The role of tachykinin receptor antagonists in the prevention of bronchial hyperresponsiveness, airway inflammation and cough

C. Advenier*, V. Lagente**, E. Boichot**

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ABSTRACT: Several recent observations suggest that tachykinins, such as substance P and neurokinin A, might be involved in the pathogenesis of bronchopulmonary alterations. Progress in investigations on the physiological and pathological roles of tachykinins has been greatly facilitated by the availability of a number of highly selective nonpeptide antagonists for tachykinin neurokinin 1, 2 and 3 (NK1, NK2, and NK3) receptors.

The use of selective tachykinin NK2 receptor antagonists suggests that tachykinin NK2 receptor stimulation plays an important role in the development of airway hyperresponsiveness in the guinea-pig. Others studies have also indicated that tachykinin NK1-receptors are involved in immediate or delayed neurogenic inflammation including microvascular leakage and the subsequent increase in plasma protein extravasation. A role for the sensory neuropeptide system has also been proposed in cough, as shown by the observation that the antitussive effect of tachykinin NK2 receptor antagonists has clearly been demonstrated in several experimental conditions, but the effect of tachykinin NK1 receptor antagonists is still debated.

Taken together, the results obtained with the various selective receptor antagonists provide pharmacological evidence that tachykinins play a role in delayed bronchopulmonary alterations and suggest that tachykinin receptor antagonists may be useful for investigating mechanisms and possibly reducing airway functional alterations in asthmatic patients.

The excitatory nonadrenergic noncholinergic (NANC) system, involving various neuropeptides of the tachykinin family, such as substance P (SP), neurokinin A (NKA), neurokinin B (NKB) and calcitonin gene-related peptide (CGRP), as transmitters, has now been well characterized. In airways, SP, NKA and CGRP are co-localized in the sensory unmyelinated C-fibres, which innervate all compartments of the airway wall from the trachea down to the bronchioles. C-fibre endings are found within the epithelium. They form a dense plexus in the subepithelial lamina propria, supply the glands, ramify within the smooth muscle layer and make direct contacts with postganglionic parasympathetic neurons, located in the local ganglion. In the trachea, this sensory innervation is almost exclusively derived from sensory vagal neurons, supplied by the jugular ganglion, whilst that of the lung is of mixed origin with a predominating vagal and a smaller spinal contribution [1–5]. The NANC system can be activated by different stimuli, which affect the chemosensitive C-fibre afferents in airways and lead to a local release of tachykinins that are responsible for several biological effects in the bronchopulmonary system: bronchospasm; increase in vascular permeability from postcapillary venules; stimulation of glandular secretion; facilitation of cholinergic neurotransmission; and recruitment and activation of some types of inflammatory cells. Sensory nerves also mediate respiratory defence reflexes, such as coughing, sneezing and secretion of mucus (fig. 1).

From these data, it has been hypothesized that abnormal stimulation of the sensory nerve terminals, e.g. induced by epithelial shedding as seen in asthma, results in enhanced release of tachykinins in the airway wall with subsequent exaggeration of inflammation. This concept of "neurogenic inflammation" introduces sensory nerve fibres as important components in the pathogenesis of asthma.

The biological actions of tachykinins are mediated via three types of receptors, denoted neurokinins 1–3 (NK1, NK2 and NK3), which have the highest affinity for SP, NKA and NKB, respectively. This receptor classification has been established from receptor-binding and functional studies. It has now been recognized that the expression of tachykinin NK1 receptors is confined mainly to the central and peripheral nervous system, whilst tachykinin NK2 and tachykinin NK3 receptors are expressed both in the central and peripheral nervous system and in target organs, including airways [5–10]. According
to the present state of our knowledge, SP and NKA seem to play an important role in the respiratory system. Therefore, the presence of tachykinin NK₁ and tachykinin NK₂ receptors on different target cells ultimately determines the biological consequences of the activation of the NANC system, although an activation of tachykinin NK₃ receptors is not completely excluded [11, 12].

Progress in investigations on the physiological and pathological roles of tachykinins and on tachykinin receptor classification has been greatly facilitated by the availability of a number of highly selective nonpeptide antagonists for tachykinin NK₁, NK₂ and NK₃ receptors (table 1 and fig. 2) [28]. These compounds can be regarded as suitable tools for the investigation of the pharmacological effect of tachykinins. Moreover, tachykinin NK₁ and NK₃ dual receptor antagonists, FK 224, S.16474 and MDL 105,212A, have been described [25–27]. With the development of newer and more selective ligands for the various receptors, it has become possible to clarify the respective contribution of tachykinin NK₁, NK₂ and NK₃ activation to the pharmacological effects of tachykinins (table 2).

Airway hyperresponsiveness, an enhanced bronchoconstrictor response to many different stimuli, is a key feature of asthma and relates closely to the severity of the disease, the frequency of symptoms, and the need for treatment [31–33]. There is some evidence that hyperresponsiveness is associated with inflammation in the airways. Histopathological studies carried out on asthmatics who died during asthma attacks have demonstrated marked inflammation in the airways, with infiltration of inflammatory cells, particularly eosinophils, alteration of the airway epithelium, and plugging of the airway lumen by viscous secretions [34].

It is increasingly apparent that different cells are involved in the pathogenesis of asthma, and that these cells produce a variety of mediators that interact in a complex way to produce a number of pathological effects.

Table 1. – Tachykinin receptor antagonists

<table>
<thead>
<tr>
<th>Type</th>
<th>Code</th>
<th>First author</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK₁ selective</td>
<td>CP 96,345</td>
<td>Snider</td>
<td>[13]</td>
</tr>
<tr>
<td></td>
<td>RP 67,580</td>
<td>Garrett</td>
<td>[14]</td>
</tr>
<tr>
<td></td>
<td>FK 888</td>
<td>Fuji</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>SR 140333</td>
<td>Emonds-Alt</td>
<td>[16]</td>
</tr>
<tr>
<td></td>
<td>(nolpitantium)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>LY 303870</td>
<td>Gitter</td>
<td>[17]</td>
</tr>
<tr>
<td></td>
<td>GR 203040</td>
<td>Beattie</td>
<td>[18]</td>
</tr>
<tr>
<td>NK₂ selective</td>
<td>MEN 10.376</td>
<td>Maggi</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>SR 48968</td>
<td>Emonds-Alt</td>
<td>[20]</td>
</tr>
<tr>
<td></td>
<td>(saredutant)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>MEN 10.627</td>
<td>Maggi</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td>GR 159897</td>
<td>Ball</td>
<td>[22]</td>
</tr>
<tr>
<td>NK₃ selective</td>
<td>SR 142801 (osanetant)</td>
<td>Emonds-Alt</td>
<td>[23]</td>
</tr>
<tr>
<td>Dual NK₁ + NK₂</td>
<td>FK 224</td>
<td>Morai</td>
<td>[24]</td>
</tr>
<tr>
<td></td>
<td>S.16474</td>
<td>Robingau</td>
<td>[25]</td>
</tr>
<tr>
<td></td>
<td>MDL 105,212A</td>
<td>Kudlacz</td>
<td>[26]</td>
</tr>
</tbody>
</table>

NK: neurokinin.
which, together, contribute to bronchial hyperresponsiveness. Among these mediators, tachykinins appear to play a major role, since they contribute to the development of "neurogenic inflammation" [3, 35]. Moreover, several mediators involved in the development or maintenance of the inflammatory response, could also enhance the production or the activity of tachykinins [36–39].

A role for the sensory neuropeptide system has also been proposed in cough. According to the recent review by Widdicombe [40], the cough reflex is usually considered to be mediated by intraepithelial nerves and by two types of sensory receptors, the pulmonary and bronchial C-fibre receptors with nonmyelinated afferents, and the rapidly adapting pulmonary stretch receptors.

Table 2. – Receptors involved in the pharmacological effects of tachykinins (substance P, neurokinin A and neurokinin B) in the airways

<table>
<thead>
<tr>
<th>Effects</th>
<th>Receptor subtypes</th>
<th>NK₁</th>
<th>NK₂</th>
<th>NK₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nerve activation</td>
<td>Increase in ganglionic transmission</td>
<td>+</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Bronchial smooth muscle</td>
<td>Contraction of ferret trachea</td>
<td>+</td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Contraction of hamster trachea</td>
<td></td>
<td>+++ (NK₂B)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Contraction of guinea-pig trachea</td>
<td>++</td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Contraction of guinea-pig bronchus</td>
<td></td>
<td>+</td>
<td>+++ (NK₂A)</td>
</tr>
<tr>
<td></td>
<td>Contraction of human bronchus</td>
<td>+</td>
<td></td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>Relaxation of rat trachea*</td>
<td></td>
<td></td>
<td>++</td>
</tr>
<tr>
<td>Vascular permeability</td>
<td>Plasma protein extravasation</td>
<td>+++</td>
<td>+/++</td>
<td></td>
</tr>
<tr>
<td>Recruitment and activation of inflammatory cells</td>
<td>Chemotaxis (guinea-pig, human)</td>
<td>+++</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lymphocyte proliferation (human)</td>
<td>+++</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Increase in neutrophil motility</td>
<td>+++</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Monocyte/macrophage stimulation</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mast cell activation²</td>
<td>?</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>Stimulation of secretion</td>
<td>Mucus in guinea-pig trachea</td>
<td>+++</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mucus in ferret trachea</td>
<td>±</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mucus in human bronchus</td>
<td>+++</td>
<td></td>
<td>±</td>
</tr>
<tr>
<td></td>
<td>Cl⁻ from epithelial cells</td>
<td>±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchial hyperresponsiveness</td>
<td>Increase of ACh-induced bronchoconstriction (guinea-pig)</td>
<td>+++</td>
<td>+++</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(mouse)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Increase of histamine-induced microvascular leakage (guinea-pig)</td>
<td>+++</td>
<td></td>
<td>+</td>
</tr>
<tr>
<td>Cough</td>
<td></td>
<td>+</td>
<td>+++</td>
<td>?</td>
</tr>
</tbody>
</table>

*: contractile effect has also been reported [29], †: a nonreceptor effect has been suggested [30]. NK: neurokinin; ACh: acetylcholine. Receptor subtypes involvement: +++: very strong; ++: strong; +: moderate; ±: doubtful; ?: questionable.
(RARs), sometimes called irritant receptors, with small diameter (A 6) myelinated fibres. The evidence that RARs cause cough is clearly established, and is based on their localization at the sites of the Airways most sensitive to cough (larynx and carina) [41–43], and on the fact that all the mechanical and chemical stimuli that lead to cough also excite them [40, 44]. In contrast, the role of pulmonary and bronchial C-fibre endings and tachykinins in cough is not yet clearly established, but some evidence suggests that the stimulation of such receptors elicits an increased sensitivity of the afferent nervous pathways associated with the stimulation of RARs [40].

The aim of the present review is to describe the involvement of tachykinins in airway inflammation, bronchial hyperresponsiveness and cough, and to describe a potential therapeutic use of new antagonists.

**Tachykinins, airway inflammation and bronchial hyperresponsiveness**

**Airway hyperresponsiveness**

Airway hyperresponsiveness is an important feature of asthma and is characterized by a nonspecific exaggerated response to bronchoconstrictor agents, such as histamine and acetylcholine [31–33]. Experimentally, bronchial hyperresponsiveness is expressed by the leftward shift of the concentration-response curves following aerosol administration of histamine or methacholine. In asthmatic patients, bronchial hyperresponsiveness results in a significant decrease in the provocative concentration of histamine or methacholine causing a 20% decrease in forced expiratory volume in one second (PC20).

Bronchial hyperresponsiveness is the expression of an exaggerated bronchopulmonary response associated with airway inflammation, involving vascular alterations, increase in bronchial secretions, recruitment and activation of inflammatory cells.

**Involvement of tachykinins in airway hyperresponsiveness**

Several observations suggest that tachykinins, such as SP and NKA, might be involved in the pathogenesis of airway hyperresponsiveness. Indeed, recent studies have reported that exposure of guinea-pigs to a single aerosol of either capsaicin (the pungent extract of red pepper, which releases endogenous sensory neuropeptides) [45] or SP elicited airway hyperresponsiveness to exogenous bronchoconstrictor agents [46–50]. NKA also enhanced methacholine response for up to 4 weeks in monkeys [51]. In asthmatic patients, exposure to SP enhanced maximal airway narrowing to methacholine 24 h later [52]. Conversely, chronic treatment with high doses (i.p.) of capsaicin, which depletes tachykinins from NANC nerves, eliminated airway hyperresponsiveness induced by acute capsaicin [48], citric acid [53], ovalbumin [54, 55] toluene diisocyanate [56], endotoxin [57], platelet-activating factor (PAF) [58], respiratory viral infection [59], and ozone [60] in guinea-pigs, dinitro-fluorobenzene [61] and toluene diisocyanate [62] in mice, and *Alternaria tenuis* aerosol in rabbits [63].

**Tachykinin receptor antagonists and bronchial hyperresponsiveness**

The involvement of tachykinins in the development of airway hyperresponsiveness has also been demonstrated using tachykinin receptor antagonists. Indeed, a single treatment with the tachykinin NK2 receptor antagonist, SR 48968 (Saredutant) [64], or with the dual tachykinin NK1 and NK2 receptor antagonists, MDL 105,212 [65] or FK 224 [66], prevented the antigen-induced airway hyperresponsiveness in the guinea-pig, whereas the tachykinin NK1 receptor antagonists, SR 140333 [64] (fig. 3) or FK 888 [66], did not. Inhaled SP in phosphoramidon-pretreated guinea-pig also induced bronchial hyperresponsiveness [49]. In this model again, SR 48968, but not SR 140333, suppressed the leftward shift of the dose-response curve to acetylcholine observed after exposure of phosphoramidon-pretreated guinea-pigs to SP [67], and these data also support a role for tachykinin NK2 receptor stimulation in the development of airway hyperresponsiveness. Similar conclusions were reported by YOSHIHARA et al. [68], who showed that SR 48968 prevented the potentiation of antigen-induced bronchoconstriction by cold air in guinea-pigs; and by PERRETTI et al. [69], who reported that the specific and long-acting peptidic antagonist, MEN 10,627, inhibited PAF-induced airway hyperresponsiveness in the guinea-pig. Finally, TOCKER et al. [70] reported that vagal stimulation in the presence of atropine potentiated pulmonary anaphylaxis in sensitized perfused guinea-pig lung; this potentiation was abolished by SR 48968, whereas NKA, but not SP, was able to mimic the effects of vagal stimulation.

**Fig. 3.** Effect of: a) SR 48968; and b) SR 140333 on antigen-induced airway hyperresponsiveness. Hartley guinea-pigs were sensitized by ovalbumin (OA) aerosol. After 15–20 days they were challenged by exposure to successive solutions of 10, 100, 1000, 5000 and 10000 OA µg·mL⁻¹ for 15 min each. The bronchopulmonary response of anaesthetized and ventilated guinea-pigs was assessed 48 h after exposure to either OA challenge or saline. After 10 min, successive administrations of 50, 100, 200 and 500 µg·mL⁻¹ acetylcholine (ACh) aerosol were given for 1 min each at 10 min intervals. The bronchopulmonary response was expressed as mean±SEM percentage of that obtained by clamping the tracheal cannula. Sensitized guinea-pigs were treated, 30 min before OA exposure with 1 mg·kg⁻¹ i.p. of SR 48968 or of SR 140333. —: saline (n=5); – ovalbumin (n=8); —: SR 48968 + OA (n=6); —: SR 140333 + OA (n=6). Significance of differences: saline vs OA, p<0.001; OA vs SR 48968 + OA, p<0.01; SR 48968 + OA vs saline, NS; OA vs SR 140333 + OA, NS; SR 140333 + OA vs saline. (Reproduced, with permission, from [64]).
In contrast, in another study, interleukin-8 (IL-8)-induced bronchial hyperresponsiveness in the guinea-pig could not be reduced either by FK224 or FK888 [71]. In these conditions, the bronchial hyperresponsiveness induced by intranasal administration of IL-8 was closely associated with recruitment of neutrophils, but not eosinophils, and involved thromboxane A2 (TXA2) as a main mediator [72]. Differences in the mechanisms of the development of bronchial hyperresponsiveness in the various experimental models of bronchopulmonary alterations in the guinea-pig could also explain the discrepancies between the effectiveness of tachykinin receptor antagonists. However, the results obtained with selective tachykinin NK2 receptor antagonists, such as SR 48968 and MEN 10,627, strongly suggest that tachykinins are involved in the development of airway hyperresponsiveness, and that tachykinin NK2 receptor stimulation plays an important role in this phenomenon, in the guinea-pig. It was reported that tachykinins are essential for the development of tracheal hyperreactivity induced by toluene diisoyxanate in mouse airways [62]. In contrast to the prevention of airway hyperresponsiveness by the tachykinin NK2 receptor antagonist in guinea-pig, the hyperresponsiveness observed in the mouse was completely blocked by the tachykinin NK1 receptor antagonist, RP 67,580 [62].

Since the actions of tachykinins are terminated by proteolytic cleavage due mainly to neutral endopeptidase (NEP) E.C. 3.4.24.11, it has been proposed that this enzyme plays a regulatory role in the development of bronchial hyperresponsiveness and airway inflammation. Blockade of NEP by phosphoramidon potentiates airway responses to exogenous and endogenous neuropeptides [73, 74]. Moreover, bronchial hyperresponsiveness to SP has been reported after viral infection or cigarette smoke exposure [75–78], which altered NEP activity. Furthermore, only guinea-pigs pretreated with the NEP inhibitor, phosphoramidon, elicited a significant increase in airway response to SP after antigen challenge [79], and to acetylcholine (ACh) after SP or citric acid exposure [49, 53].

**Tachykinins and microvascular leakage**

Among the biological effects elicited by tachykinins, which might be involved in the alteration of pulmonary responses, microvascular leakage and the subsequent increase in plasma protein extravasation, a component of "neurogenic inflammation" might play an important role. Pharmacological control of vascular leakage may be of interest in asthma, because airway oedema contributes not only to airway narrowing but also to airway hyperresponsiveness [80]. Several studies have indicated that tachykinin NK1 receptors are involved in neurogenic inflammation in the central airways of guinea-pig and rat [81–83]. SP alone has been shown to induce microvascular leakage when administered intravenously [84, 85], or by aerosol [86], in various animal species, including guinea-pigs and rats. Hence, the activity of SP on microvascular leakage is potentiated by pretreatment of the guinea-pig with a NEP inhibitor [87]. It has been demonstrated that SP-induced plasma protein extravasation is mediated mainly through tachykinin NK1 receptor stimulation.

Indeed, in guinea-pig airways, the tachykinin NK1 receptor antagonist, CP 96,345, has been reported to reduce microvascular leakage induced by exogenous SP, capsaicin, electrical field stimulation (EFS) or bradykinin [81, 88]. Inhalation of antigen by guinea-pigs leads to plasma extravasation of the trachea and nasal mucosa [83]. Interestingly, after an early phase of extravasation, release of neuropeptides from sensory nerves occurs, with subsequent increase in extravasation via activation of tachykinin NK1 receptors, as demonstrated by the inhibitory activity of CP 96,345 [83]. NK1 receptor stimulation has also been reported to be involved in the delayed-type hypersensitivity-induced increase in vascular permeability in the mouse small intestine [89], in the SP-induced inflammatory responses in guinea-pig skin [90] and in capsaicin-induced mouse ear oedema [91].

A role for tachykinin NK1 receptors in microvascular leakage cannot be excluded, since SR 48968 inhibited NKA-induced microvascular leakage in guinea-pigs 24 h later [67]. In these conditions, SR 140333 has been shown to markedly reduce the SP-induced potentiation of microvascular leakage induced by histamine, whereas SR 48968 had no preventative effects [67]; strengthening the role of the tachykinin NK1 receptor in the microvascular leakage following tachykinin stimulation. Similar results were obtained in animals exposed to aerosolized citric acid and challenged 24 h later with histamine [93].

**Tachykinins and inflammatory cells**

Several effects of SP on inflammatory cells have been described [94]. For example, SP elicited granulocyte adhesion and infiltration in skin [90, 95]. However, the infiltration of inflammatory cells appears to be mediated via the release of secondary mediators, possibly mast cell-derived mediators with 5-lipoxygenase products [90, 96].

Tachykinins have not been demonstrated to directly induce eosinophil chemotaxis in vivo [49] and in vitro [97], which suggests that SP-induced bronchial hyperresponsiveness is not closely related to eosinophil infiltration in airways [49]. Moreover, capsaicin or SR 48968 pretreatment prevented antigen challenge-induced airway hyperresponsiveness in sensitized guinea-pigs, but not the recruitment of eosinophils in airways [54, 55, 98]. Such data have recently been confirmed by Van Oosterhout et al. [50] studying the involvement of interleukin-5 (IL-5) and SP in the development of airway hyperreactivity to histamine in guinea-pigs. Indeed, in vivo administration of either IL-5 or SP induced the development of airway hyperreactivity, whereas administration of IL-5, but not SP, induced a significant increase in the number of eosinophils and eosinophil peroxidase
activity in bronchoalveolar lavage (BAL) cells. Moreover, the simultaneous administration of IL-5 and SP did not potentiate the hyperreactivity and eosinophilia observed with IL-5 alone. These data suggest that IL-5 is important in the recruitment of eosinophils, whereas both IL-5 and SP are involved in the induction of airway hyperreactivity [50].

In rabbits immunized to Alternaria tenuis, chronic treatment with capsaicin induced a reduction of bronchial hyperresponsiveness, without inhibitory effect on the pulmonary recruitment of eosinophils and neutrophils [63]. In contrast, in primates, dual antagonism by tachykinin NK1 receptor antagonist (CP 99,994) plus tachykinin NK2 receptor antagonist (SR 48968) markedly reduced the eosinophil recruitment in BAL fluids induced by antigen challenge, whereas each antagonist used alone was ineffective [99]. More recently, KALTREIDER et al. [100] reported that the tachykinin NK1 receptor antagonist, CP 96,345, moderately, but significantly, reduced the total numbers of leucocytes, lymphocytes and granulocytes retrieved by BAL after antigen challenge of sensitized mice. This result suggests that tachykinins may be secreted locally during pulmonary immune responses, and are recognized by leucocytes infiltrating lung tissue [100].

Although few data are available on the activation of eosinophils by SP, KROEGEL et al. [101] demonstrated that SP can induce eosinophil peroxidase release from guinea-pig eosinophils. When eosinophils from allergic donors were pretreated with SP, the chemotactic responses to PAF or leukotriene B4 (LTB4) were enhanced [97]. Although SP is known to stimulate the chemotaxis of human monocytes [102] and rabbit neutrophils [103], a moderate chemotactic activity of SP on neutrophils both from healthy subjects and asthmatic patients has been observed [104]. Interestingly, SP- or interleukin 1 (IL-1)-induced polymorphonuclear leucocyte accumulation was prevented by a tachykinin NK1, but not a NK2, receptor antagonist [105]. Furthermore, the release of endogenous tachykinins, possibly SP, may occur following IL-1 injection in vivo [105].

Tachykinins have other effects in vitro, including stimulation of human T- and B- lymphocytes and fibroblast proliferation [106–109]. Tachykinins may also modulate inflammatory cell activation through the release of various cytokines. LOTZE et al. [110] reported that SP induced the release of IL-1, interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) from human monocytes. SP has also been demonstrated to release IL-8 from human polymorphonuclear leucocytes and to enhance the IL-8 release induced by other stimuli, such as N-formyl-methionyl-leucyl-phenylalanine (fMLP) [111]. Finally, in association with human T-lymphocyte activation, an increase in IL-2 messenger ribonucleic acid (mRNA) expression by SP has been reported by CALVO et al. [112].

The role of tachykinin NK1 receptors in tachykinin-induced leucocyte activation in airways has recently been strengthened by the observation that the adhesion of leucocytes, induced in the venules of rat trachea by SP or capsaicin, can be reduced by a selective tachykinin NK1 receptor antagonist, CP 96,345, and thus appears to be mediated by tachykinin NK1 receptors [113]. However, the role of tachykinin NK1 receptor stimulation in the neutrophil chemotaxis is not completely established, since the tachykinin NK2 receptor antagonist, SR 48968, has been found to inhibit tachykinin-induced chemotaxis of human neutrophils [114].

Bronchial hyperresponsiveness induced by aerosolized SP in phosphoramidon-pretreated guinea-pigs was described as not being associated with recruitment of granulocytes in the airways [49]. In contrast, an enhanced chemiluminescence and an increase in arachidonate release from alveolar macrophages of guinea pigs exposed to SP was observed, in comparison to alveolar macrophages of guinea-pigs pretreated only with phosphoramidon [49, 98]. These results suggest an ex-vivo activation of alveolar macrophages by SP when administered by aerosol, since no alteration of the cell composition in the BAL was observed, which probably indirectly modifies the reactivity of macrophages through their phagocytic properties. SP also stimulates guinea-pig macrophages in vitro to induce the release of superoxide anions [115], through stimulation of tachykinin NK1 receptors and, to a lesser extent, tachykinin NK2 receptors [116]. Interestingly, this effect was enhanced in cells taken from antigen-sensitized guinea-pigs [117]. More recently, SP has been shown to induce gelatinase production by alveolar macrophages through tachykinin NK2 receptor activation [118]. In contrast, alveolar macrophages both from asthmatic patients and healthy subjects were only poorly or not activated at all by SP in vitro [119].

TAM et al. [120] reported that SP or EFS degranulate tracheal mast cells, contributing to the neurogenic responses in the trachea [120]. This result is consistent with the release of histamine by SP and capsaicin from guinea-pig airway mast cells [121]. Moreover, in these conditions, the mechanism of histamine release depends predominantly on the activation of tachykinin NK1 and NK2 receptors, as suggested by the inhibition of SP-induced histamine release both by tachykinin NK1 and NK2 receptor blockade [121]. In contrast, HUA et al. [122] reported that SP may increase sensitivity of mast cells to EFS-discharged mediators or facilitate the release of mast cell-stimulating mediators from autonomic nerves, rather than a direct stimulating effect of SP on mast cell degranulation, as previously suggested by DEVILLIER et al. [30] and MOUSLY et al. [123]. However, the stimulation of mast cells by SP may strongly contribute to the airway effects of tachykinins.

Influence of inflammatory mediators on tachykinin responses

In addition to their direct activity on the airways, many inflammatory mediators may influence the responses of various tachykinins. It was previously demonstrated, in anaesthetized dog, that histamine administered by aerosol induced an increase in the activity of C-fibres [37]. Antigen challenge induced an enhancement of noncholinergic contractile response to vagus nerves and EFS in guinea-pig isolated trachea [124], or the response to SP in the isolated airways of immunized rabbits [125]. In addition, prostaglandin E2 (PGE2), an inflammatory mediator derived from the cyclo-oxygenase pathway of arachidonic acid metabolism, enhanced the pulmonary chemoreflex (apnoea, bradycardia and hypotension) [36],
and has been shown to increase the sensitivity of the capsaicin-induced cough reflex in healthy human volunteers [126]. Furthermore, high doses of PGE2, administered either by inhalation or injection, can stimulate bronchopulmonary C-fibre endings [127, 128]. Similarly, LTD$_4$ has been shown to cause the release of SP from guinea-pig isolated trachea, or to potentiate the tachykinin-mediated response in the guinea pig isolated airways evoked by the threshold electrical stimulation of the vagus nerve or EFS [129, 130]. In Fisher (F344) rats, tachykinins cause bronchoconstriction and extravasation of plasma protein by indirect mechanisms involving the activation of tachykinin NK$_1$ receptors, release of serotonin (5-HT) and mast cell activation [29, 131]. Recently, tachykinins have been reported to be involved in KCl-induced contraction of guinea-pig trachea [132].

Recently, studies have documented possible changes in the inflammatory process, the electrical stimulation of afferent fibres is markedly modified, tachykinin synthesis by these nerves is increased, and tachykinin receptor expression may be enhanced. Indeed, using sensitized guinea-pigs, Riccio and co-workers [133, 134] reported an approximative fourfold increase of the mechanical sensitivity of A$\delta$ afferent fibres following exposure to antigen. Moreover, chronic airway inflammation after allergen challenge in the guinea-pig increases excitatory NANC nerve function, possibly by enhancing sensory neuro-peptide production and/or release [124, 135]. An increase in the synthesis of tachykinins from these fibres was demonstrated by Fischer et al. [4], following the inflammatory process of the airways after allergic reaction. In this study, 24 h after allergen exposure in sensitized guinea-pigs, there was a three- to fourfold enhancement of tissue concentrations of NKA, SP and CGRP in the lung, but not in the trachea. An increase in local tachykinin synthesis was not demonstrated, but neuropeptides measured in the lung were probably synthesized in the cell bodies of neurons located outside the lung, and then passed into the organ via axonal transport [4]. These authors also observed that 12 h after antigen stimulation, preprotachykinin mRNA was increased by 20% in nodose ganglia, but they did not detect significant quantitative changes in jugular ganglia, which was surprising, since nodose ganglia do not send tachykinin-containing axons to the airways in healthy animals.

An increase in receptor synthesis and/or expression has also been reported in rats in a model of chronic inflammatory disease produced by Mycoplasma pulmonis infection [136, 137]. In this study, the rat airways infected with M. pulmonis became abnormally sensitive to tachykinin, as revealed by the increase in plasma leakage evoked by exposure to SP. Using an antibody to rat tachykinin NK$_1$ receptor, Baluk et al. [138] demonstrated a dramatic increase in the number of tachykinin NK$_1$ receptors of endothelial cells, of postcapillary venules, and of new capillary-size vessels following inflammatory reaction due to M. pulmonis infection. These results suggest that synthesis of tachykinins undergoes marked change in the development of inflammation. Similar results have been obtained in other organs, such as skin [139]. McCarrison and Krause [140] demonstrated that tachykinin NK$_1$ and NK$_3$ receptor mRNA expression in the rat spinal cord dorsal horn is increased during adjuvant or formalin-induced nociception.

**Tachykinins and asthma**

Whilst there appears to be convincing evidence that sensory nerves and the subsequent tachykinin release play a role in bronchial hyperresponsiveness in various animal models, is there any evidence that sensory nerves play a role in asthma? In asthmatic subjects, SP exposure enhanced, maximal airway narrowing to methacholine 24 h later [52]. In allergic rhinitis, tachykinins partially mimicked the immediate nasal response to antigen by inducing nasal obstruction, recruitment of polymorphonuclear cells and leakage of albumin [141]. Immunohistochemical studies revealed conflicting evidence of an increase in SP-containing nerves in asthma [142–144]. Using high performance liquid chromatography, a reduction in SP-like immunoreactivity was observed in central airways of subjects who died of asthma or who were undergoing thoracotomy, compared with age-matched, nondiseased subjects [145]. Because of the rapid enzymatic cleavage in the extracellular microenvironment, the tissue content of neuropeptides reflects a balance between synthesis and release. In this condition, SP may be released during a severe asthmatic episode, and may then rapidly be degraded and not detected by the immunoassay [145]. This is consistent with an increase in SP-like immunoreactivity detected in the BAL fluid [146] and sputum [147] of asthmatics. This would suggest that neuropeptides can be released within the airway wall and, depending on the degree of stimulation, lead to a reduction in neuropeptide tissue content.

Recently, studies have documented possible changes in neurokinin receptor expression in asthma. There appears to be an increase in mRNA transcripts for tachykinin NK$_1$ [148] and NK$_3$ [149] receptors in lung tissue from asthmatics compared with nonasthmatics. The local release of neuropeptides may induce neuropeptide receptor tachyphylaxis that leads to increased synthesis of mRNA transcripts for these receptors. However, evidence of an increase in the expression of neuropeptide mRNA in sensory nerves and/or an increase in afferent activity awaits documentation in humans. Finally, it is also evident that several drug classes already in therapeutic use may interfere with sensory nerve function [150].

**Tachykinins and cough**

**Involvement of tachykinins in cough**

The involvement of C-fibre receptors in cough is based on experiments with capsaicin or with citric acid which...
can both stimulate pulmonary and bronchial C-fibre receptors [45, 151–154]. When given by aerosol, capsaicin and citric acid are powerful tussigenic agents in humans and other animals, and are now used as standard methods to study cough in preclinical and clinical studies [155]. Other examples showing that C-fibre receptor activation may cause cough were reported by FORSBERG et al. [153], who studied cough induced in guinea-pigs by inhalation of citric acid, by capsaicin, nicotine and mechanical stimulation of the trachea. Administration of large doses of capsaicin blocked the cough reflex due to citric acid and capsaicin, but not that due to nicotine and mechanical stimulation [153]. These authors concluded that the first two stimuli (citric acid and capsaicin) acted via receptors and the last two (nicotine and mechanical stimulation) via RARs. However, as suggested by WIDDICOMBE [40], capsaicin is probably not very specific and selective for C-fibre, and can stimulate RARs leading to cough [156, 157]. In addition, large doses of capsaicin can damage or destroy A δ myelinated fibres as well as C-fibres [158]. In contrast, due to peripheral and central nervous interactions, stimulation of C-fibres may inhibit cough in some circumstances [40].

Controversial reports have proposed tachykinins as tussive agents by themselves in guinea-pigs [159–161]. In humans, SP aerosols given to healthy subjects or to patients with asthma did not cause cough, but evoked a sensation of tightness in the chest of asthmatics, possibly secondary to bronchoconstriction, indicating that some sensory nerves were being stimulated [162]. In another study, SP aerosols caused cough in patients with upper airway infection but not in healthy subjects [163]. However, if tachykinins do not induce cough, they can elicit a marked sensitizing effect on the cough reflex, through enhanced activation of RARs. Such an action was first established by recordings of single fibres from RARs in rabbits by PRABHAKAR et al. [164], who showed that systemic SP not only caused reflex changes characteristic of stimulation of lung RARs but also increased the impulse frequency in vagal single fibres coming from RARs. This has recently been confirmed by FOX et al. [161], who reported that SP, when given by aerosol at concentrations up to 100 μM in the presence of the peptidase inhibitors, phosphoramidon and captopril, did not evoke cough by itself. In electrophysiological studies, SP applied directly onto receptive fields in the trachea did not activate either single C-fibres or A δ-fibres. In contrast, prior exposure of guinea-pigs to SP (10 nM) markedly enhanced citric acid-induced cough.

In guinea-pigs, chronic treatment with the angiotensin-converting enzyme (ACE) inhibitor, captopril, added to drinking water and given for 2 weeks, can induce an enhancement of citric acid-induced cough [165]. This effect appears to be mediated via accumulation of bradykinin, since the bradykinin B1 receptor antagonist, Hoe 140 (Icatibant) inhibits this potentiation [166]. It was proposed that the effect of bradykinin was likely to be due to C-fibre sensitization and/or to a release of tachykinins [166–168].

When given by aerosol, bradykinin, in the presence of phosphoramidon and captopril, led to a marked increase in citric acid-induced cough response; and when used in

**Effects of tachykinin receptor antagonists in cough**

The view that tachykinins are involved in cough is also supported by the observation that tachykinin antagonists block cough in several experimental conditions. The antitussive effect of tachykinin NK1 receptor antagonists has been clearly demonstrated, but the effect of tachykinin NK2 receptor antagonists is still debated. Indeed, SR 48968 inhibits, in a dose-dependent manner, citric acid- [172–174] or capsaicin- [175] induced cough in the unanaesthetized guinea-pig. This compound is approximately 150 times more potent than codeine and, in contrast to the latter, the effect of SR 48968 is not inhibited by naloxone [172]. It must be noted that both SR 48968 and codeine exert only a partial inhibition of the cough response (approximately 60–70%) [172–174]. The inhibitory effect of SR 48968 is not dependent on the reduction of citric acid-induced bronchoconstriction, since in guinea-pigs pretreated with bronchodilator doses of salbutamol, which did not reduce cough, the effect of SR 48968 was still present [173]. Moreover, a dissociation between cough and bronchoconstriction has been clearly demonstrated by FORSBERG et al. [176], who, in agreement with FULLER and COLLIER [177] and JACKSON [178], have shown that sodium cromoglycate inhibited bronchoconstriction, but not citric acid-induced cough, whereas lidocaine inhibited cough but not bronchoconstriction. The antitussive effect of tachykinin NK2 receptor antagonists has also been shown with the compound MEN 10627 against cough induced by allergen challenge in guinea-pigs sensitized with ovalbumin [179]. However, FOX et al. [161] did not observe any effect of SR 48968 on cough induced by citric acid in the guinea-pig, and LALLOO et al. [166] observed only a nonsignificant reduction.

Regarding the effect of tachykinin NK1 antagonists, various studies have shown no inhibitory activity. Such results were reported using 140333 [173] or CP 99,994 [161] on citric acid-induced cough in the guinea-pig. Similar observations have been reported in asthmatic patients using CP 99,994 against cough induced by inhalation of saline (increased osmolarity) [180]. In contrast, UIHE et al. [181] and YASUMITSU et al. [174] reported that FK 888, an antagonist of tachykinin NK1 receptors, inhibited cough induced by phosphoramidon, tobacco smoke, SP or citric acid. The reason for this discrepancy is unclear. Recent pharmacological and biochemical studies have suggested that two isoforms or two subtypes of the tachykinin NK1 receptor could exist [182–184]. One hypothesis proposes that one isoform or subtype of the tachykinin NK1 receptor, to which some tachykinin NK1 receptor antagonists could bind with higher affinity, would be implicated in cough control. Both GIRARD et al. [173] and YASUMITSU et al. [174] have observed that tachykinin NK1 receptor antagonists, SR 140333 and FK 888, were able to potentiate the effect of SR 48968, in terms of maximal effect.
The question of a central or peripheral effect for the inhibitory activity of tachykinin antagonists is not clear, since central administration of these antagonists was not performed. Yasumitsu et al. [174] have, however, suggested that the effects of the tachykinin NK1 receptor antagonist, FK 888, could be attributed to its peripheral action. Indeed, intracerebroventricular (i.c.v) injection of a tachykinin NK1 receptor agonist induced foot-tapping in gerbils, which could be inhibited by the central nervous system-penetrant tachykinin NK1 receptor antagonist [186]. The fact that FK 888 did not inhibit SP (i.c.v. injection)-induced foot-tapping in gerbils even at 10 mg·kg⁻¹ i.v., might suggest that this compound may penetrate only poorly into the central nervous system, but did not exclude a possible central effect for other compounds [174].

A possible interaction between RARs and C-fibre receptors proposed by Wisecombe [40] suggests a new hypothesis for the mechanism of action of tachykinin antagonists. As discussed above, activation of RARs induces cough reflex, and stimulation of C-fibres with a release of tachykinin that leads to the facilitation of nerve transmission associated with RAR stimulation. This explains why tachykinins, and especially SP, are only moderate tussive agents or may have no action at all, but dramatically potentiate cough induced by citric acid. It is also suggested that citric acid and capsaicin act both on RARs and C-fibre receptors, and are efficient inducers of cough through the activation of RARs, but this was observed through the increased stimulation of C-fibre receptors. This might explain the partial inhibition of the effects of tachykinin antagonists on citric acid-induced cough [172–174], since these compounds inhibit the effect of tachykinins released by C-fibres but do not alter the stimulation of RARs. Therefore, tachykinin antagonists interact with the amplification phenomenon induced by citric acid. The same observation could apply to the inhibitory activity of codeine, since it was previously described as an inhibitor of tachykinin release [187, 188].

The sensitizing effect of C-fibre on the activation of RARs is also suggested by the experiments of Lalloo et al. [166], who showed that SR 48968 moderately reduced citric acid-induced cough in guinea-pigs, but abolished the enhancement of citric acid-induced cough caused by exposure to ozone at 1 ppm for 3 h.

Conclusion

The mechanism of the development of bronchial hyperresponsiveness is unclear. It is generally accepted that pulmonary inflammation, mainly associated with a recruitment of inflammatory cells and increased release of inflammatory mediators inducing bronchoconstriction and plasma protein extravasation, plays a key role in the development of bronchial hyperresponsiveness [34, 189]. However, SP-induced airway hyperresponsiveness in the guinea-pig is not associated with eosinophil infiltration in the lung tissue [49], suggesting a dissociation between recruitment of inflammatory cells in the airways and bronchopulmonary alterations, as was previously observed for antigen-induced bronchial hyperresponsiveness [190–192]. Furthermore, it is also of interest that the eosinophilia associated with bronchial hyperresponsiveness induced by PAF or allergen was not inhibited by capsaicin [63, 193]. Hence, exposure of phosphoramidon-pretreated guinea-pigs to SP is followed by an increase in superoxide anion production and arachidonate release by alveolar macrophages, suggesting that these cells may play a key role in the development of bronchial hyperresponsiveness induced by SP [49, 98]. SP also induced an increase in microvascular leakage, allowing the plasma protein extravasation which may be involved in the bronchopulmonary alterations following allergic reaction [189].

The present review suggests a specialization of tachykinin NK1 versus tachykinin NK2 receptors in mediating the development of microvascular leakage hypersensitivity versus development of airway hyperresponsiveness by exposure to SP or citric acid in guinea-pigs. Whether or not the same situation applies exactly to human airways is not known, although it is suggested by some results. Thus, only tachykinin NK2 receptors mediate contraction of human isolated airways [194], and NKA, but not SP, produces bronchoconstriction in asthmatics [162]. Moreover, in allergic rhinitis, tachykinins induce nasal obstruction mainly through tachykinin NK1 receptor activation, whereas albumin leakage and recruitment of inflammatory cells probably involve tachykinin NK1 and NK2 receptors [141]. This suggests that an agonist with mixed (and possibly balanced) affinity for tachykinin NK1 and NK2 receptors could be of interest in a wide investigation of the various components of airway hyperresponsiveness and possible associations with pulmonary inflammation. Joos et al. [195] recently reported that the dual antagonist FK224 did not offer protection against NKA-induced bronchoconstriction in a group of mild asthmatic patients. Since FK224 is a moderate tachykinin receptor antagonist and was used at doses that did not displace concentration-response curves of NKA in asthmatics, further studies have to be conducted with selective antagonists to provide a final statement on the therapeutic interest of such compounds. SR 48968 might be a candidate, since it has been reported to be able to displace concentration-response curves in asthmatics [196]. The question of a role of tachykinin NK1 and NK2 receptors will have to be further considered, since it was recently reported that the tachykinin NK1 receptor antagonist, SR 142801, markedly reduced the bronchial hyperresponsiveness and the increased microvascular leakage after exposure of guinea-pigs to SP [12].

Thus, taken together, the results obtained with the various selective receptor antagonists provide pharmacological evidence that tachykinins play a role in delayed bronchopulmonary alterations and suggest that tachykinin receptor antagonists may be useful for investigating mechanisms and possibly reducing airway functional alterations in asthmatic patients.

Acknowledgement: The authors thank G. Bouër for his assistance in preparing the manuscript.

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