaglandin E_2 and leukotriene B_4 sensitise afferent C fibres thus lowering their threshold for release of neuropeptides (Hua and Yaksh, 1993). Furthermore, both serotonin and histamine cause release of substance P and CGRP from unmyelinated C fibres (Martin et al., 1988; Michaelis et al., 1998; Hua and Yaksh, 1993). One of the major cytokines released by mast cells under inflammatory conditions is tumour necrosis factor α (TNF-α). It has been demonstrated that application of TNF-α (0.001–0.01 ng/ml) along nociceptive primary afferent fibres in skin elicited an aberrant electrophysiological activity (Sorkin et al., 1997). Very recently, we have demonstrated that TNF-α plays an important role in delayed-type hypersensitivity-induced tracheal hyperpermeability responses in mice. In this study, TNF-α has a priming effect on excitatory NANC nerves leading to an enhanced tracheal vascular permeability (Van Houwelingen et al., 2000).

6. Macrophages and excitatory NANC nerves

Macrophages that exist in the bronchioles and large airways are likely to be exposed to C-afferent fibre-derived neuropeptides considering that the airways are heavily innervated by substance P-, CGRP-, and neurokinin A-containing NANC nerves (Widdicombe, 1986). In asthma, where the integrity of the epithelial lining in the large airways is lost (Laitinen et al., 1985), neuropeptide and airway macrophage interactions may contribute to the pathology of the disease. Macrophages from various species have receptors for, and respond to substance P, in vitro. Interestingly, Germonpre et al. (1999) recently demonstrated that the human monocytic U-937 cell line and sputum macrophages from normal subjects express mRNA for substance P and for the tachykinin NK_1 receptor, indicating that human macrophages are able to produce substance P and its receptor. Substance P stimulates macrophage phagocytic and chemotactic capacity as well as increased macrophage interleukin-1, interleukin-6, TNF-α, superoxide anion, prostaglandin E_2, and thromboxane B_2 production (Chancellor-Freeland et al., 1995; Cozens and Rowe, 1987; Hartung, 1988; Lotz et al., 1988). Substance P may therefore have an important autocrine effect on human macrophages. Substance P greatly enhances lipopolysaccharide-induced peritoneal macrophage TNF-α and interleukin-6 production from stressed animals but produces relatively little effect on macrophages from control animals (Chancellor-Freeland et al., 1995; Zhu et al., 1996). Airway macrophages collected from ovalbumin sensitised guinea pigs display an enhanced responsiveness to tachykinins as demonstrated by increased superoxide production, which is likely mediated through protein kinase C-dependent mechanisms (Brunelleschi et al., 1996). Furthermore, preprotachykinin gene-I mRNA has been demonstrated in rat alveolar macrophages which can be upregulated by lipopolysaccharide and inhibited by dexamethasone (Killingsworth et al., 1997). Taken together, these data indicate that substance P is involved in stress-induced responses of the macrophage and in pulmonary immune function.

Apart from the direct effects of neuropeptides in inflammatory cells, C-afferent fibres also play a role in the central responses of stress within the lungs. Alveolar macrophage secretory products such as cytokine and lipid mediators may activate excitatory NANC nerve fibres, for instance. In other systems primarily evaluating pain mechanisms, prostaglandin E_2 and leukotriene B_4 are known to sensitise C-afferent excitatory NANC nerve fibres and lower their threshold for firing (Ahlgren et al., 1997; Lee and Morton, 1995; Martin et al., 1987).

Rat alveolar and peritoneal macrophages express functional receptors for CGRP (Vignery et al., 1991; Hastings and Hua, 1995), and human CGRP dose-dependently stimulates cAMP production in mouse alveolar- and bone marrow-derived macrophages, which is abolished by the addition of human CGRP fragment (8-37), a selective antagonist for GRP receptors (Owan and Ibaraki, 1994). CGRP pretreatment of murine airway macrophages for 24 h dose-dependently suppressed DNA synthesis induced by granulocyte–macrophage colony-stimulating factor (Owan and Ibaraki, 1994), and recombinant CGRP was shown to inhibit lipopolysaccharide-induced TNF-α production from murine resident peritoneal macrophages via activation of cyclic AMP responses (Feng et al., 1997). Also, CGRP potently stimulated cyclic AMP levels in cultured airway macrophages (Pittner, 1997), as well as enhanced phagocytosis by peritoneal mouse macrophages, also via an increase in cAMP (Ichinose et al., 1996a,b). CGRP inhibits antigen presentation by Langerhans cells and macrophages, and macrophages and Langerhans cells are anatomically associated with CGRP-containing epidermal nerves in the skin (see Asahina et al., 1995). The suppression of antigen-presenting function by CGRP is mediated, at least in part, by changes in cytokine expression (Torii et al., 1997).

7. Eosinophils and excitatory NANC nerves

Evidence has been obtained that in addition to the migration of eosinophils, their activation is a crucial step to the induction of airway hyperresponsiveness found in asthma (Pretolani et al., 1994). However, the precise mechanism by which eosinophils induce bronchial hyperresponsiveness is, at present, unknown. Excitatory NANC neuropeptides could be important mediators in this process, since it has been demonstrated that airway nerves are surrounded by and infiltrated with eosinophils after antigen challenge (Elbon et al., 1995). Studies by Weinstock et al. (1988) indicate that human and murine eosinophils are able to produce substance P. Several studies have reported that exposure of guinea pigs to an aerosol of either capsaicin or substance P elicited airway hyperresponsiveness to bronchoconstrictor agents (Boichot et al., 1993; Hsiue et al., 1992; Ladenius et al., 1995; Matsuse et al., 1991; Van Oosterhout et al., 1996; Kraneveld et al., 1997). Thus, excitatory NANC neuropeptides possibly form the link between eosinophil activation and the development of airway hyperreactivity. Substance P can also prime eosinophils for chemotaxis by other agents such as platelet activating factor, leukotriene B_4, and interleukin-5 (Numao and Agrawal, 1992; El-Shazly et al., 1996). Also, CGRP is capable of causing an eosinophilia in the rat lung, in vivo (Bellibas, 1996). In addition, tachykinins may be involved in the activation of the accumulated eosinophils. Substance P induces the release of eosinophil cationic protein and superoxide anion by human eosinophils (Iwamoto et al., 1993). Kroegel et al. (1990) have demonstrated that substance P can induce peroxidase release from isolated guinea pig eosinophils, however, this was likely not mediated via a neurokinin-receptor dependent mechanism. Eosinophil

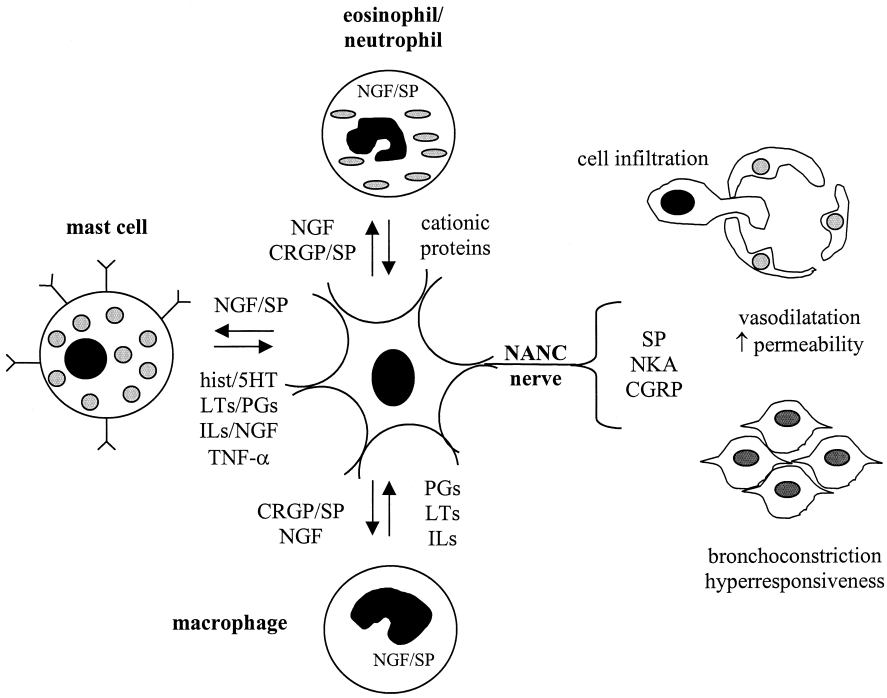

Fig. 1. Possible interaction between excitatory NANC nerves and inflammatory cells leading to airway hyperresponsiveness, bronchoconstriction, vasodilatation and increased vascular permeability, and cellular infiltration (abbreviations: SP: substance P, NKA: neurokinin A, CGRP: calcitonin gene-related peptide, NGF: nerve growth factor, ILs: interleukins, PGs: prostaglandins, LTs: leukotrienes, hist: histamine, 5HT: serotonin, TNF-α, tumour necrosis factor α).

granule proteins have been shown to induce airway hyperresponsiveness when incubated with guinea pig trachea in vitro (Hamann et al., 1993). These cationic proteins may induce functional changes of the epithelial layer such as a decreased neutral endopeptidase activity or a diminished release of epithelium-derived relaxing factor (O'Byrne, 1988). Furthermore, eosinophil-derived cationic proteins can directly activate excitatory NANC nerves and can induce epithelial damage resulting in exposure of excitatory NANC nerves in the airway lumen. Garland et al. (1993) have shown that mediators released from activated eosinophils directly stimulated tachykinin release from excitatory NANC nerves in cell culture. Released cationic granule proteins are likely important eosinophilic mediators, since it has been shown that they induced the release of neuropeptides in human bronchi, indicating the activation of NANC nerves (Coyle et al., 1994). A number of in vivo observations suggest that excitatory NANC nerves are involved in airway hyperreactivity as a consequence of eosinophil accumulation and activation. First, intra-airway application of substance P induced airway hyperresponsiveness in the guinea pig that was not associated with changes in the number of eosinophils or eosinophil activation status in vivo, suggesting that substance P is not causing its effect by activation of eosinophils (Kraneveld et al., 1997). Secondly, we have shown that capsaicin induced excitatory NANC neuropeptide depletion resulted in a complete inhibition of the ovalbumin-induced airway hyperresponsiveness in guinea pigs (Ladenius and Nijkamp, 1993; Matsuse et al., 1991), while the presence of eosinophils was still evident in these animals after allergen challenge. Similar findings were obtained after a viral infection (Ladenius et al., 1995). Thirdly, in guinea pigs, we have further demonstrated that interleukin-5-induced airway hyperresponsiveness to histamine, but not the eosinophilia, was completely blocked by pretreatment of animals with a tachykinin NK_2 receptor antagonist. These findings indicate that excitatory NANC neuropeptides could be the final, more downstream common pathway after eosinophil infiltration and activation in inducing airway hyperresponsiveness due to allergic asthma or viral infections (see Fig. 1).

8. Neutrophils and lymphocytes and excitatory NANC nerves

Substance P increased the expression of intracellular adhesion molecule-1 on human endothelial cells (Nakagawa et al., 1993) pointing to its role in the recruitment of immune cells into the lung. In addition, it has been shown that substance P activates neutrophils, an effect that appears to be mediated by tachykinin NK_1 receptors (Tanabe et al., 1996). Stimulation of human polymorphonuclear cells by substance P leads to superoxide anion production (Iwamoto et al., 1993; Tanabe et al., 1996), interleukin-8

release, increased antibody-dependent cell mediated cytotoxicity (Wozniak et al., 1993) and adherence and chemotaxis of human neutrophils (Wiedermann et al., 1989; Iwamoto et al., 1993; Zimmerman et al., 1992). Substance P fragments appear to induce varying cellular effects, which may depend to some extent upon the cell type in question. For instance, neutrophil chemotactic properties of substance P has been shown to reside in the carboxyterminal portion of the molecule (Iwamoto et al., 1990), while Wiedermann et al. (1989) have shown that the locomotion of polymorphonuclear leukocytes is induced by aminoterminal substance P. Presently, most data concerning the effects of tachykinins on lymphocytes are not related to the lung. Tachykinin NK_1 receptors have been demonstrated on human and murine lymphocytes (Cook et al., 1994; Payan et al., 1984) and substance P has been shown to stimulate chemotaxis (Schratzberger et al., 1997), proliferation and activation of such cells (Payan et al., 1983; Calvo et al., 1992). T-lymphocyte proliferation induced by substance P occurred both in the absence and presence of other stimuli and was mediated via the tachykinin NK_1 receptor (Payan et al., 1983). Substance P also enhances the production of interleukin-2 by human T-lymphocytes (Calvo et al., 1992) and finally, substance P modulates immunoglobulin production (Carucci et al., 1995; Eglezos et al., 1991; Pascual et al., 1991). Substance P causes an increase in the proliferation of Peyer's patch lymphocytes as well as immunoglobulin (especially IgA) synthesis (Bienenstock et al., 1989).

The role of CGRP on immune function within the lung requires further investigation, however, studies do demonstrate CGRP effects on immune cells. CGRP inhibits human peripheral blood mononuclear cell proliferation, in part through the release of interleukin-10 (Asahina et al., 1995; Fox et al., 1997). In antigen stimulated non-adherent splenocytes and helper T cell clones CGRP inhibits interferon-γ production, but had no effect on interleukin-4 production (Kawamura et al., 1998). Substance P had no effect on interferon-γ production, but substance P enhanced interleukin-4 production slightly but consistently (Kawamura et al., 1998). Since asthma is believed to be a Th2-driven immune response, neuropeptides might play a role in influencing the direction of the Th1/Th2 cascade and may therefore be important in the airway pathology.

9. NGF, NANC nerves, and immune cells in asthma

Neurotrophins could participate in the pathogenesis of asthma in several ways: as NGFs inducing neuropeptide synthesis and neuronal hyperreactivity, and as factors influencing the allergic immune response and inflammation (reviewed by Braun et al., 2000).

Communication between the immune and the nervous system is not restricted to 'signalling molecules'. NGF, a member of the neurotrophin family, is essential for the survival of sympathetic excitatory NANC afferent neurones and the development, growth and differentiation of neural crest-derived excitatory NANC nerve cells (Thoenen and Edgar, 1985; Levi-Montalcini and Calissano, 1986). NGF has been extensively studied in relation to neurite outgrowth. White et al. (1987) suggested that substance P may modulate the availability of NGF in the microenvironment of the regenerating nerve fibre endings. The increase in basal NGF levels following nerve injury may be a consequence of peptide depletion, suggesting that under normal conditions, NGF production and release is regulated by a neurogenic negative feedback mechanism. In addition to neurones, non-neuronal cells such as connective tissue mast cells (Leon et al., 1994), macrophages (Otten et al., 1987), T-cells (Ehrhard et al., 1993), B-cells (Torcia et al., 1996), lymphocytes (Barouch et al., 2000) fibroblasts (Hattori et al., 1994), eosinophils (Solomon et al., 1998) and epithelial cells (Fox et al., 1998) produce NGF. Therefore, NGF is likely an important molecule that mediates communication between the nervous and the immune systems. The effects of NGF are mediated by binding to the high affinity ($K_d = 10^{-11}$) glycoprotein receptor tyrosine kinase A (TrK A) or the low affinity ($K_d = 10^{-9}$) pan neurotrophin receptor p75. Rat mast cells express the functional high affinity NGF receptor TrK A (Horigome et al., 1994). NGF acts as a chemoattractant (Sawada et al., 2000) and thereby causes an increase in the number of mast cells in different tissues as well as their rapid degranulation (Horigome et al., 1994). In addition, NGF stimulates differentiation of granulocytes and macrophages (Susaki et al., 1996; Kannan et al., 1993; Matsuda et al., 1991; Kannan et al., 1992), promotes proliferation of B- and T-cell subsets (Otten et al., 1989; Thorpe and Perez-Polo, 1987), enhances vascular permeability in the skin (Otten et al., 1984) and induces differentiation of activated B-cells into Ig-secreting plasma cells (Kimata et al., 1991a,b; Brodie and Gelfand, 1994; Otten et al., 1994). Matsuda et al. (1988) showed that NGF causes a significant stimulation of granulocyte colonies grown from human peripheral blood. NGF appears to act in a relatively selective fashion to induce the differentiation of eosinophils and basophils/mast cells (Matsuda et al., 1988). Depletion experiments showed that these effects were T-cell dependent suggesting synergistic effects of NGF on human colony growth with cytokines. Recently, Kawamoto et al. (1995) showed that NGF also prevents apoptosis of rat peritoneal mast cells through the p140TrK tyrosine phosphorylation.

It could be postulated that eosinophils might serve as a functional link between mast cells and the activation of excitatory NANC afferent fibres. Cationic granule proteins, which are strong activators of mast cells (Mousli et al., 1994; Patella et al., 1996), may stimulate release of mast cell-derived NGF, which then sensitises excitatory NANC neurones and induces the expression of excitatory NANC neuropeptides. On the other hand, it has been

recently shown that NGF is preformed in human peripheral blood eosinophils and also activates eosinophils (Solomon et al., 1998), which may have consequences for the direct interactions between eosinophils and excitatory NANC nerves. Furthermore, NGF enhances the survival and cytotoxic activity of eosinophils (Hamada et al., 1996).

Mast cell activation by neuropeptides occurs in particular with the serosal mast cells (Mousli et al., 1994). NGF receptors on mast cells may act as autoreceptors, thus regulating mast cell NGF synthesis and release, while at the same time also being sensitive to NGF released from other cell types within the microenvironment (see Fig. 1). Several reviews have focussed on the interaction between NGF and mast cells from various body sites, and the role of NGF in certain pathologies (Williams et al., 1995; Levi-Montalcini and Calissano, 1986; Levi-Montalcini et al., 1996).

In addition, macrophages appear to regulate NGF synthesis in non-neuronal cells such as Schwann cells, and likely play a role in the maintenance and repair of excitatory NANC neurones (Brown et al., 1991; Heumann et al., 1987; Lindholm et al., 1987). Cultured explants of rat sciatic nerve containing NGF-responsive excitatory NANC and sympathetic neurones, produce NGF-mRNA when conditioned media of activated macrophages is added, the effect of which is mediated by interleukin-1 (Lindholm et al., 1987). NGF also enhances phagocytosis and interleukin-1 beta production from murine peritoneal macrophages and monocyte-macrophage J774A.1 cells via the activation of a tyrosine kinase (p140 Trk) receptor (Susaki et al., 1996). Interestingly, macrophages are also cellular sources of NGF (Otten et al., 1987). The precise effects of macrophage-derived products on NGF synthesis and maintenance of excitatory NANC neurones in the lung however, remains to be investigated.

Inflammation can lead to an enhanced production and release of NGF. Inflammatory mediators, including interleukin-1, interleukin-4, interleukin-5, TNF-α and interferon-γ have been shown to induce the release of NGF (Hattori et al., 1994; Yoshida et al., 1992). NGF induces expression of neuropeptides in excitatory NANC neurones and sensitises neurones thus lowering their threshold for firing (Lindsay and Harmar, 1989). During inflammation, NGF increases hyperalgesia (Woolf, 1996; Woolf et al., 1996). This effect may be the consequence of an increase in sensitivity of the peripheral terminals of high threshold nociceptors due to activation of TrK A receptors on excitatory NANC fibres by NGF or indirectly via the release of sensitising mediators from TrK A expressing inflammatory cells and postganglionic sympathetic neurones. Additionally, NGF may alter the phenotype of excitatory NANC nerves, resulting in structural changes leading to hyperinnervation. An enhanced innervation of predominantly excitatory NANC nerves producing substance P can be found in the airways of transgenic mice overexpressing NGF (Hoyle et al., 1998). Interestingly, an enhanced expression of tachykinin mRNA in the nodose ganglia in a guinea pig model for asthma has been demonstrated (Fischer et al., 1996a,b), and it could therefore be postulated that NGF might play a role in this allergic model. It is known that NGF changes the properties of excitatory NANC nerve endings by inducing a very fast accumulation of second messenger in synaptosomes (Knipper et al., 1993), by sensitising the nerve terminal (Woolf, 1996) and/or by altering neuropeptide levels in excitatory NANC nerves (Lindsay and Harmar, 1989). NGF might bind to and activate TrK A receptors on the peripheral terminals of primary excitatory NANC nerves leading to the phosphorylation of critical transduction-related proteins or ion channels, thereby sensitising the peripheral terminal (Knipper et al., 1993; Woolf et al., 1996).

In severe allergic asthmatic patients, NGF values are strongly increased and these values positively correlated with total IgE antibody titer (Bonini et al., 1996). Neurotrophins, among which NGF, are increased in bronchoalveolar lavage fluid after segmental allergen provocation in patients with asthma (Virchow et al., 1998). In a murine model of allergic asthma as well as in mild asthmatic patients, Braun et al. (1998, 1999) recently demonstrated enhanced levels of NGF in serum and bronchial alveolar lavage fluids after allergen provocation. Splenic mononuclear cells from the allergen-sensitised mice produced NGF in response to allergen (Braun et al., 1998). These cells also responded to exogenously added NGF with the production of Th2-type cytokines. Furthermore, nasal application of anti-NGF antibody to allergen-sensitised mice significantly reduced interleukin-4 production and prevented the development of airway hyperreactivity (Braun et al., 1998).In the human study group, NGF levels in bronchoalveolar lavage fluid after allergen provocation were correlated significantly with baseline FEV1 levels (Braun et al., 1999). We recently showed that intravenously administered NGF potentiates the histamine-induced bronchoconstriction with a maximum of over 200% in anaesthetised spontaneously breathing guinea pigs (De Vries et al., 1999). The tachykinin NK_1 receptor antagonist SR 140333 completely blocked the NGF-induced hyperresponsiveness, pointing to a role for tachykinins. Given the potential of NGF effects on collateral sprouting and growth of excitatory NANC and sympathetic nerves as well as the release of neuropeptides by these excitatory NANC nerves, mast cells (via the release of NGF) could regulate the sensitivity of excitatory NANC nerves towards stimuli and thereby contribute to the pathogenesis and symptoms of asthma (see Fig. 1).

10. Clinical relevance of excitatory NANC neuropeptide antagonists in asthma

Very recently, Advenier et al. (1999) have reviewed clinical studies with tachykinin antagonists in asthma.

Only four neurokinin receptor antagonists have been studied in clinical trials: FK224, a peptide tachykinin receptor antagonist for tachykinin NK_1 and NK_2 receptors, CP-99994, a non-peptide tachykinin NK_1 receptor antagonist, FK888, a peptide tachykinin NK_1 receptor antagonist and SR 48968, a non-peptide tachykinin NK_2 receptor antagonist.

In 1992, Ichinose et al. have demonstrated that the non-specific NK receptor antagonists FK224 (4 mg by inhalation) protected against bradykinin-induced bronchoconstriction and cough in nine asthmatic patients. Joos et al. (1996) examined the effect of inhaled FK224 on neurokinin A-induced bronchoconstriction in 10 mild asthmatics. This group demonstrated that inhalation of 4 mg FK224 did not change baseline lung function in patients. Moreover, FK224 did not offer protection against neurokinin A-induced bronchoconstriction in asthmatics (Joos et al., 1996). FK 224 was also studied using a 4-week treatment period (4 mg by inhalation q.i.d.) in patients with moderate asthma (Lunde et al., 1994). No beneficial effects of FK224 on symptoms and lung function were observed.

Fahy et al. (1995) showed that the specific tachykinin NK_1 receptor antagonists CP 99994 at a dose of 250 $\mu g/kg$ body weight (intravenous administration) did not significantly inhibit hypertonic saline-induced bronchoconstriction or cough in 14 patients with mild asthma. Currently, it has not been investigated whether this tachykinin NK_1 receptor antagonist is able to inhibit airway responses by inhaled substance P or neurokinin A.

More promising results have been found in a clinical study examining the tachykinin NK_1 receptor antagonist FK888 in exercise-induced asthma (Ichinose et al., 1996a,b). In this double blind, placebo-controlled crossover trail in nine asthmatic patients, FK888 improved exercise-induced airway narrowing by profoundly reducing recovery time. Only in some patients, this tachykinin NK_1 receptor antagonism attenuates the maximal decrease in lung function (Ichinose et al., 1996a,b). FK888 had no effect on baseline lung function. These results suggest that the tachykinin NK_1 receptor-mediated mechanisms be involved in the recovery phase of exercise-induced asthma.

The effects of the tachykinin NK_2 receptor antagonist SR48968 on neurokinin A-induced bronchoconstriction have recently been studied in 12 asthmatic patients (Van Schoor et al., 1998). Oral pretreatment with 100 mg/kg SR48968 caused a significant inhibition of neurokinin A-induced airway narrowing. This finding constitutes the first evidence of inhibition of excitatory NANC neuropeptide-induced bronchoconstriction by a selective neurokinin receptor antagonist in humans.

To date, no clinical studies have examined the effects of tachykinin NK_1 or NK_2 receptor antagonists on allergen-induced bronchoconstriction or airway hyperreactivity as well as on inflammatory response (vasodilatation, plasma extravasation, mucus production and cellular infiltration/activation). The present development of potent and selective neurokinin receptor antagonists will allow further definition of the role of excitatory NANC neuropeptides in the pathogenesis of asthma.

11. Conclusions

Both animal and human data strongly suggest that excitatory NANC neuropeptides (especially the tachykinins) are important in the pathogenesis of asthma. There is convincing evidence for the presence of excitatory NANC nerves and neuropeptides in human airways. Studies on autopsy tissue, lung lavage fluid and sputum suggest that tachykinins are present in increased amount in asthmatic airways. The tachykinins are potent bronchoconstrictors of human airways with asthmatics being more sensitive for the effects of tachykinins compared to healthy persons. In addition, excitatory NANC neuropeptides have strong inflammatory effects such as vasodilatation, plasma extravasation, and mucus secretion that are also involved in the pathology of asthma.

Excitatory NANC neuropeptides do not only play a role in asthma via a direct action on bronchial smooth muscle or vasculature, but a lot of evidence has been obtained in recent years showing that neuropeptides are modulators of immune cells such as mast cells, eosinophils, macrophages, neutrophils and lymphocytes. The relationship between excitatory NANC nerves and immune cells seems to be bi-directional since neuropeptides influence the activity of these cells and mediators released from mast cells, macrophages, eosinophils and lymphocytes (such as histamine, serotonin, NGF, eosinophils cationic proteins and cytokines) are able to activate excitatory NANC nerves leading to the release of neuropeptides (see Fig. 1).

Many animal studies show that capsaicin-induced depletion of excitatory NANC neuropeptides or treatment with neurokinin receptor antagonists prevent an airway hyperresponsiveness and pulmonary inflammation. For instance, airway hyperresponsiveness induced by ovalbumin immediate type hypersensitivity, platelet-activating factor, toluene diisocyanate, delayed type hypersensitivity or respiratory viral infection can be attenuated by interruption or antagonism of the excitatory NANC system. These data support the concept that excitatory NANC neuropeptides are a common downstream step in the pathway leading to airway obstruction and hyperresponsiveness as is seen in asthma. Selective excitatory NANC neuropeptide receptor antagonists or drugs that inhibit the release of neuropeptides could be expected to have a beneficial effect in the pathogenesis of asthma by reducing the neurogenic component of this disease. However, the future therapeutic potential of such pharmacological agents remains to be investigated in the clinic.

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