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Evidence that tachykinins relax the guinea-pig trachea *via* nitric oxide release and by stimulation of a septide-insensitive NK_1 receptor

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1 This study investigated the possibility that tachykinins relax the guinea-pig isolated trachea by releasing nitric oxide (NO) from the epithelium. The types of tachykinin receptor mediating both relaxation and contraction of the trachea were also studied. Isometric tension was recorded in isolated tracheal tube preparations precontracted with acetylcholine (10 μM) in which compounds were administered intraluminally in the presence of phosphoramidon and indomethacin (both 1 μM) and the tachykinin NK_2 receptor antagonist, SR 48,968 ((S)-N-methyl-N[4-(4-acetyl amino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide), 0.1 μM).

2 In the presence of the inactive enantiomer of an NO-synthase inhibitor, N^G-monomethyl-D-arginine (D-NMMA, 100 μM), substance P (SP), neurokinin A (NKA), neurokinin B (NKB) and the selective NK_1 receptor agonist, [Sar^9, $Met(O_2)^{11}$]-SP, (0.1–10 nM) relaxed tracheal tube preparations. This relaxation was changed into a contraction by pretreatment with the NO-synthase inhibitor, N^G-monomethyl-L-arginine (L-NMMA, 100 μM). The effect of L-NMMA on SP- and [Sar^9, $Met(O_2)^{11}$]-SP-induced responses was reversed by L-arginine (L-Arg, 1 mM), but not by D-Arg (1 mM). After removal of the epithelium SP, NKA and NKB and [Sar^9, $Met(O_2)^{11}$]-SP (0.1–10 nM) evoked contractile responses in the presence of either L-NMMA (100 μM) or D-NMMA (100 μM). The effects of SP and [Sar^9, $Met(O_2)^{11}$]-SP obtained in the presence of another NO-synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) or its inactive enantiomer, N^G-nitro-D-arginine methyl ester (D-NAME, 100 μM) were similar to those observed with L-NMMA or D-NMMA, respectively.

3 The selective NK_1 receptor agonist, [$pGlu^6$, Pro^9]-SP(6-11) (septide, 0.1–10 nM) evoked contractile responses of tracheal tube preparations in the presence of either D-NMMA (100 μM) or L-NMMA (100 μM). The log concentration-response curve to septide obtained in the presence of L-NMMA was similar to that obtained in the presence of D-NMMA. [Sar^9, $Met(O_2)^{11}$]-SP (0.1–10 nM) relaxed tracheal tube preparations precontracted with septide (1 μM), whereas septide (0.1 nM–1 μM) further contracted tracheal tube preparations precontracted with [Sar^9, $Met(O_2)^{11}$]-SP (1 μM).

4 Relaxant and contractile responses evoked by SP, NKA, NKB and by [Sar^9, $Met(O_2)^{11}$]-SP (0.1–10 nM) were not affected by a combination of the histamine H_1 (pyrilamine, 1 μM) and H_2 (cimetidine, 1 μM) receptor antagonists, but were abolished by the tachykinin NK_1 receptor antagonist, CP-99,994 ((2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine, 1 μM), though not by its inactive enantiomer CP-100,263 (1 μM). Contractile responses evoked by septide (10 nM and 1 μM) were also abolished by CP-99,994 (1 μM) but not by CP-100,263 (1 μM).

5 These results demonstrate that tachykinins relax guinea-pig tracheal tube preparations by releasing NO *via* the stimulation of epithelial NK_1 receptors by a mechanism independent of histamine release. The NK_1 receptor type involved is sensitive to SP, NKA, NKB and [Sar^9, $Met(O_2)^{11}$]-SP but not to septide, and is pharmacologically distinct from the NK_1 receptor that mediates contraction, which is stimulated by all the agonists, including septide.

Keywords: Tachykinin NK_1 receptor; nitric oxide; trachea; airway epithelium; relaxation; septide; substance P; neurokinin

Introduction

The mammalian tachykinins substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) are members of a family of structurally related peptides, which exert their effects by activating three distinct receptor types, NK_1, NK_2 and NK_3 receptors (Maggi *et al.*, 1993b; Otsuka & Yoshioka, 1993; Regoli *et al.*, 1994). Tachykinins produce a variety of responses in the airways of various mammals, including man, by stimulation of NK_1 and NK_2 receptors (Barnes *et al.*, 1991; Joos *et al.*, 1994). In particular, tachykinins are amongst the most potent spasmogens of the airways smooth muscle (Barnes *et al.*, 1991; Joos *et al.*, 1994), and although in most species this effect is due to NK_2 receptor activation, in guinea-pigs, NK_1 receptors are also involved (Bertrand *et al.*, 1993). Recently, pharmacological evidence for heterogeneity of tachykinin NK_1 receptors has been reported in guinea-pig ileum (Petitet *et al.*, 1992; Chassaing *et al.*, 1992), where the selective tachykinin NK_1 receptor agonist, [$pGlu^6$, Pro^9]-SP(6-11) (septide) (Laufer *et al.*, 1986), was proposed to act on a new type of tachykinin NK_1 receptor. Further evidence for differences between the 'septide-selective' and 'classical' tachykinin NK_1 receptors has been

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obtained in several rat, rabbit and guinea-pig tissues (Hall *et al.*, 1994; Meini *et al.*, 1994; Zeng & Burcher, 1994).

In guinea-pig bronchus Zeng & Burcher (1994) showed that the pA_2 values of several nonpeptide tachykinin NK_1 receptor antagonists differed, depending on whether septide or other selective NK_1 receptor agonists, ([Sar^9, Met(O$_2$)^{11}]-SP or [Pro^9]-SP), were used, suggesting receptor heterogeneity. In addition, removal of the epithelium increased the potencies of [Sar^9, Met(O$_2$)^{11}]-SP or [Pro^9]-SP, but not that of septide. Since these experiments (Zeng & Burcher, 1994) were performed in the presence of indomethacin and phosphoramidon, the epithelial factor(s) that increased the potency of [Sar^9, Met(O$_2$)^{11}]-SP or [Pro^9]-SP seems unlikely to be a prostanoid or neutral endopeptidase (NEP,E.C.3.4.24.11). Evidence has been provided for the existence, in the epithelium of the rodent airways, including the guinea-pig (Frossard *et al.*, 1989), of tachykinin receptors that relax the tracheobronchial smooth muscle *via* the release of prostanoids, and that these receptors belong to the NK_1 type. Recent findings indicate that histamine (Nijkamp *et al.*, 1993) and bradykinin (Ricciardolo *et al.*, 1994; Schempler & Calixto, 1994; Figini *et al.*, 1995), in addition to their contractile action, may also relax guinea-pig airways *via* nitric oxide (NO) release from the epithelium.

The aims of the present study were two fold: firstly, to determine whether the tachykinin-evoked relaxation of the guinea-pig isolated trachea results from stimulation of an epithelial tachykinin receptor and subsequent release of NO; secondly, to investigate whether this tachykinin receptor is pharmacologically distinct from the NK_1 receptor that mediates the contraction. The motor response to naturally-occurring mammalian tachykinins and to selective NK_1 receptor agonists was studied in guinea-pig isolated tracheal tube preparations perfused through the lumen. This system was chosen because it was used successfully to show that histamine (Nijkamp *et al.*, 1993) and bradykinin (Figini *et al.*, 1995) counteract their own constrictor action by releasing NO from the tracheal epithelium. Experiments were performed in the presence of the tachykinin NK_2 receptor antagonist, SR 48,968 ((S)-N-methyl-N[4-(4-acetyl-amino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) (Emonds-Alt *et al.*, 1992), to exclude any influence of these receptors on the responses to tachykinins.

Methods

Male guinea pigs (350–400 g) were killed with sodium pentobarbitone (80 mg kg^{-1}, i.p.) and the trachea isolated and perfused with a Krebs solution of the following composition (mM): NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, and glucose 8.3. The solution was maintained at 37°C and was aerated continuously by bubbling with a mixture of 95% O_2-5% CO_2, which maintained a pH of 7.4. The experiments were performed with apparatus and techniques similar to those reported previously (Nijkamp *et al.*, 1993). Briefly, proximal ends of the trachea were used for the experiments. An organ bath was constructed that permitted independent circulation of fluid within the lumen of the tracheal segment or around the exterior of the tracheal segment. Two hooks were passed through the trachea wall around two adjacent cartilaginous rings as close as possible to the muscle. The lower hook was fixed; the upper hook was connected to an isometric transducer (Basile, Italy). The tracheal tension was set at an optimal counterweight of 2 g. The inside of the trachea was perfused with Krebs solution at a constant flow rate of 2 ml min^{-1} with a peristaltic pump. All drugs were administered intraluminally. Every 15 min the Krebs buffer was changed on both sides for 90 min. Thereafter, the intraluminal side of the trachea was perfused for 30 min with N^G-nitro-L-arginine methyl ester (L-NAME, 100 μM) or N^G-monomethyl-L-arginine (L-NMMA) and N^G-nitro-D-arginine methyl ester (D-NAME, 100 μM) or N^G-monomethyl-D-arginine (D-NMMA). In some experiments L-arginine (L-Arg, 1 mM) or D-Arg (1 mM) were co-incubated with L-NMMA.

Tracheal tubes were precontracted with intraluminal perfusion of acetylcholine (ACh, 10 μM). The contraction in response to ACh remained stable for at least 25 min. As soon as a stable contraction to ACh was obtained, tachykinin NK_1 receptor agonists (0.1 nM–1 μM) were added intraluminally. In each trachea only one challenge with ACh tone was performed. Cumulative agonist concentration-response curves were constructed by adding increasing concentrations of the agonists as soon as a plateau to the previous concentration was reached. In some experiments tracheal tube preparations precontracted with septide (1 μM) received increasing concentrations of [Sar^9, Met(O$_2$)^{11}]-SP (0.1 nM–1 μM), or tracheae precontracted with [Sar^9, Met(O$_2$)^{11}]-SP (1 μM) received increasing concentrations of septide (0.1 nM–1 μM). In experiments in which antagonists were used these were added intraluminally 15 before ACh administration.

In a separate set of experiments the epithelial layer was removed by a cotton swab (Nijkamp *et al.*, 1993). To verify that the tissues were denuded of epithelium, histological examinations were performed. The tissues were fixed by immersion in formaldehyde (4%) and embedded in paraffin blocks. Sections measuring 5 μm were cut and stained with haematoxylin and eosin for histological evaluation. Histological examination showed that the epithelial layer was completely removed, whereas no damage was observed to the lamina propria (data not shown).

Drugs

L-Arg, D-Arg, D-NMMA, L-NMMA, D-NAME, L-NAME, acetylcholine, cimetidine, pyrilamine, indomethacin and sodium nitroprusside were obtained from Sigma Chemical (U.S.A.). SP, NKA, NKB, septide ([pGlu^6, Pro^9]-SP(6-11)) and [Sar^9, Met(O$_2$)^{11}]-SP were purchased from Bachem (Switzerland). Phosphoramidon was obtained from Peninsula (U.S.A.). CP-99,994 ((2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine) and its inactive enantiomer, CP-100,263, were kind gifts from Dr J.A. Lowe III (Pfizer Inc. U.S.A.). SR 48,968 was a gift of Dr X. Emonds-Alt (Sanofi Recherche, France). All peptides and SR 48,968 were dissolved in dimethylsulphoxide. All the other drugs were dissolved in 0.9% saline. Stock solution of peptides were stored at −20°C. L-Arg, D-Arg, L-NAME, D-NAME, acetylcholine, sodium nitroprusside and tachykinin and histamine receptor antagonists were freshly prepared for each experiment.

Statistical analysis

Values in the text and figures are the mean ± s.e.mean. Statistical comparisons were performed with Student's t test for unpaired values. In all cases, a P value of less than 0.05 was considered significant.

Results

Naturally-occurring tachykinins

Addition of the selective tachykinin NK_2 receptor antagonist, SR 48,968 (0.1 μM), and the NEP inhibitor, phosphoramidon (1 μM), through the lumen did not affect the tone of isolated guinea-pig tracheal tube preparations (data not shown). After the addition of D-NAME (100 μM for 30 min) or D-NMMA (100 μM for 30 min) no appreciable change in tone was observed (data not shown). However, after the addition of L-NAME (100 μM for 30 min) or L-NMMA (100 μM for 30 min) tracheal tube preparations developed a sustained increase in tone that reached a maximum at 20 min (183 ± 21 mg, $n=5$, and 204 ± 27 mg, $n=5$ over baseline, respectively). Contractions induced by ACh (10 μM) were similar in preparations pretreated with D-NMMA (456 ± 71 mg, $n=6$) or L-NMMA (523 ± 63 mg, $n=6$, $P>0.05$). Similarly, contractions produced

by ACh were not affected by pretreatment with D-NAME (421±56, $n=6$) or L-NAME (493±50, $n=6$, $P>0.05$). In tracheal tube preparations precontracted with ACh in the presence of D-NMMA, addition of increasing concentrations of SP (0.1–10 nM) caused a moderate, concentration-related relaxation. A higher concentration of SP (0.1 μM) either caused no further change of tracheal tone or evoked a moderate contraction, and 1 μM SP induced a marked contraction (Figures 1 and 2a). When the same series of experiments were performed in the presence of L-NMMA, SP caused a concentration-dependent contraction at all the concentrations tested (Figures 1 and 2a). Pretreatment with L-Arg (1 mM, 30 min), but not D-Arg (1 mM, 30 min) in the presence of L-NMMA (100 μM, 30 min) reversed the contractile response to SP into a relaxation (Figure 2b). Similar results were obtained with NKA and NKB ($n=4$ each, data not shown).

Selective tachykinin NK_1 receptor agonists

In the presence of D-NMMA (100 μM, 30 min) the selective tachykinin NK_1 receptor agonist [Sar^9, $Met(O_2)^{11}$]-SP (0.1–10 nM) caused a moderate, concentration-dependent relaxation in tracheal tubes precontracted with ACh (10 μM), whereas higher concentrations of peptide caused either no change in tracheal tone or a moderate contraction (0.1 μM) or a marked contraction (1 μM) (Figures 1 and 3a). In the presence of L-NMMA (100 μM, 30 min) [Sar^9, $Met(O_2)^{11}$]-SP (0.1 nM–1 μM) caused a concentration-dependent contraction

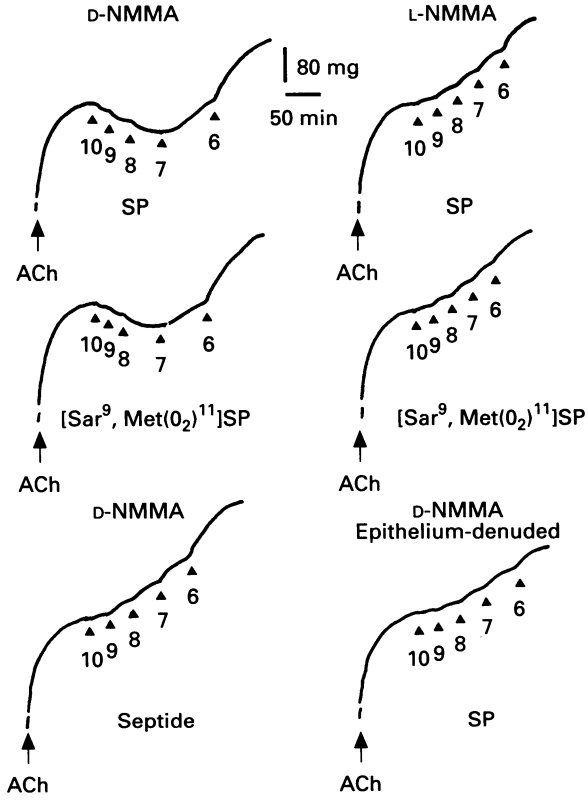

Figure 1 Typical original tracings of the motor response to substance P (SP), [Sar^9, $Met(O_2)^{11}$]-SP and [$pGlu^6$, Pro^9]-$SP_{(6-11)}$ (septide) in guinea-pig isolated tracheal tube preparations precontracted with acetylcholine (ACh, 10 μM). Experiments were performed in the presence of indomethacin (1 μM), phosphoramidon (1 μM) and SR48,968 (0.1 μM). The nitric oxide synthase inhibitor, L-NMMA (100 μM) or its inactive enantiomer, D-NMMA (100 μM) were applied 30 min before tachykinin challenge. All the drugs were applied intraluminally. Peptide agonists were applied as indicated. Similar tracings to those illustrated were obtained in at least 5 separate experiments.

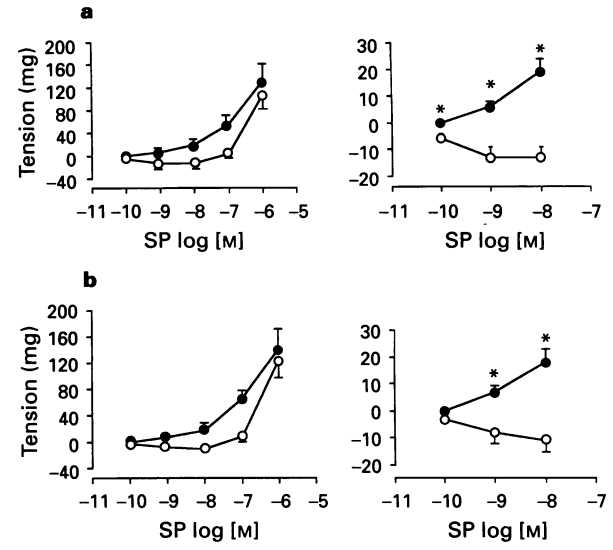

Figure 2 Involvement of nitric oxide in the motor response to substance P (SP) in guinea-pig isolated tracheal tube preparations precontracted with acetylcholine (ACh, 10 μM). In (a), the effect of D-NMMA (100 μM; ○) or L-NMMA (100 μM; ●) on the motor response to substance P. *$P<0.05$ vs D-NMMA. In (b), the effect of D-Arg (1 mM; ●) or L-Arg (1 mM; ○) on the motor response to SP in the presence of L-NMMA (100 μM). *$P<0.05$ vs D-Arg. Experiments were performed in the presence of indomethacin (1 μM), phosphoramidon (1 μM) and SR48,968 (0.1 μM) and data are shown as mean±s.e.mean for 6 experiments. The right hand panels show data obtained with the lowest concentrations of the agonist, on an expanded scale.

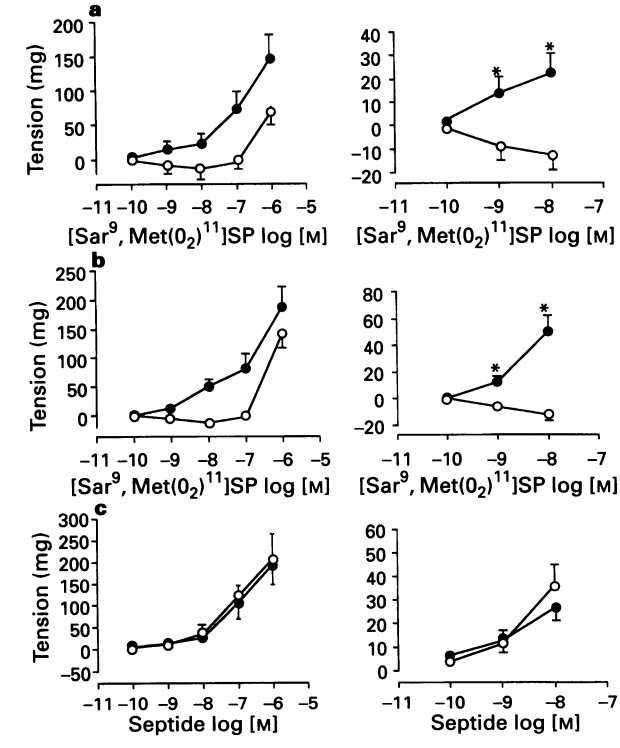

Figure 3 Involvement of nitric oxide on the motor response to [Sar^9, $Met(O_2)^{11}$]-SP and [$pGlu^6$, Pro^9]-SP(6-11) (septide) in guinea-pig isolated tracheal tube preparations precontracted with acetylcholine (ACh, 10 μM). Effect of D-NMMA (100 μM; ○) or L-NMMA (100 μM; ●) on the motor response to [Sar^9, $Met(O_2)^{11}$]-SP (a) or septide (c). $P<0.05$ vs D-NMMA. In (b), the effect of D-Arg (1 mM); ○) or L-Arg (1 mM; ●) on the motor response to [Sar^9, $Met(O_2)^{11}$]-SP in the presence of L-NMMA (100 μM). Experiments were performed in the presence of indomethacin (1 μM), phosphoramidon (1 μM) and SR48,968 (0.1 μM) and data are shown as mean±s.e.mean for 6 (a and c) or (b) experiments. The right hand panels show data obtained with the lowest concentrations of the agonist, on an expanded scale.

at all the concentrations tested (Figures 1 and 3a). Similar results were obtained with L-NAME and D-NAME ($n=4$ each, data not shown). Pretreatment with L-Arg (1 mM for 30 min) but not D-Arg (1 mM for 30 min) reversed the contraction produced by [Sar^9, Met(O$_2$)^{11}]-SP (0.1 – 10 nM) in the presence of L-NMMA into a relaxation (Figure 3b). In contrast to [Sar^9, Met(O$_2$)^{11}]-SP, septide (0.1 nM – 1 μM) caused a concentration-related contraction at all the concentrations tested either in the presence of D-NMMA or L-NMMA (Figures 1 and 3c). Very low concentrations of septide (1 – 10 pM) evoked either no affect or a moderate contraction ($n=4$, data not shown). In another series of experiments, in preparations precontracted with septide (1 μM) and in the presence of D-NMMA, [Sar^9, Met(O$_2$)^{11}]-SP, (0.1 – 10 nM) caused a concentration-dependent relaxation, whereas in the presence of L-NMMA [Sar^9, Met(O$_2$)^{11}]-SP, (0.1 – 10 nM) induced a contraction (Figure 4a). In contrast, preparations precontracted with 1 μM [Sar^9, Met(O$_2$)^{11}]-SP responded to septide (0.1 – 10 nM) with a concentration-dependent contraction either in the presence of D-NMMA or L-NMMA (Figure 4b).

Epithelium removal, CP-99,994, pyrilamine and cimetidine

In epithelium-denuded tracheal tubes, ACh (10 μM) caused a contraction (492 ± 62 mg, $n=6$) that was similar to the contraction evoked in epithelium-intact preparations (456 ± 71, $n=6$, $P>0.05$). After removal of the epithelium SP (Figure 1 and 5a) or [Sar^9, Met(O$_2$)^{11}]-SP (Figure 5b) (0.1 nM – 1 μM) caused a concentration-related contraction at all the concentrations tested both in the presence of D-NMMA (100 μM, 30 min) and L-NMMA (100 μM, 30 min). Addition of the selective tachykinin NK_1 receptor antagonist, CP-99,994 (1 μM) and the inactive enantiomer, CP-100,263 (1 μM) had no effect on baseline tension (data not shown). In epithelium-intact preparations and in the presence of D-NMMA, pretreatment with CP-99,994 (1 μM), but not with the inactive enantiomer, CP-100,263 (1 μM), abolished the relaxation induced by SP, NKA, NKB or [Sar^9, Met(O$_2$)^{11}]-SP (all 10 nM) (Figure 6a). CP-99,994 also abolished contractile responses evoked by septide (10 nM) (Figure 6a). Contractile responses induced by SP, NKA, NKB, [Sar^9, Met(O$_2$)^{11}]-SP and septide (all 1 μM) were significantly inhibited by CP-99,994 (1 μM), but not by CP-100,263 (1 μM) (Figure 6b). The relaxation produced by sodium nitroprusside (100 μM, 232 ± 35 mg, $n=5$) in tracheal tubes precontracted with ACh (10 μM) was not affected by CP-99,994 (1 μM) (243 ± 31 mg, $n=5$, $P>0.05$, data not shown). Pretreatment with a combination of the histamine H_1 antagonist, pyrilamine (1 μM) and H_2 antagonist, cimetidine (1 μM) did not affect the motor response induced in tracheal tube preparations by SP or [Sar^9, Met(O$_2$)^{11}]-SP ($n=5$, data not shown).

Discussion

The major finding of the present paper is the demonstration of a tachykinin NK_1 receptor that mediates relaxation of the trachea *via* NO release, and the observation that this NK_1 receptor is apparently not stimulated by the NK_1 receptor selective agonist, septide.

Tachykinin NK_1 receptors release NO from the tracheal epithelium

Naturally-occurring tachykinins, and the tachykinin NK_1-selective agonist, [Sar^9, Met(O$_2$)^{11}]-SP, relaxed tracheal tube preparations in a concentration-related manner. The observation that the presence of NO-synthase inhibitors changed the relaxation produced in tracheal tube preparations evoked by low concentrations of SP, NKA, NKB and [Sar^9, Met(O$_2$)^{11}]-SP, into a contraction suggests that tachykinins release NO, or a molecule related to NO, which relaxes the tracheal smooth muscle. The observation that the effect of NO-synthase inhibitors was reversed by L-Arg, but not by D-Arg further supports this hypothesis. Release of NO which counteracts the contractile effect of histamine (Nijkamp *et al.*, 1993)

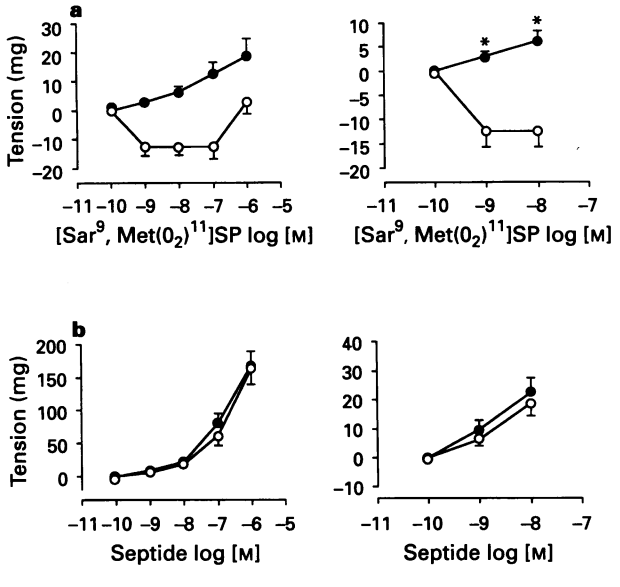

Figure 4 Effect of the nitric oxide synthase inhibitor, L-NMMA, on the motor response to [Sar^9, Met(O$_2$)^{11}]-SP and [pGlu^6, Pro^9]-SP$_{(6-11)}$ (septide) in guinea-pig isolated tracheal tube preparations precontracted with septide or [Sar^9, Met(O$_2$)^{11}]-SP, respectively. In (a), responses to [Sar^9, Met(O$_2$)^{11}]-SP were obtained in preparations precontracted with septide and in (b), responses to septide were obtained in preparations precontracted with [Sar^9, Met(O$_2$)^{11}]-SP. Responses were obtained in the presence of D-NMMA (100 μM; ○) or L-NMMA (100 μM; ●). *$P<0.05$ vs D-NMMA. Experiments were performed in the presence of indomethacin (1 μM), phosphoramidon (1 μM) and SR48,968 (0.1 μM) and data are shown as mean ± s.e.mean for 5 experiments. The right hand panels show data obtained with the lowest concentrations of the agonist, on an expanded scale.

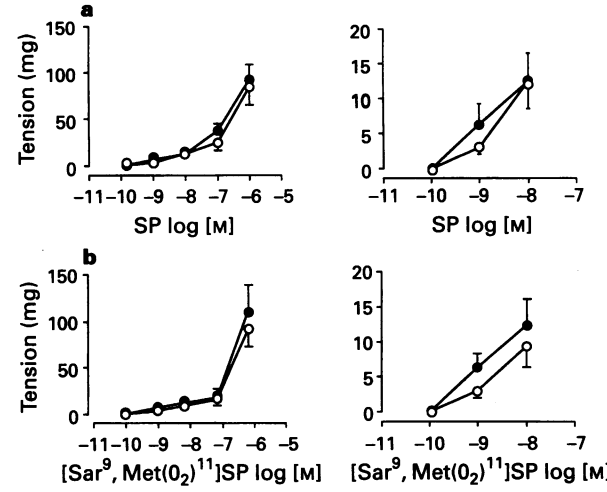

Figure 5 Effect of the nitric oxide synthase inhibitor, L-NMMA, on the motor response to substance P (SP) and [Sar^9, Met(O$_2$)^{11}]-SP in epithelium-denuded tracheal tube preparations precontracted with acetylcholine (ACh; 10 μM). Responses to substance P (a) or [Sar^9, Met(O$_2$)^{11}]-SP (b) were obtained either in the presence of L-NMMA (100 μM; ●) or D-NMMA (100 μM; ○). Experiments were performed in the presence of indomethacin (1 μM), phosphoramidon (1 μM) and SR48,968 (0.1 μM) and data are shown as mean ± s.e.mean for 5 experiments. The right hand panels show data obtained with the lowest concentrations of the agonist, on an expanded scale.

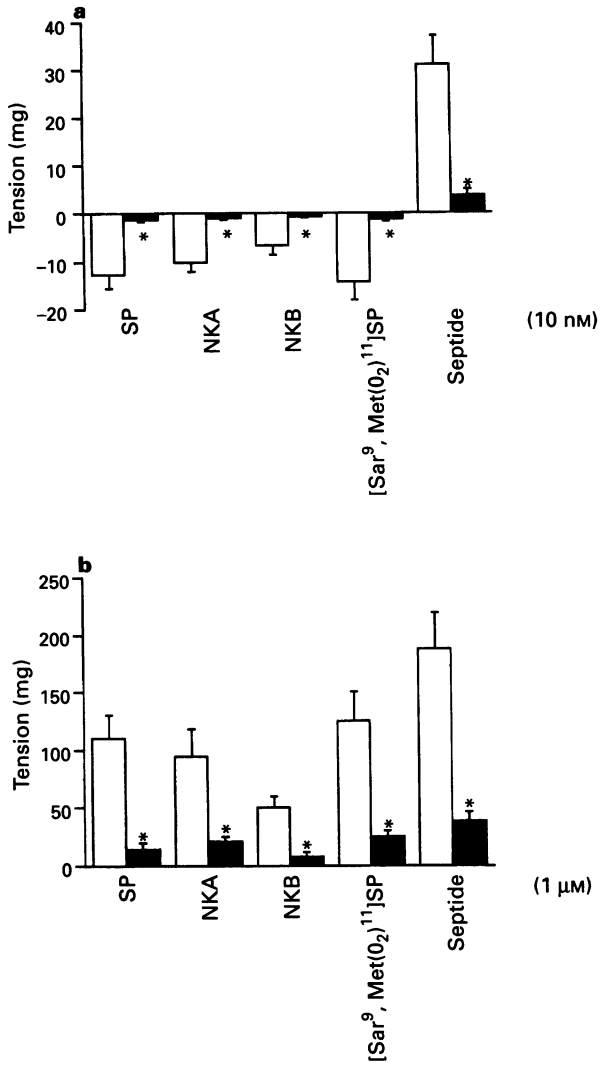

Figure 6 Effect of the NK_1 receptor antagonist, CP-99,994, on the motor response to tachykinins and analogues in guinea-pig isolated tracheal tube preparations precontracted with acetylcholine (ACh 10 μM). Motor responses to 10 nM (a) and 1 μM (b) substance P (SP), neurokinin A (NKA), neurokinin B (NKB), [Sar^9, $Met(O_2)^{11}$]-SP and [$pGlu^6$, Pro^9]-SP(6-11) (septide) were obtained either in the presence of CP-99,994 (1 μM; solid columns) or its enantiomer CP-100,263 (1 μM; open columns). $^*P < 0.01$ vs CP-100, 263. Experiments were performed in the presence of indomethacin (1 μM), phosphoramidon (1 μM) and SR48,968 (0.1 μM) and data are shown as mean ± s.e.mean for 5 experiments.

or bradykinin (Schempler & Calixto, 1994; Figini *et al.*, 1995) has previously been shown in guinea-pig tracheal tube preparations and bronchial rings. Altogether these findings indicate that in the guinea-pig airways the motor effect of several autacoids or neurotransmitters, including tachykinins, results from the opposing forces that they stimulate: a direct or indirect contraction of the smooth muscle and an indirect epithelium-dependent and NO-mediated relaxation. There is evidence that the loss of the NO-mediated relaxation is the basis for the hyperesponsiveness to histamine after virus infection of the guinea-pig airways (Folkerts *et al.*, 1995). However, the physiological and pathophysiological relevance of this mechanism is, at present, unknown.

Since the selective tachykinin NK_1 receptor antagonist, CP-99,994 (Mclean *et al.*, 1993), abolished the relaxation produced by SP, NKA, NKB and [Sar^9, $Met(O_2)^{11}$]-SP, we suggest that the receptor involved in the tachykinin-mediated NO release is of the NK_1 type. We discard the possibility that non specific action of CP-99,994, including the Ca^{2+} antagonist activity (Mclean *et al.*, 1993), may be involved in the inhibition of tachykinin-induced relaxation since an identical concentration of the enantiomer, CP-100,263, that does not interact with the NK_1 receptor, was completely inactive, and since CP-99,994 did not inhibit relaxant responses to sodium nitroprusside. Finally, the possible role of tachykinin NK_2 receptors in the tachykinin-induced NO-mediated relaxation of the guinea-pig trachea is excluded because of the presence of SR 48,968, a high affinity antagonist of these receptors (Emonds-Alt *et al.*, 1992). Since histamine-induced contraction of tracheal tube is markedly potentiated by NO synthase inhibitors (Nijkamp *et al.*, 1993), the hypothesis may be advanced that relaxation in response to SP or [Sar^9, $Met(O_2)^{11}$]-SP is due to the known ability of these peptides to release histamine from mast cells (Mousli *et al.*, 1990). Four different observations exclude this possibility. Thus the relaxant effect of SP or [Sar^9, $Met(O_2)^{11}$]-SP was: (a) observed at low peptide concentrations, that usually are not sufficient to release histamine (Mousli *et al.*, 1990); (b) blocked by the selective NK_1 receptor antagonist, CP-99,994, whereas histamine release from mast cells is generally considered to result from a non-receptor mediated mechanism; (c) evoked by NKA and NKB that do not release histamine from mast cells (Devillier *et al.*, 1985); (d) not affected by histamine H_1 and H_2 receptor antagonists.

Removal of the epithelium converted the relaxation in response to SP, NKA, NKB and [Sar^9, $Met(O_2)^{11}$]-SP into a contraction. Therefore, it is likely that the NO released by tachykinins derives from the epithelium. An epithelial origin for the NO released by histamine (Nijkamp *et al.*, 1993) and bradykinin (Figini *et al.*, 1995; Schempler & Calixto, 1994) has been shown previously. The presence of both constitutive and inducible NO-synthase has been described in the airway epithelium of various mammals, including guinea-pigs (Fischer *et al.*, 1993). Previously, the presence of epithelial tachykinin NK_1 receptors that relax the guinea-pig airways *via* release of prostaglandins has been proposed (Frossard *et al.*, 1989). However, this is the first direct demonstration of the existence of an epithelial NK_1 receptor in the guinea-pig airways that relaxes the airway smooth muscle *via* NO release.

Possible tachykinin NK_1 receptor subtypes in the guinea-pig trachea

The current data suggest that the tachykinin receptor that mediates relaxation is pharmacologically distinct from the tachykinin receptor that mediates contraction. The contractile response appears to be mediated by stimulation of a 'classical' NK_1 receptor type, thus contractile responses were evoked by naturally-occurring tachykinins as well as the NK_1 selective agonists [Sar^9, $Met(O_2)^{11}$]-SP and septide. Furthermore, these contractile responses were inhibited by a low concentration of the NK_1-selective antagonist CP-99,994. In contrast, although relaxant responses were evoked, in a concentration-related manner, by naturally-occurring tachykinins and the NK_1 selective agonist [Sar^9, $Met(O_2)^{11}$]-SP and were inhibited by the NK_1-selective antagonist, CP-99,994, septide was unable to evoke relaxation. This along with the observation that septide (1 pM – 1 μM), in the presence of D-NMMA, did not cause any measurable relaxation of tracheal tube preparations precontracted with ACh, but instead caused a contraction suggests that the NK_1 epithelial receptors mediating relaxation are not stimulated by septide. This hypothesis is supported by the observations that concentration-response curves to septide in the presence of D-NMMA or L-NMMA were similar, and that [Sar^9, $Met(O_2)^{11}$]-SP relaxed tracheae precontracted with septide, whereas septide caused further contraction of tracheal preparations precontracted with [Sar^9, $Met(O_2)^{11}$]-SP. Furthermore, the potency of septide, which is unable to stimulate the relaxant NK_1 epithelial receptor, is unaffected by epithelium denudation. In contrast, the potency of [Sar^9, $Met(O_2)^{11}$]-SP, which stimulates NK_1 receptors which release NO from the epithelium, is increased by the removal of the epithelium.

The existence of 'atypical' tachykinin NK_1 receptors has

previously been reported by several groups (Chassaing *et al.*, 1992; Petitet *et al.*, 1992; Maggi *et al.*, 1993a; Hall *et al.*, 1994; Zheng & Burcher, 1994). For example, Petitet *et al.* (1992) reported pharmacological and radioligand binding data which were interpreted as supporting the existence of novel 'septide-sensitive' tachykinin receptors, which are activated by septide. In contrast, the data reported in the present study indicate the existence of a septide-insensitive receptor mediating relaxation of the guinea-pig trachea. The hypothesis is supported indirectly by previous reported observations; thus, Zeng & Burcher (1994) showed that epithelium removal increased the pD_2 of [Sar^9, $Met(O_2)^{11}$]-SP and [Pro^9]SP, but not of septide in contracting the guinea-pig bronchi.

In order to confirm the existence of two distinct receptor types (septide-insensitive relaxant receptor and 'classical' NK_1 contractile receptor) determination of affinities of selective NK_1 receptor antagonists, such as CP-99,994, is required. However, in view of the fact that naturally-occurring tachykinins and [Sar^9, $Met(O_2)^{11}$]-SP stimulate both the relaxant and the contractile receptors on tracheal tube preparations it is not possible to determine accurately the affinity of CP-99,994 for the relaxant receptor. Indeed, it has been previously shown that various NK_1 receptor antagonists were more potent in inhibiting contractile responses to septide than those induced by other NK_1 receptor agonists, for example [Pro^9]SP or [Sar^9, $Met(O_2)^{11}$]-SP in guinea-pig isolated airways (Longmore *et al.*, 1994; Zeng & Burcher, 1994). This observation may be explained by the fact that the potency of antagonists depends on the relative contribution of contractile and relaxant responses evoked by the individual agonist under study.

Evidence of intraspecies pharmacological heterogeneity of tachykinin NK_1 receptor has been reported, although only one gene encoding for the NK_1 receptor has been found in each species so far. Difference in receptor glycosylation (Burcher *et al.*, 1991) or alternative splicing resulting in different receptor isoforms (Fong *et al.*, 1992) have been proposed as possible explanations of affinity differences of agonists and antagonists. Although it must be emphasized that in guinea-pig only one NK_1 receptor gene has been cloned so far (Gorbulev *et al.*, 1992), a possible explanation of our present data would be the existence of two pharmacologically distinct tachykinin NK_1 receptor subtypes in the guinea-pig trachea. One receptor is stimulated by natural tachykinins, [Sar^9, $Met(O_2)^{11}$]-SP and septide and contracts the smooth muscle, the other receptor is stimulated by natural tachykinins, and [Sar^9, $Met(O_2)^{11}$]-SP, but not by septide, and *via* NO release, relaxes the trachea.

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