

PHARMACOLOGICAL REVIEW

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

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The role of tachykinin receptor antagonists in the prevention of bronchial hyperresponsiveness, airway inflammation and cough. C. Advenier, V. Lagente, E. Boichot. ©ERS Journals Ltd 1997.

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.

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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 sub-epithelial 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 secre-

tion; 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 NK₃ receptors is confined mainly to the central and peripheral nervous system, whilst tachykinin NK₁ and tachykinin NK₂ receptors are expressed both in the central and peripheral nervous system and in target organs, including airways [5–10]. According

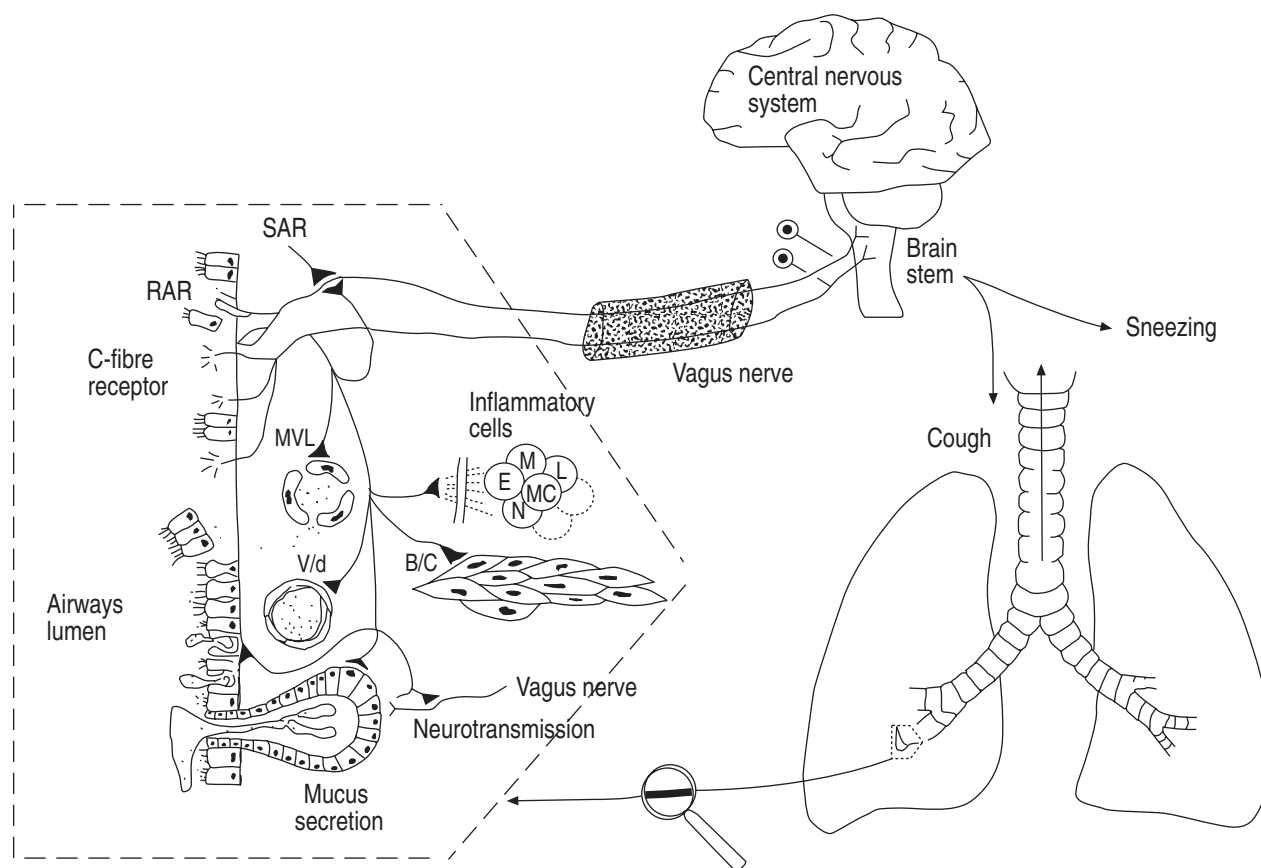


Fig. 1. – Schematic representation of the nonadrenergic noncholinergic (NANC) nerves of airways. SAR: slowly adapting pulmonary stretch receptor; RAR: rapidly adapting pulmonary stretch receptor; MVL: microvascular leakage; V/d: vasodilatation; B/C: bronchoconstriction; E: eosinophils; M: monocytes/macrophages; L: lymphocytes; MC: mast cells; N: neutrophils. (Adapted from [1–5]).

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 NK₂ 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

Type	Code	First author	[Ref.]
NK ₁ selective	CP 96,345	SNIDER	[13]
	RP 67,580	GARRET	[14]
	FK 888	FUJII	[15]
	SR 140333	EMONDS-ALT	[16]
	(nolpitantium)		
	LY 303870	GITTER	[17]
NK ₂ selective	GR 203040	BEATTIE	[18]
	MEN 10,376	MAGGI	[19]
	SR 48968	EMONDS-ALT	[20]
	(saredutant)	ADVENIER	[21]
	MEN 10,627	MAGGI	[22]
	GR 159897	BALL	[23]
NK ₃ selective	SR 142801 (osanetant)	EMONDS-ALT	[24]
Dual NK ₁ + NK ₂	FK 224	MURAI	[25]
	S.16474	ROBINGAU	[26]
	MDL 105,212A	KUDLACZ	[27]

NK: neurokinin.

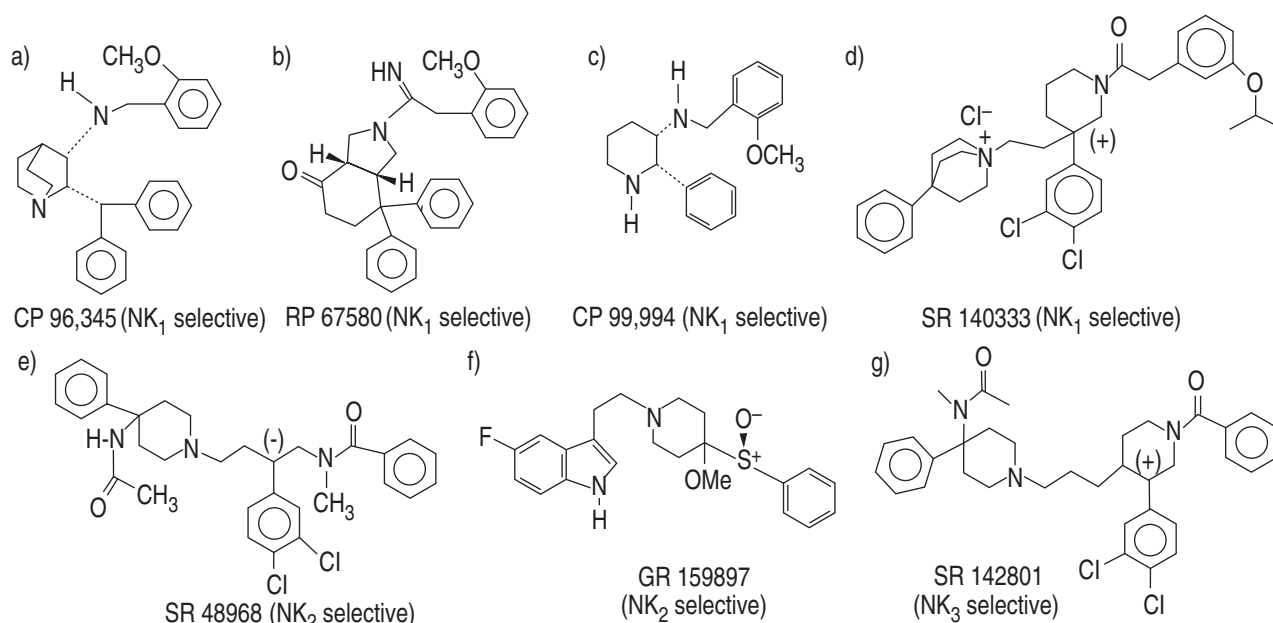


Fig. 2. — Chemical structures of nonpeptide tachykinin receptor antagonists. a) CP 96,345: [2S,3S]-cis-2-(diphenylmethyl)-N-[2-methoxyphenyl]-methyl-1-azabicyclo[2.2.2]octane-3-amine; b) RP 67580: (3aR,7aR)-7,7-diphenyl-2-[1-imino-2-(2-methoxyphenyl)-ethyl]perhydroisoindol-4-one; c) CP 99,994: (+)-(2S,3S)-3-(2-methoxy-benzylamino)-2-phenylpiperidine; d) SR 140333: (S)-1-[2-[3-(3,4-Dichlorophenyl)-1-(3-isopropoxyphenylacetyl)piperidino-3-yl]ethyl]-4-phenyl-1-azoniabicyclo [2.2.2]octane; e) SR 48968: (S)-N-methyl-N[4-acetylamino-4-phenylpiperidino]-2-(3,4-dichlorophenyl)butyl-benzamide; f) GR 159897: (R)-1-[2-(5-fluoro-1H-indol-3-yl)ethyl]-4-methoxy-4-[(phenylsulfinyl)methyl]-piperidine; g) SR 142801: (R)-(N)-(1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidino-3-yl)propyl)-4-phenylpiperidino-4-yl)-N-methylacetamide. NK₁, NK₂, NK₃: neurokinins 1, 2 and 3, respectively.

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

Effects		Receptor subtypes		
		NK ₁	NK ₂	NK ₃
Nerve activation	Increase in ganglionic transmission	+	+	+++
Bronchial smooth muscle	Contraction of ferret trachea	+	+++	
	Contraction of hamster trachea		+++ (NK _{2B})	
	Contraction of guinea-pig trachea	++	+++	
	Contraction of guinea-pig bronchus	+	+++ (NK _{2A})	
	Contraction of human bronchus	+	+++	
	Relaxation of rat trachea*	++		
	Plasma protein extravasation	+++	+/++	
Vascular permeability	Chemotaxis (guinea-pig, human)	+++		
Recruitment and activation of inflammatory cells	Lymphocyte proliferation (human)	+++	+	
	Increase in neutrophil motility	+++	+	
	Monocyte/macrophage stimulation	++	++	
	Mast cell activation‡	?	?	
	Mucus in guinea-pig trachea	+++	±	
Stimulation of secretion	Mucus in ferret trachea	+++	±	
	Mucus in human bronchus	+++	±	
	Cl ⁻ from epithelial cells	+++	±	
	Increase of ACh-induced bronchoconstriction (guinea-pig)		+++	+++
Bronchial hyperresponsiveness	(mouse)	++		
	Increase of histamine-induced microvascular leakage (guinea-pig)	+++		+++
Cough		+	+++	?

*: 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 δ) 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 *Altenaria 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.

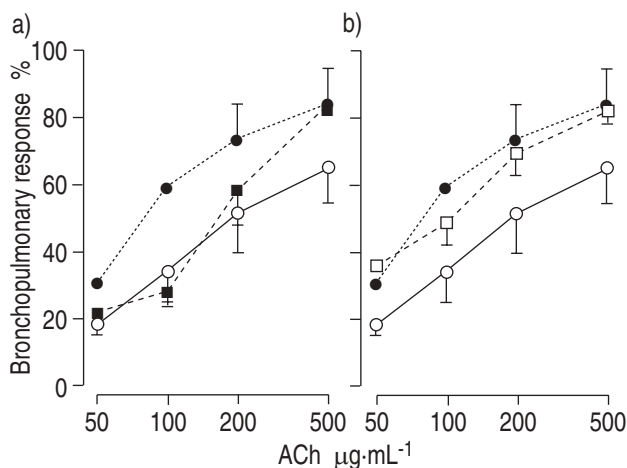


Fig. 3. — Effect of: a) SR 48968; and b) SR 140333 on antigen-induced airway hyperresponsiveness. Hartley guinea-pigs were sensitized by ovalbumin (OA) aerosol. After 15–20 days they were challenged by exposure to successive solutions of 10, 100, 1000, 5000 and 10000 OA $\mu\text{g}\cdot\text{mL}^{-1}$ 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 $\mu\text{g}\cdot\text{mL}^{-1}$ acetylcholine (ACh) aerosol were given for 1 min each at 10 min intervals. The bronchopulmonary response was expressed as mean \pm SEM percentage of that obtained by clamping the tracheal cannula. Sensitized guinea-pigs were treated, 30 min before OA exposure with 1 $\text{mg}\cdot\text{kg}^{-1}$ *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 diisocyanate 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 NK₂ receptors in microvascular leakage cannot be excluded, since SR 48968 inhibited NKA-induced microvascular leakage in guinea-pigs [88]; however, NKA is considerably less potent and efficient than SP for inducing this effect. TOUSIGNANT *et al.* [92] recently reported that [β-Ala⁸]NKA(4–10) may induce plasma protein extravasation in guinea-pig secondary bronchi and intraparenchymal airways, *via* tachykinin NK₂ receptor stimulation.

We recently showed that aerosolized SP, in addition to its direct effects, potentiates histamine-induced microvascular leakage in phosphoramidon-pretreated 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. LOTZ *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 proliferation, 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 NK₂ receptors and, to a lesser extent, tachykinin NK₁ 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, LTD₄ 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 NK₁ 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]. Finally, bradykinin may also be involved in the effects of tachykinin, since Fox *et al.* [38] reported that bradykinin caused sensitization of airway sensory nerves and an enhancement of the cough reflex in conscious guinea-pigs [38].

Change in afferent innervation and tachykinin receptor expression in the airway: correlation with airway hyperresponsiveness

Various studies have suggested that following 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 δ 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 neuropeptide 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 ganglions, but they did not detect significant quantitative changes in jugular ganglions, which was surprising, since nodose ganglions 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 NK₁ receptor, BALUK *et al.* [138] demonstrated a dramatic increase in the number of tachykinin NK₁

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]. McCARSON and KRAUSE [140] demonstrated that tachykinin NK₁ and NK₃ 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 NK₁ [148] and NK₂ [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 μ M 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* an accumulation of bradykinin, since the bradykinin B₂ 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

in vitro studies, bradykinin produced an increase of 100–400% in the firing of single vagal C-fibres stimulated by capsaicin [165, 169]. Interestingly, patients treated with ACE inhibitors developed cough, and cough response to capsaicin was enhanced in these patients [170]. Finally, cough sensitivity to capsaicin in humans increases during viral infections [171].

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 NK₂ receptor antagonists has been clearly demonstrated, but the effect of tachykinin NK₁ 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, UJIE *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, CP 99,994 [185], but not by a nonpenetrant 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 WIDDICOMBE [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 antagonist 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 reconsidered, since it was recently reported that the tachykinin NK₃ 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.

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References

1. Lembeck F, Holzer P. Substance P as neurogenic mediator of antidromic vasodilation and neurogenic plasma

- extravasation. *Naunyn Schmiedebergs Arch Pharmacol* 1970; 310: 175–183.
2. Lundberg JM, Saria A. Polypeptide-containing neurons in airway smooth muscle. *Ann Rev Physiol* 1987; 49: 557–572.
3. Ellis JL, Udem BJ. Pharmacology of nonadrenergic noncholinergic nerves in airway smooth muscle. *Pulm Pharmacol* 1994; 7: 205–223.
4. Fischer A, McGregor GP, Saria A, Philippin B, Kummer W. Induction of tachykinin gene and peptide expression in guinea-pig nodose primary afferent neurons by allergic airway inflammation. *J Clin Invest* 1996; 98: 2284–2291.
5. Lundberg JM. Pharmacology of co-transmission in the autonomic nervous system: integrative aspects on amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide. *Pharmacol Rev* 1996; 48: 113–178.
6. Guard S, Watson SP. Tachykinin receptor types: classification and membrane signalling mechanisms. *Neurochem Int* 1991; 18: 149–165.
7. Frossard N, Advenier C. Tachykinin receptors and the airways. *Life Sci* 1991; 49: 1941–1953.
8. Maggi CA. Tachykinin receptors and airway pathophysiology. *Eur Respir J* 1993; 6: 735–742.
9. Baluk P, Bunnett NW, McDonald DM. Localization of tachykinin NK-1, NK-2 and NK-3 receptors in airways by immunohistochemistry. *Am J Respir Crit Care Med* 1996; 153: A161.
10. Buell G, Schulz MF, Arkinstall SJ, et al. Molecular characterisation, expression and localisation of human neurokinin-3 receptor. *FEBS Lett* 1992; 299: 90–95.
11. Myers A, Udem B. Electrophysiological effects of tachykinins and capsaicin on guinea-pig bronchial parasympathetic ganglion neurones. *J Physiol* 1993; 470: 665–679.
12. Advenier C, Daoui S, Cui YY, Lagente V, Emonds-Alt X. Inhibition by the tachykinin NK₃ receptor antagonist, SR 142801, of substance P-induced microvascular leakage hypersensitivity and airway hyperresponsiveness in guinea-pigs. *Am J Respir Crit Care Med* 1996; 153: A163.
13. Snider RM, Constantine JW, Lowe III JA, et al. A potent nonpeptide antagonist of the substance P (NK-1) receptor. *Science* 1991; 251: 435–437.
14. Garret C, Carruette A, Fardin V, et al. Pharmacological properties of a potent and selective nonpeptide substance P antagonist. *Proc Natl Acad Sci USA* 1991; 88: 10208–10211.
15. Fujii T, Murai M, Morimoto H, et al. Pharmacological profile of a high affinity dipeptide NK₁ receptor antagonist, FK 888. *Br J Pharmacol* 1992; 107: 785–789.
16. Emonds-Alt X, Doutremepuich JD, Heaulme M, et al. *In vitro* and *in vivo* biological activities of SR 140333, a novel potent nonpeptide tachykinin NK₁ receptor antagonist. *Eur J Pharmacol* 1993; 250: 403–413.
17. Gitter BD, Bruns RF, Howbert JJ, et al. Pharmacological characterization of LY303870: a novel, potent and selective nonpeptide substance P (neurokinin-1) receptor antagonist. *Br J Pharmacol* 1995; 275: 737–744.
18. Beattie DT, Beresford IJM, Connor HE, et al. The pharmacology of GR203040, a novel, potent and selective nonpeptide tachykinin NK₁ receptor antagonist. *Br J Pharmacol* 1995; 116: 3149–3157.
19. Maggi CA, Giuliani S, Ballati L, et al. *In vivo* evidence for tachykininergic transmission using a new NK₂ receptor selective antagonist, MEN 10,376. *J Pharmacol Exp Ther* 1991; 257: 1172–1178.
20. Emonds-Alt X, Vilain P, Goulaouic P, et al. A potent and selective nonpeptide antagonist of the neurokinin A (NK₂) receptor. *Life Sci* 1992; 50: PL101–PL106.
21. Advenier C, Rouissi N, Nguyen QT, et al. Neurokinin A (NK₂) receptor revisited with SR 48968, a potent nonpeptide antagonist. *Biochem Biophys Res Commun* 1992; 184: 1418–1424.
22. Maggi CA, Astolfi M, Giuliani S, et al. MEN 10,627, a novel polycyclic peptide antagonist of tachykinin NK₂ receptors. *J Pharmacol Exp Ther* 1994; 271: 1495–1500.
23. Ball DI, Beresford IJM, Wren GPA, et al. *In vitro* and *in vivo* pharmacology of the nonpeptide antagonist at tachykinin NK₂-receptors, GR159897. Abstract. *Br J Pharmacol* 1994; 112: 48P.
24. Emonds-Alt X, Bichon D, Ducoux JP, et al. SR 142801, the first potent nonpeptide antagonist of tachykinin NK₃ receptor. *Life Sci* 1994; 56: PL27–PL32.
25. Murai M, Morimoto H, Maeda Y, Kiyotoh S, Nishikawa M, Fujii T. Effect of FK 224, a novel compound NK₁ and NK₂ receptor antagonist, on airway constriction and airway edema induced by neurokinins and sensory stimulation in guinea-pigs. *J Pharmacol Exp Ther* 1992; 261: 403–408.
26. Robineau P, Longchamps M, Kucharczyk N, et al. *In vitro* and *in vivo* pharmacology of S.16474, a novel dual tachykinin NK₁ and NK₂ receptor antagonist. *Eur J Pharmacol* 1995; 294: 677–684.
27. Kudlacz EM, Shatzer SA, Knippenberg RW, et al. *In vitro* and *in vivo* characterization of MDL 105,212A, a nonpeptide NK-1/NK-2 tachykinin receptor antagonist. *J Pharmacol Exp Ther* 1996; 277: 840–851.
28. Regoli D, Boudon A, Fauchère JL. Receptors and antagonists for substance P and related peptides. *Pharmacol Rev* 1994; 46: 551–599.
29. Joos GF, Lefebvre RA, Kips JC, Pauwels RA. Tachykinins contract trachea from Fischer 344 rats by interaction with a tachykinin NK₁ receptor. *Eur J Pharmacol* 1994; 271: 47–54.
30. Devillier P, Renoux M, Giroud JP, Regoli D. Peptides and histamine release from rat peritoneal mast cells. *Eur J Pharmacol* 1985; 117: 89–96.
31. Boushey HA, Holtzman MJ, Sheller JR, Nadel JA. Bronchial hyperreactivity. *Am Rev Respir Dis* 1980; 121: 389–413.
32. O'Byrne PM. Allergen-induced airway hyperresponsiveness. *J Allergy Clin Immunol* 1988; 81: 119–127.
33. Barnes PJ. New concepts in the pathogenesis of bronchial hyperresponsiveness and asthma. *J Allergy Clin Immunol* 1989; 83: 1013–1026.
34. Holgate ST, Djukanovic R, Wilson J, Roche W, Howarth PH. Inflammatory processes and bronchial hyperresponsiveness. *Clin Exp Allergy* 1991; 21 (Suppl. 1): 30–36.
35. Bozic CR, Lu B, Höpken UE, Gerard C, Gerard NP. Neurogenic amplification of immune complex inflammation. *Science* 1996; 273: 1722–1725.
36. Lee LY, Morton RF. Pulmonary chemoreflex sensitivity is enhanced by prostaglandin E₂ in anaesthetized rats. *J Appl Physiol* 1995; 79: 1679–1686.
37. Lee LY, Morton RF. Histamine enhances vagal pulmonary C-fiber responses to capsaicin and lung inflation. *Respir Physiol* 1993; 93: 83–96.
38. Fox AJ, Laloo UG, Belvisi MG, Bernareggi M, Chung KF, Barnes PJ. Bradykinin-evoked sensitization of airway sensory nerves: a mechanism for ACE-inhibitor cough. *Nature Med* 1996; 2: 815–817.

39. Hay DWP, Torphy TJ, Undem BJ. Cysteinyl leukotrienes in asthma: old mediators up to new tricks. *Trends Pharmacol Sci* 1995; 6: 304–339.
40. Widdicombe JG. Neurophysiology of the cough reflex. *Eur Respir J* 1995; 8: 1193–1202.
41. Widdicombe JG. Receptors in the trachea and bronchi of the cat. *J Physiol* 1954; 123: 71–104.
42. Widdicombe JG. Sensory innervation of the lungs and airways. In: Cervero F, Morrison JFB, eds. *Progress in Brain Research*. Vol. 67. Visceral Sensation. Amsterdam, Elsevier, 1986; pp. 49–64.
43. Das RM, Jeffery PK, Widdicombe JG. The epithelial innervation of the lower respiratory tract of the cat. *J Anat* 1978; 126: 123–131.
44. Coleridge HM, Coleridge JCG. Reflex evoked from the tracheobronchial tree and lungs. In: Cherniack NS, Widdicombe JG, eds. *Handbook of Physiology*. 3. The Respiratory System. Vol. II. Control of Breathing. Bethesda, American Physiological Society, 1986; pp. 395–429.
45. Lundberg JM, Hokfelt T, Martling CR, Saria A, Cuella C. Substance P immunoreactive sensory nerves in the lower respiratory tract of various mammals including man. *Cell Tissue Res* 1984; 235: 251–261.
46. Omini C, Brunelli G, Hernandez A, Daffonchio L. Bradykinin and substance P potentiate acetylcholine-induced bronchospasm in guinea-pig. *Eur J Pharmacol* 1989; 163: 195–197.
47. Umeno E, Hirose T, Nishima S. Pretreatment with aerosolized capsaicin potentiates histamine-induced bronchoconstriction in guinea-pigs. *Am Rev Respir Dis* 1992; 146: 159–162.
48. Hsuie T, Garland A, Ray DW, Hershenson MB, Leff AR, Solway J. Endogenous sensory neuropeptide release enhances nonspecific airway responsiveness in guinea-pigs. *Am Rev Respir Dis* 1992; 146: 148–153.
49. Boichot E, Lagente V, Paubert-Braquet M, Frossard N. Inhaled substance P induces activation of alveolar macrophages and increases airway responses in the guinea-pig. *Neuropeptides* 1993; 25: 307–313.
50. Van Oosterhout AJM, van Ark I, Hofman G, Van der Linde HJ, Fattah D, Nijkamp FP. Role of interleukin-5 and substance P in development of airway hyperreactivity to histamine in guinea-pigs. *Eur Respir J* 1996; 9: 493–499.
51. Tamura G, Sakai K, Taniguchi Y. Neurokinin A-induced bronchial hyperresponsiveness to methacholine in Japanese monkeys. *J Exp Med* 1989; 159: 69–73.
52. Cheung D, Van Der Veen H, Den Hartig J, Dijkman JH, Sterk PJ. Effects of inhaled substance P on airway hyperresponsiveness to methacholine in asthmatic subjects *in vivo*. *J Appl Physiol* 1994; 77: 1325–1332.
53. Girard V, Yavo JC, Emonds-Alt X, Advenier C. The tachykinin NK₂ receptor antagonist, SR 48968, inhibits citric acid-induced airway hyperresponsiveness in guinea-pigs. *Am J Respir Crit Care Med* 1996; 153: 1496–1502.
54. Matsuse T, Thomson RJ, Chen XR, Salari H, Schellenberg RR. Capsaicin inhibits airway hyperresponsiveness but not lipoxygenase activity or eosinophilia after repeated aerosolized antigen in guinea-pigs. *Am Rev Respir Dis* 1991; 144: 366–372.
55. Ladenius AR, Nijkamp FP. Capsaicin-pretreatment of guinea-pigs *in vivo* prevents ovalbumin-induced tracheal hyperreactivity *in vitro*. *Eur J Pharmacol* 1993; 235: 127–131.
56. Sheppard D, Scypinski L. A tachykinin receptor antagonist inhibits and an inhibitor of tachykinin metabolism potentiates toluene diisocyanate-induced airway hyperresponsiveness in guinea-pigs. *Am Rev Respir Dis* 1988; 138: 547–551.
57. Jarreau PH, D'Ortho MP, Boyer V, Harf A, Macquini-Mavier I. Effects of capsaicin on the airway responses to inhaled endotoxin in the guinea-pig. *Am J Respir Crit Care Med* 1994; 149: 128–133.
58. Perretti F, Manzini S. Activation of capsaicin-sensitive sensory fibers modulates PAF-induced bronchial hyperresponsiveness in anaesthetized guinea-pigs. *Am Rev Respir Dis* 1993; 148: 927–931.
59. Ladenius AR, Folkerts G, Van der Linde HJ, Nijkamp FP. Viral respiratory infection potentiates ovalbumin-induced guinea-pig tracheal hyperresponsiveness: role of tachykinins. *Br J Pharmacol* 1995; 115: 1048–1052.
60. Koto H, Aizawa H, Takata S, Inoue H, Hara N. An important role of tachykinins in ozone-induced airway hyperresponsiveness. *Am Rev Respir Crit Care Med* 1995; 151: 1763–1769.
61. Buckley TL, Nijkamp FP. Airways hyperreactivity and cellular accumulation in a delayed-type hypersensitivity reaction in the mouse: modulation by capsaicin-sensitive nerves. *Am J Respir Crit Care Med* 1994; 149: 400–407.
62. Scheerens H, Buckley TL, Muis T, Van Loveren H, Nijkamp FP. The involvement of sensory neuropeptides in toluene diisocyanate-induced tracheal hyperreactivity in the mouse airways. *Br J Pharmacol* 1996; 119: 1665–1671.
63. Herd CM, Gozzard N, Page CP. Capsaicin pretreatment prevents the development of antigen-induced airway hyperresponsiveness in neonatally immunised rabbits. *Eur J Pharmacol* 1995; 282: 111–119.
64. Boichot E, Germain N, Lagente V, Advenier C. The tachykinin NK₂ receptor antagonist, SR 48968, prevents substance P-induced airway hyperresponsiveness in guinea-pigs. *Br J Pharmacol* 1995; 114: 259–261.
65. Kudlacz EM, Knippenberg RW, Logan DE, Burkholder TP. Effect of MDL 105,212, a nonpeptide NK₁/NK₂ receptor antagonist, in an allergic guinea-pig model. *J Pharmacol Exp Ther* 1996; 279: 732–739.
66. Mizuguchi M, Fujimara M, Amemiya T, Nishi K, Ohka T, Matsuda T. Involvement of NK₂ receptors rather than NK₁ receptors in bronchial hyperresponsiveness induced by allergic reaction in guinea-pigs. *Br J Pharmacol* 1996; 117: 443–448.
67. Boichot E, Biyah K, Germain N, Emonds-Alt X, Lagente V, Advenier C. Involvement of tachykinin NK₁ and NK₂ receptors in substance P-induced microvascular leakage hypersensitivity and airway hyperresponsiveness in guinea-pigs. *Eur Respir J* 1996; 9: 1445–1450.
68. Yoshihara S, Geppetti P, Linden A, Hara M, Chan B, Nadel JA. Tachykinins mediate the potentiation of antigen-induced bronchoconstriction by cold air in guinea-pigs. *J Allergy Clin Immunol* 1996; 97: 756–760.
69. Perretti F, Ballati L, Manzini S, Maggi CA, Evangelista S. Antibronchospastic activity of MEN 10,627, a novel tachykinin NK₂ receptor antagonist, in guinea-pig airways. *Eur J Pharmacol* 1995; 273: 129–135.
70. Tocker JE, Gertner SB, Welton AF, Selig WM. Vagal stimulation augments pulmonary anaphylaxis in the guinea-pig lung. *Am J Respir Crit Care Med* 1995; 151: 461–469.
71. Fujimura M, Tsujiura M, Nomura M, Mizuguchi M, Matsuda T, Matsushima K. Sensory neuropeptides are not directly involved in bronchial hyperresponsiveness induced by interleukin-8 in guinea-pigs *in vivo*. *Clin Exp Allergy* 1996; 26: 357–362.

72. Xiou Q, Fujimura M, Nomura M, *et al.* Bronchial hyper-responsiveness and airway neutrophil accumulation induced by interleukin-8 and the effects of thromboxane A₂ antagonist, S-1452, in guinea-pigs. *Clin Exp Allergy* 1995; 25: 51–59.
73. Stimler-Gerard NP. Neutral endopeptidase-like enzyme controls the contractile activity of substance P in guinea-pig lung. *J Clin Invest* 1987; 79: 1819–1825.
74. Thompson JE, Sheppard D. Phosphoramidon potentiates the increase in lung resistance mediated by tachykinins in guinea-pigs. *Am Rev Respir Dis* 1988; 137: 337–340.
75. Dusser DJ, Djokic TD, Borson DB, Nadel JA. Cigarette smoke induces bronchoconstrictor hyperresponsiveness to substance P and inactivates airway neutral endopeptidase in the guinea-pig: possible role of free radicals. *J Clin Invest* 1989; 84: 900–906.
76. Dusser DJ, Jacoby DB, Djokic TD, Rubinstein I, Borson DB, Nadel JA. Virus induces airway hyperresponsiveness to tachykinins: role of neutral endopeptidase. *J Appl Physiol* 1989; 67: 1504–1511.
77. Daffonchio L, Hernandez A, Gallico L, Omini C. Airway hyperreactivity induced by active cigarette smoke exposure in guinea-pigs: possible role of sensory neuropeptides. *Pulm Pharmacol* 1990; 3: 161–166.
78. Elwood W, Lötvall JO, Barnes PJ, Chung KF. Airway hyperresponsiveness to acetylcholine and to tachykinins after respiratory virus infection in the guinea-pig. *Ann Allergy* 1993; 70: 231–236.
79. Boichot E, Lagente V, Le Gall G, *et al.* Bronchial responses to substance P after antigen challenge in the guinea-pig: *in vivo* and *in vitro* studies. *Med Inf* 1992; 1: 207–212.
80. Erjefält I, Persson CGA. Pharmacologic control of plasma exudation into tracheobronchial airways. *Am Rev Respir Dis* 1991; 143: 1008–1014.
81. Lei YH, Barnes PJ, Rogers DF. Inhibition of neurogenic plasma exudation in guinea-pig airways by CP-96,345, a new nonpeptide NK₁ receptor antagonist. *Br J Pharmacol* 1992; 105: 261–262.
82. Bertrand C, Geppeti P, Graf PD, Foresi A, Nadel JA. Involvement of neurogenic inflammation in antigen-induced bronchoconstriction in guinea-pigs. *Am J Physiol* 1993; 265: L507–L511.
83. Bertrand C, Geppeti P, Baker J, Yamawaki I, Nadel JA. Role of neurogenic inflammation in antigen-induced vascular extravasation in guinea-pig trachea. *J Immunol* 1993; 150: 1479–1485.
84. Rogers DF, Belvisi MG, Aursudkij B, Evans TW, Barnes PJ. Effects and interactions of sensory neuropeptides on airway microvascular leakage in guinea-pigs. *Br J Pharmacol* 1988; 95: 1109–1116.
85. Lazzaret F, Chauveau M, Weber S, Lockhart A, Frossard N. Inhibition of substance P-induced microvascular leakage by inhaled methoxamine in rat airways. *Br J Pharmacol* 1994; 113: 649–655.
86. Lötvall JO, Lemenn RJ, Hui KP, Barnes PJ, Chung KF. Airflow obstruction after substance P aerosol: contribution of airway and pulmonary oedema. *J Appl Physiol* 1990; 69: 1473–1478.
87. Umeno E, Nadel JY, Huang HT, McDonald DM. Inhibition of neutral endopeptidase potentiates neurogenic inflammation in the rat trachea. *J Appl Physiol* 1989; 66: 2647–2652.
88. Qian Y, Emonds-Alt X, Advenier C. Effects of capsaicin, (±)-CP-96,345 and SR 48968 on the bradykinin-induced airways microvascular leakage. *Pulm Pharmacol* 1993; 6: 63–67.
89. Kraneveld AD, Buckley TL, van Heuven-Holsen Y, van Schaik Y, Koster AS, Nijkamp FP. Delayed-type hypersensitivity-induced increase in vascular permeability in the mouse small intestine: inhibition by depletion of sensory neuropeptides and NK₁ receptor blockade. *Br J Pharmacol* 1995; 114: 1483–1489.
90. Walsh DT, Weg VB, Williams TJ, Noursharg S. Substance P-induced inflammatory responses in guinea-pig skin: the effect of specific NK₁ receptor antagonists and the role of endogenous mediators. *Br J Pharmacol* 1995; 114: 1343–1350.
91. Inoue H, Nagata N, Koshihara Y. Effect of the tachykinin receptor antagonists, SR 140333, FK 888 and SR 142801, on capsaicin-induced mouse ear oedema. *Inflamm Res* 1996; 45: 303–307.
92. Tousignant C, Chan CC, Guevremont D, *et al.* NK₂ receptors mediate plasma extravasation in guinea-pig lower airways. *Br J Pharmacol* 1993; 108: 383–386.
93. Biyah K, Molimard M, Emonds-Alt X, Advenier C. SR 140333, prevents potentiation by citric acid of plasma exudation induced by histamine in airways. *Eur J Pharmacol* 1996; 308: 325–328.
94. Joos GF, Germonpre PR, Kips JC, Peleman RA, Pauwels RA. Sensory neuropeptides and the human lower airways: present state and future directions. *Eur Respir J* 1994; 7: 1161–1171.
95. Smith CH, Barker JNWN, Morris RW, McDonald DM, Lee TH. Neuropeptides induce rapid expression of endothelial cell adhesion molecules and elicit granulocyte infiltration in human skin. *J Immunol* 1993; 151: 3274–3282.
96. Iwamoto I, Tomoe S, Tomioka H, Yoshida S. Leukotriene B₄ mediates substance P-induced granulocyte infiltration in mouse skin. *J Immunol* 1993; 151: 2116–2123.
97. Numao T, Agrawal DK. Neuropeptides modulate human eosinophil chemotaxis. *J Immunol* 1992; 149: 3309–3315.
98. Boichot E, Germain N, Emonds-Alt X, Lagente V, Advenier C. Effects of the tachykinin NK₁ (SR 140333) and NK₂ (SR 48968) receptor antagonists on antigen and substance P-induced activation of guinea-pig alveolar macrophages. *Am J Respir Crit Care Med* 1996; 153: A417.
99. Turner CR, Andresen CJ, Patterson DK, *et al.* Dual antagonism of NK₁ and NK₂ receptors by CP 99,994 and SR 48968 prevents airway hyperresponsiveness in primates. *Am J Respir Crit Care Med* 1996; 153: A160.
100. Kaltreider HB, Ichikawa S, Byrd PK, *et al.* Upregulation of neuropeptide receptors in a murine model of immune inflammation in lung parenchyma. *Am J Respir Cell Mol Biol* 1997; 16: 133–144.
101. Kroegel C, Giembycz MA, Barnes PJ. Characterization of eosinophil cell activation by peptides: differential effects of substance P, mellitin and f-met-leu-phe. *J Immunol* 1990; 145: 2581–2587.
102. Ruff MR, Wahl SM, Pert CB. Substance P receptor-mediated chemotaxis of human monocytes. *Peptides* 1985; 6: 107–111.
103. Marasco WA, Showell HJ, Becker EL. Substance P binds to the formylpeptide chemotaxis receptor on the rabbit neutrophil. *Biochem Biophys Res Commun* 1981; 99: 1065–1072.
104. Rabier M, Damon M, Chanez P, *et al.* Neutrophil chemotactic activity of PAF, histamine and neuromediators in bronchial asthma. *J Lip Med* 1991; 4: 265–275.
105. Perretti M, Ahluwalia A, Flower RJ, Manzini S. Endogenous tachykinins play a role in IL-1-induced neutrophil accumulation: involvement of NK-1 receptors. *Immunology* 1993; 80: 73–77.

106. Payan DG, Goetzl EJ. Substance P receptor-dependent responses of leukocytes in pulmonary inflammation. *Am Rev Respir Dis* 1987; 136: S39–S46.
107. Payan DG, Levine JD, Goetzl EJ. Modulation of immunity and hypersensitivity by sensory neuropeptides. *J Immunol* 1984; 132: 1601–1604.
108. Laurenzi MA, Persson MA, Dalsgaard CJ, Ringden O. Stimulation of B-lymphocyte differentiation by the neuropeptides substance P and neurokinin A. *Scand J Immunol* 1989; 30: 695–701.
109. Harrison NK, Dawes KF, Kwon OJ, Barnes PJ, Laurent GJ, Chung KF. Effects of neuropeptides on human lung fibroblast proliferation and chemotaxis. *Am J Physiol* 1995; 268: L278–L283.
110. Lotz M, Vaughan JH, Carson DA. Effect of neuropeptides on production of inflammatory cytokines by human monocytes. *Science* 1988; 241: 1218–1221.
111. Serra MC, Calzetti F, Ceska M, Cassatella MA. Effect of substance P on superoxide anion and IL-8 production by human PMNL. *Immunology* 1994; 82: 63–69.
112. Calvo CF, Chavanel G, Senik A. Substance P enhances IL-2 expression in activated human T-cells. *J Immunol* 1992; 148: 3498–3504.
113. Baluk P, Bertrand C, Geppetti P, McDonald D, Nadel JA. NK₁ receptors mediate leukocyte adhesion in neurogenic inflammation in the rat trachea. *Am J Physiol (Lung Cell Mol Physiol)* 1995; 268: L263–L269.
114. Lenique F, Jarreau PH, Boyer V, Harf A, Macquin-Mavier I. Tachykinins induce human neutrophil chemotaxis: involvement of NK₂ receptors. Abstract. *Fund Clin Pharmacol* 1993; 7: 369.
115. Hartung HP, Toyka KV. Activation of macrophages by substance P: Induction of oxidative burst and thromboxane release. *Eur J Pharmacol* 1983; 89: 301–305.
116. Brunelleschi S, Vanni L, Ledda F, Giotti A, Maggi CA, Fantozzi R. Tachykinins activate guinea-pig alveolar macrophages: involvement of NK₂ and NK₁ receptors. *Br J Pharmacol* 1990; 100: 417–420.
117. Brunelleschi S, Parenti A, Ceni E, Giotti A, Fantozzi R. Enhanced responsiveness of ovalbumin-sensitized guinea-pig alveolar macrophages to tachykinins. *Br J Pharmacol* 1992; 107: 964–969.
118. D'Ortho MP, Jarreau PH, Delacourt C, et al. Tachykinins induce gelatinase production by guinea-pig alveolar macrophages: involvement of NK₂ receptors. *Am J Physiol* 1996; 269: L631–L636.
119. Pujol JL, Bousquet J, Grenier J, et al. Substance P activation of bronchoalveolar macrophages from asthmatic patients and normal subjects. *Clin Exp Allergy* 1989; 19: 625–628.
120. Tam EK, Aufderheide J, Hua XY. Chymotryptic activity in perfusates of isolated rat trachea: correlation with mucosal and connective tissue mast cell secretion. *Am J Respir Cell Mol Biol* 1994; 11: 321–328.
121. Lilly CM, Hall AE, Rodger IW, Kobzik L, Haley KJ, Drazen JM. Substance P-induced histamine release in tracheally-perfused guinea-pig lungs. *J Appl Physiol* 1995; 78: 1234–1241.
122. Hua XY, Back SM, Tam EK. Substance P enhances electrical field stimulation-induced mast cell degranulation in rat trachea. *Am J Physiol* 1996; 270: L985–L991.
123. Mously M, Bueb JL, Bronner C, Rouot B, Landry Y. G protein activation: a receptor-independent mode of action for cationic amphiphilic neuropeptides and venom peptides. *Trends Pharmacol Sci* 1990; 11: 358–362.
124. Ellis JL, Undem BJ. Antigen-induced enhancement of noncholinergic contractile responses to vagus nerve and electrical field stimulation in guinea-pig isolated trachea. *J Pharmacol Exp Ther* 1992; 262: 646–653.
125. Colasurdo GN, Loader JE, Graves JP, Larsen GL. SP-induced contraction of airway smooth muscle in normal and allergen-sensitized rabbits: mechanism of action. *J Appl Physiol* 1995; 78: 428–432.
126. Choudry NB, Fuller RW, Pride NB. Sensitivity of the human cough reflex: effect of inflammatory mediators, prostaglandin E₂, bradykinin and histamine. *Am Rev Respir Dis* 1989; 140: 137–141.
127. Coleridge HM, Coleridge JCG, Ginzl KH, Baker DG, Banzett RB, Morrison MA. Stimulation of "irritant" receptors and afferent C-fibres in the lung by prostaglandins. *Nature (Lond)* 1976; 264: 451–453.
128. Coleridge JCG, Coleridge HM. Afferent vagal C fibre innervation of the lungs and airways and its functional significance. *Rev Physiol Biochem Pharmacol* 1984; 99: 1–110.
129. Bloomquist EI, Kream RM. Release of substance P from guinea-pig trachea leukotriene D₄. *Exp Lung Res* 1990; 16: 645–659.
130. Ellis JL, Undem BJ. Role of peptidoleukotrienes in capsaicin-sensitive sensory fibre-mediated responses in guinea-pig airways. *J Physiol (Lond)* 1991; 436: 469–484.
131. Germonpre PR, Joos GF, Everaert E, Kips JC, Pauwels RA. Characterization of neurogenic inflammation in the airways of two highly inbred rat strains. *Am J Respir Crit Care Med* 1995; 152: 1796–1804.
132. Mitchell RW, Ndukwu IM, Herrnreiter A, et al. Differential tachykinin receptor subtype activation in capsaicin and KCl contractions of guinea-pig trachealis. *Am J Physiol* 1995; 269: L837–L842.
133. Riccio MM, Myers AC, Undem BJ. Immunomodulation of afferent neurons in guinea-pig isolated airway. *J Physiol* 1996; 491: 499–509.
134. Riccio MM, Proud D, Undem BJ. Enhancement of afferent nerve excitability in the airways by allergic inflammation. *Pulm Pharmacol* 1995; 8: 181–186.
135. Kageyama N, Ichinose M, Igarashi A, et al. Repeated allergen exposure enhances excitatory nonadrenergic noncholinergic nerve-mediated bronchoconstriction in sensitized guinea-pigs. *Eur Respir J* 1996; 9: 1439–1444.
136. McDonald DM, Schoeb TR, Lindsey JR. *Mycoplasma pulmonis* infections cause long-lasting potentiation of neurogenic inflammation in the respiratory tract of the rat. *J Clin Invest* 1991; 87: 787–799.
137. McDonald DM. Upregulation of tachykinin receptors in chronic airway inflammation. *Pulm Pharmacol* 1995; 8: 203–206.
138. Baluk P, Bowden JJ, Lefevre PL, McDonald DM. Increased expression of substance P (NK₁) receptors on airway blood vessels of rats with *Mycoplasma pulmonis* infection. *Am J Respir Crit Care Med* 1995; 151: A719.
139. Galeazza MT, Garry MG, Yost HJ, Strait KA, Hargreaves KM, Seybold VS. Plasticity in the synthesis and storage of substance P and calcitonin gene-related peptide in primary afferent neurons during peripheral inflammation. *Neurosci* 1995; 66: 443–458.
140. McCarron KE, Krause JE. NK₁ and NK₃ type tachykinin receptor mRNA expression in the rat spinal cord dorsal horn is increased during adjuvant or formyl-induced nociception. *J Neurosci* 1994; 14: 712–720.
141. Braunstein G, Fajac I, Lacroque J, Frossard N. Clinical and inflammatory responses to exogenous tachykinins in allergic rhinitis. *Am Rev Respir Dis* 1991; 144: 630–635.

142. Ollerenshaw SL, Jarvis D, Sullivan CE, Woolcock AJ. Substance P-immunoreactive nerves in airways from asthmatics and nonasthmatics. *Eur Respir J* 1991; 4: 673–682.
143. Howarth PH, Djukanovic HR, Wilson JW, Holgate ST, Springall DR, Polak JM. Mucosal nerves in endobronchial biopsies in asthma and nonasthma. *Int Arch Allergy Appl Immunol* 1991; 94: 330–333.
144. Howarth PH, Springall DR, Redington AE, Djukanovic R, Holgate ST, Polak JM. Neuropeptide-containing nerves in endobronchial biopsies from asthmatic and nonasthmatic subjects. *Am J Respir Cell Mol Biol* 1995; 13: 288–296.
145. Lilly CM, Bai TR, Shore SA, Hall AE, Drazen JM. Neuropeptide content of lungs from asthmatic and nonasthmatic patients. *Am J Respir Crit Care Med* 1995; 151: 548–553.
146. Nieber K, Baumgarten CR, Rathsack R, Furkert J, Oehme P, Kunkel G. Substance P and endorphin-like immunoreactivity in lavage fluids of subjects with and without allergic asthma. *J Allergy Clin Immunol* 1992; 90: 646–652.
147. Tomaki M, Ichinose M, Miura M, *et al.* Elevated substance P content in induced sputum from patients with asthma and patients with chronic bronchitis. *Am J Respir Crit Care Med* 1995; 151: 613–617.
148. Adcock IM, Peters M, Gelder C, Shirasaki H, Brown CR, Barnes PJ. Increased tachykinin receptor gene expression in asthmatic lung and its modulation by steroids. *J Mol Endocrinol* 1993; 11: 1–7.
149. Bai TR, Zhou D, Weir T, *et al.* Substance P (NK₁) and neurokinin A (NK₂) receptor gene expression in inflammatory diseases. *Am J Physiol* 1995; 269: L309–L317.
150. Spina D, Page CP. Airway sensory nerves in asthma: targets for therapy? *Pulm Pharmacol* 1996; 9: 1–18.
151. Lundberg JM, Saria A. Capsaicin-induced desensitization of airway mucosa to cigarette smoke, mechanical and chemical irritants. *Nature* 1983; 302: 251–253.
152. Satoh H, Lou YP, Lundberg JM. Inhibitory effects of capsazepine and SR 48968 on citric acid-induced bronchoconstriction in guinea-pigs. *Eur J Pharmacol* 1993; 236: 367–372.
153. Forsberg K, Karlsson J-A, Theodorssont E, Lundberg JM, Persson CGA. Cough and bronchoconstriction mediated by capsaicin-sensitive sensory neurons in the guinea-pig. *Pulm Pharmacol* 1988; 1: 33–39.
154. Laloo UG, Fox AJ, Belvisi MG, Chung KF, Barnes PJ. Capsazepine inhibits cough induced by capsaicin and citric acid but not hypertonic saline in guinea-pigs. *J Appl Physiol* 1995; 79: 1082–1087.
155. Laude EA, Higgins KS, Morice AH. A comparative study of the effects of citric acid, capsaicin and resiniferatoxin on the cough challenge in guinea-pig and man. *Pulm Pharmacol* 1993; 6: 171–175.
156. Armstrong DJ, Luck JC. A comparative study of irritant and type J receptors in the cat. *Respir Physiol* 1974; 21: 47–60.
157. Mohammed SP, Higenbottam TW, Adcock JJ. Effects of aerosol-applied capsaicin, histamine and prostaglandin E₂ on airway sensory receptors of anaesthetized cats. *J Physiol* 1993; 6: 51–66.
158. Jansco G. Pathobiological reactions of C-fibre primary sensory neurones to peripheral nerve injury. *Exp Physiol* 1992; 77: 405–431.
159. Takahama K, Fuchikama T, Isohama Y, Kai H, Miyata T. Neurokinin A but not neurokinin B and substance P induces codeine-resistant coughs in awake guinea-pigs. *Regul Pept* 1993; 42: 236–237.
160. Kohrogi H, Graf PD, Sekizawa K, Borson DB, Nadel JA. Neutral endopeptidase inhibitors potentiate substance P and capsaicin-induced cough in awake guinea-pigs. *J Clin Invest* 1988; 82: 2063–2068.
161. Fox AJ, Bernareggi M, Laloo UG, Chung KF, Barnes PJ, Belvisi MG. The effect of substance P on the cough reflex and airway sensory nerves in guinea-pigs. *Am J Respir Crit Care Med* 1996; 153: A161.
162. Joos GF, Pauwels RA, Van Der Straeten ME. Effect of inhaled substance P and neurokinin A on the airways of normal and asthmatic subjects. *Thorax* 1987; 42: 779–783.
163. Katsumata U, Sekizawa K, Inoue H, Sasaki H, Takishima T. Inhibitory actions of procaterol, a beta₂-stimulant, on substance P-induced cough in normal subjects during upper respiratory tract infection. *J Exp Med* 1989; 158: 105–106.
164. Prabhakar NR, Runold M, Yamamoto Y, Lagercrantz H, Cherniack NS, von Euler C. Role of the vagal afferents in substance P-induced respiratory responses in anaesthetized rabbits. *Acta Physiol Scand* 1987; 131: 63–71.
165. Laloo UG, Fox AJ, Bernareggi M, Belvisi MG, Chung KF, Barnes PJ. Bradykinin sensitisation of airway sensory nerves: a mechanism for captopril-induced enhancement of the cough reflex. *Am J Respir Crit Care Med* 1996; 153: A162.
166. Laloo UG, Koto H, Bernareggi M, Salmon M, Barnes PJ, Chung KF. Ozone-induced sensitisation of citric acid-induced cough reflex in awake guinea-pigs: role of bradykinin B₂ and tachykinin NK₂ receptors. *Am J Respir Crit Care Med* 1996; 153: A162.
167. Kaufman MP, Coleridge HM, Coleridge JCG, Baker DG. Bradykinin stimulates afferent vagal C-fibres in intrapulmonary airways of dogs. *J Appl Physiol: Respirat Environ Exercise Physiol* 1980; 48: 511–517.
168. Saria A, Lundberg JM, Skofitsch G, Lembeck F. Vascular protein leakage in various tissues induced by substance P, capsaicin, bradykinin, serotonin, histamine and by antigen challenge. *Naunyn Schmiedebergs Arch Pharmacol* 1983; 324: 212–218.
169. Belvisi MG, Bernareggi M, Laloo UG, Chung KF, Barnes PJ, Fox AJ. Inhibitory effect of nedocromil on sensitization of the cough reflex in guinea-pigs. *Am J Respir Crit Care Med* 1996; 153: A159.
170. Fuller RW, Choudry NH. Increased cough reflex associated with angiotensin converting enzyme inhibitor cough. *Br Med J* 1987; 295: 1025–1026.
171. O'Connell F, Thomas VE, Studham JM, O'Neill TP, Fuller RW, Pride NB. Cough sensitivity to inhaled capsaicin during naturally occurring upper respiratory tract infection. *Am Rev Respir Dis* 1993; 147: A714.
172. Advenier C, Girard V, Naline E, Vilain P and Emonds-Alt X. Antitussive effect of SR 48968, a nonpeptide tachykinin NK₂ receptor antagonist. *Eur J Pharmacol* 1993; 250: 169–171.
173. Girard V, Naline E, Vilain P, Emonds-Alt X, Adenier C. Effect of the two tachykinin antagonists, SR 48968 and SR 140333, on cough induced by citric acid in the unanaesthetized guinea-pig. *Eur Respir J* 1995; 8: 1110–1114.
174. Yasumitsu R, Hirayama Y, Imai T, Miyayasu K, Hiroi J. Effects of specific tachykinin receptor antagonists on citric acid-induced cough and bronchoconstriction in unanaesthetized guinea-pigs. *Eur J Pharmacol* 1996; 300: 215–219.
175. Robineau P, Petit C, Staczek J, Peglion JL, Brion JD, Canet E. NK₁ and NK₂ receptors involvement in capsaicin-induced cough in guinea-pigs. *Am J Respir Crit Care Med* 1994; 149: A186.

176. Forsberg K, Karlsson JA, Zackrisson C, Persson CGA. Selective inhibition of cough and bronchoconstriction in conscious guinea-pig. *Respiration* 1992; 59: 72–76.
177. Fuller RW, Collier JC. Sodium cromoglycate and atropine block the fall in FEV₁ but not the cough induced by hypotonic mist. *Thorax* 1984; 39: 766–770.
178. Jackson DM. The effect of nedocromil sodium, sodium cromoglycate and codeine phosphate, on citric acid-induced cough in dogs. *Br J Pharmacol* 1988; 93: 609–612.
179. Evangelista S, Ballati J, Perretti F. MEN 10,627, a new selective NK₂ receptor antagonist inhibits antigen-induced bronchoconstriction in sensitized guinea-pigs. *Neuropeptides* 1994; 26 (Suppl. 1): 39–40.
180. Fahy JV, Wong HH, Geppetti P, Nadel JA, Boushey HA. Effect of an NK₁ receptor antagonist (CP 99,994) on hypertonic saline-induced bronchoconstriction and cough in asthmatic subjects. *Am J Respir Crit Care Med* 1994; 149: A1057.
181. Ujiie Y, Sekizawa K, Aikawa T, Sasaki H. Evidence for substance P as an endogenous substance causing cough in guinea-pigs. *Am Rev Respir Dis* 1993; 148: 1628–1632.
182. Fong TM, Anderson SA, Yuh H, Huang RRC, Strader C. Differential activation of intracellular effector by two isoforms of human neurokinin-1 receptor. *Mol Pharmacol* 1992; 41: 24–30.
183. Mantyh PW, Rogers SD, Ghilardi JR, Maggio JE, Mantyh CR, Vigna SR. Differential expression of two isoforms of the neurokinin-1 (substance P) receptor *in vivo*. *Brain Res* 1996; 719: 8–13.
184. Tian Y, Wu L, Pu Y, Huang CC, Chung FZ. Structural motifs encoded by individual exons of the human neurokinin-1 receptor gene interact differentially with selective agonists and antagonists. *J Neurochem* 1996; 67: 1191–1199.
185. McLean S, Ganong P, Seymour PA, *et al*. Pharmacology of CP 99,994; a nonpeptide antagonist of the tachykinin neurokinin-1 receptor. *J Pharmacol Exp Ther* 1993; 267: 472–479.
186. Rupniak NMJ, Williams AR. Differential inhibition of foot-tapping and chromodacryorrhoea in gerbils by CNS penetrant and nonpenetrant tachykinin NK receptor antagonists. *Eur J Pharmacol* 1994; 265: 179–185.
187. Barnes PJ. Modulation of neurotransmission in airways. *Physiol Rev* 1992; 72: 699–729.
188. Frossard N, Barnes PJ. μ -Opioid receptors modulate non-cholinergic nerves in guinea-pig airways. *Eur J Pharmacol* 1987; 141: 519–521.
189. Persson CGA. Role of plasma exudation in asthmatic airways. *Lancet* 1986; ii: 1126–1128.
190. Boichot E, Lagente V, Carre C, Waltmann P, Mencia-Huerta JM, Braquet P. Bronchial hyperresponsiveness and cellular infiltration in the lung of guinea-pig sensitized and challenged by aerosol. *Clin Exp Allergy* 1991; 21: 67–76.
191. Boichot E, Richard MP, Paubert-Braquet M. Effect of cefadroxil on antigen-induced bronchial hyperresponsiveness and eosinophil accumulation in lung from sensitized guinea-pigs. *Int Arch Allergy Immunol* 1993; 102: 87–93.
192. Lagente V, Boichot E, Carre C, Guinot P, Mencia-Huerta JM, Braquet P. Effects of the platelet-activating factor antagonists, BN 52021 and BN 50730, on antigen-induced bronchial hyperresponsiveness and eosinophil infiltration in lung from sensitized guinea-pigs. *Clin Exp Allergy* 1993; 23: 1002–1010.
193. Spina D, McKenniff MG, Coyle AJ, *et al*. Effect of capsaicin on PAF-induced bronchial hyperresponsiveness and pulmonary cell accumulation in the rabbits. *Br J Pharmacol* 1991; 103: 1268–1274.
194. Advenier C, Naline E, Toty L, *et al*. Effects on the isolated human bronchus of SR 48968, a potent and selective nonpeptide antagonist of the neurokinin A (NK₂) receptors. *Am Rev Respir Dis* 1992; 146: 1171–1181.
195. Joos GF, Scoor JV, Kips JC, Pauwels RA. The effect of inhaled FK224, a tachykinin NK-1 and NK-2 receptor antagonist, on neurokinin A-induced bronchoconstriction in asthmatics. *Am J Respir Crit Care Med* 1996; 153: 1781–1784.
196. Van Schoor J, Joos GF, Chasson B, Brouard RJ, Pauwels RA. The effect of SR 48968, a nonpeptide neurokinin-2 receptor antagonist on neurokinin A-induced bronchoconstriction in asthmatics. *Eur Respir J* 1996; 9: 289s.