

Neural mechanisms in asthma

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Summary

Advances in the understanding of neural mechanisms in asthma may provide novel therapeutic approaches in the treatment of asthma. Excessive activity of cholinergic nerves may be important in asthma. Dysfunction of M₂ muscarinic receptors in asthma may lead to excessive bronchoconstriction and mucus secretion and can be induced in animal models by a range of stimuli including allergen, viral infection, ozone, eosinophil products and cytokines. Cholinergic mechanisms may be especially important in certain types of patients and anticholinergic agents provide protection against bronchospasm due to psychogenic factors or beta₂-blockers. Non-adrenergic non-cholinergic (NANC) mechanisms, both inhibitory (i-NANC) and excitatory (e-NANC), may play a significant role in the pathophysiology of asthma. The putative neurotransmitters, vasoactive intestinal polypeptide (VIP) and nitric oxide (NO), mediate neural bronchodilation in human airways. There does not appear to be a defect in the i-NANC system in moderate or severe asthma. e-NANC is mediated by the sensory neuropeptides substance P (SP) and the more potent bronchoconstrictor neurokinin A (NKA). Various studies suggest that the SP content of human airways is increased in asthma. Tachykinins are not only present in sensory nerves, but also are produced by inflammatory cells such as alveolar macrophages, dendritic cells, eosinophils, lymphocytes and neutrophils. They can be released into the airways by stimuli such as allergen and ozone. Evidence suggests that in addition to smooth muscle contraction, which is mediated mainly by NK₂ receptors, tachykinins also cause mucus secretion, plasma extravasation and stimulate inflammatory and immune cells. These effects are mediated by NK₁ receptors. Recent studies have shown that NK₂ receptor antagonists such as saredutant partially inhibit NKA-induced bronchoconstriction in asthmatics. Thus, tachykinin receptor antagonists have potential as therapies for asthma.

Keywords: asthma, neural mechanisms, cholinergic, NANC, tachykinins, nitric oxide, substance P, neurokinin receptor antagonists

Introduction

Neural mechanisms are involved in asthma and advances in the understanding of these mechanisms may provide novel approaches to the treatment of this disease.

Cholinergic mechanisms

Cholinergic mechanisms are the predominant bronchoconstrictor neural pathway in human airways. There is

increased airway tone and vagal nerve activity in patients with asthma compared with healthy individuals. Studies with anticholinergic agents have taught us that cholinergic mechanisms are very important in beta-blocker-induced bronchospasm and in the bronchoconstriction induced by emotions and suggestions. Other stimuli such as SO₂, bradykinin, irritant gases and ozone induce some vagal reflexes, but the importance of the cholinergic system in allergen- and exercise-induced asthma is unclear.

There has been much investigation, mainly in animal models, examining the importance of the three different types of muscarinic receptor (M₁, M₂, M₃). Dysfunction of the M₂ receptor has been proposed as a mechanism leading to excessive bronchoconstriction and mucus secretion in

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asthma [1]. Dysfunction of the M2 receptor in guinea pigs can be induced by exposure to various stimuli such as allergen, viral infection, ozone, eosinophil products such as major basic protein (MBP) and eosinophil peroxidase (EPO), and other factors such as transforming growth factor (TGF) β 1, tumour necrosis factor (TNF) α and interleukin (IL)-1 β .

Non-adrenergic, non-cholinergic (NANC) mechanisms

Another important system, which is the only neural bronchodilator mechanism in human airways, is the inhibitory NANC (i-NANC) system. Over the past 20 years, several putative neurotransmitters of this system have been proposed including purines, vasoactive intestinal polypeptide (VIP) and nitric oxide (NO). *In vivo* and *in vitro* studies have shown that there is no defect of the i-NANC system either in mild or severe asthma [2–4]. Some studies have suggested that the amount of VIP is reduced in more severe asthma, but others have not reproduced this [5]. Lucchini *et al.* [7] have shown that there are more VIP immunoreactive nerves around the submucosal glands in chronic bronchitis, but again it is a rather limited effect.

Neurogenic inflammation in the airways

Figure 1 presents the scheme of neurogenic inflammation in the airways. The sensory nerves innervate the airways and can release substance P and related tachykinins including neurokinin A (NKA) by local axon reflexes. These tachykinins can then induce various changes in the airways.

Various studies have suggested that the substance P content of human airways is increased in asthma (Table 1). The amount of substance P in the trachea of asthmatics that have died of their disease is less than in normal subjects. In an interesting study of the amount of substance P recovered from sputum, both asthmatics and patients with chronic bronchitis had more substance P in their sputum than normal subjects [8]. Similarly, bronchoalveolar lavage (BAL) studies have shown that there is more substance P in asthmatics compared with normal individuals [9]. The amount of substance P in BAL fluid can be increased further by instilling allergen into the airways or by inhalation of ozone [9,10]. However, when human and guinea pig airways are compared, it is evident that there are few substance P containing nerves in the lower human airways. Recent studies in rodents have suggested that substance P is not only produced by nerves but also by inflammatory

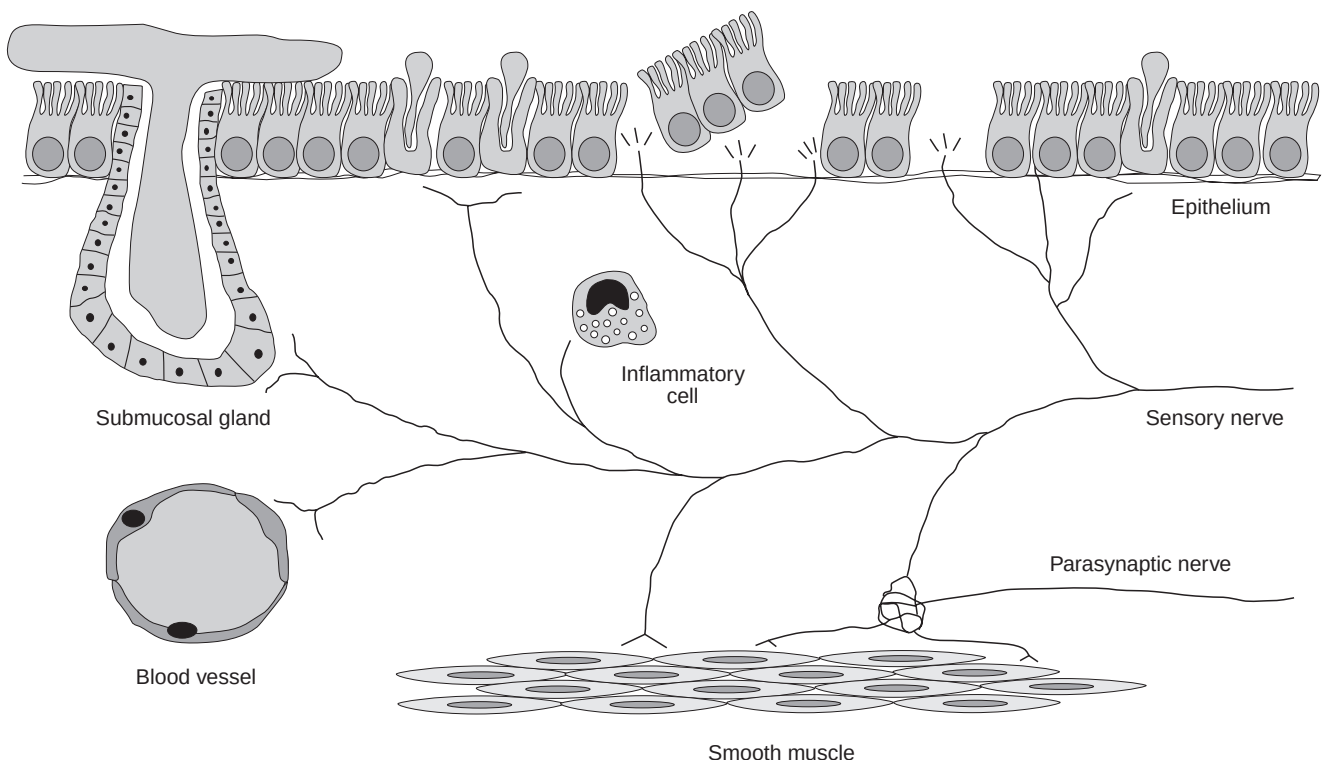


Fig. 1. Neurogenic inflammation in the airways.

	Trachea (pmol/g)	Sputum (fmol/mL)	BAL-fluid (fmol/mL)	BAL-fluid post Ag challenge
Normals	13 ± 5	1.1 ± 0.4	30 ± 7	37 ± 15
Asthmatics	3 ± 3	17.7 ± 2.4	185 ± 22	260 ± 33

Table 1. Substance P content in human airways [6–8]

References: Lilly *et al.* 1995, Tomaki *et al.* 1995, Nieber *et al.* 1992.

cells. Therefore, we probably have to reconsider the localization of tachykinins in the airways. They are present in sensory nerves, but there is a lot of data showing that tachykinins are produced by alveolar macrophages, dendritic cells, eosinophils, lymphocytes and neutrophils. For example, Killingsworth *et al.* [11] found that the expression of preprotachykinin (PPT) mRNA in rat alveolar macrophages was upregulated by administration of the pro-inflammatory stimulus lipopolysaccharide (LPS). This corresponded with the

demonstration of substance P-like immunoreactivity in those cells. Ho *et al.* [12] demonstrated that human peripheral blood monocytes and macrophages contain both the PPT-I mRNA and substance P itself. Additionally, substance P was released from these inflammatory cells by capsaicin, which is generally considered to be a neurotoxin releasing substance P from the sensory nerves. Moreover, mRNA for neurokinin-1 receptor was present in these human peripheral blood monocytes and macrophages.

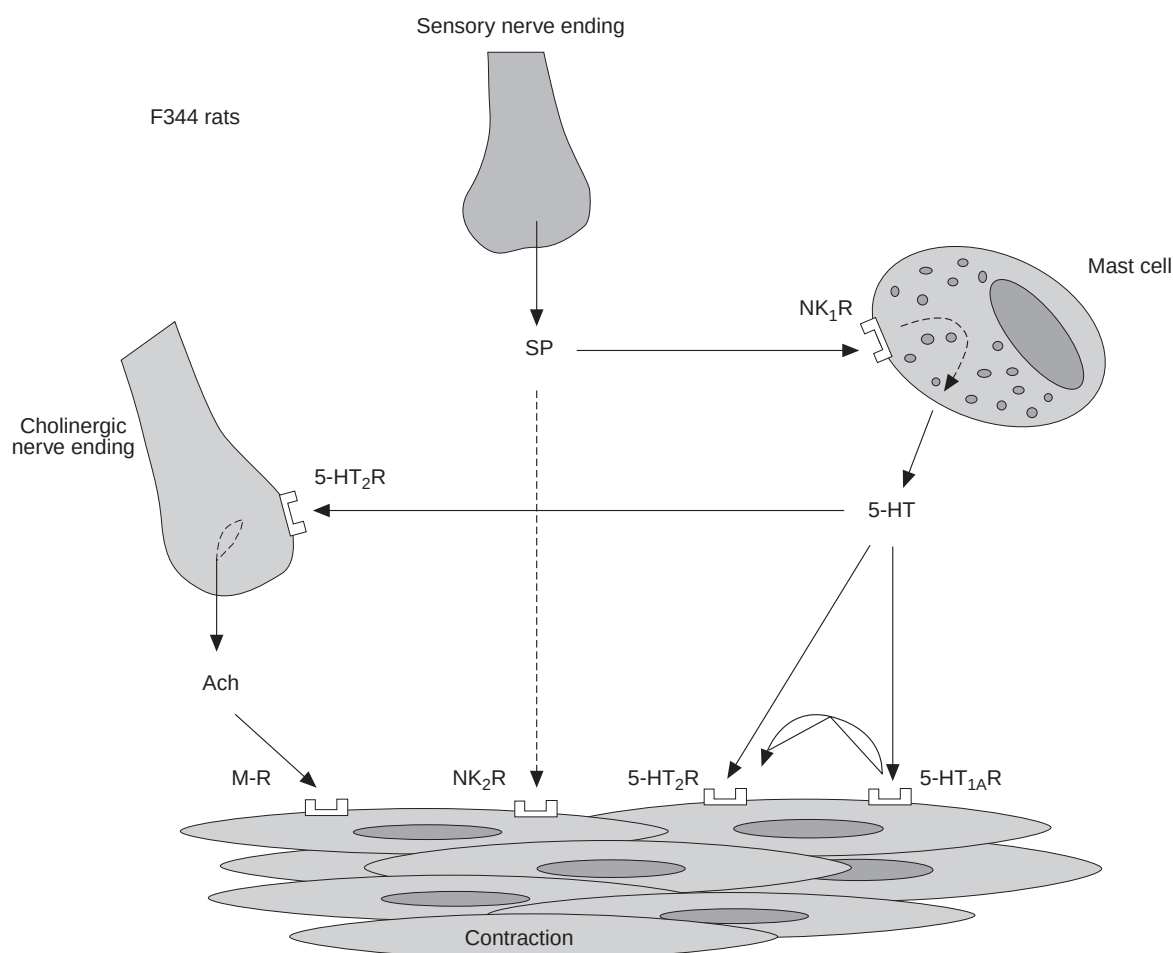


Fig. 2. Mechanisms of substance P-induced airway contraction in F344 rats. Reproduced with permission of the British Pharmacological Society from ref [15].

Using an antibody against substance P, Germonpré *et al.* [13] showed that human sputum macrophages contain substance P. In addition, NK1 receptor mRNA was present. Other studies have demonstrated the presence of tachykinins or PPT in rat and mouse dendritic cells [14]. In mice, there was an important interaction between dendritic cells and the stimulation of lymphocytes. Tachykinins play a role in the mixed leukocyte reaction because it can be reduced by adding a NK1 antagonist. Thus, tachykinins are present in neurons and inflammatory cells in the airways, and can be released into the airways by stimuli such as ozone and allergen.

Effects of tachykinins relevant to asthma

Tachykinins have a range of effects that are relevant to asthma. They not only cause smooth muscle contraction, which is mediated mainly by the NK2 receptor, but also several pro-inflammatory effects that are mediated by the NK1 receptor. These are: plasma extravasation, mucus secretion, potentiation of acetylcholine release, inflammatory cell chemotaxis, activation of inflammatory cells and smooth muscle proliferation.

We have performed animal studies looking at the

mechanisms involved in the bronchoconstriction to substance P. *In vitro* studies using trachea from the Fisher 344 rat, which is hyperresponsive to substance P, have demonstrated that the bronchoconstrictor effect of substance P is not due to a direct effect on smooth muscle but is mainly due to an indirect effect through stimulation of mast cells, cholinergic nerves and release of 5-hydroxytryptamine (5-HT) [Fig. 2] [15]. There is evidence for the presence of NK1 receptors on these mast cells.

Substance P has many pro-inflammatory effects (Fig. 3). It not only degranulates mast cells and releases TNF from them, it can activate macrophages, monocytes, lymphocytes, neutrophils and eosinophils. In addition, substance P plays a role in airway remodelling as it can induce proliferation of smooth muscle *in vitro* and can produce proliferation of fibroblasts.

The effects of tachykinins therefore are not limited to airway smooth muscle contraction. At least in animal models, tachykinins are a major component of neurogenic inflammation (vasodilatation, plasma exudation) as well as airway hypersecretion, regulation of neural transmission, and attraction and activation of inflammatory cells. Many of these effects are mediated through the NK1 receptor.

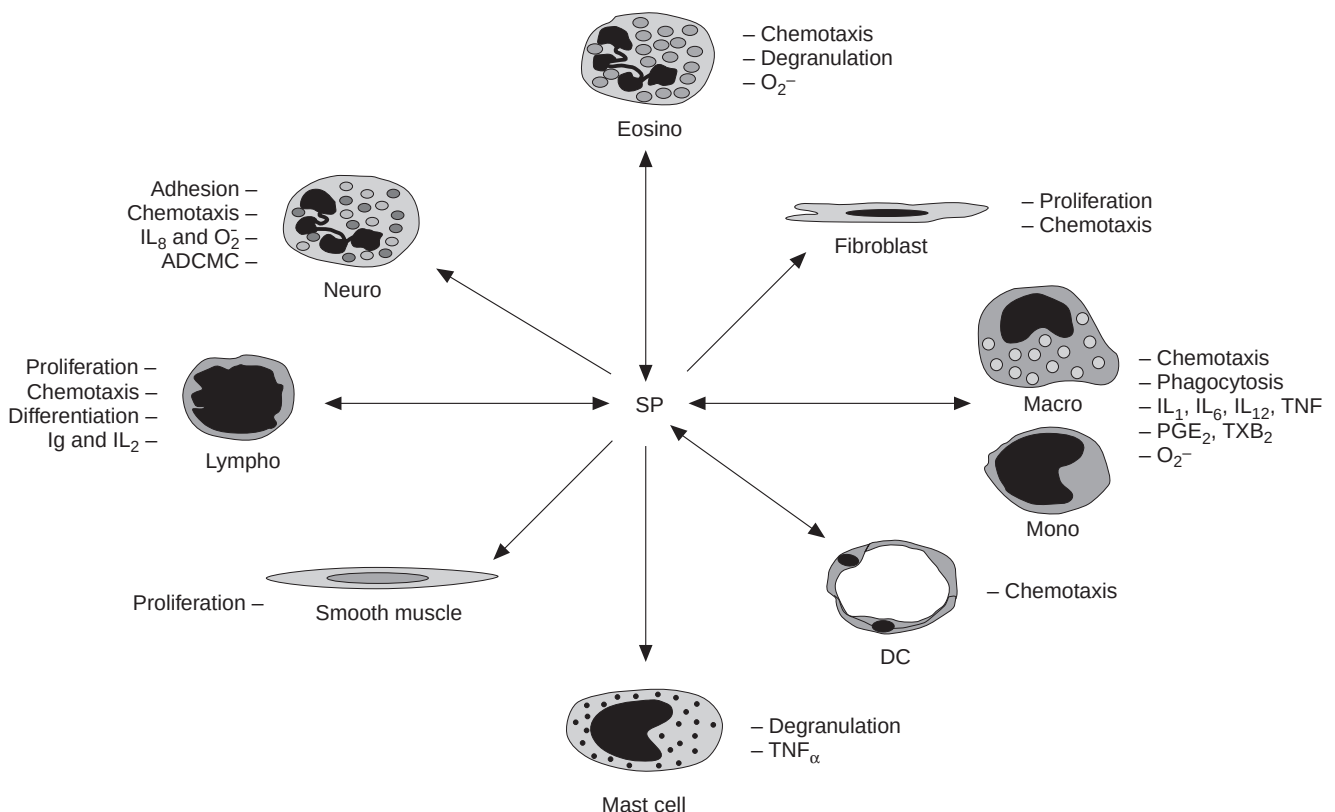


Fig. 3. Immunomodulation by substance P.

NK1 selective	NK2 selective	NK3 selective	Dual NK1 + NK2
CP 96,345	MEN 10,378	SR 142801 (osanetant)	FK224
RP 67,580	SR 48988 (saregutant)		S.16474
FK 888	MEN 10,627		MDL 105.212A
SR 140333	MEN 11420 (nepadutant)		
LY 303870	GR 159897		
GR 203040			

Table 2. Tachykinin receptor antagonists [16]

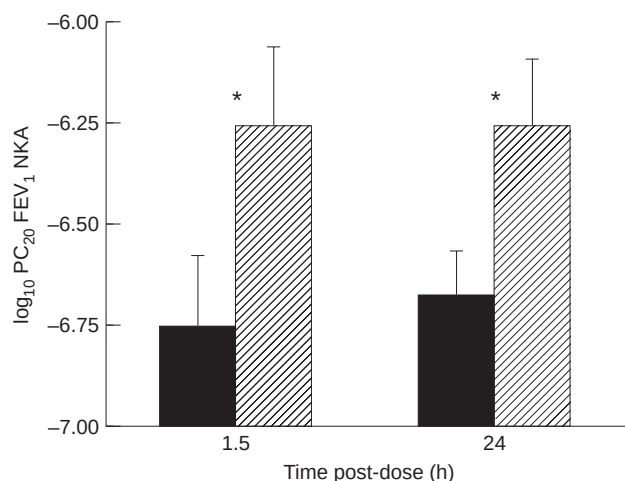


Fig. 4. Shift of the bronchoconstrictor dose-response curve to neurokinin A by SR48968 (■ placebo; ▨ SR48968) (reproduced with permission from [22]).

Tachykinin receptor antagonists

There is increasing interest in the development of tachykinin receptor antagonists as a possible new therapeutic approach to asthma. Selective antagonists for NK1, NK2, NK3 receptors have been developed, as have dual NK1 and NK2 antagonists (Table 2) [16]. To date, few of these compounds have been studied in human asthma. Two studies have examined the effects of the combined NK1 and NK2 antagonist FK-224, which has a rather low potency. FK-224 protected against bradykinin-induced bronchoconstriction in patients with asthma [17]. However, when used at the same dose, FK-224 had no effect against NKA-induced bronchoconstriction in asthmatics [18]. In addition, a 4-week study performed in Sweden found that FK-224 was ineffective in clinical asthma [19].

Few NK1 antagonists have been tested in asthma. Fahy *et al.* [20] found that CP-99 994 did not protect against hypertonic saline-induced bronchoconstriction. However,

another compound, FK-888, was able to shorten the recovery time in exercise-induced bronchoconstriction, but did not attenuate the maximal fall in sGaw [21]. It is likely that other more potent and longer-acting drugs will be developed. In the last year, NK1 antagonists have been found to have antiemetic efficacy in chemotherapy-induced emesis.

The first clinical study of an NK2 tachykinin receptor antagonist in asthmatics was reported recently by Van Schoor *et al.* [22]. They found that 1.5 and 24 h after administering SR 48968 (saregutant), there was a small but significant shift in the bronchoconstrictor dose-response curve to NKA (Fig. 4).

Conclusions

There remains a lot of work to do in this field. Several models must be tested before any conclusions can be made about the role of tachykinin antagonists in the treatment of asthma. Firstly, such antagonists have to block the airway effects of NKA or substance P. Secondly, tachykinin antagonists must be tested in various clinical models such as exercise-induced bronchoconstriction, hypertonic saline-induced bronchoconstriction, cold air challenge and allergen-induced bronchoconstriction. Finally, such compounds must demonstrate effects in clinical asthma.

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