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Review

Airway inflammation and tachykinins: prospects for the development of tachykinin receptor antagonists

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Abstract

The tachykinins substance P and neurokinin A are contained within sensory airway nerves. Immune cells form an additional source of tachykinins in inflamed airways. Elevated levels of tachykinins have been recovered from the airways of patients with asthma and chronic obstructive pulmonary disease. Airway inflammation leads to an upregulation of tachykinin NK_1 and NK_2 receptors. Preclinical studies have indicated a role for the tachykinin NK_1, NK_2 and NK_3 receptors in bronchoconstriction, airway hyperresponsiveness and airway inflammation caused by allergic and nonallergic stimuli. Compounds that are able to block two or three tachykinin receptors hold promise for the treatment of airways diseases such as asthma and/or chronic obstructive pulmonary disease.

Keywords: Substance P; Neurokinin A; Tachykinin receptor; Asthma; Airway inflammation

1. Introduction

The tachykinins are potent vasodilators and contractors of smooth muscle. In studies on rodent airways, substance P and neurokinin A 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 and neurogenic inflammation (Lundberg and Saria, 1982; Barnes, 1986).

Substance P and neurokinin A 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, chemo-attraction of eosinophils and neutrophils and the vascular adhesion of neutrophils (Joos et al., 2000). Substance P and neurokinin A interact with the different targets in the airways by stimulation of tachykinin NK_1, NK_2 and NK_3 receptors (Maggi, 2000). In the present article, an update on the role of tachykinins and their receptors in asthma and chronic obstructive pulmonary disease is given. In addition, preclinical data on the evaluation of tachykinin receptor antagonists in animal models of asthma are discussed.

2. Presence of tachykinins in normal and diseased airways

2.1. Airway nerves containing sensory neuropeptides

Substance P and neurokinin A are localized in a distinct subpopulation of primary afferent nerves that are characterized by sensitivity to capsaicin. Studies in rodents have shown that release of tachykinins from sensory nerves can be evoked by a variety of stimuli such as capsaicin, electrical nerve stimulation, low pH, ether, formalin, toluene diisocyanate, histamine, bradykinin, methacholine, prostaglandins, leukotrienes and cigarette smoke (reviewed in Lundberg, 1996; Joos et al., 2000).

In guinea pig, airway sensory nerves containing tachykinins are easily demonstrated. However, it has proven more difficult to demonstrate excitatory nonadrenergic, noncholinergic nerves (e-NANC) in human airways. Substance P- and neurokinin A are present in nerve profiles, found beneath and within the epithelium, around blood vessels and submucosal glands and within the bronchial smooth muscle layer (Lundberg et al., 1984;

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Luts et al., 1993; Sheldrick et al., 1995). Tachykinins have been measured in bronchoalveolar lavage fluid, induced sputum and plasma. Various studies have shown that tachykinins can be released into the airways after exposure to allergen, ozone or hypertonic saline. Nieber et al. found a significantly larger amount of substance P in bronchoalveolar lavage fluid of atopic compared to nonallergic subjects. After intrasegmental provocation with allergen, a significant increase in substance P levels was observed in bronchoalveolar lavage fluid (Nieber et al., 1992). Heaney et al. also recovered neurokinin A from bronchoalveolar lavage fluid from normal and asthmatic patients. Neurokinin A was increased in the asthmatic patients 4 h after inhalation challenge with house dust mite (Heaney et al., 1998). Exposure of healthy individuals to ozone increased the concentration of substance P in bronchoalveolar lavage fluid (Hazbun et al., 1993) and decreased the immunoreactivity for substance P, assessed on bronchial biopsies (Krishna et al., 1997). Nasal application of hypertonic saline also induced release of substance P (Baraniuk et al., 1999).

Substance P has been measured in sputum induced by inhalation of hypertonic saline. In comparison to healthy subjects, the concentrations of substance P were significantly higher in patients with asthma and chronic bronchitis. In all subjects, the concentration of substance P in induced sputum was related to the degree of airway obstruction (Tomaki et al., 1995). Plasma levels of substance P-like immunoreactivity were significantly higher in patients with an acute exacerbation of asthma compared to healthy controls (Cardell et al., 1994).

Although increased amounts of tachykinins have been recovered from airways, there has been debate about a possible upregulation of substance P-containing nerves in patients with asthma or chronic obstructive pulmonary disease. In tissue obtained at autopsy, after lobectomy and at bronchoscopy, both the number and the length of substance P-immunoreactive nerve fibres were increased in airways of subjects with asthma, when compared to airways from subjects without asthma (Ollerenshaw et al., 1991). However, Howarth et al. (1995) could not identify any substance P-containing nerves in endobronchial biopsies from patients with mild asthma. More recently, in an extensive study on 49 asthmatic patients, including 16 patients with severe asthma, Chanez et al. did not find evidence for an upregulation of substance P-containing nerves in asthma. Nerves were present in most of the endobronchial biopsies, as demonstrated by the general neural marker protein gene product (PGP) 9.5, and were found within and below the epithelium and adjacent to smooth muscle, glands and blood vessels. However, nerves positive for substance P and calcitonin gene-related peptide were rarely found in the biopsy specimens and no increase in patients with asthma was observed (Chanez et al., 1998). On the other hand, it was observed that substance P-like immunoreactivity was decreased in tracheal tissue of asthmatic subjects studied at autopsy. This may reflect augmented release of substance P, followed by degradation (Lilly et al., 1995). Lucchini et al. studied lung tissue obtained at thoracotomy from patients with chronic bronchitis. The lung tissue in chronic bronchitis did not contain more nerves, and did not show a change in the amount of substance P- or calcitonin gene-related peptide containing nerves. In contrast, the density of vasoactive intestinal peptide-containing nerves was significantly higher in the glands of patients with chronic bronchitis compared to control subjects (Lucchini et al., 1997). Data on patients with cough are limited. O'Connell et al. (1995) reported that in patients with persistent unexplained cough, intraepithelial airway nerves were found to contain increased quantities of the sensory neuropeptide calcitonin gene-related peptide, but not substance P.

2.2. Nonneuronal sources of tachykinins in the airways

In recent years, it has become clear from both animal and human studies that immune cells may form an additional source of tachykinins (Maggi, 1997; Joos and Pauwels, 2000). Evidence for the production of substance P by eosinophils (mouse, man), monocytes and macrophages (rat, man), lymphocytes (man) and dendritic cells (mice) has been reported. Inflammatory stimuli such as lipopolysaccharide can upregulate the concentration of tachykinins in these cells (Germonpré et al., 1999; Lambrecht et al., 1999). These findings can help to explain the paradox between the relatively few substance P- and neurokinin A-containing nerves observed in human airways and the recovery of substance P and neurokinin A from sputum and bronchoalveolar lavage (Tomaki et al., 1995; Heaney et al., 1998). Thus, increased amounts of immune cells attracted to the inflamed airways might be responsible for an increased content of substance P and neurokinin A in patients with asthma or chronic obstructive pulmonary disease. Moreover, the release of tachykinins from these inflammatory cells might further stimulate and activate these cells in an autocrine or paracrine fashion. Indeed, these inflammatory cells produce and secrete substance P and possess tachykinin NK_1 receptors on their membrane (Germonpré et al., 1999).

It is also possible that substance P is produced by epithelial or endothelial cells (Maggi, 1997). Chu et al. reported staining for substance P in the epithelium on biopsy specimens. In comparison to control subjects, patients with asthma showed a significantly higher expression of substance P in the epithelium but not in the submucosa. It is of interest to note that in their study, the epithelial expression of substance P was correlated with the epithelial mucus content (Chu et al., 2000).

2.3. Tachykinin receptors in normal and diseased airways

Using an RNase protection assay, Bai et al. detected the presence of mRNA for the tachykinin NK_1 and NK_2

receptors (but not the tachykinin NK_3 receptor) in human cartilaginous and membranous bronchi and subpleural lung. Tachykinin NK_1 and NK_2 receptor mRNAs were expressed with similar relative abundance in central airways and peripheral lung. In lung samples containing membranous airways, tachykinin NK_2 receptor mRNA expression was increased fourfold in asthmatics compared with nonsmoking controls, whereas tachykinin NK_1 receptor mRNA levels were similar in the two groups (Bai et al., 1995). Adcock et al. however, reported a 50% increase in tachykinin NK_1 receptor mRNA in asthmatic lung compared with non-asthmatic control tissue. In situ hybridization indicated that the tachykinin NK_1 receptor mRNA was expressed in submucosal glands and airway epithelial cells. The tachykinin NK_2 and NK_3 receptor mRNAs were not detectable using the in situ hybridization technique (Adcock et al., 1993). Glucocorticoids are able to reduce tachykinin NK_1 and NK_2 receptor mRNA expression (Adcock et al., 1993; Katsunuma et al., 1998). In bovine tracheal smooth muscle, they were found to decrease the rate of tachykinin NK_2 receptor gene transcription (Katsunuma et al., 1998).

In a study on surgical specimens and using antibodies to the tachykinin NK_1 and NK_2 receptors, Mapp et al. found expression of both tachykinin receptors in bronchial glands, bronchial vessels, and bronchial smooth muscle. Receptors were occasionally found in nerves (NK_1) and in inflammatory cells (NK_2) such as T lymphocytes, macrophages, and mast cells (Mapp et al., 2000). In this study, the distribution of both tachykinin NK_1 and NK_2 receptors was similar in the tissues examined from nonsmokers, asymptomatic smokers, symptomatic smokers with normal lung function and symptomatic smokers with chronic airflow limitation. In a study on endobronchial biopsies by Chu et al., immunoreactivity for the tachykinin NK_1 receptor was found in the epithelium and submucosa. The tachykinin NK_1 receptor expression was mainly seen on cell surfaces of the upper half of the epithelial layer. Goblet cells appeared to be the cells with the strongest staining. In the submucosa, the tachykinin NK_1 receptor was primarily localized on the endothelial cells of the blood vessels, the surfaces of inflammatory cells, and some smooth muscle cells. In this study, the expression of substance P as well as the tachykinin NK_1 receptor was significantly higher in the epithelium of asthmatic subjects (Chu et al., 2000).

3. Bronchoconstrictor effect of tachykinins: which mechanism(s) and which tachykinin receptor?

3.1. In vitro contraction of human bronchi

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 substance P and neurokinin A has been studied extensively. Substance P contracts human bronchi and bronchioli (Advenier et al., 1987; Finney et al., 1985), but is less potent than histamine or acetylcholine (Martling et al., 1987). Neurokinin A is a more potent constrictor of human bronchi than substance P and was reported to be, on a molar base, 2–3 orders of magnitude more potent than histamine or acetylcholine. In contrast to guinea pigs, neurokinin B had no contractile effect on human airways (Advenier et al., 1987). Noncholinergic pathways might be relatively more important in the smaller airways. Indeed, neurokinin A and substance P were found to contract small airways to a larger extent and at lower concentrations compared to large airways (Frossard and Barnes, 1991).

It has long been thought that only tachykinin NK_2 receptors are involved in contraction of isolated human airways (Advenier et al., 1992). However, in small-diameter bronchi ($\sim$1 mm in diameter), in addition to a robust tachykinin NK_2 receptor-mediated contraction, tachykinins also cause contraction via tachykinin NK_1 receptor stimulation. The tachykinin NK_1-mediated contraction of small bronchi appears to be mediated by prostanoids (Naline et al., 1996). Pretreatment with interleukin-1β potentiates the contraction of these small human bronchi to a specific tachykinin NK_1 receptor agonist (Barchasz et al., 1999). In an extensive study on medium-sized human isolated bronchi (2–5 mm in diameter), the specific tachykinin NK_1 receptor agonist [Sar^9,$Met(O_2)^{11}$]-substance P was found to induce contraction in about 60% of the preparations, an effect which was not mediated by prostanoids, but from a direct activation of smooth muscle receptors and release of inositol phosphate (Amadesi et al., 2001). So, it appears that in both small- and medium-sized human bronchi part of the contraction induced by tachykinins is mediated by tachykinin NK_1 receptors.

Passive sensitization has been reported to influence the in vitro contractile effect of substance P and neurokinin A on human bronchi (Ben-Jebria et al., 1993). Human bronchi incubated overnight with serum from asthmatic patients atopic to *Dermatophagoides pteronyssinus* showed an enhanced sensitivity and an enhanced maximal contractile response to substance P and neurokinin A, an effect that 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, both with regard to the potency of the agonist and the mechanism of induced contraction (Björck et al., 1992).

3.2. In vivo bronchoconstrictor effect of tachykinins in humans

The in vivo bronchoconstrictor effect of substance P and neurokinin A, administered by inhalation or intra-

venous infusion, has been reported by several groups. In these studies, neurokinin A was found to be a more potent bronchoconstrictor than substance P. Patients with asthma are hyperresponsive to substance P and neurokinin A (reviewed in Joos et al., 1994). The bronchial response to inhaled neurokinin A is reproducible (Joos et al., 1996). The bronchoconstrictor effect of inhaled substance P and neurokinin A in asthmatics can be prevented by pretreatment with sodium cromoglycate and nedocromil sodium (Crimi et al., 1988; Joos et al., 1989). This has led us to postulate that substance P and neurokinin A are indirect bronchoconstrictors in man. This indirect bronchoconstrictor effect could arise from an effect on inflammatory cells (e.g. mast cells) and/or nerves. In experimental animals, tachykinins are able to cause acetylcholine release from postganglionic cholinergic airway nerve endings (Joos et al., 1988b; Szarek et al., 1993; Tanaka and Grunstein, 1984) and to activate mast cells (Fewtrell et al., 1982; Joos et al., 1997). The exact mechanism of tachykinin-induced bronchoconstriction in man is however not yet known. In some patients, cholinergic mechanisms do play a role (Crimi et al., 1990; Joos et al., 1988a). Histamine however does not seem to be involved as pretreatment with different histamine H_1 receptor antagonists (astemizole and terfenadine) did not inhibit neurokinin A induced bronchoconstriction (Crimi et al., 1990, 1993). We recently were able to demonstrate that leukotrienes mediate part of the bronchoconstrictor effect of neurokinin A: the $CysLT_1$ receptor antagonist zafirlukast partially inhibited bronchoconstriction induced by inhaled neurokinin A in patients with asthma (Joos et al., 2001a).

3.3. Release of acetylcholine from airway cholinergic nerve endings by tachykinins

Substance P has a facilitatory role in the release of acetylcholine from postganglionic cholinergic nerves in guinea pig (Hall et al., 1989), rabbit (Colasurdo et al., 1995) and human airways (Black et al., 1990; Joos et al., 1988a). By the use of specific tachykinin receptor antagonists, it has been suggested that tachykinin NK_1 receptors may be involved in this facilitation (Watson et al., 1993). In a recent study, we evaluated the role of the tachykinin NK_1 receptor on cholinergic neurotransmission in mouse trachea in vitro. In comparison to wild type mice, electrical field stimulation induced a significant lower contraction of the trachea in tachykinin NK_1 receptor knockout mice. This study provides direct evidence for a role of the tachykinin NK_1 receptor in the augmentation of cholinergic neurotransmission in mouse trachea (De Swert et al., 2001a) (Fig. 1) and is in accordance with experiments in rabbits, where a significant release of acetylcholine was observed upon application of substance P, an effect that could be blocked by CP 96345 ((2*S*-*cis*)-2-(diphenylmethyl)-*N*-[(2-methoxyphenyl)methyl]-1-azabicyclo[2.2.2]octan-3-amine), a tachykinin NK_1 receptor antagonist (Colasurdo et al., 1995). In a recent study on guinea pig trachea, tachykinin NK_2 receptors were also found to be involved in the facilitation of acetylcholine release (D'Agostino et al., 2000).

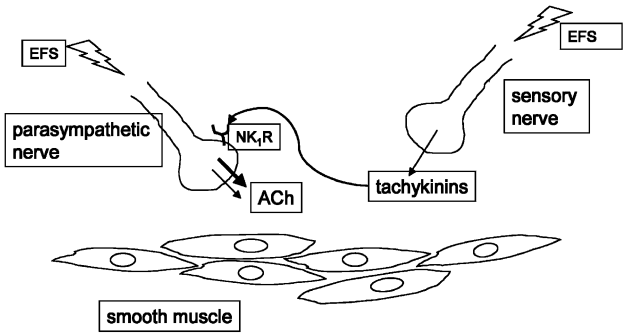

Fig. 1. Electrical field stimulation (EFS) of mouse trachea causes contraction mediated by cholinergic nerves. Concomitant release of tachykinins from sensory nerves enhances cholinergic contraction by interaction with tachykinin NK_1 receptors (NK_1R), causing a positive feedback on acetylcholine (ACh) release from cholinergic nerve endings (De Swert et al., 2001a).

3.4. Role of mast cell activation in the bronchoconstrictor effect of tachykinins

Substance P degranulates mast cells, leading to the release of histamine and 5-HT (Cross et al., 1996; Fewtrell et al., 1982; Heaney et al., 1995). Substance P induces tumor necrosis factor-α (TNF-α) production and secretion in a murine mast cell line and in murine peritoneal mast cells (Ansel et al., 1993). Picomolar concentrations of substance P trigger electrical responses in rat peritoneal mast cells without degranulation, resulting in priming of these cells (Janiszewski et al., 1994).

Both a receptor-independent and receptor-dependent mechanism has been reported for mast cell activation induced by substance P. Higher (micromolar) concentrations of substance P cause a direct activation of G-proteins. It has been proposed that the amino-terminus of substance P is responsible for this receptor-independent mechanism of mast cell activation (Mousli et al., 1992). However, lower (nanomolar) concentrations of substance P cause mast cell activation by interaction with tachykinin receptors. The histamine release from the murine MC/9 mast cell line is mediated by tachykinin NK_2 receptors (Krumins and Broomfield, 1993), while the mast cell degranulating of substance P in Fisher 344 rat airways involves activation of tachykinin NK_1 receptors (Germonpré et al., 1995; Joos and Pauwels, 1993; Joos et al., 1997; Okada et al., 1999). In Fisher 344 rats, mast cell activation mediated by stimulation of tachykinin NK_1 receptors and involving release of the mast cell mediator serotonin (5-HT) explains the hyperresponsiveness of the airways to the sensory

neuropeptides substance P and neurokinin A (Germonpré et al., 1998).

4. Plasma extravasation

Ablation of sensory nerves by capsaicin pretreatment abolishes airways plasma extravasation induced by a variety of mediators and stimuli, including histamine, serotonin and cigarette smoke (Lundberg and Saria, 1983; Saria et al., 1983). Tachykinin NK_1 receptors that mediate neurogenic plasma extravasation have been visualized on postcapillary endothelial cells (Bowden et al., 1994). Once plasma extravasation occurs, leukocytes initiate a process that results in slowing down their velocity, rolling on and adhering to the venular endothelium. Tachykinin NK_1 receptor antagonists reduce all these phenomena caused by different stimuli, including cigarette smoke (Baluk et al., 1996), hypertonic saline and cold air (Pedersen et al., 1998; Piedimonte et al., 1993; Yoshihara et al., 1995). It is not known how much of this effect is due to a tachykinin NK_1 receptor-mediated effect on leukocytes/endothelial cells or merely the consequence of inhibition of tachykinin NK_1 receptor-mediated plasma extravasation.

The involvement of tachykinin NK_1 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 tachykinin NK_1 receptors are also implicated in the extravasation caused by hypertonic saline, bradykinin, antigen challenge or acetylcholine. Tachykinin NK_2 receptors mediate part of the neurogenic plasma extravasation in the secondary bronchi and intraparenchymal airways of the guinea pig (Bertrand et al., 1993b; Delay-Goyet et al., 1992; Qian et al., 1993; Abelli et al., 1991; Eglezos et al., 1991; Hirayama et al., 1993; Tousignant et al., 1993).

Using antibodies directed against the tachykinin NK_1 receptor protein, Bowden et al. have demonstrated that in the rat trachea, tachykinin NK_1 receptors are present on the endothelial cells of the postcapillary venules. These receptors are internalized in endosomes upon binding with substance P. They also observed an increase in the number of tachykinin NK_1 receptor immunoreactive endosomes when the vagus nerve is electrically stimulated, indicating that substance P released by activation of the sensory nerves has a direct effect on the tachykinin NK_1 receptors on postcapillary venular endothelium (Bowden et al., 1994).

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 (Delaunois et al., 1993a,b). Neurogenic plasma protein extravasation in rat airways involves the activation of tachykinin NK_1 receptors. In Fisher 344 but not in BDE rats, an additional indirect mechanism, involving mast cell activation, 5-HT release and stimulation of $5\text{-}HT_2$ receptors, participates in this process (Germonpré et al., 1995, 1997) (Fig. 2).

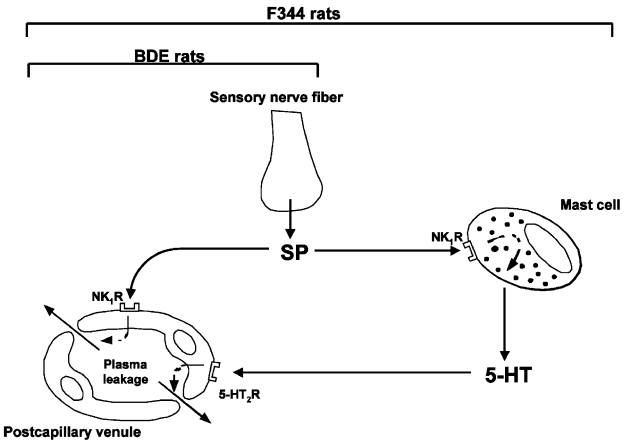

Fig. 2. Schematic representation of the cellular mechanisms and receptors involved in the neurogenic inflammation in the trachea of BDE and Fisher 344 rats (Germonpré et al., 1995, 1997). NK_1R = tachykinin NK_1 receptor; $5\text{-}HT_2R$ = serotonin $5\text{-}HT_2$ receptor; SP = substance P.

5. Mucus secretion

Neurogenic mucus secretion results from the contribution of different components and shows marked variation among species. Adrenergic and cholinergic agonists may stimulate mucus secretion in the ferret and human airways. Tachykinins cause also marked mucus secretion in the ferret, an effect exclusively mediated by tachykinin NK_1 receptors. Endogenous tachykinins mediate mucus secretion induced by electrical stimuli and part of the response produced by exposure to antigen via tachykinin NK_1 receptor activation (Khan et al., 2001).

6. Plasticity of tachykinergic airway innervation and role of neurotrophins

Allergic airway inflammation may increase the amount of substance P and neurokinin A in the airways by increasing the number of nerves, and/or by increasing the non-neuronal sources of these tachykinins. In a guinea pig model, a three to fourfold increase of substance P, neurokinin A and calcitonin gene-related peptide measured in lung tissue was seen 24 h following antigen challenge. Moreover, increased levels of preprotachykinin I mRNA were found in the nodose ganglia of these animals (Fischer et al., 1996).

One of the factors known to increase the expression of substance P is nerve growth factor. Nerve growth factor

belongs to the family of neurotrophins. Neurotrophins control the survival, differentiation and maintenance of neurons in the peripheral and central nervous system. Under physiological conditions, neurotrophins are produced by nerve-associated cells like glia cells or Schwann cells and by nerve cells themselves, while during inflammation, neurotrophins can also be produced by fibroblasts, mast cells, macrophages, and T and B cells (for review see Bonini et al., 1999; Braun et al., 2000). In a study performed by Virchow et al. (1998), a significant increase in the neurotrophins nerve growth factor, brain-derived neurotrophic factor, and neurotrophin-3 was observed in bronchoalveolar lavage fluid, obtained 18 h after segmental allergen provocation in patients with asthma. Mice with nerve growth factor overexpression from a lung-specific promoter were shown to have an increased number of tachykinin-containing sensory nerve fibres in the airways (Hoyle et al., 1998). When nerve growth factor is injected in the tracheal wall of guinea pigs, the substance P expression in airway neurons is enhanced. Moreover, a phenotypic switch in the nature of vagal sensory neurons producing this neuropeptide was observed: large, capsaicin-insensitive nodose neurons become substance P positive (Hunter et al., 2000).

Another factor thought to be involved in neuronal plasticity is leukemia inhibitory factor. Leukemia inhibitory factor is a member of the interleukin-6 cytokine family, which is released from a variety of lung cells, including fibroblasts, bronchial smooth muscle cells and epithelial cells. Leukemia inhibitory factor can be upregulated and released by interleukin-1β, interleukin-6 and anti-IgE (Knight et al., 1999). Leukemia inhibitory factor has been shown to increase the expression of tachykinin receptor mRNA as well as the synthesis and release of tachykinins. In a guinea pig tracheal explant model, leukemia inhibitory factor augmented the contractile responses to both endogenous and exogenous tachykinins. Receptors for leukemia inhibitory factor have been localized by immunohistochemistry to both cholinergic and sensory nerves (Knight et al., 2000).

7. Involvement of tachykinins in animal models of nonspecific bronchial hyperresponsiveness

In animal models, tachykinins and their receptors have been involved in airway responses to nonspecific stimuli. Both the tachykinin NK_1 and NK_2 receptors have been involved in airway contraction induced by cold-air (Yang et al., 1997; Yoshihara et al., 1996), hyperventilation and cigarette smoke (Wu and Lee, 1999), in plasma extravasation induced by hypertonic saline (Pedersen et al., 1998; Piedimonte et al., 1993), and in airway hyperresponsiveness induced by viruses (Jacoby et al., 2000; Piedimonte et al., 1999), interleukin-5 and nerve growth factor (De Vries et al., 1999; Kraneveld et al., 1997). In addition, the tachykinin NK_3 receptor was found to be involved in citric acid-induced cough and enhanced bronchial responsiveness (Daoui et al., 1998, 2000).

8. Involvement of tachykinins in antigen-induced airway changes

Tachykinins have been found to be involved in antigen-induced bronchoconstriction, airway inflammation and enhanced bronchial responsiveness in various animal models. A combination of a tachykinin NK_1 receptor antagonist, CP-96,345 (Snider et al., 1991), and a tachykinin NK_2 receptor antagonist, SR 48968 ((*S*)-*N*-methyl-*N*-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl-benzamide) (Emonds-Alt et al., 1992), inhibited bronchoconstriction produced by ovalbumin challenge in sensitized guinea pigs (Bertrand et al., 1993c). The tachykinin NK_1 receptor antagonist, CP-96,345, was also able to limit antigen-induced plasma extravasation (Bertrand et al., 1993a). By using tachykinin NK_1/NK_2 receptor blockade, the involvement of endogenous tachykinins in antigen-induced bronchial hyperresponsiveness was demonstrated (Kudlacz et al., 1996).

The relative contribution of the tachykinin NK_1 and NK_2 receptors in antigen-induced airway changes has now been studied in guinea pigs and rats. In conscious, unrestrained guinea pigs, the tachykinin NK_1 receptor is involved in both the development of antigen-induced airway hyperresponsiveness to histamine and the antigen-induced infiltration of eosinophils, neutrophils and lymphocytes (Schuiling et al., 1999b). On the other hand, the tachykinin NK_2 receptor is involved in the development of the antigen-induced late reaction (Schuiling et al., 1999a). The involvement of both tachykinin receptors in allergic airway inflammation has also been reported in the Brown Norway rat model (Maghni et al., 2000). Expression of tachykinin NK_1 and NK_2 receptors was demonstrated in lung tissue of both naïve and ovalbumin-sensitized Brown Norway rats. Substance P was found to increase 2.4-fold in bronchoalveolar lavage after challenge with ovalbumin. The tachykinin NK_1 receptor antagonist CP99,994 (((1)-(2*S*, 3*S*)-3-methoxybenzyl amino)-2-phenylpiperidine) and the tachykinin NK_2 receptor antagonist SR 48968 were not able to reduce the early airway response to ovalbumin, but both antagonists reduced the ovalbumin-induced late airway responses. An interesting finding in this study was that the tachykinin NK_2 receptor antagonist decreased the number of eosinophils in bronchoalveolar lavage fluid and decreased the expression of both Th1 (interferon-γ) and Th2 (interleukin-4 and interleukin-5) cytokines in bronchoalveolar lavage cells. So, from animal studies, it seems that both the tachykinin NK_1 and NK_2 receptors are involved in antigen-induced airway effects. In addition, it is possible that tachykinin NK_3 receptors are also involved in this

process: aerosol administration of the tachykinin NK_3 receptor antagonist SR142801 ((*R*)-(*N*)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propyl)-4-phenylpiperidin-4-yl-*N*-methylacetamide)) caused a significant reduction in neutrophil and eosinophil influx in the airways of ovalbumin sensitized and challenged mice (Nénan et al., 2001).

The majority of airway sensory innervation originates from afferent neurons whose somata reside in vagal (nodose and jugular) ganglia. After antigenic activation of nodose ganglion mast cells in vitro, many nodose neurons reveal depolarizing responses to substance P that are reversibly abolished by a tachykinin NK_2 receptor antagonist. The tachykinin NK_2 receptor expression can occur within 5 min of mast cell activation by the antigen and can last for 3.5 days. This phenomenon has been designated as "unmasking" (Weinreich et al., 1997). The mast cell mediator serotonin (5-HT) acting through 5-HT_3 receptors is able to induce similar effects (Moore et al., 1999). Unmasking of the tachykinin NK_2 receptor-mediated responses in vagal afferents has now also been described in vivo. Unmasking of tachykinin NK_2 receptor-mediated responses in nodose neurons was observed 24 h after antigen inhalation by guinea pigs. The antigen-induced unmasking of tachykinin responses was significantly attenuated by vagotomy, indicating that the nerve fibres connecting the airways and the vagal somata are involved in the transduction of a signal essential for unmasking tachykinin responses (Moore et al., 2000).

In addition to the ovalbumin model that mimics the anaphylactic response, tachykinin receptor antagonists were studied in other pathophysiologically relevant models of asthma. Toluene diisocyanate is known to cause occupational asthma. Many of the proinflammatory actions of toluene diisocyanate are mediated by stimulation of sensory nerves (Mapp et al., 1992). Toluene diisocyanate-induced bronchoconstriction and induction of hyperresponsiveness in mice are in part mediated by tachykinins released from sensory nerves (Scheerens et al., 1996).

9. Tachykinin NK_1 receptor knockout mice in the study of lung and airway inflammation

Bozic et al. reported that gene-targeted disruption of the tachykinin NK_1 receptor protected the lung from immune

Table 1
Role of tachykinin NK_1 receptors in inflammation: findings from NK_1 receptor (NK_1R) knockout mice

Experimental model	Main findings	Reference
• Immune complex-mediated lung injury	• Reduced neutrophil accumulation in tachykinin NK_1 receptor knockout mice	Bozic et al., 1996
• Air-pouch model	• Reduced neutrophil extravasation in response to interleukin-1β in tachykinin NK_1 receptor knockout mice	Ahluwalia et al., 1998
• Skin edema formation in dorsal skin	• Edema formation induced by tachykinins absent in tachykinin NK_1 receptor knockout mice • Compound 48/80 induced greater edema in the tachykinin NK_1 receptor knockout mice (compensatory mechanism?)	Cao et al., 1999
• Neutrophil accumulation and edema formation in skin	• Added substance P, or tachykinin NK_1 agonist septide, potentiated interleukin-1β induced neutrophil accumulation in wild type, but not in tachykinin NK_1 receptor knockout mice	Cao et al., 2000
• Antigen-induced cystitis	• Significant attenuation of congestion and edema of the mucosa, as well as reduced infiltration with polymorphonuclear cells, in response to antigen challenge in tachykinin NK_1 receptor knockout mice • Fourfold increased number of mast cells in subepithelial layers, within smooth muscle and around blood vessels in tachykinin NK_1 receptor knockout mice	Saban et al., 2000
• Clostridium difficile-induced enteritis	• Tachykinin NK_1 receptor knockout mice are protected from the secretory and inflammatory changes, as well as from the epithelial cell damage induced by toxin A	Castagliuolo et al., 1998
• Caerulein-induced pancreatitis	• The magnitude of hyperamylasemia, hyperlipasemia, neutrophil sequestration in the pancreas, and pancreatic-acinar cell necrosis, was reduced in tachykinin NK_1R knockout mice • Pancreatitis-associated lung injury (sequestration of neutrophils and increased pulmonary microvascular permeability) was reduced in tachykinin NK_1 receptor knockout mice	Bhatia et al., 1998; Grady et al., 2000

complex injury. The lung tissues of immune complex-challenged tachykinin NK_1 receptor knockout mice, appeared to be no different from those of control animals (Bozic et al., 1996). In Table 1, an overview is given on the use of tachykinin NK_1 receptor knockout mice in the study of inflammation in various organs. We recently evaluated the role of the tachykinin NK_1 receptor in antigen-induced airway inflammation by using these tachykinin NK_1 receptor knockout mice (De Swert et al., 2001b). Despite all available evidence for the role of this receptor in airway inflammation, we were not able to point out any proinflammatory action of the tachykinin NK_1 receptor in our mouse model. Indeed, analysis of different inflammatory parameters (antigen-specific serum IgE, evaluation of cells in bronchoalveolar lavage fluid) revealed no differences between the tachykinin NK_1 receptor knockout and the wild type animals.

10. Role of tachykinins in virus-induced airway changes

Various neural mechanisms are involved in virus-induced airway changes (Folkerts et al., 1998). Epithelial damage can expose sensory nerves that may be more easily stimulated by inhaled particles and inflammatory mediators. Epithelial damage may also enhance the effect of tachykinins by a reduction in the activity of airway neutral endopeptidase. The muscarinic M_2 receptors on the vagus nerves in the lung, which normally inhibit acetylcholine release, are no longer functional in guinea pigs infected with parainfluenza virus. This virus-induced dysfunction of the muscarinic M_2 receptor can be prevented by administration of a tachykinin NK_1 receptor antagonist (Jacoby et al., 2000). Infection with respiratory syncytial virus in Fischer 344 rats results in increased inflammatory responses to substance P and an upregulation of tachykinin NK_1 receptor mRNA (Piedimonte et al., 1999).

11. Role of tachykinins in cough

The reflex arch that mediates the cough response is made up by a sensory and efferent arm that synapses in brainstem areas, including the nucleus tractus solitarius. Mediators and mechanisms that modulate the cough reflex in the sensory arm of the reflex pathways are not completely understood, although they seem to offer interesting novel therapeutic opportunities. Glutamate is likely the main excitatory mediator released in the first synapse in the nucleus tractus solitarius (De Biasi and Rustioni, 1998). However, there is pharmacological evidence that substance P may also contribute (Mutoh et al., 2000). The role of tachykinins in the regulation of the cough response appears to be complex since antagonists for the three tachykinin receptors (NK_1, NK_2 and NK_3) have antitussive activity in preclinical models (Daoui et al., 1998; Girard et al., 1995; Moreaux et al., 2000; Ujiie et al., 1993). In guinea pigs, substance P locally applied into the airways has been shown to produce (Kohrogi et al., 1988) or potentiate cough produced by other stimuli (Moreaux et al., 2000). These findings support a role for substance P at a peripheral site of action, probably promoting a local, perineural inflammation. In support of this hypothesis, both peripheral and central sites of action has been reported in guinea pigs for the antitussive effect of tachykinin NK_1 and NK_2 receptor antagonists (Bolser et al., 1997). Species variations may be of importance, as tachykinins appear to act at a site located in the central nervous system in the cat (Bolser et al., 1997).

12. Conclusions

Several lines of evidence indicate a role for the tachykinins in airways diseases. Elevated levels of tachykinins have been recovered from the airways of patients with asthma and chronic obstructive pulmonary disease. Airway inflammation leads to an upregulation of the tachykinin NK_1 and NK_2 receptors. Preclinical studies have indicated a role for the tachykinin NK_1 and NK_2 receptors in bronchoconstriction, airway hyperresponsiveness and airway inflammation caused by allergic and nonallergic stimuli. The possible role of the tachykinin NK_3 receptor in cholinergic neurotransmission and allergic airway inflammation has recently been reported. As most of the effects of the tachykinins in the airways are mediated by more than one tachykinin receptor type, blocking either the tachykinin NK_1 or the NK_2 receptor is probably insufficient (Joos and Pauwels, 2001). Recently, dual tachykinin NK_1/NK_2 receptor antagonists have been developed that deserve clinical evaluation in patients with asthma or chronic obstructive pulmonary disease (Joos et al., 2001b).

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