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Involvement of NK_1 and NK_2 Receptors in Pulmonary Responses Elicited by Non-adrenergic, Non-cholinergic Vagal Stimulation in Guinea-pigs

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Abstract

Previous studies from our laboratory using exogenously administered neurokinin (NK) agonists have shown that both NK_1- and NK_2-receptor subtypes are involved in plasma extravasation in the guinea-pig airways. In the present study, we have extended these observations using antidromic vagal stimulation to stimulate sensory c-fibres as a means of eliciting the release of endogenous tachykinins in propranolol- and atropine-treated guinea-pigs.

Antidromic vagal stimulation (5 ms, 30 s) induced frequency-dependent (1–10 Hz) bronchoconstriction that was completely abolished by co-administration of the NK_1-selective antagonist CP-99,994 ((2s-methoxy-benzyl)-(2-phenyl-piperidin-3s-yl)-amine), and the NK_2-selective antagonist SR-48,968 ((*S*)-*N*-methyl-*N*-[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl) butyl]benzamide), each at a dose sufficient to block NK_1 and NK_2 receptors, respectively (each at 0·3 mg kg^{-1}, i.v.). In contrast, SR-48,968 when given alone only partially blocked the vagal stimulation-induced bronchospasm, whereas CP-99,994 had no effect. Significant increases (2–3-fold) in plasma extravasation of [^{125}I]fibrinogen in the trachea, main bronchi, distal airways and oesophagus following vagal stimulation (5 Hz, 5 min, 10 V, 5 ms) were observed. Pretreatment with the neutral endopeptidase inhibitor, thiorphan (1 mg kg^{-1}, i.v.), and the angiotensin-converting enzyme inhibitor, enalapril (1 mg kg^{-1}, i.v.), potentiated both vagal stimulation-induced bronchoconstriction and plasma leakage in all tissues examined. This potentiation was due to reduced metabolism of endogenously released tachykinins since enhanced plasma overflow of immunoreactive substance P was observed following vagal stimulation in thiorphan- and enalapril-treated guinea-pigs. CP-99,994 substantially blocked plasma leakage in all parts of the airways and in the oesophagus. In comparison, SR-48,968 had no significant effect in the trachea and the oesophagus but partially inhibited plasma leakage in the main bronchi and distal airways. Co-administration of both CP-99,994 and SR-48,968 abolished the residual plasma leakage in these two regions.

These results support the hypothesis that both NK_1 and NK_2 receptors are involved in tachykinin-induced pulmonary responses in the airways.

Neurokinin A and substance P are small peptides belonging to the tachykinin family which share the common C-terminal sequence Phe-X-Gly-Leu-Met-NH_2. They have different amino-terminal sequences for recognition by specific receptors (for reviews see Casale 1991; Guard & Watson 1991). These two tachykinins are encoded by a single gene, the preprotachykinin A gene, which produces mRNAs for three precursors α-, β- and γ-preprotachykinin A (Nawa et al 1983; Krause et al 1987), from which substance P and neurokinin A are derived. It is now known that in mammalian systems, substance P and neurokinin A are present in abundance in the central nervous system and also in some peripheral tissues to mediate various functions via activation of specific receptors. The receptors for substance P and neurokinin A, designated NK_1 and NK_2, respectively, have been established by pharmacological and radioligand-binding studies. More recently, the rat and human NK_1 receptors have been cloned and characterized (Yokota et al 1989; Gerard et al 1991).

It has been suggested that tachykinins mediate important physiological functions in peripheral tissues including the lung and may play a role in the pathogenesis of asthma (Barnes 1991; Maggi et al 1991). In human and animal studies, substance P and neurokinin A have both been shown to induce bronchoconstriction, plasma extravasation and mucus production in the airways. Tachykinins are believed to be involved in non-adrenergic non-cholinergic (NANC) nerve pathways associated with stimulation of sensory c-fibres. Substance P immunoreactive nerves have been demonstrated in asthmatic and non-asthmatic airways (Ollerenshaw et al 1991) and, indeed, it has been proposed that substance P immunoreactive nerves may be modulated in asthmatics (Adcock et al 1993). The discovery of potent, selective, non-peptide neurokinin antagonists (reviewed by Watling 1992) such as CP-96,345 (*cis*-2-(diphenylmethyl)-N-[(2-methoxyphenyl)-methyl]-1-azabicyclo-[2.2.2]octan-3-amine) and CP-99,994 ((2s-methoxy-benzyl)-(2-phenyl-piperidin-3s-yl)-amine) for NK_1 receptors (Snider et al 1991; McLean et al 1993) and SR-48,968 ((*S*)-*N*-methyl-*N*-[4-(4-acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl) butyl]benzamide) for NK_2 receptors (Advenier et al 1992) has provided pharmacologists with powerful tools to probe the significance of the tachykinin neuropeptide system. Using these tools and a sensitive marker for microvascular leakage, [^{125}I]fibrinogen, we have reported previously that both NK_1 (Tousignant et al 1993b) and NK_2 (Tousignant et al 1993a) receptors are involved in plasma

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extravasation induced by tachykinin agonists given exogenously to the guinea-pig airways. In the present study, we have extended our observations in the guinea-pig lung to include release of endogenous tachykinins elicited by antidromic vagal nerve stimulation.

Materials and Methods

Plasma extravasation

All experimental protocols were approved by the Animal Care Committee at the Merck Frosst Centre for Therapeutic Research in accordance with the guidelines established by the Canadian Council on Animal Care. Plasma extravasation of [^{125}I]fibrinogen in the guinea-pig airways was measured using the same procedures described previously (Tousignant et al 1993a). Briefly, male guinea-pigs, 350–400 g, were anaesthetized with ketamine hydrochloride (25 mg kg^{-1}, i.m.)/xylazine hydrochloride (5 mg kg^{-1}, i.m.) and were ventilated with room air using an animal ventilator (Harvard Apparatus) following injection of succinylcholine chloride (5 mg kg^{-1}, i.m.). The body temperature was maintained at 37°C using a thermostatically controlled heating blanket. The right vagus nerve was sectioned at the level of the fifth tracheal cartilage and the caudal end was placed carefully around a palladium electrode (Harvard Apparatus). Following stabilization for 5–10 min, the animals were routinely given propranolol (1 mg kg^{-1}, i.v.) and atropine (1 mg kg^{-1}, i.v.) to block the adrenergic and the cholinergic systems, respectively. Five minutes later, [^{125}I]fibrinogen was injected intravenously at 50 μCi kg^{-1}. When applied, SR-48,968 (0·3 mg kg^{-1}), CP-99,994 (0·1 mg kg^{-1}), the neutral endopeptidase inhibitor thiorphan (1 mg kg^{-1}), the angiotensin converting enzyme inhibitor enalapril (1 mg kg^{-1}), or their combination, was also administered intravenously at this time point. Five minutes after drug administration, the vagus nerve was stimulated at 5 Hz, 5 ms, 10 V for 5 min using an Ortec stimulator (model 4710). The frequency and amplitude of the electrical pulses were monitored via an oscilloscope connected in parallel to the stimulator. The animals were killed 2 min after the cessation of vagal stimulation by intravenous injection of a lethal dose of sodium pentobarbitone. Thirty seconds before the guinea-pigs were killed, a blood sample was taken for quantitation of radioactivity in the plasma. Heparin (1400 units kg^{-1}) was injected intravenously to prevent non-specific fibrin formation during dissection of the airway tissues.

The heart and lungs were then quickly removed and perfused with 50 mL gravity-fed saline (0·9% NaCl) via the pulmonary artery. The airways were dissected free of connective tissues and separated into three parts—trachea, main bronchi, and distal airways (tracheal tissues from the first branch off the main bronchi to the small airways embedded in the parenchyma). A section of the oesophagus was also removed for comparison. Radioactivity in these tissues was determined and microvascular leakage was expressed as μL plasma equivalent per 100 mg tissue. Non-specific, background leakage was determined in tissues obtained from a group of sham-operated guinea-pigs.

Bronchoconstriction

In separate experiments, bronchoconstriction induced by vagal stimulation was monitored using a pulmonary mechanics data-acquisition system (Modular Instruments M100 data acquisition system with Biowindows software, Malvern, PA, USA). Guinea-pigs were prepared as described above and the vagus nerve was stimulated at 1–60 Hz, 5 ms, 10 V, for 30 s. Increases in transpulmonary pressure were monitored using a Validyne transducer (DP45, ± 55 cm H_2O) connected via a side arm to the tracheal tube. The other side of the transducer was connected to the pleural space via a polyethylene tubing and an 18-gauge needle. After a 5–10-min stabilization period, the animals were given atropine (1 mg kg^{-1}, i.v.) and propranolol (1 mg kg^{-1}, i.v.). Thiorphan and enalapril were not administered in these experiments. A paired protocol was used to evaluate the effects of SR-48,968 (0·3 mg kg^{-1}), CP-99,994 (0·3 mg kg^{-1}) or their combination. Bronchoconstriction was obtained following a first period of vagal stimulation at 3 and 10 Hz for 30 s. After the transpulmonary pressure had returned to baseline (or had stabilized), the test compounds were administered intravenously. Five minutes later, a second period of vagal stimulation at 3 and 10 Hz was performed. Matching vehicle controls were obtained from a separate group of guinea-pigs (Fig. 1).

Substance P immunoreactivities in plasma

Substance P immunoreactivity (i[SP]) in plasma was measured using a commercial enzyme immunoassay kit (substance P EIA kit, Cayman Chemical) after purification with C-18 reverse phase SepPak cartridges (Waters). Arterial blood samples were obtained immediately following vagal stimulation (5 Hz, 5 ms, 10 V for 5 min) in vehicle- or thiorphan- (1 mg kg^{-1}) and enalapril-treated (1 mg kg^{-1}) guinea-pigs. Corresponding groups of sham-operated guinea-pigs were used as controls. The plasma samples were purified using the following procedure. Plasma sample (200 μL) was mixed with 4 vols 4% acetic acid and passed through a C-18 SepPak cartridge which was preconditioned with 5 mL HPLC grade methanol and 5 mL deionized water. The cartridge was washed with 10 mL 4% acetic acid and the i[SP] was eluted with 3 mL ethanol:water:acetic acid (90:10:0.4). The organic solvent was evaporated by passing a gentle stream of nitrogen gas over the surface of the samples. The samples were then reconstituted in the same volume of EIA buffer (200 μL) and were subjected to EIA for i[SP]. Recovery of i[SP] was determined in each sample by adding a known amount of [^{3}H]substance P (about 5000 counts min^{-1}). The recovery was between 50 and 60%.

Materials

SR-48,968 and CP-99,994 were synthesized by the Medicinal Chemistry departments at Merck Research Laboratories at Terlings Park, UK, and at Rahway, New Jersey, USA. Other chemicals and their sources were: [^{125}I]fibrinogen (McMaster University, Hamilton, Ontario, Canada); [^{3}H]substance P (Dupont/NEN, Mississauga, Ontario, Canada); ketamine hydrochloride (Ayerst, Montreal, Quebec, Canada); xylazine hydrochloride (Haver, Etobicoke, Ontario, Canada); thiorphan (Sigma Chemicals, St Louis, MO, USA); enalapril (Merck Frosst Canada, Kirkland, Québec, Canada).

Data analysis and statistics

Microvascular leakage in different parts of airways was expressed as μL plasma per 100 mg tissue. Specific leakage was obtained by subtracting the background leakage (in the sham-operated group) from the total leakage. All values are presented as mean ± s.e.m. Differences between the means of a treated and a control group were compared using Student's *t*-test. A P value $< 0{\cdot}05$ was considered significant.

Results

Vagal stimulation-induced bronchoconstriction

Guinea-pigs responded to vagal stimulation at 1, 3 and 10 Hz with increases in transpulmonary pressure in a frequency-dependent manner. The responses reached a plateau at 10 Hz and declined at 30 and 60 Hz (data not shown). Fig. 1 shows the typical tracings from two guinea-pigs using the paired protocol to evaluate the effects of SR-48,968 and CP-99,994. In the control guinea-pig, there was no evidence

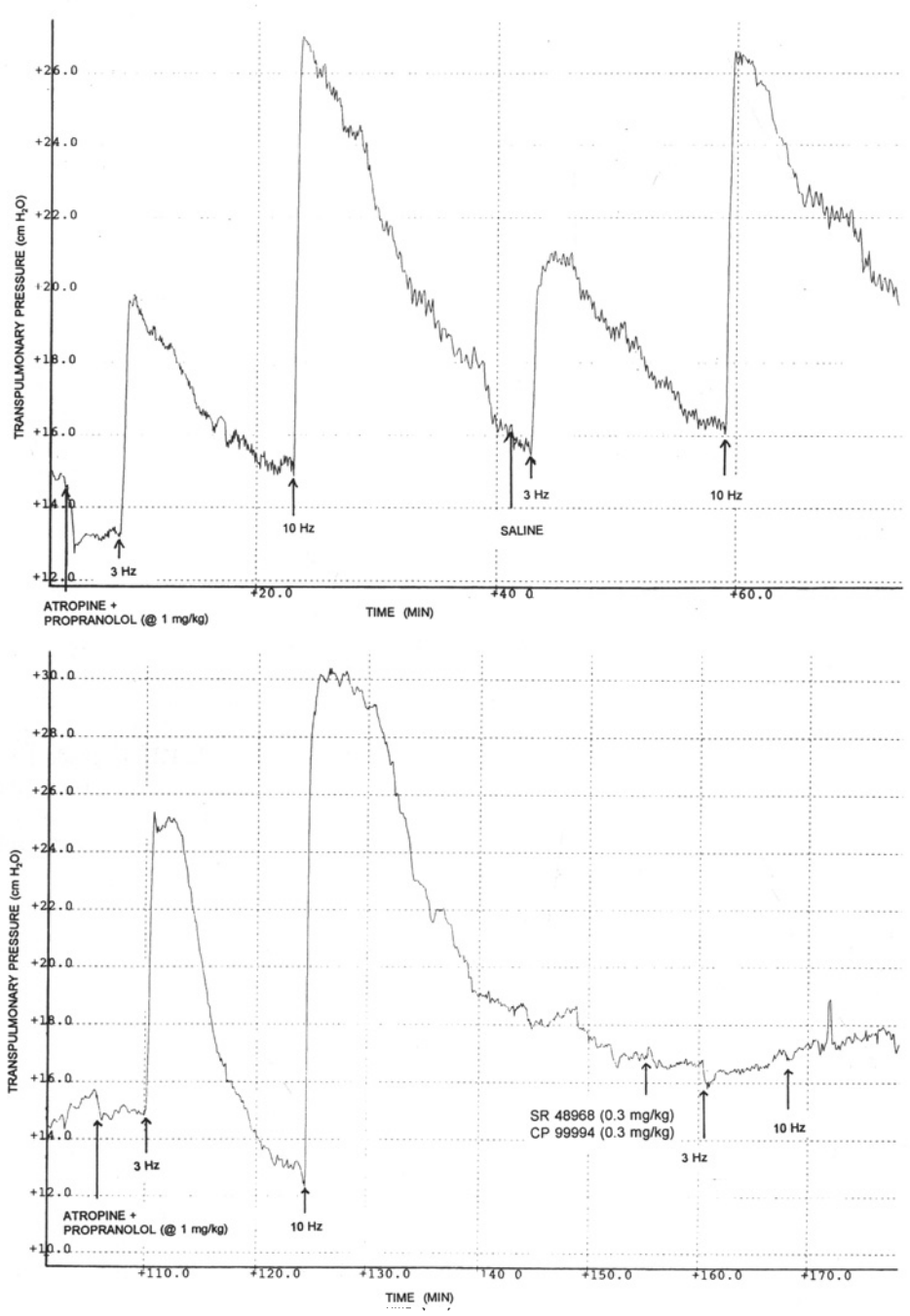

FIG. 1. Typical tracings showing the effect of co-administration of SR-48,968 and CP-99,994 (each at 0·3 mg kg^{-1}, i.v.) on vagal stimulation-induced bronchoconstriction. Upper panel: tracing obtained from a saline control guinea-pig showing reproducible bronchoconstriction induced by repeated vagal stimulation at 3 or 10 Hz (5 ms, 10 V, 30 s). Lower panel: tracing showing complete blockade of bronchoconstriction by CP-99,994 and SR-48,968. These animals were given atropine (1 mg kg^{-1}, i.v.) and propranolol (1 mg kg^{-1}, i.v.) but did not receive thiorphan or enalapril.

Table 1. Effects of CP-99,994 (0·3 mg kg^{-1}, i.v.), SR-48,968 (0·3 mg kg^{-1}, i.v.) or their co-administration on vagal stimulation-induced bronchoconstriction using a paired stimulation protocol as shown in Fig. 1.

Experiment	Peak transpulmonary pressure (% baseline)			
	Before		After	
	3 Hz	10 Hz	3 Hz	10 Hz
Saline	151·7 ± 21·7	206·2 ± 2·7	159·3 ± 5·9	208·7 ± 6·6
SR-48,968	146·1 ± 17·9	211·6 ± 16·1	139·1 ± 13·6	162·8 ± 15·1
CP-99,994	117·5 ± 8·5	214·6 ± 20·6	140·9 ± 19·0	192·7 ± 34·5
SR-48,968 + CP-99,994	139·9 ± 11·1	176·8 ± 9·4	99·3 ± 7·1	105·4 ± 5·7

The baseline response was 15·0 ± 0·7 cm H_2O, n = 15. For other experiments n = 4–5.

of tachyphylaxis to vagal stimulation at 3 and 10 Hz when repeated about 30 min apart. In contrast, the second period of vagal stimulation-induced responses was abolished in the guinea-pig treated with both SR-48,968 and CP-99,994 (each at 0·3 mg kg^{-1}, i.v.) (lower panel). When administered alone at 0·3 mg kg^{-1}, SR-48,968 only partially inhibited the bronchoconstriction induced by vagal stimulation, whereas CP-99,994 had no effect (Table 1).

Effects of thiorphan and enalapril on vagal stimulation-induced responses

Bronchoconstriction. Table 2 shows the effects of thiorphan (1 mg kg^{-1}, i.v.), enalapril (1 mg kg^{-1}, i.v.) or their combination on vagal stimulation-induced bronchoconstriction. Neither compound had an effect on baseline transpulmonary pressure. However, thiorphan, but not enalapril, significantly potentiated the responses to vagal stimulation.

Vagal stimulation-induced overflow of plasma i[SP]. In untreated guinea-pigs, vagal stimulation at 5 Hz for 5 min did not result in an increase in overflow of plasma i[SP] over the background levels (441 ± 10 pg mL^{-1} compared with 414 ± 10 pg mL^{-1} in sham-operated animals). In guinea-pigs treated with both thiorphan and enalapril, however, significantly more i[SP] was detected in the plasma following vagal stimulation (620 ± 70 pg mL^{-1} compared with 450 ± 33 pg mL^{-1}, $P < 0·05$).

Vagal stimulation-induced plasma extravasation

Vagal stimulation at 5 Hz for 5 min induced a slight but significant increase in extravasation of [^{125}I]fibrinogen in all parts of the airways and the oesophagus (Table 3). In the sham-operated group, the background leakage was 5·1 ± 0·3, 2·7 ± 0·5, 2·9 ± 0·6 and 0·9 ± 0·1 μL plasma/100 mg tissue (n = 7) in the trachea, main bronchi, distal airways and oesophagus, respectively. Pretreatment of the guinea-pigs with thiorphan (1 mg kg^{-1}, i.v.) and enalapril (1 mg kg^{-1}, i.v.) significantly enhanced the vagal stimulation-induced plasma extravasation. In subsequent experiments, the effects of CP-99,994, SR-48,968, or their combination, at a dose sufficient to block NK_1- or NK_2-mediated leakage, respectively (Tousignant et al 1993a, b), was examined in thiorphan- and enalapril-treated guinea-pigs. SR-48,968 (0·3 mg kg^{-1}, i.v.) significantly reduced plasma extravasation in the main bronchi and distal airways but had no effect in the trachea and oesophagus (Table 4). In contrast, CP-99,994 (0·1 mg kg^{-1}, i.v.) completely blocked plasma extravasation in the trachea and oesophagus, and had a significant inhibitory effect in the main bronchi and distal airways. Co-administration of SR-48,968 and CP-99,994 abolished plasma extravasation induced by vagal stimulation in all parts of the airways and oesophagus.

Table 2. Effects of enalapril (1 mg kg^{-1}, i.v.), thiorphan (1 mg kg^{-1}, i.v.) or their co-administration on vagal stimulation-induced bronchoconstriction (5 Hz, 5 ms, 10 V, 5 min).

Experiment	Transpulmonary pressure (cm H_2O)	
	Baseline	Vagal stimulation
Vehicle	15·8 ± 0·8	25·2 ± 1·3
Enalapril	15·2 ± 0·8	25·7 ± 1·0
Thiorphan	13·8 ± 0·7	34·1 ± 2·7*
Enalapril + thiorphan	16·9 ± 0·9	36·4 ± 1·8*

The responses were obtained from the same groups of guinea-pigs used in the plasma extravasation experiments. n = 6–10. *$P < 0·05$ compared with the control group.

Discussion

We have reported previously, using selective neurokinin agonists and antagonists, that plasma extravasation in guinea-pig airways is mediated by both NK_1 and NK_2 receptors (Tousignant et al 1993a, b). In the present study, we have extended these observations to include antidromic vagal stimulation to stimulate sensory c-fibres under the protection of atropine and propranolol, thereby revealing only the NANC component. Although the precise function of the NANC nervous system in man is not yet clearly defined, animal studies have shown that the NANC system participates in the regulation of a variety of lung functions (Barnes et al 1991; Lundberg 1993). In concurrence with this idea, we have shown that NANC vagal stimulation induces significant increases in both bronchospasm and plasma extravasation in the guinea-pig airways. Both direct and indirect evidence supports the conclusion that these responses are mediated by the release of neuropeptides such as substance P.

Substance P is rapidly metabolized in-vivo by a number of peptidases. In the lung, neutral endopeptidase (EC 3.4.24.11) and angiotensin-converting enzyme (EC 3.14.5.1) are two major enzymes responsible for the degra-

Table 3. Potentiation of vagal stimulation-induced (5 Hz, 5 ms, 10 V, 5 min) plasma extravasation of [^{125}I]fibrinogen in the trachea, main bronchi, distal airways and oesophagus by enalapril (1 mg kg^{-1}, i.v.) and thiorphan (1 mg kg^{-1}, i.v.).

Site	Specific plasma extravasation (μL plasma/100 mg tissue)			
	Trachea	Main bronchi	Distal airways	Oesophagus
Vehicle	1·82 ± 0·03	2·71 ± 0·24	2·32 ± 0·01	5·04 ± 1·84
Thiorphan	3·44 ± 0·43	4·48 ± 0·54	4·00 ± 0·53	7·56 ± 1·87
Enalapril	1·29 ± 0·10	2·00 ± 0·28	1·66 ± 0·59	8·25 ± 2·23
Thiorphan + enalapril	4·55 ± 0·58	8·03 ± 0·69*	8·18 ± 1·11*	16·48 ± 2·26*

Specific plasma extravasation was obtained by subtracting the background leakage from the total leakage. n = 6–10. *$P < 0·05$ compared with thiorphan alone.

dation of substance P. Neutral endopeptidase is present in abundance in the tracheal epithelium and angiotensin-converting enzyme is present in the vascular endothelium (reviewed by Erdös 1979; Nadel 1992). In the present study, inhibition of these two enzymes, particularly neutral endopeptidase, potentiated bronchoconstriction and airway leakage induced by NANC vagal stimulation. Since the site of leakage of macromolecules is the postcapillary venules which are devoid of neural input (McDonald et al 1988), substance P released from the sensory nerve terminals must travel down from the arterioles to reach the site of action. Thus, it is not surprising that inhibition of angiotensin-converting enzyme (predominantly on the vascular side) has an additive effect on inhibition of neutral endopeptidase (predominantly on the tracheal side) in enhancing airway leakage. In other species such as the rat, an enhancing effect of a neutral endopeptidase inhibitor on vagal stimulation-induced plasma extravasation in the airways has also been demonstrated (Umeno et al 1989), consistent with the concept that neutral endopeptidase plays a role in regulating the activities of endogenously released neuropeptides. Furthermore, a role of neutral endopeptidase in neurogenic inflammation has been proposed and it has been suggested that neutral endopeptidase deficiency in man can potentiate neurogenic inflammation (Nadel 1992). In both healthy and asthmatic subjects, inhaled thiorphan increases bronchoconstrictor responses to neurokinin A without changing the responses to methacholine (Cheung et al 1992a, b), suggesting that neutral endopeptidase may regulate the bronchial reactivity to exogenous neurokinin A in both healthy and asthmatic subjects.

Direct evidence that substance P is released following vagal stimulation in-vivo was obtained using a sensitive EIA. Significant increases in vagal stimulation-induced plasma overflow of i[SP] were observed in the guinea-pigs treated with thiorphan and enalapril but not in the untreated group, confirming that endogenously released substance P is rapidly metabolized by neutral endopeptidase and angiotensin-converting enzyme, presumably at the site of release and in the blood circulation. This is in contrast to the observation of Saria et al (1988) who have shown that vagal stimulation in isolated guinea-pig lung induces a significant increase in i[SP] in the lung perfusate even without inhibition of either neutral endopeptidase or angiotensin-converting enzyme. Obviously, the conditions used in the lung perfusion study are different from the present in-vivo study. In particular, the lung was perfused with cell-free buffer. Since it has been proposed that neutrophils also contain neutral endopeptidase activities (Iwamoto et al 1990), the lack of leucocytes in the perfusate may result in a longer half-life of the released i[SP], which may account, at least in part, for the different results.

The involvement of tachykinins in the responses to NANC vagal stimulation was confirmed using the non-peptide NK_1 antagonist CP-99,994 and the NK_2 antagonist SR-48,968. These compounds were intentionally used at maximally effective doses to block the NK_1 (McLean et al 1993) and NK_2 (Emonds-Alt et al 1992) receptors. SR-48,968 alone only partially blocked the NANC vagal stimulation-induced bronchospasm, suggesting that such a response is not solely mediated by NK_2 receptors. Interestingly, complete blockade was achieved when both SR-48,968 and CP-99,994 were given together, showing an additive effect of these two compounds. A similar additive interaction between NK_1- and NK_2-receptor antagonists has also been demonstrated in isolated guinea-pig trachea in response to resiniferatoxin challenge (Foulon et al 1993) and electrical field stimulation (Martin et al 1992).

Table 4. Effects of CP-99,994 (CP, 0·1 mg kg^{-1}, i.v.), SR-48,968 (SR, 0·3 mg kg^{-1}, i.v.) or their co-administration on vagal stimulation-induced (5 Hz, 5 ms, 10 V, 5 min) plasma extravasation of [^{125}I]fibrinogen in the trachea, main bronchi, distal airways and oesophagus. Thiorphan (1 mg kg^{-1}, i.v.) and enalapril (1 mg kg^{-1}, i.v.) were given in all animals.

Site	Specific plasma extravasation (μL plasma/100 mg tissue)			
	Trachea	Main bronchi	Distal airways	Oesophagus
Vehicle	11·17 ± 1·96	9·66 ± 2·33	6·93 ± 0·75	15·53 ± 2·15
SR-48,968	7·32 ± 2·11	4·28 ± 0·87*	1·50 ± 0·11*	14·75 ± 4·11
CP-99,994	0·38 ± 0·10*	0·66 ± 0·14*	1·46 ± 0·08*	0·43 ± 0·03*
SR-48,968 + CP-99,994	0·19 ± 0·05*	0·36 ± 0·10*	0·49 ± 0·07*	0·73 ± 0·23*

n = 5–11 in each panel. *$P < 0·05$ compared with the control (5 Hz) group.

With regard to plasma extravasation in the guinea-pig airways, we have shown previously that CP-99,994 and SR-48,968, at the doses used in this study, completely block NK_1- and NK_2-mediated responses, respectively (Tousignant et al 1993a, b). In contrast to the general view, we have demonstrated that plasma extravasation in the guinea-pig is mediated by both NK_1 and NK_2 receptors. The smaller NK_2 component is manifested mainly in the lower part of the airways and is revealed only with the use of the sensitive permeability marker [^{125}I]fibrinogen. In the present study, the results obtained with vagal stimulation are entirely consistent with our previous observation. Thus, antagonism of NK_2 receptors by SR-48,968 had no effect on plasma extravasation in the trachea (where extravasation is mediated via NK_1 receptors only), but it reduced responses significantly in the main bronchi and maximally in the distal airways. In contrast, the NK_1 antagonist CP-99,994 reduced the responses maximally in the trachea and somewhat less in the other parts of the airways. Administered in combination, SR-48,968 and CP-99,994 completely inhibited plasma extravasation in the main bronchi and the distal airways, which was in accord with their effect on bronchoconstriction. In the oesophagus, vagal stimulation-induced plasma leakage was not affected by SR-48,968 but was abolished by CP-99,994, suggesting that extravasation in this tissue is mediated solely by NK_1 receptors.

In conclusion, the present findings support the view that both NK_1 and NK_2 receptors are involved in the bronchoconstrictor and plasma extravasation responses induced by tachykinins in guinea-pig airways (Martin et al 1992; Foulon et al 1993; McKee et al 1993; Sakamoto et al 1993; Solway et al 1993; Tousignant et al 1993a, b; Chan et al 1994). Thus, by implication, if neurogenic inflammation caused by aberrant activity of the NANC pathway plays a role in airway diseases such as asthma, then pharmacological antagonism of both neurokinin receptor subtypes may be necessary in order to achieve therapeutic efficacy. Such a conclusion is derived from studies in small laboratory animals, notably guinea-pigs and rats, and remains to be confirmed in man. In this context, a peptide antagonist of both NK_1 and NK_2 receptors, FK-224, has been shown to block bronchospasm induced by inhalation of bradykinin (which stimulates sensory c-fibres) in asthmatic subjects (Ichinose et al 1992). Development of more potent, preferably non-peptide, NK_1/NK_2 antagonists will ultimately help to clearly define the roles of tachykinins in asthma.

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