REVIEW

Sensory neuropeptides and the human lower airways: present state and future directions

G.F. Joos, P.R. Germonpre, J.C. Kips, R.A. Peleman, R.A. Pauwels

**ABSTRACT:** The sensory neuropeptides, substance P and neurokinin A, are present in human airway nerves, beneath and within the epithelium, around blood vessels and submucosal glands, and within the bronchial smooth muscle layer. Studies on autopsy tissue, bronchoalveolar lavage and sputum suggest that in asthma the substance P content of the airways may be increased. Neurokinin A is a more potent bronchoconstrictor than substance P. Asthmatics are hyperresponsive to endogenously released and exogenously administered substance P and neurokinin A, both in normal and asthmatic subjects. As for other indirect bronchoconstrictor stimuli, the effect of neurokinin A on airway calibre in asthmatics can be inhibited by pretreatment with nedocromil sodium.

Evidence is accumulating, not only from studies in animals but also from experiments on human airways, that tachykinins may also cause mucus secretion and plasma extravasation. They also have important proinflammatory effects, such as the chemotraction of eosinophils and neutrophils, the adhesion of neutrophils, and the stimulation of lymphocytes, macrophages and mast cells. The tachykinins interact with the targets on the airways by specific tachykinin receptors. The NK subtypes have been characterized in human airways, both pharmacologically and by cloning. The NK receptor is responsible for the effect of neurokinin A on airway calibre in asthmatics can be inhibited by pretreatment with nedocromil sodium.

Because of their presence in the airways and because of their ability to mimic the various pathological features of asthma, substance P and neurokinin A are presently considered as possible mediators of asthma. The present development of potent and selective tachykinin antagonists will allow us to further define the role of tachykinins in the pathogenesis of asthma.

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Numerous peptides have been demonstrated in airway nerves and endocrine cells [1]. Among the best studied are substance P (SP) and neurokinin A (NKA), which are neuropeptides contained within sensory nerves. SP and NKA are members of the tachykinin peptide family, consisting of mammalian and nonmammalian tachykinins. At the present time, five tachykinin peptides have been identified in mammalian nervous tissues: substance P (SP), neurokinin A (NKA), neuropeptide K (NPK), neuropeptide-gamma (NP-gamma) and neurokinin B (NKB) (table 1) [2]. The tachykinins are potent vasodilators andcontractors of smooth muscle. In studies on rodent airways, SP and NKA have been implicated as the neurotransmitters mediating the excitatory part of the nonadrenergic, noncholinergic (NANC) nervous system. These noncholinergic excitatory nerves can be activated by mechanical and chemical stimuli, generating antidromic impulses and a local axon reflex, which leads to noncholinergic bronchoconstriction, plasma extravasation and vasodilatation [1, 3–5].

SP and NKA have various effects that could contribute to the changes observed in asthmatic airways, including smooth muscle contraction, submucosal gland secretion, vasodilatation, increase in vascular permeability, stimulation of cholinergic nerves, stimulation of mast cells, stimulation of B- and T-lymphocytes, stimulation of macrophages, chemotraction of eosinophils and neutrophils and the vascular adhesion of neutrophils. In view of their potential airway effects, the sensory neuropeptides have been implicated in the pathogenesis of asthma. Various review articles on this topic have been published in the past few years, focusing mainly on animal models [1, 3–8]. In the present article, we review the knowledge which has been obtained from studies on human airways.
and discuss the arguments which favour a possible role for SP and NKA in the pathogenesis of asthma. Where appropriate, reference is made to animal studies.

**Presence of sensory neuropeptides in human airways**

SP and NKA are present in the human lung. By radioimmunoassay (RIA), the SP content in segmental bronchi was reported to be around 3 pmol·g⁻¹ tissue, a value which is in the same range as found in corresponding tissues of the guinea-pig [9]. The content of NKA was reported to be lower, around 0.3 pmol·g⁻¹ tissue [10]. By immunocytochemistry, nerve fibres containing SP-like immunoreactivity (SPL-IR) have been described beneath and within the airway epithelium, around blood vessels and submucosal glands, within the bronchial smooth muscle layer and around local tracheobronchial ganglion cells. These nerve fibres are found in and around bronchi, bronchioles and more distal airways, occasionally extending into the alveoli [9]. In a recent paper, LUTS et al. [11] confirmed the presence of SP nerve fibres in normal larynx, trachea and bronchi, but pointed out that they were relatively few compared to nerve fibres containing peptides such as vasoactive intestinal peptide (VIP), and that the respiratory epithelium lacked SP nerve fibres. NKA and, probably, other related peptides are also present in human airway nerves. Using an antiserum raised against the nonmammalian tachykinin, kassinin, MARTLING et al. [10] reported the presence of tachykinin-like immunoreactivity in human bronchi: characterization of the immunoreactive material by high performance liquid chromatography (HPLC) revealed that the tachykinin-like immunoreactivity was heterogeneous and consisted of NKA, NPK and an eledoisin-like component. No immunoreactive material corresponding to NKB was found.

**Table 1. – Mammalian tachykinin peptides**

<table>
<thead>
<tr>
<th>Gene</th>
<th>PPT mRNA</th>
<th>Peptide</th>
<th>Sequence*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SP/NKA (PPT I)</strong></td>
<td>alpha, beta, gamma</td>
<td>Substance P</td>
<td>Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH₂</td>
</tr>
<tr>
<td>SP/NKA (PPT I)</td>
<td>beta, gamma</td>
<td>Neurokinin A</td>
<td>His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH₂</td>
</tr>
<tr>
<td></td>
<td>gamma</td>
<td>Neuropeptide gamma</td>
<td>Asp-Ala-Gly-His-Gly-Gln-Ile-Ser-His-Lys-Arg-His-Lys-Thr-Ser-Phe-Val-Gly-Leu-Met-NH₂</td>
</tr>
<tr>
<td></td>
<td>beta, gamma</td>
<td>Neurokinin A (3–10)</td>
<td>Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH₂</td>
</tr>
<tr>
<td><strong>NKB (PPT II)</strong></td>
<td>gamma</td>
<td>Neurokinin B</td>
<td>Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH₂</td>
</tr>
</tbody>
</table>

SP: substance P; NKA: neurokinin A; NKB: neurokinin B; PPT, I, II preprotachykinin, I, II. *: the amino acids common to the C-terminal and have been underlined.

The content of SP in human lung may decrease with age and after denervation. In a comparative study of autopsy tissue from infants (0–3.5 yrs), children (8–11 yrs) and adults (17–24 yrs), relatively fewer peptide-containing nerve fibres, containing SP and calcitonin gene-related peptide (CGRP), were found in the adult bronchioli, suggesting that the content of SP in human lung may decrease with age [12]. In a study on transplanted human respiratory tracts, which were removed at retransplantation, the content of peptide-immunoreactive nerves was lower than in control lung. Some immunoreactivity for the sensory neuropeptides CGRP and SP persisted, although the nerve fibres were more sparse compared to nontransplanted tissue, suggesting that no reinnervation occurred [13].

Asthmatic lung tissue may contain more SP compared to that of normal subjects. In tissue obtained at autopsy, after lobectomy and at bronchoscopy, both the number and the length of SP-immunoreactive nerve fibres was increased in airways of subjects with asthma, when compared to airways from subjects without asthma [14]. These findings need confirmation, however. Indeed, SP-immunoreactive (SP-IR) nerves were not invariably found in bronchial biopsies from patients with mild asthma [15].

SP has been measured in bronchoalveolar lavage (BAL) fluid. NIEMBER et al. [16] examined six atopic subjects with grass pollen allergy and six nonallergic, healthy volunteers. A significantly larger amount of SP was found in the atopic compared to the nonallergic subjects. After intrasegmental provocation with allergen, a significant increase in BAL SP levels was observed in the allergic subjects. A similar release of SP has now also been demonstrated during allergen reactions occurring in the nose [17]. SP has also been measured in sputum induced by inhalation of hypertonic saline: SP-immunoreactivity was detected in patients with asthma and chronic bronchitis, but not in healthy subjects [18].

**Bronchoconstrictor effect of sensory neuropeptides**

The in vitro bronchoconstrictor effect of SP and NKA

The in vitro contractile effect of SP and NKA has been studied extensively, and is summarized in table 2. SP
contracts human bronchi and bronchioli [19, 20], being less potent than histamine or acetylcholine [21, 22]. NKA is a more potent constrictor of human bronchi than SP and was reported to be, on a molar base, 2–3 orders of magnitude more potent than histamine or acetylcholine [22]. In contrast to guinea-pigs, NKB had no contractile effect on human airways [22]. Noncholinergic pathways might be more important in the smaller airways. Indeed, NKA and SP were found to contract small airways to a larger extent and at lower concentrations compared to the large airways [23].

Recently, the effect of passive sensitization on the in vitro contractile effect of SP and NKA on human bronchi was reported [25]. Human bronchi incubated overnight with serum from asthmatic patients atopic to Dermatophagoides pteronyssinus showed an enhanced sensitivity and an enhanced maximal contractile response to SP and NKA, an effect which was independent of changes in the activity of neutral endopeptidase.

It is important to stress that all the studies described above have been performed on airways obtained at thoracotomy, mostly from current or ex-smokers with lung cancer. To our knowledge, no studies have been reported on the effect of tachyklinins on isolated asthmatic bronchi. It has been demonstrated for adenosine that such an approach could reveal important differences between normal and asthmatic persons, both with regard to the potency of the agonist and the mechanism of induced contraction [26].

The in vivo bronchoconstrictor effect of SP and NKA

The in vivo bronchoconstrictor effect of SP and NKA, administered by inhalation or intravenous infusion, has been reported by several groups, as summarized in table 3. In these studies, NKA was found to be a more potent bronchoconstrictor than SP, and asthmatics were found to be hyperresponsive to SP and NKA.

Table 2. – Contractile effect of tachyklinins: studies on isolated human airways

<table>
<thead>
<tr>
<th>Author [Ref.]</th>
<th>Agonist</th>
<th>Tissue</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lundberg et al. [19]</td>
<td>SP</td>
<td>Bronchi</td>
<td>SP less potent than Ach</td>
</tr>
<tr>
<td>Finney et al. [20]</td>
<td>SP</td>
<td>Bronchioli</td>
<td></td>
</tr>
<tr>
<td>Advener et al. [22]</td>
<td>SP, NKA, NKB</td>
<td>Bronchi</td>
<td>pD2 NKA 7.10; pD2 SP 5.63; NKB inactive</td>
</tr>
<tr>
<td>Martling et al. [21]</td>
<td>SP, NKA, NPK</td>
<td>Bronchioli</td>
<td>NKA 1000 fold more active than SP; NKA unaffected by atropine, mepyramine and cimetidine</td>
</tr>
<tr>
<td>Black et al. [24]</td>
<td>SP, NKA, E, P</td>
<td>Bronchi</td>
<td>NKA and SP had a greater constrictor effect in bronchioli than in bronchi</td>
</tr>
<tr>
<td>Frossard and Barnes [23]</td>
<td>SP, NKA</td>
<td>Bronchioli</td>
<td></td>
</tr>
</tbody>
</table>

SP: substance P; NKB: neurokinin B; E: eleoisin; NKA: neurokinin A; NPK: neuropeptide K; P: physalaemin; Ach: acetylcholine; pD2: apparent affinity.

Intravenous infusion of SP (0.2–3.3 pmol·kg⁻¹·min⁻¹) caused a significant fall in diastolic blood pressure (8.5±2.9 mmHg) and an increase in heart rate. At low infusion rates, a small fall was observed in airflow at 30% of vital capacity during a partial flow-volume manoeuvre (Vp₃₀) (123±45 to 111±47 l/min⁻¹) [27]. Comparing the effect of intravenous SP and NKA, Evans et al. [28] reported that in six normal subjects SP was more potent than NKA in increasing skin temperature and heart rate. In this study, intravenous SP caused bronchodilatation, whilst NKA up to 64 pmol·kg⁻¹·min⁻¹ caused a maximal fall in Vp₃₀ of 79%.

We have studied the effect of inhaled SP and NKA on airway calibre in normal and asthmatic airways [29]. Inhalation of SP and NKA by six healthy, nonsmoking subjects did not cause a significant change in specific airways conductance (sGaw). Inhalation of SP, up to 10⁶ moles·ml⁻¹, caused no change in sGaw in asthmatics, a finding also reported by Fuller et al. [27]. After inhalation of NKA by the asthmatics, a concentration-dependent bronchoconstriction was observed, with a mean decrease in sGaw of 48%, at a concentration of 5×10⁻⁷ moles·ml⁻¹. The bronchoconstriction occurred rapidly and sGaw returned to the baseline within 30 min in most patients. All patients reported chest tightness. No cough and no change in heart rate or blood pressure was noted [29]. In a group of 19 asthmatics, we found a weak but nonsignificant correlation between the bronchial responsiveness to methacholine and neurokinin A, suggesting that different mechanisms are involved in the bronchoconstrictor action of these two agents [30]. In a recent study, we determined the degree of reproducibility of the bronchial response to neurokinin A in a group of nine mild asthmatics who were on inhaled sympathomimetics only. In 8 of the 9 asthmatics, the provocative concentration producing a 35% fall in sGaw (PC₃₅sGaw NKA) performed with an interval of at least one week, was reproducible, differing by less than 0.5 log units from each other (Joos et al., unpublished observations).

The in vivo bronchoconstrictor effect of inhaled sensory neuropeptides in man has been confirmed and extended by other groups. Studying patients with a more pronounced bronchial responsiveness, Cram et al. [31] demonstrated that inhaled SP is also able to cause bronchoconstriction in asthmatic subjects. High doses of SP, up to 8 mg·ml⁻¹, caused a significant decrease in forced exhalation.
expiratory volume in one second (FEV₁) but also a significant fall in blood pressure and increase in heart rate [32]. Using an efficient nebulizer system and a sensitive measure of airway calibre, CHEUNG and co-workers [33, 34] found that NKA caused bronchoconstriction not only in asthmatics but also in normal persons, the asthmatics being more sensitive than the normal subjects. Aerosolized SP was also shown to cause bronchoconstriction in children with asthma, an effect which was dependent on the severity of the asthma [35].

Mechanisms involved in the bronchoconstrictor effect of tachykinins in man

In guinea-pig airways and isolated large human airways, the constrictor effect of the tachykinins was reported to be direct, as antihistamines and muscarinic receptor antagonists did not affect the reaction [19]. However, in rabbit airways the bronchoconstrictor action of SP was partially inhibited by pretreatment with atropine. In Fisher 344 rats, tachykinins cause bronchoconstriction mainly by indirect mechanisms, namely activation of cholinergic nerves and mast cells [38, 39]. The effect of nedocromil sodium and the anticholinergic agent oxitropium bromide on NKA-induced bronchoconstriction in mild asthmatics has been studied. Nedocromil sodium prevented the bronchoconstriction caused by NKA [36] and substance P [31]. Oxitropium bromide offered some protection against the bronchoconstrictor effect of NKA, an effect which was evident in 4 out of 11 asthmatics [37]. In seven moderate asthmatic patients, ipratropium bromide caused a small, but significant, rightward shift in the dose-response curve to substance P [40]. The protective effect offered by nedocromil sodium against tachykinin-induced bronchoconstriction in asthmatics indicates that SP and NKA cause bronchoconstriction by an indirect mechanism. This could arise from an effect on inflammatory cells (e.g. mast cells) and/or nerves. An alternative explanation is that nedocromil sodium and sodium cromoglycate act as a tachykinin antagonist, a suggestion which was raised from a study with sodium cromoglycate in human skin [41].

In experimental animals, tachykinins are able to cause acetylcholine release from postganglionic cholinergic airway nerve endings [42, 43]. In human isolated airways, NKA, but not SP, potentiated the contractile response to cholinergic neural stimulation in the presence of K⁺ channel blockade with 4-aminopyridine (4-AP). Because neither 4-AP itself nor the combination of 4-AP and NKA potentiated the postjunctional effects of acetylcholine, it was suggested that the potentiation of the neural response by NKA was occurring at the level of the postganglionic nerve terminal [44].

Substance P causes histamine and serotonin release from rat peritoneal and pleural mast cells in a dose-dependent way. This is a rapid phenomenon, occurring within 15 s, and with more than 90% of the response occurring within one minute [45]. Histamine release by SP has also been documented in rat lung in vitro [46]. The protective effect of nedocromil sodium against NKA-induced bronchoconstriction in asthmatics suggests a possible involvement of mast cells in the human airway response towards tachykinins. Although SP released histamine in the human skin and from dispersed human skin mast cells, it did not cause histamine release from

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Table 3. – Contractile effect of tachykinins: studies on human airways in vivo

<table>
<thead>
<tr>
<th>Author [Ref.]</th>
<th>Agonist</th>
<th>Mode of administration</th>
<th>Lung function</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>JOOS et al. [29, 30, 36, 37]</td>
<td>SP/NKA normal/asthma</td>
<td>Aerosol</td>
<td>sGaw</td>
<td>SP had no effect in normals and asthmatics; NKA caused a dose-dependent bronchoconstriction in asthmatics</td>
</tr>
<tr>
<td>FULLER et al. [27]</td>
<td>SP normal/asthma</td>
<td>i.v./aerosol</td>
<td>Vp₃₀/sGaw</td>
<td></td>
</tr>
<tr>
<td>EVANS et al. [28]</td>
<td>SP, NKA normal</td>
<td>i.v.</td>
<td>Vp₅₀</td>
<td></td>
</tr>
<tr>
<td>CRIMI and co-workers [31]</td>
<td>SP asthma</td>
<td>Aerosol</td>
<td>FEV₁</td>
<td>SP caused bronchoconstriction in moderate asthmatics</td>
</tr>
<tr>
<td>NAKAI et al. [35]</td>
<td>SP, asthmatic children</td>
<td>Aerosol</td>
<td>FEV₁/V₅₀</td>
<td>No change in bronchial responsiveness to methacholine 24 h after NKA</td>
</tr>
<tr>
<td>CHEUNG and co-workers [32–34]</td>
<td>NKA, normal NKA, asthma</td>
<td>Aerosol</td>
<td>Vₑₛ</td>
<td>Inhaled SP caused a drop in diastolic blood pressure</td>
</tr>
</tbody>
</table>

sGaw: specific airways conductance; Vₑₛ: expiratory flow at 50% of vital capacity; FEV₁: forced expiratory volume in one second; V₅₀: expiratory flow from partial expiratory flow-volume curve; V₃₀: airflow at 70% of vital capacity measured from total lung capacity after a forced partial expiratory flow manoeuvre. For further abbreviations see legend to table 2.
human lung mast cells that were obtained from patients undergoing pneumonectomy for lung cancer [46, 47]. However, a preliminary report suggests that SP can release histamine from mast cells recovered from BAL fluid [48]. The lack of effect of pretreatment with astemizole and terfenadine, two potent and specific H1-antagonists, on NKA-induced bronchoconstriction in asthmatics would tend to suggest that histamine does not play a major role in NKA-induced bronchoconstriction in man [40, 49].

During the last decade, direct evidence for a close contact between mast cells and nerves has been obtained. A large proportion of rat intestinal mucosal cells from normal and infected rats were in direct contact with nerve profiles, some of which contained SP or another sensory neuropeptide, CGRP [50]. Such an association has now also been found in rat lung [51]. Although this contact between mast cells and neuropeptide containing nerve fibres has been shown to be present in the human intestine [52] and skin [53], it is not known whether it also occurs in the human lung.

**Other effects of sensory neuropeptides**

**Mucus secretion**

Receptors for SP have been demonstrated by autoradiographic labelling on human airway submucosal glands [54]. SP was found to be more potent than NKA in inducing mucus secretion in human bronchi [55].

**Vascular permeability**

In rodents, tachykinins released from sensory nerves cause microvascular leakage. No direct measurement of this effect on human lower airways has been described, but some studies have been performed on the nose. The application of capsaicin to human nasal mucosa caused a significantly larger secretory response in patients with vasomotor rhinitis than in normal controls. The secretory response was almost completely blocked by pretreatment with a muscarinic receptor antagonist, suggesting that this response occurs through cholinergic parasympathetic reflexes [56]. SP and NKA caused an increase in nasal protein output in patients with allergic rhinitis, which was taken as evidence for microvascular leakage [57].

**Proinflammatory effects**

It is now widely accepted that mucosal inflammation plays a central role in the pathogenesis of asthma. In the past few years, a vast amount of knowledge has been gained on the interaction between neuropeptides and the cells proposed to play a central role in asthmatic airway inflammation.

The first evidence for a direct effect of SP on lymphocytes was the demonstration that nanomolar concentrations of SP alone could stimulate human peripheral blood lymphocyte proliferation and could enhance the response to the T-cell mitogens, phytohaemaglutinin A (PHA) and concanavalin A (con A) [58]. SP can act as a cosignal with PHA and phorbol myristate acetate (PMA) to enhance the expression of interleukin-2 (IL-2) messenger ribonucleic acid (mRNA) and IL-2 secretion [59]. High affinity receptors for SP have been characterized on cultured human IM-9 lymphoblasts. SP can enhance immunoglobulin secretion by human B-cells in the presence of a second stimulus [60]. SP is also able to stimulate macrophage, neutrophil and eosinophil function. SP has a potent chemotactic effect on human monocytes. This effect is gradient-dependent and can be blocked by a SP antagonist. The production of cytokines by macrophages is also influenced by SP; nanomolar concentrations stimulate the secretion of interleukin-1 (IL-1), tumour necrosis factor-α (TNF-α) and interleukin-6 (IL-6) [61]. In contrast, SP in concentrations up to 10−4 M was not able to stimulate the release of thromboxane B2 (TXB2) from bronchoalveolar macrophages derived both from normal and asthmatic subjects [62]. SP is also a chemoattractant for neutrophils, an effect which probably occurs through a subset of Pertussis toxin-sensitive G proteins not coupled to classical phospholipases [63]. Recently, SP was found to induce a rapid influx of neutrophils and eosinophils in human dermis, effects which occurred in parallel with translocation of P-selectin and a significant upregulation of E-selectin, suggesting that SP can induce endothelial adhesion molecule expression [64]. NUMAO and AGRAWAL demonstrated that eosinophils from allergic and normal subjects differed with regard to their chemotactic response to SP. SP alone was not chemotactic for eosinophils, whereas the chemotactic response to platelet-activating factor (PAF) and leukotriene B4 (LTB4) of eosinophils derived from asthmatic but not normal subjects was enhanced by pretreatment with nanomolar quantities of SP [65]. As has been described for isolated mast cells, high concentrations of SP were also found to induce degradation of eosinophils [66].

**NANC responses in human airways**

The question of whether endogenous neuropeptides can be released from human airway nerves has been approached in two different ways: electrical field stimulation (EFS) and administration of the neurotoxin, capsaicin. Noncholinergic excitatory nerves have been demonstrated in guinea-pig airways; an atropine-resistant contraction which comprises about 60% of the contractions that can be induced by EFS is found in this animal species [1]. This noncholinergic bronchoconstrictor response is absent in animals pretreated with capsaicin, a neurotoxin that damages unmyelinated C-fibres, depleting them of neuropeptides. These noncholinergic excitatory nerves can also be stimulated by mechanical irritation and chemical irritants, such as ether, formalin, capsaicin and cigarette smoke. A noncholinergic bronchoconstrictor has not been consistently demonstrated in human airways. It has been shown repeatedly that human bronchus responds to EFS
In the metabolism of SP and NKA is concerned, much operating in human lower airways is lacking at present. Fibres, reduced the Evans blue extravasation to nerve capsaicin, destroying nonmyelinated sensory airway nerve fibres, increased vascular permeability. Pretreatment with ether, formalin, bradykinin or capsaicin also induced an increase in vascular permeability [1]. This effect was resistant to extravasation of Evans blue, indicating an increased permeability of noncholinergic excitatory nerves in human airways. Moreover, up to 45% of isolated human bronchi contracted spontaneously (up to 20% of the maximal contraction to carbachol), when peptidase inhibitors were added to the organ bath, a finding which also suggests the release of an endogenous tachykinin or another substance susceptible to degradation by peptidases [69].

Capsaicin has also been shown to stimulate mucus secretion in surgically resected human bronchi in vitro, an effect which could be blocked by morphine [70]. From the available in vitro data, it is unclear whether NANC mechanisms exist in human airways. In order to explore this further in vivo, capsaicin has been administered by inhalation to human subjects. Inhalation of capsaicin caused dose-dependent coughing in normal volunteers and subjects with mild asthma. It also caused a dose-dependent fall in sGaw in normal and asthmatic subjects, an effect that was significantly reduced after pretreatment with ipratropium bromide. The time course of this bronchoconstriction (maximal decrease in sGaw within 20 s of exposure and lasting less than 60 s) and its reduction by an anticholinergic agent, has been taken as an indication that the bronchoconstrictor response to capsaicin inhalation is mainly due to a vagally-mediated cholinergic reflex and probably not to a local axon reflex with release of neuropeptides [71].

Neurogenic inflammation has been described in the airways of rodents. In anaesthetized guinea-pigs and rats, electrical stimulation of the vagus nerves caused extravasation of Evans blue, indicating an increased vascular permeability [1]. This effect was resistant to atropine and hexamethonium pretreatment, indicating that noncholinergic nerve fibres were stimulated. Cigarette smoke and light mechanical or local chemical irritation by ether, formalin, bradykinin or capsaicin also induced an increase in vascular permeability. Pretreatment with capsaicin, destroying nonmyelinated sensory airway nerve fibres, reduced the Evans blue extravasation to nerve stimulation. It is difficult to approach this problem in humans, and direct evidence for such a mechanism operating in human lower airways is lacking at present.

**Role of neutral endopeptidase**

The physiological actions of neuropeptides are normally terminated by extracellular metabolism [72]. As far as the metabolism of SP and NKA is concerned, much attention has been paid to the role of the neutral endopeptidase 24.11 (also called NEP, enkephalinase, CALLA, CD10 or E.C.3.4.24.11) and to the role of peptidyl dipeptidase-A-15.1, better known as angiotensin-converting enzyme (ACE, kininase II or E.C.3.4.15.1). A series of potent and relative selective inhibitors of NEP are available, such as the thiol inhibitors (e.g. thiorphan), and phosphorus-containing inhibitors (e.g. phosphoramidon) [73].

The role of NEP in the airways, and its relation to NANC airway innervation, has been studied mainly in animal airways [74, 75]. NEP has been localized in the lung of different animal species both by biochemical studies (i.e. measurement of enzyme activity) and by immunohistochemistry. NEP is present in the basal cells of the epithelium, in alveolar cells type II, in submucosal glands, airway smooth muscle, postcapillary venules and nerves. It was also found in neutrophils. An increase in NEP activity and in NEP immunolabelling has been found in adult compared to foetal lung [76].

Using thiorphan or phosphoramidon, various authors have confirmed that inhibition of NEP enhances various airway effects of exogenously administered SP and tachykinins, including airway smooth muscle contraction, plasma extravasation and airway mast cell activation [39, 74, 75]. Moreover, the airway effects of sensory neuropeptides endogenously released by vagal electrical stimulation or capsaicin were shown to be enhanced in the presence of a NEP inhibitor [77]. In guinea-pigs, it was shown that NEP and also ACE participate in the metabolism of SP when administered intravascularly, whilst the tachykinins administered by aerosol are degraded by NEP only [78].

It has also been shown in animal models that NEP activity can be modulated [74, 75]. A variety of environmental irritants, such as toluene diisocyanate (TDI), cigarette smoke and hypochlorous acid, and pathogens, such as the human influenza virus A/Taiwan, the Sendai virus and *Mycoplasma pulmonis*, decrease airway NEP and increase the response of the airways to the SP and NKA. On the other hand, glucocorticoids, well-known for their suppression of inflammation, are able to upregulate the synthesis of peptidases. It is of interest to note that corticosteroids are able to reduce the magnitude of plasma extravasation produced in rat trachea, an effect which seems to be mediated, at least in part, by an increase of NEP activity [79]. Direct evidence for an upregulation of the expression of NEP by corticosteroids (dexamethasone and budesonide) has been obtained in transformed human tracheal epithelial cells grown in culture [80].

It was shown that the *in vitro* contractile response of human bronchi to NKA, SP and the nonmammalian tachykinins, eledoisin and physalaemin, was potentiated by phosphoramidon [24]. Moreover, phosphoramidon was shown not only to potentiate the SP-induced contraction, but also to increase and prolong the contraction induced by capsaicin [68]. This suggested that NEP regulates the contractile effect of substance P, but also of endogenous substances, probably tachykinins, which are released from capsaicin-sensitive nerves.

The effect of an inhaled inhibitor of NEP on the bronchoconstrictor effect of NKA in man has recently
been studied [33, 34]. Cheung and co-workers [33] demonstrated that the inhalation of thiorphan (0.5 ml of 2.5 mg·ml⁻¹) 10 min before NKA challenge enhanced the bronchoconstrictor effect of NKA, both in normal [33] and asthmatic subjects [34]. Interestingly, the leftward shift of the NKA dose response curve was not significantly different in the asthmatic compared to nonasthmatic subjects. Thiorphan had no effect on baseline lung function or bronchial responsiveness to methacholine in either group. Using another inhibitor of NEP, phosphoramidon, Crimi et al. [81] have confirmed these findings. They found that the inhalation of phosphoramidon by six asthmatics had no effect on baseline airway tone but caused a significant leftward shift of the dose response curve to inhaled NKA.

These two in vivo studies offer functional proof that NEP is involved in the in vivo breakdown of inhaled NKA in man, confirming findings obtained on isolated human airways [23, 24, 68, 82]. As inhibition of NEP offers a similar shift of the dose-response curve both in normal and nonsmoking stable, mild to moderate asthmatics, it can be argued that the functional activity of NEP is not significantly decreased in stable asthmatics. Indeed, it has been hypothesized that the epithelial damage frequently observed in asthmatic airways could result in decreased NEP activity, enhancing neurogenic airway inflammation. Moreover, as neither thiorphan nor phosphoramidon influenced baseline lung function, it can be assumed that tachykinins (or another peptide susceptible to breakdown by NEP), unlike histamine, leukotrienes or acetylcholine, are not involved in the maintenance of basal airway tone.

The studies by Cheung and co-workers [33, 34] and Crimi et al. [81] would, therefore, tend to argue against an important role for endogenously released tachykinins in the pathogenesis of asthma. Obviously, several caveats remain in the interpretation and generalization of these findings. Higher doses of potent NEP inhibitors might be necessary to unmask subtle quantitative differences in NEP activity between normal subjects and mild asthmatics. Moreover, it is not known whether a more pronounced loss of NEP activity occurs in more severe asthma. Since it has been shown that viral infections and exposure to occupational agents, such as TDI result in loss of NEP activity, this mechanism may be important in well-defined subsets of asthma or during exacerbations.

Inhibition of NEP is now also used as an indirect measure of the involvement of tachykinins in the airway contractile response to stimuli, such as metabisulphite [83] or leukotriene D₄ [84]. However, caution in the interpretation of these studies is warranted; as NEP is also able to cleave other peptides (such as bradykinin, endothelin, atrial natriuretic peptide...), an enhancing effect of thiorphan does not necessarily mean that tachykinins are involved.

**Tachykinin receptors in human airways**

Three receptors for the tachykinins, respectively, the NKC₁, NKC₂ and NKC₃ receptors, have been characterized pharmacologically [85]. Moreover, the three NK-receptors have been cloned both in animal and human tissue [86].

**Pharmacological characterization in human bronchi**

Based on the rank order of potency of the endogenous tachykinins SP, NKA and NKB, three tachykinin receptor types are recognized in mammalian tissues: the NK₁ (SP>NKA>NKB), NK₂ (NKA>SP>NKB) and NK₃ (NKB>NKA>SP) receptor. Receptor selective agonists and specific and potent receptor antagonists are now available [85]. Pharmacological evidence for heterogeneity of the tachykinin receptors amongst species has also been presented. Based on differences in affinity for the nonpeptide NK₁ receptor antagonists CP96,345 and RP67,580, the NK₁ receptor can be divided into two broad categories: the human, bovine, guinea-pig and rabbit NK₁ receptor for which CP96,345 is more potent; and the rat, mouse and chicken NK₁ receptor for which RP67,580 is more potent. A similar species-dependent heterogeneity is evident for the NK₂ receptor, whereby the NK₂ A/B classification based on differences in rank order of potency of NK₂ receptor selective antagonists has been introduced by Maggi [85] to distinguish the pharmacological characteristics of the NK₂ receptor found in man, bovine, guinea-pig and rabbit (NK₂-2A) versus the pharmacological characteristics found in hamster and rat (NK₂-2B).

From studies on animal airways, NK₁ receptors, and to a lesser extent NK₂ receptors, have been shown to be involved in bronchoconstriction, whereas NK₃ receptors were found to be involved in mucus secretion, microvascular leakage, vasodilation and most of the effects on inflammatory cells [85, 87]. Data on human airways are scarce at the moment. The human airway tachykinin receptor involved in bronchoconstriction, whereas NK₁ receptors were found to be involved in mucus secretion, microvascular leakage, vasodilation and most of the effects on inflammatory cells [85, 87]. Data on human airways are scarce at the moment. The human airway tachykinin receptor involved in bronchoconstriction has at the present time been characterized in vitro only. Naline et al. [82] demonstrated that the NK₁ receptor selective agonist [Nle^10]NKA(4–10) was a potent contractor of isolated human bronchi, whereas agonists selective for the NK₂ and NK₃ receptor were almost inactive. The recently described potent and specific nonpeptide receptor antagonists have been described and evaluated in isolated human bronchi. Advendier et al. [88] showed that the potent and specific NK₁ receptor antagonist, SR 48968, displayed competitive antagonism for the contraction induced by [Nle^10]NKA(4–10). No effect on acetylcholine, histamine, KCl or prostaglandin F₂α (PGF₂α)-induced contractions was seen. SR 48968 also shifted the dose-response curve for SP to the right. The specific NK₂ receptor antagonist CP-96,345 had no effect. These in vitro results suggest that the contraction induced by SP and NKA in large, normal airways is mediated by NK₂ receptors.

At present, potent and specific NK₁ and NK₂ receptor antagonists are being developed, and it can be expected that clinical trials will be performed with some of them within the next years [89]. At the present time, the only compound which has gone into clinical trials is FK224, a cyclic peptide tachykinin antagonist which blocks both the NK₁ and the NK₂ receptors. FK224, 4 mg given...
by metered dose aerosol, was shown to inhibit the bradykinin-induced bronchoconstriction and cough in nine asthmatics [90].

**Molecular biology of airway tachykinin receptors**

Recently the three mammalian tachykinin receptor genes and complementary deoxyribonucleic acid (cDNA) have been cloned from several species, including man [86]. The amino acid sequence of each receptor type is well conserved among species, with the greatest differences observed at the NH₂- and C-termini. The overall homology for the NK₂ receptor among species (bovine, man, rat, murine) is greater than 85%. For the NK₁ receptor (man, rat, mouse, guinea-pig) and NK₃ (man, rat) receptor the homology is approx 90%. Fong et al. [91] recently reported that the molecular basis for the species selectivity of the two nonpeptide NK₁ antagonists CP96,345 and RP67,580 resides in two residues (116 and 206) in the transmembrane domain of the NK₁ receptor, probably by altering the local helical packing of the receptor, rather than by direct interaction with the antagonists [91].

The tachykinin receptors are members of the superfamily of guanine nucleotide binding protein-coupled receptors. All members of this family of receptors are glycoproteins with seven putative alpha-helical transmembrane segments, an extracellular amino-terminus and an intracellular carboxyl tail. They all interact with one or more G-proteins to promote high-affinity binding and signal transduction. The human NK₁ receptor has been cloned from IM-9 lymphoblasts, and lung tissue. The human NK₂ receptor has been cloned from lung, trachea, jejunum and gastric tissue, and the human NK₃ receptor from brain tissue [86, 92–94]. Comparison of the three human tachykinin receptors indicates a somewhat closer relationship between the NK₁ receptor and the NK₂ receptor (51% homology), than between either the NK₁ and NK₃ receptors or the NK₂ and NK₃ receptors (respectively, 41 and 47% sequence identity).

Bai et al. [95] detected mRNA for the NK₁ receptor and the NK₂ receptor both in central airways and peripheral lungs, with similar relative abundance of the mRNA levels at both sites [95]. In situ hybridization indicates that the NK₁ receptor mRNA is localized predominantly in airway epithelium and vascular endothelium; and there is evidence that NK₁ receptor gene expression is increased in asthmatic lung disease [96].

**Are SP and NKA mediators of asthma?**

There is now convincing evidence for the presence of SP and NKA in human airway nerves. Studies on autopsy tissue, as well as on BAL fluid and sputum, suggest that SP may be present in increased amounts in the asthmatic airway. SP and NKA are potent bronchoconstrictors of human airways, asthmatics being more sensitive than normal persons. The major enzyme responsible for the degradation of the tachykins, neutral endopeptidase (NEP), is present in the airways and is involved in the breakdown of exogenously administered SP and NKA, both in normal and asthmatic persons. Other, less well-documented airway effects of SP and NKA, include mucus secretion, vasodilation and plasma extravasation, as well as the chemotraction and stimulation of the various cells presumed to be involved in asthmatic airway inflammation. Hence, SP and NKA fulfill two of the three criteria to which a presumed mediator of asthma has to respond: their presence and release in the airways and their ability to mimic various features of asthma. An alternative hypothesis has been put forward, in which a role is suggested for tachykinin containing capsaicin-sensitive nerves in the production of nonproductive cough, but not asthma [97]. The present development of potent and selective tachykinin antagonists will allow us to further define the role of the sensory neuropeptides, SP and NKA, in the pathogenesis of asthma [89].

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**References**

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