

Review article

Role of tachykinins in asthma

The sensory neuropeptides substance P (SP) and neurokinin A (NKA) are localized to sensory airway nerves, from which they can be released by a variety of stimuli, including allergen, ozone, or inflammatory mediators. Sensory nerves containing these peptides are relatively scarce in human airways, but it is becoming increasingly evident that inflammatory cells such as eosinophils, macrophages, lymphocytes, and dendritic cells can produce the tachykinins SP and NKA. Moreover, immune stimuli can boost the production and secretion of SP and NKA. SP and NKA have potent effects on bronchomotor tone, airway secretions, and bronchial circulation (vasodilation and microvascular leakage) and on inflammatory and immune cells. Following their release, tachykinins are degraded by neutral endopeptidase (NEP) and angiotensin-converting enzyme. The airway effects of the tachykinins are largely mediated by tachykinin NK₁ and NK₂ receptors. Tachykinins contract smooth muscle mainly by interaction with NK₂ receptors, while the vascular and proinflammatory effects are mediated by the NK₁ receptor. In view of their potent effects on the airways, tachykinins have been put forward as possible mediators of asthma, and tachykinin receptor antagonists are a potential new class of antiasthmatic medication.

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Introduction

A large number of biologically active peptides have been identified and characterized (for recent review, see refs. 1, 2). Since the discovery of vasoactive intestinal peptide (VIP), first in the lung and intestine, then in the brain, numerous other neuropeptides have been identified in the lung, or otherwise found to influence its function. Most of these peptides are neuropeptides, as they are synthesized in neurons and released from nerve terminals (e.g., substance P [SP] and the tachykinins, VIP). A few are present in neuroendocrine cells (e.g., bombesin) and others have been localized to the endothelium (e.g., endothelin). Moreover, it has recently become evident that neuropeptides may also be produced and released by inflammatory cells (3).

The tachykinins SP and neurokinin A (NKA) are potent bronchoconstrictors, vasoactive and proinflammatory substances, which have been widely studied in animal and human airways. They are the focus of this review, which aims to assemble new data on the role of tachykinins in the pathogenesis of asthma, including the role of tachykinins in neurogenic inflammation, the interaction of tachykinins with inflammatory cells, and recent data on the development of tachykinin antagonists.

Tachykinins: synthesis

Tachykinins have been isolated from both nonmammalian (amphibian) and mammalian species. The mammalian tachykinins are also called neurokinins. At present, five mammalian tachykinins have been identified: SP, NKA, neurokinin B (NKB), and the N-terminally extended forms of NKA, neuropeptide K (NPK) and neuropeptide γ (NP γ) (Table 1). The tachykinins are a family of neuropeptides that share a common carboxyl-terminal sequence: Phe-X-Gly-Leu-Met-NH₂, where X is either an aromatic (Tyr or Phe) or a branched aliphatic (Val or Ile) amino acid (4). The common carboxyl-terminal sequence is essential for receptor subtype specificity (5).

The mammalian tachykinins are derived from two distinct genes: the preprotachykinin-I gene (PPT-I, also called PPT-A) encodes for SP, NKA, NPK, and NP γ , while the PPT-II gene (also called PPT-B) encodes for NKB (6) (see Fig. 1). The PPT-I gene has seven exons that can be alternately spliced into one of four transcripts: α -, β -, γ -, or δ -PPT-I. SP and NKA are encoded by exons 3 and 6, respectively. Exon 3 is present in each transcript, and exon 6 is present in the β - and γ -PPT-I transcripts (7). Therefore, while SP is derived from each of four transcripts, the other related peptides are derived from selected transcripts. This

Table 1. Chemical structure of five mammalian tachykinins

Peptide	Sequence
Substance P	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH ₂
Neurokinin A	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH ₂
Neuropeptide K	Asp-Ala-Asp-Ser-Ser-Ile-Glu-Lys-Gln-Val-Ala-Leu-Leu-Lys-Ala-Tyr-Gly-His-Gly-Gln-Ile-Ser-His-Lys-Arg-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH ₂
Neuropeptide γ	Asp-Ala-Gly-His-Gly-Gln-Ile-Ser-His-Lys-Arg-His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH ₂
Neurokinin B	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH ₂

Amino acids common to carboxyl-terminal are italicized.

suggests that SP may have a higher probability of being produced whenever the PPT-I gene is expressed (7).

SP and NKA are produced by a distinct subpopulation of primary afferent nerves characterized by their sensitivity to capsaicin, the pungent agent in hot peppers. The tachykinins and calcitonin gene-related peptide (CGRP) are stored in large, dense-cored vesicles and transported to the peripheral endings of the sensory nerves, where they are released by a classical exocytotic process (reviewed in refs. 9–11). The release of tachykinins from sensory nerves can be evoked by a variety of stimuli such as the neurotoxin capsaicin, electrical nerve stimulation, low pH, ether, formalin, SO₂, toluene diisocyanate, histamine, bradykinin, methacholine, prostaglandins, leukotrienes, and cigarette smoke (reviewed in refs. 11, 12).

Source of tachykinins in the airways

Tachykinins have been shown to be present in airway sensory nerves and in secretions recovered from human airways (Table 2). An enhanced amount may be present within the asthmatic airways. By radioimmunoassay, the SP content in segmental bronchi was reported to be around 3 pmol/g tissue, a value which is in the same range as found in corresponding tissues of the guinea pig (12). Lilly et al. determined the SP content in HPLC-purified extracts from tracheal and lung parenchymal tissue by an enzyme-linked immunosorbent assay (ELISA) method. They found around 13.0 and 5.4 pmol/g tissue in tracheal and peripheral lung tissue,

Table 2. Concentrations of substance P in human airways

	Healthy control	Asthma	Reference
Trachea (pmol/g)	13.0 $\pm$ 5.0	3.0 $\pm$ 3.0	(13)
Sputum (fmol/ml)	1.1 $\pm$ 0.4	17.7 $\pm$ 2.4	(18)
Bronchoalveolar lavage fluid (fmol/ml)	30.0 $\pm$ 7.0	185.0 $\pm$ 22.0	(15)
Bronchoalveolar lavage fluid, after instillation of allergen (fmol/ml)	37.7 $\pm$ 15.0	260.0 $\pm$ 33.0	(15)

respectively (13). The content of NKA was reported to be lower, around 0.3 pmol/g tissue (14).

Tachykinins are released into the airways after exposure to allergen or ozone. Nieber et al. found a significantly larger amount of SP in the bronchoalveolar lavage fluid (BAL) fluid of atopic than nonallergic subjects (15). After intrasegmental provocation with allergen, a significant increase in BAL SP levels was observed. In a study by Heaney et al., NKA was recovered from BAL fluid and was found to be increased in asthmatic patients 4 h after inhalation challenge with house-dust mite (15a). Exposure of healthy individuals to ozone increased the concentration of SP in BAL fluid (16) and decreased the immunoreactivity for SP, assessed in bronchial biopsies (17).

SP has been measured in sputum induced by inhalation of hypertonic saline: SP concentrations were significantly higher in patients with asthma and chronic bronchitis than in healthy subjects. In all subjects, there was an inverse correlation between the SP concentration in induced sputum and an index of airway obstruction, the FEV₁/FVC (18). The plasma levels of SP-like immunoreactivity were significantly higher in patients with acute exacerbation of asthma than in healthy controls (19).

Neuronal sources of tachykinins

By immunohistochemistry, nerve fibers containing SP, NKA, and CGRP were demonstrated to be present from the pharynx down to the bronchioli, occasionally extending into the alveoli. They are located beneath

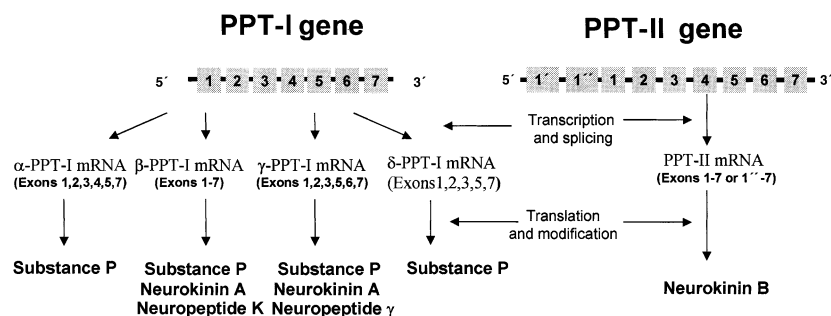


Figure 1. Synthesis of tachykinins from PPT-I and PPT-II.

and within the respiratory epithelium; among bundles of smooth muscle; around blood vessels, submucosal glands, and local tracheobronchial ganglion cells; and in close association with lymphoid aggregates and 5-hydroxytryptamine (5-HT)-positive cells (Fig. 2). Studies in animals have shown that the trachea and stem bronchi receive a tachykinergic afferent innervation mainly from the right vagus nerve, whereas the lungs receive a tachykinin-containing afferent innervation from both vagal parasympathetic and spinal sympathetic ganglia. Capsaicin pretreatment of animals causes an almost total depletion of SP-, NKA-, and CGRP-immunoreactivity in the airway nerves (14, 20, 21).

Luts et al. confirmed the presence of SP nerve fibers in normal human larynx, trachea, and bronchi, but pointed out that they were relatively few compared to nerve fibers containing peptides such as VIP, and that the respiratory epithelium lacks SP-containing nerve fibers (22). In human airways, SP- and CGRP-containing nerve fibers make up only $\pm 1\%$ of the total number of nerve fibers, compared to 60% in guinea pigs (23). NKA and probably other related peptides are also present in human airway nerves. Sheldrick et al. described the presence of numerous NKA-like immunoreactive nerves around intrinsic neurons of local bronchial ganglia and within the bronchial smooth-muscle layer (24).

The content of SP in human lung may vary with age, as relatively fewer SP- and CGRP-containing nerve fibers were found in the bronchioli of adults than infants and children (25). In a study on transplanted human respiratory tracts, which were removed at retransplantation, the content of peptide-immunoreactive nerves was lower than in control lungs. Some immunoreactivity for the sensory neuropeptides CGRP and SP persisted, although the nerve fibers were more sparse than in nontransplanted tissue, suggesting that no reinnervation occurred (26).

Allergic airway inflammation may increase the presence of SP and NKA in the airways. In a guinea-pig model, a three- to fourfold increase of SP, NKA, and CGRP measured in lung tissue was seen 24 h after antigen challenge. Moreover, increased levels of PPT-I

mRNA were found in the nodose ganglia of these animals (27). One of the factors known to increase the expression of SP is nerve growth factor (NGF). Mice with NGF overexpression from a lung-specific promoter were shown to have an increased number of tachykinin-containing sensory nerve fibers in the airways (28). Whether the expression of SP-containing nerves is upregulated in the airways of asthmatic individuals is a controversial issue. In tissue obtained at autopsy, after lobectomy, and at bronchoscopy, both the number and the length of SP-immunoreactive nerve fibers were increased in the airways of subjects with asthma, when compared to airways from subjects without asthma (29). However, Howarth et al. could not identify any SP- or CGRP-containing nerves in endobronchial biopsies from patients with mild asthma (30). In a large study on 49 asthmatic patients, including 16 patients with severe asthma, Chanez et al. did not find evidence for an upregulation of SP-containing nerves in asthma. In their studies, nerves were present in most of the biopsies (as demonstrated by the neural marker PGP 9.5) and were found within and below the epithelium and adjacent to smooth muscle, glands, and blood vessels. However, nerves positive for SP and CGRP were rarely found in the biopsy specimens, and no increase in patients with asthma was observed (31). On the other hand, it was observed that SP-like immunoreactivity was decreased in the tracheal tissue of asthmatic subjects studied at autopsy (13). This may reflect augmented release of SP, followed by degradation.

Nonneuronal sources of tachykinins

Although SP is generally considered a peptide of neuronal origin, there is now evidence for its production by eosinophils, monocytes-macrophages, lymphocytes, neutrophils, and dendritic cells.

In 1988–9, studies by Weinstock and coworkers indicated that eosinophils are able to produce SP. Eosinophils extracted from *Schistosoma mansoni*-induced liver granulomas were found to produce SP (32, 33). Moreover, eosinophils within the human intestine were found to produce SP (34).

Rat peritoneal and alveolar macrophages have been shown to produce SP (35, 36). Killingsworth et al. (1997) found that rat alveolar macrophages express PPT-I mRNA, which is upregulated by lipopolysaccharide (LPS). Ho et al. (1997) reported that human peripheral blood monocyte-derived macrophages express both the peptide SP, and the message PPT-I mRNA. Moreover, mRNA for the tachykinin NK₁ receptor was found to be expressed in these cells. It is of interest to note that the neurotoxin capsaicin, reported to release tachykinins from sensory nerves, is able to release SP from both human monocytes and macrophages (37). We have shown that sputum macrophages from normal subjects and monocytic-like cells from the

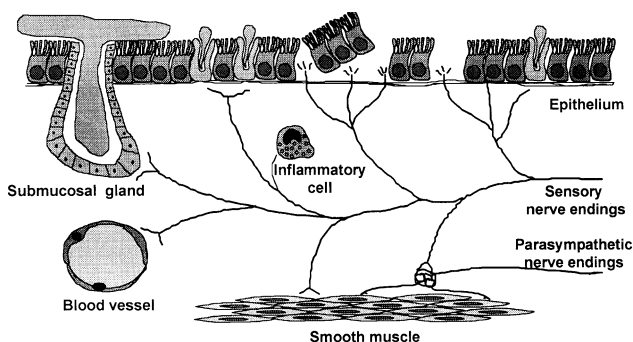


Figure 2. Sensory airway innervation by tachykinins.

U-937 cell line express the mRNA for SP and SP-immunoreactive material. Differentiated U-937 cells and sputum macrophages also express the mRNA for the NK₁ receptor. LPS was able to upregulate the NK₁ mRNA in the U-937 cells (38).

Lymphocytes are also able to produce SP. Lai et al. (1988) demonstrated the presence of the β -, γ -, and δ -transcripts of the SP PPT-I gene in human peripheral blood lymphocytes. At the protein level, the presence of SP was demonstrated and, as reported for monocytes, the neurotoxin capsaicin was shown to release pre-formed SP from lymphocytes (39, 40). By combining flow cytometry and immunohistochemistry, de Giorgio et al. (1998) showed that lymphocytes were markedly labeled for SP, particularly when activated (41).

Our group has recently demonstrated the production of SP by dendritic cells (42). RT-PCR analysis on purified mouse bone-marrow-derived dendritic cells, using primers for the mouse PPT-A gene, revealed the presence of the gamma-transcript of the gene coding for SP and NKA. Immunostaining revealed expression of SP in a perinuclear distribution. The cellular content of SP in purified dendritic cells was 345 ± 16 pg per 10^6 cells. The induction of maturation by LPS or tumor necrosis factor- α (TNF- α) increased the content to 572 ± 57 and 462 ± 37 pg, respectively. The addition of a specific NK₁ tachykinin receptor antagonist to the mixed leukocyte reaction induced by dendritic cells partially inhibited T-cell proliferation. Therefore, dendritic cells have the capacity to produce SP as part of their costimulatory activity for lymphocytes (42).

Tachykinin receptors in the airways: structure and localization

Pharmacology and molecular biology of tachykinin receptors

According to the rank order of potency of the endogenous tachykinins, three receptors are recognized: SP preferentially binds to the neurokinin-1 (NK₁) receptor, NKA to the neurokinin-2 (NK₂) receptor, and NKB to the neurokinin-3 (NK₃) receptor (for review, see refs. 43, 44).

The three mammalian tachykinin receptor genes and complementary deoxyribonucleic acid (cDNA) have been cloned from several species, including man. These 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 α -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 (reviewed in ref. 6).

The amino-acid sequence of each receptor type is well conserved among species, with the greatest differences observed at the NH₂- and COOH-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 approximately 90% (45).

The human NK₁ receptor has been cloned from IM-9 lymphoblasts and lung tissue. The human NK₂ receptor has been cloned from lung, tracheal, jejunum, and gastric tissue, and the human NK₃ receptor from brain tissue (45–48). Comparison of the three human tachykinin receptors indicates a somewhat closer relationship between the NK₁ receptor and the NK₃ receptor (51% homology) than between either the NK₂ and NK₃ receptors or the NK₂ and NK₁ receptors (respectively, 41% and 47% sequence identity). The signal transduction mechanisms of the three tachykinin receptors have been studied in transfected Chinese hamster ovary cells. The three receptors showed a marked stimulation of inositol phosphate (IP) formation and an increase in cyclic AMP (cAMP) formation. However, the activation of adenylate cyclase was less efficient than the activation of phospholipase C (PLC) and required about one order of magnitude higher concentrations of tachykinin peptides (49).

Although each neurokinin receptor subtype has its own preferred endogenous ligand, all naturally occurring tachykinins have high affinity for and act as full agonists on the three receptors, presumably because of the common carboxyl terminal. Receptor-selective agonists have been obtained by structural modifications of the carboxyl-terminal sequences. As a result of random screening of large compound libraries and/or rational drug-design strategies, a number of potent and selective nonpeptide neurokinin receptor antagonists have become available (Table 3). The first selective, high-affinity nonpeptide NK₁ receptor antagonists, CP 96345 (50) and RP 67580 (51), display different affinities for the NK₁ receptors from diverse species. CP 96345 has a higher affinity for the human, bovine, guinea-pig, and rabbit than for the rat and mouse NK₁ receptor, while RP 67580 displays a reverse species selectivity. Other nonpeptide NK₁ antagonists, such as the highly potent SR 140333 (52), do not distinguish between the “rat” and “human” NK₁ receptor (43).

Localization and characterization of tachykinin receptors in the airways

Morphologic studies. Binding sites for [¹²⁵I]-SP have been identified on the epithelium, on small blood vessels and glands in the lamina propria of rat and guinea-pig airways. In guinea pigs and rabbits, SP-binding was also observed over airway smooth muscle. With a selective radioligand, [¹²⁵I]-[Lys⁵, Tyr(I₂)⁷, MeLeu⁹, Nle¹⁰]-NKA (4–10), NK₂ binding sites have been demonstrated over bronchial smooth muscle (54–57).

The presence of NK₂ receptors in developing human lung has been studied with monoclonal antibodies

Table 3. Selective tachykinin receptor agonists and antagonists (modified from ref. 53)

Receptor	NK ₁ receptors	NK ₂ receptors	NK ₃ receptors
<i>Selective agonists</i>			
Endogenous	Substance P	Neurokinin A Neuropeptide γ	Neurokinin B
Synthetic analogs	[Sar ⁹ ,Met(O ₂) ¹¹]SP Septide	[β Ala ⁸]NKA(4-10) [Nle ¹⁰]NKA(4-10) [Lys ⁵ ,MeLeu ⁹ ,Nle ¹⁰] NKA(4-10)	[MePhe ⁷]NKB Senktide
<i>Stable peptide antagonists</i>	FK 888 MEN 11 149	MEN 10 376 MEN 10 627 MEN 11 420 (nepadutant)	
<i>Nonpeptide antagonists</i>	CP-96 345 CP-99 994 CP-122 721 SR 140333 (nolpitanium) RP 67580 RPR 100893 L 709210 L 732138 LY 303870 (lanepitant) GR 203040	SR 48968 (saredutant) SR 140190 GR 159897	SR 142801 (osanetant) SB 223412 PD 157652 GR 138676

against the NK₂ receptor protein. Immunostaining for the NK₂ receptor was found on airway and perivascular smooth muscle in human fetal lung (gestational age 11–22 weeks). Isolated epithelial cell staining was also noted (58).

Using a RNase protection assay, Bai et al. (1995) detected the presence of mRNA for the NK₁ and NK₂ receptors (but not the NK₃ receptor) in human cartilaginous and membranous bronchi and subpleural lung. NK₁ and NK₂ receptor mRNAs were expressed with similar relative abundance in central airways and peripheral lung. In lung samples containing membranous airways, NK₂-receptor mRNA expression was increased fourfold in asthmatics compared with non-smoking controls, whereas NK₁ receptor mRNA levels were similar in the two groups (59). However, Adcock et al. (1993) reported a 50% increase in NK₁ receptor mRNA in asthmatic lung compared with nonasthmatic control tissue. *In situ* hybridization indicated that the NK₁ receptor mRNA was expressed in submucosal glands and airway epithelial cells. The NK₂ receptor and NK₃ receptor mRNAs were not detectable by the *in situ* hybridization technique (60). Glucocorticoids are able to reduce NK₁ and NK₂ receptor expression (60, 61). In bovine tracheal smooth muscle, they were found to decrease the rate of tachykinin NK₂ receptor gene transcription (61).

In contrast to the ligand-binding experiments, Hakonarson et al. detected the presence of mRNA for the NK₁, but not the NK₂, receptor in rabbit airway smooth muscle. Incubation of the rabbit airways with

serum from atopic asthmatics caused an upregulation of the NK₁ receptor mRNA at 6 and 24 h (62).

Pharmacologic characterization. In guinea-pig airways, NK₂ receptors and, to a lesser extent, NK₁ receptors have been shown to be involved in bronchoconstriction induced by exogenous tachykinins, capsaicin, and electrical field stimulation (63–67). In rats, strain-dependent differences were observed with regard to mechanisms and receptors involved in tachykinin-induced bronchoconstriction. In the BDE rat strain, tachykinins cause bronchoconstriction by a direct NK₂ receptor-mediated effect on airway smooth muscle. In the more sensitive Fisher 344 rats, SP and NKA cause bronchoconstriction mainly by an indirect NK₁ receptor-mediated mechanism involving cholinergic nerve and mast-cell activation (68, 69).

In man, the NK₂ receptor mediates the contraction induced by tachykinins in isolated large airways. Naline et al. (1989) demonstrated the NK₂ receptor selective agonist [Nle¹⁰]-NKA(4–10) to be a potent contractor of isolated human bronchi (internal diameter 3–5 mm), whereas agonists selective for the NK₁ and NK₃ receptor were almost inactive (70). Sheldrick et al. (1995) found that NKA was the most potent contractile agonist on human bronchi. The NK₂ receptor agonist GR64349 was only threefold less potent than NKA, while SP, NKB, and a NK₁ receptor agonist were at least 100 times less potent (24). Potent and specific nonpeptide receptor antagonists have been evaluated in isolated human bronchi. Advenier et al. (1992) showed that the potent and specific NK₂ receptor antagonist SR 48968 displayed competitive antagonism for the contraction induced by the specific NK₂ receptor agonist [Nle¹⁰]-NKA(4–10). No effect on contractions induced by acetylcholine, histamine, KCl, or prostaglandin F_{2 α} was seen. SR 48968 also shifted the dose-response curve for SP to the right. The specific NK₁ receptor antagonist CP-96,345 had no effect (71). These *in vitro* results suggest that the contraction induced by SP and NKA in large, normal human airways is mediated by NK₂ receptors. However, NK₁ receptors are also present on small human bronchi (internal diameter <1 mm), and their stimulation induces a prostanoid-dependent contraction (72).

Tachykinins as contractile agonists of the airways

Tachykinins are potent contractors of airways. This has been demonstrated both *in vitro* and *in vivo*, both in animal and in human airways.

The *in vitro* contractile effect of SP and NKA has been studied extensively. SP contracts human bronchi and bronchioli (73, 74), but is less potent than histamine or acetylcholine (14). NKA is a more potent constrictor of human bronchi than SP and was reported to be, on a

molar base, two to three orders of magnitude more potent than histamine or acetylcholine. In contrast to guinea pigs, NKB had no contractile effect on human airways (74). Noncholinergic pathways might be relatively more important in the smaller airways. Indeed, NKA and SP were found to contract small airways to a larger extent and at lower concentrations than large airways (75).

Passive sensitization has been reported to influence the *in vitro* contractile effect of SP and NKA on human bronchi (76). 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 tachykinins on isolated asthmatic bronchi. It has been demonstrated for adenosine that such an approach could reveal important differences between normal and asthmatic persons, with regard both to the potency of the agonist and the mechanism of induced contraction (77).

The *in vivo* bronchoconstrictor effect of SP and NKA, administered by inhalation or intravenous infusion, has been reported by several groups. In these studies, NKA was found to be a more potent bronchoconstrictor than SP. Patients with asthma are hyperresponsive to SP and NKA (reviewed in ref. 78). The bronchial response to inhaled NKA is reproducible (79). The bronchoconstrictor effect of inhaled SP and NKA in asthmatics can be prevented by pretreatment with sodium cromoglycate and nedocromil sodium (80, 81). This has led us to postulate that SP and NKA are indirect bronchoconstrictors in man. This indirect bronchoconstrictor effect could arise from an effect on inflammatory cells (e.g., mast cells) and/or nerves. However, histamine does not seem to be involved, as pretreatment with the H₁ antagonists astemizole and terfenadine did not inhibit NKA-induced bronchoconstriction (82, 82). In experimental animals, tachykinins are able to cause acetylcholine release from postganglionic cholinergic airway nerve endings (84–86) and to activate mast cells (see below). However, the exact mechanism of tachykinin-induced bronchoconstriction in man is not yet known.

Role of tachykinins in neurogenic airway inflammation

The term “neurogenic inflammation” was first used by Jancsó et al. to describe the increase in vascular permeability, plasma extravasation, and tissue swelling

which occurs in the skin and conjunctiva when sensory nerves are stimulated by topical administration of irritants such as capsaicin, hypertonic saline, mustard oil, and formaldehyde or by antidromic electrical stimulation (87, 88).

Neurogenic inflammation in rodent airways

Assessment of the vascular permeability usually relies on measurements of extravasation of intravascular albumin, with either radiolabeled albumin or Evans blue dye as intravascular markers. Most animal studies on plasma extravasation in the airways used the Evans blue technique. Evans blue is a diazo dye that binds quantitatively to albumin *in vivo* (89, 90). Spectrophotometric measurements of the amount of dye extractable from the airways are used to quantify the plasma protein extravasation.

A wide array of stimuli can induce neurogenic inflammation in rodent airways. Lundberg and colleagues were the first to describe neurogenic inflammation in the respiratory tract. They found that in rats and guinea pigs topical application of capsaicin, inhalation of cigarette smoke, or electrical stimulation of the vagus nerve induced an increase in the plasma protein extravasation in the airways. This neurogenic plasma extravasation occurred both in the upper airways (nasal mucosa, epiglottis, vocal cords) and in the lower airways (from the trachea down to the bronchioles). The increase in vascular permeability was abolished by capsaicin pretreatment and inhibited by a SP antagonist. Administration of exogenous SP mimicked the plasma leakage induced by either capsaicin or electrical nerve stimulation. Furthermore, the effect of SP was not affected by depletion of sensory nerves with capsaicin. Consequently, they postulated that SP released from capsaicin-sensitive nerve fibers mediates the neurogenic inflammation in the airways (2, 91). Subsequent studies have shown that the plasma extravasation induced by cold air, hypertonic saline, or isocapnic hyperpnea depends on capsaicin-sensitive sensory nerves and is inhibited by tachykinin receptor antagonists. Furthermore, part of the increase in vascular permeability produced by histamine, bradykinin, acetylcholine, or antigen challenge involves tachykinins released from capsaicin-sensitive nerves (91).

The involvement of NK₁ receptors in the neurogenic plasma extravasation in the central airways of rats and guinea pigs has been demonstrated by the use of selective tachykinin receptor agonists and antagonists. The NK₁ receptors are also implicated in the extravasation caused by hypertonic saline, bradykinin, antigen challenge, or acetylcholine. NK₂ receptors mediate part of the neurogenic plasma extravasation in the secondary bronchi and intraparenchymal airways of the guinea pig (92–98).

Using antibodies directed against the NK₁ receptor protein, Bowden et al. have demonstrated that in the rat trachea NK₁ receptors are present on the endothelial cells of the postcapillary venules. These receptors are internalized in endosomes upon binding with SP. They also observed an increase in the number of NK₁ receptor immunoreactive endosomes when the vagus nerve is electrically stimulated, indicating that SP released by activation of the sensory nerves has a direct effect on the NK₁ receptors on the postcapillary venular endothelium (99).

Both direct and indirect mechanisms have been involved in neurogenic inflammation in rodent airways.

Direct mechanisms. Intravenous injection of SP causes the formation of intercellular gaps between endothelial cells in postcapillary and collecting venules in rat trachea. These gaps are formed within 20 s, and their numbers peak after 1 min (representing approximately 3% of the surface area of the postcapillary venules and 0.7% of the collecting venules), after which they decrease with a half-life of 3.2 min (100).

However, increased microvascular permeability is not the only mechanism responsible for the plasma extravasation and edema formation produced by neuropeptides released from sensory nerves. Electrical stimulation of the vagus nerve causes an increase in the negativity of interstitial fluid pressure in rat trachea. The reduction in interstitial fluid pressure occurs within 30 s of nerve activation, and reaches a stable level within 3–5 min. Depleting sensory nerves with capsaicin or degranulating mast cells with compound 48/80 or polymyxin B abolished the effect of vagal nerve stimulation on the interstitial fluid pressure. This led the authors to conclude that there is a functional innervation of the loose connective tissue in rat trachea. They also concluded that the interstitium “actively” participates in the generation of airway wall edema, since increased negativity of interstitial fluid pressure promotes fluid filtration (101).

Indirect mechanisms. In addition to the direct effects of tachykinins on the venular endothelium, indirect mechanisms involving mast-cell activation and serotonin (5-HT) release participate in the tachykinin-induced plasma exudation in the respiratory tract of some animal species.

In rabbit airways, involvement of 5-HT receptors and arachidonic acid derivatives in the tachykinin-induced increase in vascular permeability has been suggested (102, 103). Saria et al. found that intravenous administration of compound 48/80 induced an acute increase in vascular permeability in rat airways, which was inhibited by a SP antagonist or by capsaicin depletion of the sensory nerve fibers (104). These findings suggest

that mast-cell mediators may have a stimulatory effect on sensory airway nerves.

Both inhibitory and stimulatory prejunctional 5-HT and histamine receptors have been described on rodent sensory nerves. The neurogenic inflammation in intracranial blood vessels of rats and guinea pigs has been shown to be inhibited prejunctionally by 5-HT_{1B/D} receptors (105, 106). In rat trachea, the release of the sensory neuropeptide calcitonin gene-related peptide (CGRP) from capsaicin-sensitive nerves is increased by activation of 5-HT₃ receptors (107). Buckner et al. (1991) found that *in vivo* activation of 5-HT_{1like} and 5-HT₃ receptors causes release of sensory neuropeptides in guinea pig airways (108). Ward et al., on the other hand, described an inhibitory, “atypical” 5-HT receptor on sensory C-fibers in isolated guinea-pig bronchi (109). Similarly, in rat skin and in guinea-pig airways, SP release and neurogenic inflammation are inhibited by prejunctional histamine H₃ receptors and potentiated by H₁ receptors (110, 111).

These observations illustrate that the interactions between sensory nerves and mast cells and/or mast-cell mediators differ according to the species and the tissue preparation studied.

Morphine reduces the neurogenic inflammatory response in the airways, presumably through activation of prejunctional opioid receptors, resulting in an inhibition of neuropeptide release from sensory nerves (112). Similarly, forskolin (an adenylate cyclase stimulator), dibutyryl, cAMP, cilostazol (a cyclic nucleotide phosphodiesterase inhibitor), cromakalim (a K⁺ channel opener), and prostaglandin E₁ have been shown to decrease the neurogenic extravasation by prejunctional inhibition of neurotransmitter release from afferent C-fibers (113–115).

Neurogenic inflammation in human airways

Since most methods used for direct measurement of neurogenic inflammation in airways are not applicable to man, information on the mechanisms and control of microvascular permeability has been obtained largely from animal studies. However, measurements of proteins in nasal and BAL fluids have been used as indirect measurements of plasma exudation into human airways (116–118).

There have been some studies on the role of tachykinins and sensory nerves in vascular control in the nose. In normal subjects, nasal challenge with capsaicin induces nasal pain, discomfort, and rhinorrhea. This rhinorrhea is caused mainly by glandular secretion. Topical application of capsaicin has no effect on nasal airway resistance or plasma protein leakage in healthy subjects (119). Geppetti et al. (1988) proposed that the capsaicin-induced nasal secretions are mediated primarily via local neurotransmitter release from sensory nerves (119), whereas others have suggested

that the secretory effect of capsaicin is mediated via cholinergic parasympathetic reflexes (120).

After allergen challenge, SP and CGRP are released in the nasal secretions of atopic patients (121, 122). In allergic rhinitis patients, but not in normal controls, exogenous SP induces nasal obstruction (123), which is associated with inflammatory cell recruitment and plasma protein exudation in the nasal lumen (124). Application of CGRP to the nasal mucosa does not stimulate glandular secretion or plasma protein exudation (124). These findings suggest a role for SP in allergic rhinitis, but additional studies are necessary to clarify the involvement of tachykinins in this allergic disorder.

Role of neutral endopeptidase (NEP)

The physiological actions of neuropeptides are normally terminated by extracellular metabolism. 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-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). In guinea pigs, it was shown that both NEP and ACE participate in the metabolism of SP when administered intravascularly, while the tachykinins administered by aerosol are degraded by NEP only (127).

NEP mRNA and NEP-immunoreactive material has been identified in human airways (128). NEP is present in the basal cells of the epithelium, alveolar cells type II, submucosal glands, neutrophils, airway smooth muscle, postcapillary venules, and nerves (129). 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 (130). The expression of NEP is significantly increased in the airway epithelium of patients using inhaled steroids (131).

It has been hypothesized that the epithelial damage frequently observed in asthmatic airways could result in decreased NEP activity, enhancing neurogenic airway inflammation. To test this hypothesis, the effect of an inhaled inhibitor of NEP on the bronchoconstrictor effect of NKA in man has been studied by several groups.

Cheung et al. demonstrated that the inhalation of thiorphan 10 min before NKA challenge enhanced the bronchoconstrictor effect of NKA, in both normal (132) and asthmatic subjects (133). Interestingly the leftward shift of the NKA dose-response curve was not significantly different in the asthmatic from the

nonasthmatic subjects. Thiorphan had no effect on baseline lung function or bronchial responsiveness to methacholine in either group. Using phosphoramidon, another inhibitor of NEP, Crimi et al. (1994) 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 (134).

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. As inhibition of NEP offers a similar shift of the dose-response curve in both normal subjects and nonsmoking stable, mild to moderate asthmatics, it can be argued that the functional activity of NEP is not significantly decreased in stable asthmatics. 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 (135).

Immunomodulatory effects of tachykinins

Tachykinins are potent bronchoconstrictors and vasoactive agents. Moreover, it is now clear that they express a wide array of immunomodulatory effects which might contribute to inflammatory processes in the airways (Fig. 3).

Effects of tachykinins on inflammatory and mesenchymal cells

Mast cells. SP degranulates mast cells, leading to the release of histamine and 5-HT (136–138). SP induced TNF- α production and secretion in a murine mast-cell line and in murine peritoneal mast cells (139). Picomolar concentrations of SP triggered electrical responses in rat peritoneal mast cells without degranulation, resulting in priming of these cells (140).

Both a receptor-independent and a receptor-dependent mechanism have been reported for mast-cell activation induced by SP. Higher (micromolar) concentrations of SP cause a direct activation of G protein. It has been proposed that the amino-terminus of SP is responsible for this receptor-independent mechanism of mast-cell activation (141). However, lower (nanomolar) concentrations of SP cause mast-cell activation by interaction with tachykinin receptors. The histamine release from the murine MC/9 mast-cell line is mediated by NK₂ receptors (142), while the mast-cell degranulation in Fisher 344 rat airways involves activation of NK₁ receptors (143–145). In Fisher 344 rats, mast-cell activation mediated by stimulation of NK₁ receptors and involving release of the mast-cell

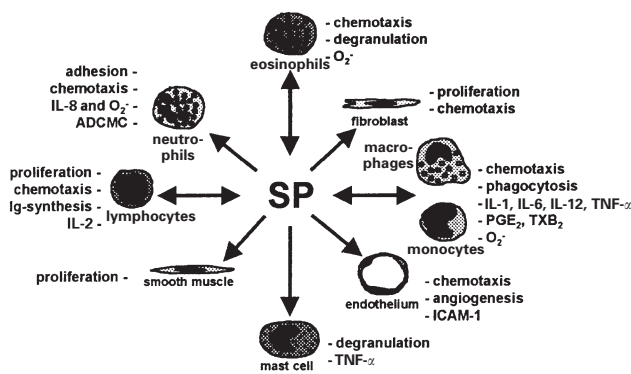


Figure 3. Immunomodulatory effects of substance P (for specific references, see text).

mediator serotonin (5-HT) explains the hyperresponsiveness of the airways to the sensory neuropeptides SP and NKA (146) (Fig. 4).

Neutrophils. Tachykinins cause adherence and chemotaxis of human neutrophils (147–150). *In vivo*, in rats, tachykinins mediate partially the recruitment of neutrophils into the airways caused by instillation of interleukin (IL)-17 (151). SP is also able to induce increased antibody-dependent cell-mediated cytotoxicity (152), superoxide anion production (153), and IL-8 release (154) by human polymorphonuclear cells.

Although SP has been shown to bind to the FLMP-receptor on rabbit neutrophils (155), the activation of human neutrophils by SP appears to be mediated by NK₁ receptors (153).

Eosinophils. Wiedermann et al. described SP-induced chemotaxis of human eosinophils, with a EC₅₀ of approximately 1 pM (156). However, others failed to detect a direct effect of SP on human eosinophil migration, but found a potentiating effect of SP

on the eosinophil chemotaxis induced by PAF, leukotriene B₄, and IL-5 (157, 158). SP also induces the release of ECP and superoxide anion by human eosinophils (148).

Lymphocytes. Several *in vitro* and *in vivo* studies have shown that SP is able to modulate the chemotaxis, proliferation, and activation of lymphocytes.

Schratzberger et al. (1997) have demonstrated that SP can induce chemotaxis of human peripheral blood lymphocytes (both B and T cells) (159). The NK₁ receptor antagonist SR 140333 inhibited this SP-induced chemotaxis, indicating the involvement of NK₁ receptors. In healthy subjects, SP and NKA enhanced the lymphocyte proliferation induced by phytohemagglutinin, concavalin A, and PMA, while, in AIDS patients, SP inhibited lymphocyte proliferation (160). SP has also been reported to enhance the proliferation of T cells in the absence of other stimuli, an effect which was inhibited by a NK₁ receptor antagonist. The production of IL-2 by human and murine T cells was enhanced by SP (161–163). Several studies have demonstrated the modulation of immunoglobulin synthesis by SP (164, 165).

Although a receptor-independent mechanism of lymphocyte activation has been suggested (166), NK₁ receptors have been demonstrated on human and murine lymphocytes (167).

Monocytes and macrophages. Tachykinins also have a number of stimulatory effects on monocytes and macrophages. SP has been shown to evoke superoxide anion production (168, 169), enhance phagocytosis (170), and cause production of IL-1 and IL-12 by macrophages (171, 172). Alveolar macrophages from ovalbumin-sensitized guinea pigs showed an enhanced responsiveness to tachykinin-induced superoxide anion and prostanoid production (173). The HIV-1 expression in peripheral blood monocyte-derived macrophages was enhanced by the addition of SP (174). Monocytes have been shown to respond chemotactically to SP (156, 175). SP induces the release of cytokines such as IL-1, IL-6, and TNF- α from peripheral blood monocytes, especially the activated ones (176, 177). More recent reports found no effect of SP alone on IL-1 or IL-6 release by monocytes. However, SP appeared to potentiate the release of cytokines induced by low concentrations of LPS (178, 179).

Although both NK₁ receptors and/or NK₂ receptors have been described on human, murine, and guinea-pig macrophages (180–183), the exact type of neurokinin receptor on monocytes has not been determined. Lucey et al. (1994) reported that binding of [¹²⁵I]-SP with high affinity and specificity (K_d = 27 to 55 nM) to a single class of receptors on monocytes was possible only in the

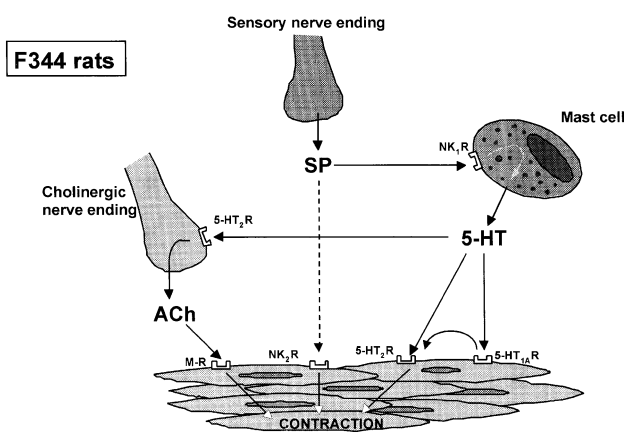


Figure 4. Schematic representation of receptors and cellular mechanisms involved in tachykinergic contraction of F344 rat isolated airways. NK₁R: NK₁ receptor; NK₂R: NK₂ receptor; M-R: muscarinic receptor; ACh: acetylcholine.

presence of excess serine protease inhibitor enzyme complex (SEC) receptor ligand. The number of SP-binding sites rapidly decreased after isolation of peripheral blood monocytes, possibly due to down-regulation of receptor expression as a result of adherence (184). Other authors described an atypical SP receptor ($K_d = 224$ nM for binding of [^{125} I]-SP) on peripheral blood monocytes that is coupled to Gi proteins, phospholipase C (PLC), phospholipase D, and MAP kinase (185, 186).

Mesenchymal airway cells. In addition to their effects on circulating and tissue leukocytes, tachykinins are also able to activate mesenchymal cells in the airways. These stimulatory effects on mesenchymal cells may play a role in the airway remodeling which is a characteristic feature of asthmatic airways.

Nilsson et al. reported that SP and NKA are able to stimulate DNA synthesis in rat arterial smooth-muscle cells and human skin fibroblasts (187). Harrison et al. (1995) showed that both SP and NKA stimulated proliferation of human lung fibroblast cell lines, but only NKA stimulated chemotaxis of these fibroblasts (188). However, others found that SP stimulated the migration of human skin fibroblasts (in both the presence and absence of a concentration gradient of SP). This migration-promoting effect of SP on human fibroblasts involved the activation of NK₁ receptors (189, 190). The SP-induced proliferation of human fibroblasts and rabbit airway smooth muscle is mediated by interaction with NK₁ receptors (191–193).

SP has been shown to induce a concentration-dependent migration of endothelial cells of bovine origin through activation of NK₁ receptors (194). Incubation of human umbilical cord vein endothelial cells with SP induced the differentiation of these cells into capillary-like structures (195). Nakagawa et al. (1993) found that SP increased the expression of ICAM-1 on human endothelial cells (196).

Involvement of tachykinins in allergic airway inflammation

There is no direct evidence for an involvement of tachykinins in human allergic airway disease. Tachykinins have been shown to be involved in the acute bronchoconstriction induced by antigen in sensitized guinea pigs (197, 198). Antigen challenge has been shown to enhance the noncholinergic contractions and to induce bronchial hyperresponsiveness to SP and NKA (199–201).

After pretreatment with capsaicin, the antigen-induced bronchial hyperresponsiveness to histamine in guinea pigs and rabbits is suppressed, suggesting the involvement of tachykinins (202, 203). In guinea pigs, pretreatment with the tachykinin NK₂ receptor antagonists FK 224 and SR 48968 prevented the IL-5-induced airway hyperresponsiveness, but not the IL-5-induced

eosinophil accumulation. Therefore, tachykinins activating NK₂ receptors are involved in the IL-5-induced airway hyperresponsiveness (204).

Both NK₁ and NK₂ tachykinin receptors have been involved in the antigen-induced airway effects. In a murine model of lung immune modulation, an increased SP content in the BAL fluid and an increased NK₁ receptor mRNA expression in BAL lymphocytes and alveolar macrophages has been observed. Furthermore, the tachykinin NK₁ receptor antagonist CP-96,345 significantly reduced the number of leukocytes, granulocytes, and lymphocytes retrieved by BAL on day 5 after antigen challenge in sensitized mice (180). Bozic et al. reported that gene-targeted disruption of the NK₁ receptor protected the lung from immune complex injury. The lung tissues of immune complex-challenged NK₁ receptor knockout mice appeared to be no different from those of control animals (205). In a model of conscious, unrestrained guinea pigs, the NK₁ receptor was found to be involved in both the development of antigen-induced airway hyperresponsiveness to histamine and the antigen-induced infiltration of eosinophils, neutrophils, and lymphocytes (206). Moreover, in the same model, the NK₂ receptor was involved in the development of the antigen-induced late reaction (207).

Clinical studies with tachykinin antagonists in asthma

At present, clinical trials with four tachykinin antagonists have been reported: FK224, a cyclic peptide tachykinin antagonist for NK₁ and NK₂ receptors; CP-99,994, a nonpeptide NK₁ tachykinin receptor antagonist; FK888, a peptidic tachykinin NK₁ receptor antagonist; and SR48968, a nonpeptide NK₂ tachykinin receptor antagonist.

FK224, 4 mg given by metered-dose inhaler, was shown to inhibit bradykinin-induced bronchoconstriction and cough in nine asthmatics (208). These findings suggested that bradykinin causes bronchoconstriction by release of tachykinins from sensory nerves. We have studied the effect of inhaled FK224 on NKA-induced bronchoconstriction in 10 mildly asthmatic patients (79). Inhaled FK224 had no effect on baseline lung function and offered no protection against NKA-induced bronchoconstriction. Lunde et al. (1994) studied this drug as a 4-week treatment (4 mg via metered-dose inhaler q.i.d.) in patients with mild to moderate asthma (209). Compared to placebo, no beneficial effects of FK224 on symptoms and lung function were found. However, as FK224 was not able to antagonize the airway effects of NKA in asthmatics, the study by Lunde et al. does not reject a possible role for sensory neuropeptides in asthma.

Fahy et al. (1995) studied the effect of the nonpeptide NK₁ tachykinin receptor antagonist CP-99,994 (250

µg/kg intravenously) on hypertonic saline-induced bronchoconstriction in 14 mild asthmatics (210). Compared to placebo, it had no effect on bronchoconstriction and cough induced by hypertonic saline. However, it remains to be proven whether this compound at the given dose is able to block the airway responses induced by inhaled SP or NKA.

The NK₁ tachykinin receptor antagonist FK888 has been studied in exercise-induced asthma (211). Inhalation of FK888 in nine asthmatic subjects had no effect on baseline lung function. While pretreatment with FK888 did not attenuate the maximal decrease in lung function, it did reduce the time of recovery from exercise-induced bronchoconstriction.

The NK₂ tachykinin receptor antagonist SR 48968 (saredutant) (100 mg *per os*) caused a significant inhibition of NKA-induced bronchoconstriction, with a mean three- to fivefold shift of the dose-response curve for NKA to the right (212). This study demonstrates that NK₂ tachykinin receptors mediate at least part of the bronchoconstriction induced by inhaled NKA in asthmatic patients.

Conclusions and future prospects

Tachykinins are present in neurons and inflammatory cells in airways. They may be released from the airways by stimuli such as ozone and allergen. The best characterized effect of tachykinins on the airways is bronchoconstriction. SP and NKA induce bronchoconstriction in man, asthmatics being more sensitive than normal subjects. The bronchoconstrictor effect of these neuropeptides is partly indirect. The exact

mechanism by which they cause bronchoconstriction is unknown. The effect of tachykinins is not limited to airway smooth-muscle contraction. Tachykinins are involved, at least in animal models, as a major component of neurogenic inflammation (vasodilation, plasma extravasation), as well as in airway hypersecretion, regulation of neural transmission, and attraction and activation of inflammatory cells.

Tachykinin NK₂ receptors are present on smooth muscle of both large and small airways. They mediate a major part of the bronchoconstrictor effect of tachykinins. Tachykinin NK₁ receptors are localized on smooth muscle of small airways, submucosal glands, blood vessels, and inflammatory cells. Thus, the potential therapeutic effects of tachykinin antagonists may not be restricted to the inhibition of tachykinin-induced bronchoconstriction. Clearly, additional clinical trials examining the effect of NK₁ and/or NK₂ receptor antagonists on different indirect stimuli of bronchial responsiveness are necessary to establish the role of tachykinins in the pathophysiology of asthmatic bronchial hyperresponsiveness. Furthermore, long-term studies with tachykinin receptor antagonists are needed to elucidate the role of sensory neuropeptides in asthma and other airway diseases.

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