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Role of nitric oxide and septide-insensitive NK_1 receptors in bronchoconstriction induced by aerosolised neurokinin A in guinea-pigs

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1 The tachykinin, neurokinin A (NKA), contracts guinea-pig airways both *in vitro* and *in vivo*, preferentially activating smooth muscle NK_2 receptors, although smooth muscle NK_1 receptors may also contribute. *In vitro* evidence suggests that NKA activates epithelial NK_1 receptors, inducing the release of nitric oxide (NO) and subsequent smooth muscle relaxation. A number of selective NK_1 receptor agonists have been reported to activate both smooth muscle and epithelial NK_1 receptors, however septide appears only to activate smooth muscle NK_1 receptors.

2 The aim of the present study was to investigate whether NKA-induced bronchoconstriction in guinea-pigs *in vivo* may be limited by NO release *via* NK_1 receptor activation, and whether selective NK_1 receptor agonists may activate this mechanism differently.

3 Aerosolized NKA caused an increase in total pulmonary resistance (RL) that was markedly reduced by the NK_2 receptor antagonist, SR 48968, and abolished by the combination of SR 48968 and the NK_1 receptor antagonist, CP-99,994. The increase in RL evoked by NKA was potentiated by pretreatment with the NO synthase (NOs) inhibitor, L-NAME, but not by the inactive enantiomer D-NAME. Potentiation by L-NAME of NKA-induced increase in RL was reversed by L-Arginine, but not by D-Arginine.

4 Pretreatment with L-NAME did not affect the increase in RL induced by the selective NK_2 receptor agonist, [β-Ala^8]NKA(4-10), and by the selective NK_1 receptor agonist, septide, whereas it markedly potentiated the increase in RL caused by a different NK_1 selective agonist, [Sar^9,Met(O$_2$)^{11}]SP. Dose-response curves showed that septide was a more potent bronchoconstrictor than [Sar^9,Met(O$_2$)^{11}]SP to cause bronchoconstriction.

5 Pretreatment with the NK_1 receptor antagonist, CP-96,994, abolished the ability of L-NAME to increase bronchoconstriction to aerosolized NKA. Bronchoconstriction to aerosolized NKA was increased by L-NAME, after pretreatment with the NK_3 receptor antagonist, SR 142801.

6 The present study shows that *in vivo* bronchoconstriction in response to the aerosolized naturally occurring tachykinin, NKA, is limited by its own ability to release relaxant NO *via* NK_1 receptor activation. This receptor is apparently insensitive to septide, thus justifying, at least in part, the high potency of septide to cause bronchoconstriction in guinea-pigs.
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Abbreviations: NKA, neurokinin A; NK_1, neurokinin$_1$ receptor; SP, substance P; RL; lung resistance

Introduction

Neurokinin A (NKA) is a decapeptide belonging to the tachykinin family of peptides, that includes also substance P (SP) and neurokinin B (NKB). Tachykinins are co-expressed in and co-released from central and peripheral endings of a subset of primary sensory neurones, characterized also for their sensitivity to capsaicin (Holzer, 1991). Biological functions of tachykinins released from peripheral terminals of sensory neurones are multiple and are mediated by three types of receptors, the NK_1, NK_2 and NK_3 receptors (Regoli *et al*., 1994). NKA and NKB preferentially bind to NK_2 and NK_3 receptors, respectively (Maggi & Schwartz, 1997). However, all the three naturally occurring tachykinins, including NKA, exhibit similar high affinity (in the nM range) for the phylogenetically more ancient tachykinin receptor, the NK_1 receptor (Maggi & Schwartz, 1997).

In the airways tachykinins increase blood flow, plasma extravasation, adhesion of leukocytes to the vascular endothelium and cause secretion from seromucous glands *via* NK_1 receptor activation (Maggi *et al*., 1993; Regoli *et al*., 1994). The powerful contraction of airway smooth muscle induced by tachykinins is, instead, mediated by NK_2 receptors (Emonds-Alt *et al*., 1992). However, in guinea-pigs there is *in vitro* (Maggi *et al*., 1991) and *in vivo* (Bertrand *et al*., 1993; Buckner *et al*., 1993; Boni *et al*., 1994) evidence that NK_1 receptors contribute to the increase in smooth muscle tone induced by endogenously released or exogenously administered tachykinins. The contribution of NK_1 receptors to the modulation of airway motility is not limited to bronchoconstriction, because NK_1 receptors also mediate epithelium- and

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prostanoid-dependent bronchorelaxation in mice (Manzini, 1992) and guinea-pigs (Frossard *et al*., 1989).

More recently, using guinea-pig isolated tracheal tube preparations it has been demonstrated that NK_1 receptors mediate relaxation of tracheal smooth muscle by releasing nitric oxide (NO) from the airway epithelium (Figini *et al*., 1996a; 1997). In tracheal tube preparations naturally occurring tachykinins and certain selective agonists of NK_1 receptors, including [Sar^9,$Met(O_2)^{11}$]SP, activate both NK_1-mediated contraction and relaxation (Figini *et al*., 1996a; 1997). However, other selective NK_1 receptor agonists, including septide, activate only the contractile component of the NK_1 mediated response (Figini *et al*., 1996a; 1997).

The aim of the present study was to investigate whether the ability of NKA to stimulate relaxant NK_1 receptors in guinea-pigs *in vitro* might influence its bronchoconstrictor response *in vivo*. For this purpose selective agonists of NK_2 receptor, [β-Ala^8]NKA(4-10), and NK_1 receptor, [Sar^9,$Met(O_2)^{11}$]SP, were used. Selective non peptide antagonists for NK_1 (CP-96,994) (McLean *et al*., 1993), NK_2 (SR 48968) (Emonds-Alt *et al*., 1992) and NK_3 (SR 142801) (Emonds-Alt *et al*., 1995) receptors were also used. Finally, the ability of septide to stimulate the relaxant NK_1 receptor was studied in *in vivo* conditions. The data indicate that the motor response to NKA in guinea-pig airways *in vivo* results from its ability to stimulate bronchoconstrictor NK_2 and NK_1 receptors and broncho-relaxant septide-insensitive NK_1 receptors.

Methods

Animals

Male Hartley guinea-pigs (Simonsen Laboratories Inc., Gilroy, CA, U.S.A.), weighing 300–350 g at the time of housing, were used in this study. They were kept in a temperature-controlled environment with standard laboratory food and water freely available. All procedures were approved by the local animal care and use committee.

Measurement of total pulmonary resistance (R_L)

Animals were anaesthetized with sodium pentobarbital (45 mg kg^{-1}, intraperitoneally; Antony Product Corp., Arcadia, CA, U.S.A.) and then ventilated artificially through a tracheal cannula, using a constant-volume ventilator (model 683; Harvard Apparatus Co., Inc., South Natick, MA, U.S.A.) at a frequency of 80 breaths min^{-1}. The tidal volume (VT) was adjusted to maintain normal arterial blood gases as described previously (Dusser *et al*., 1988). Airflow was monitored continuously with a pneumotachograph (A. Fleisch, Medical Inc., Richmond, VA, U.S.A.) connected to a differential pressure transducer (model DP45; Validyne Engineering Corp., Northridge, CA, U.S.A.). A fluid-filled polyethylene catheter was introduced into the oesophagus to measure the oesophageal pressure as an approximation of pleural pressure. Intratracheal pressure was measured with a polyethylene catheter inserted into a short tube connecting the trachea cannula to the pneumotachograph. The transpulmonary pressure (defined as the pressure difference between the intratracheal and the oesophageal pressures) was measured with a differential pressure transducer (model DP7; Validyne Engineering Corp.). Output signals representing transpulmonary pressure and airflow were amplified with an amplifier (model CD19; Validyne Engineering Corp.) and recorded on a polygraph recorder (model 1508 B Visicorder; Honeywell, Inc., Denver, CO, U.S.A.). Total pulmonary resistance (RL) was calculated as previously described (Dusser *et al*., 1988). The right jugular vein and the left carotid artery were cannulated to permit administration of drugs and to collect a sample of blood for arterial blood gas measurement, respectively.

Experimental design

Baseline RL remained stable for at least 2 h, and no significant change was produced by aerosol administration (40 breaths) or i.v. injection (1 ml kg^{-1}) of saline (0.9% NaCl) after a stabilization period of 30 min. Aerosols of NKA (10 μM; 40 breaths) were generated from an ultrasonic nebuliser (Pulmo-Sonic model 25, DeVilbiss Co., Somerset, PA, U.S.A.) and were delivered into the airways by the respirator *via* the tracheal cannula (aerosol delivery rate: 0.2 ml min^{-1}; mass median aerodynamic diameter: 1.8 μm). All animals were pretreated with the muscarinic receptor antagonist, atropine (1.4 μmol kg^{-1}, i.v., 15 min before the stimulus) and with the inhibitor of neutral endopeptidase (NEP), phosphoramidon (4.5 μmol kg^{-1}, i.v., 5 min before the stimulus). The tachykinin NK_2 receptor antagonist (SR 48968, 0.3 μmol kg^{-1}, i.v.) and NK_1 (CP-99,994; 2 μmol kg^{-1}, i.v.) were administered 15 and 5 min before the stimulus, respectively.

Dose-dependency of the response to [βAla^8]NKA(4-10), [Sar^9,$Met(O_2)^{11}$]SP and septide, was assessed by constructing dose-response curves. Each curve was obtained by the addition of increasing concentrations of the aerosolized agonist 30 min after that the response to the previous dose had returned to the baseline level.

To deliver the NOs inhibitor, we adopted a protocol used previously (Ricciardolo *et al*., 1994a): guinea-pigs inhaled ten breaths of an aerosol containing L-N^G-nitroarginine methyl ester (L-NAME: 1 mM) or its inactive enantiomer D-N^G-nitroarginine methyl ester (D-NAME: 1 mM). This procedure was repeated every 5 min for 30 min (total 60 breaths). Five minutes after the last inhalation of the NOs inhibitor or the inactive enantiomer, aerosolized NKA (10 μM; 40 breaths), [βAla^8]NKA(4-10) (10 μM; 40 breaths), [Sar^9,$Met(O_2)^{11}$]SP (0.2 mM; 40 breaths) or septide (2 μM; 40 breaths) were given. In some experiments the vehicle of L-NAME or D-NAME (0.9% saline) was given to guinea-pigs following the same procedure adopted for the NO-synthase inhibitors. In another set of experiments, ten breaths of aerosolized L-arginine (L-Arg, 3 mM) or D-arginine (D-Arg: 3 mM), were given every 5 min for 30 min, after the end of L-NAME administration. Five minutes after the last inhalation of L-Arg or its inactive enantiomer aerosolized NKA challenge (10 μM; 40 breaths) was given. As shown previously (Ricciardolo *et al*., 1994a) aerosol administration of the doses of L-NAME, D-NAME, L-Arg or D-Arg used in this study did not affect cardiovascular parameters (data not shown).

Drugs

Phosphoramidon was purchased from Peninsula Laboratories Inc. (Belmont, CA, U.S.A.). NKA, [βAla^8]NKA 4-10), [Sar^9,$Met(O_2)^{11}$]SP and septide ([$pGlu^6$,Pro^9]SP(6-11)) were purchased from Bachem (Switzerland). Atropine, L-NAME, D-NAME, L-Arg and D-Arg were obtained from Sigma Chemical (St. Louis, MO, U.S.A.). CP-99,994 (+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine) was a generous gift of Dr J.A. Lowe III (Pfizer Inc., Groton, CT, U.S.A.). SR 48968, {(S)-N-methyl-N-[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl) butyl] benzamide} and SR

142801, (S)-(N)-1-(3-(1-benzoyl-3-(3,4-dichlorophenyl)piperidin-3-yl)propryl)-4-phenilpiperidin-4-yl)-N-methylacetamide, were kindly provided by Dr X. Emonds-Alt (Sanofi Recherche, Montpellier, France). Drugs were dissolved in 0.9% saline with the exception of SR 48968, [βAla^8]NKA (4-10) and septide that were dissolved in dimethyl sulphoxide (10 mM). Further dilutions were made in 0.9% saline. SR 142801 was dissolved in ethanol and saline (Daoui *et al*., 1998). All drugs were freshly prepared each time.

Statistical analysis

Values in the text and figures are mean ± standard error of the mean (s.e.mean) from at least five experiments. Statistical comparisons were performed using a one-way analysis of variance and Dunnett's test or bilateral unpaired Student's *t*-tests, when appropriate. In all cases, a P value of less than 0.05 was considered significant.

Results

Bronchoconstriction by NKA

In guinea-pigs pretreated with atropine (1.4 μmol kg^{-1}, i.v.) and phosphoramidon (4.5 μmol kg^{-1}, i.v.) baseline RL was 0.18 ± 0.02 cmH$_2$O ml^{-1} s^{-1} ($n=12$). Aerosolization of 0.9% saline (40 breaths) did not change the baseline value of RL (data not shown). The combination of the vehicles of SR 48968 and CP-99,994 did neither significantly change baseline value of RL (Figure 1) nor affect bronchoconstriction caused by inhalation of NKA (10 μM; 40 breaths) (data not shown). Aerosolized NKA (10 μM; 40 breaths) significantly increased RL to 0.46 ± 0.04 cmH$_2$O ml^{-1} s^{-1} ($P<0.05$). Pretreatment with SR 48968 (0.3 μmol kg^{-1}, i.v.) markedly reduced the increase in RL induced by NKA (Figure 1). The increase in RL caused by NKA was completely abolished by the combination of SR 48968 (0.3 μmol kg^{-1} i.v.) and CP-99,994 (2 μmol kg^{-1}, i.v.) (Figure 1).

Aerosolized L-NAME (1 mM) or D-NAME (1 mM) did not change baseline RL value (data not shown). Pretreatment with aerosolized L-NAME significantly increased NKA-induced bronchoconstriction (0.87 ± 0.07 cmH$_2$O ml^{-1} s^{-1}, $n=6$) in comparison with the effect obtained in animals pretreated with the inactive enantiomer, D-NAME (0.44 ± 0.04 cmH$_2$O ml^{-1} s^{-1}, $n=6$, $P<0.05$) (Figure 2). Aerosolization of D-Arg (3 mM) or L-Arg (3 mM) did not change baseline value of RL (data not shown). Pretreatment with L-Arg (3 mM), but not with D-Arg (3 mM), reversed the potentiation caused by L-NAME on NKA-induced bronchoconstriction (Figure 2).

Studies with tachykinin receptor agonists

Aerosolization of the vehicles of [βAla^8]NKA (4-10) or septide (10% dimethyl sulphoxide in 0.9% saline) (40 breaths) did not change baseline RL value (data not shown). Aerosolized of increasing doses of [βAla^8]NKA (4-10) (40 breaths), septide (40 breaths) or [Sar^9,Met(O$_2$)^{11}]SP (40 breaths) induced a dose-dependent increase in RL (Figure 3).

Pretreatment with aerosolized D-NAME did not affect the increase in RL induced by [Sar^9,Met(O$_2$)^{11}]SP (0.2 mM), septide (2 μM) or [βAla^8]NKA (4-10) (10 μM) (Figure 4). However, pretreatment with L-NAME (1 mM) markedly potentiated the increase in RL caused by aerosolized [Sar^9,Met(O$_2$)^{11}]SP (0.2 mM: 40 breaths), but not the increase in RL produced by aerosolized [βAla^8]NKA (4-10) (10 μM) or septide (2 μM) (Figure 4).

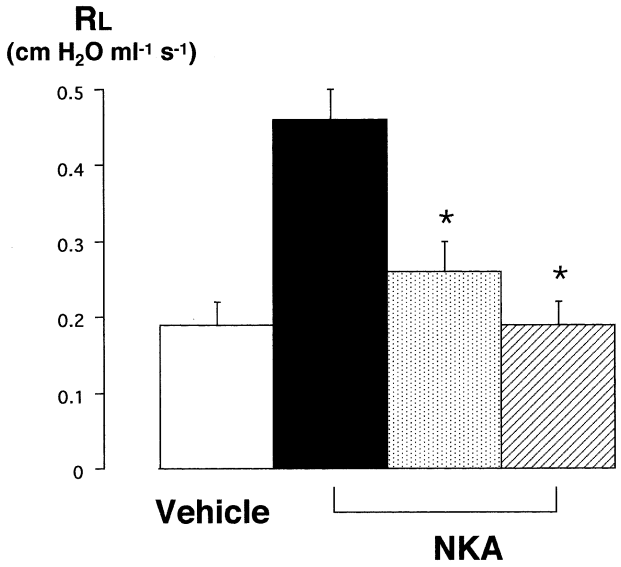

Figure 1 Effect of the tachykinin NK_2 receptor antagonist, SR 48968 (0.3 μmol kg^{-1}, i.v., dotted column), or the combination of SR 48968 and the tachykinin NK_1 receptor antagonist, CP-99,994 (2 μmol kg^{-1}; hatched column) on the maximum increase in total pulmonary resistance (RL) evoked by aerosolized NKA (10 μM; 40 breaths) in anaesthetized and artificially ventilated guinea-pigs, pretreated with phosphoramidon (4.5 μmol kg^{-1}, i.v.) and atropine (1.4 μmol kg^{-1}, i.v.). Filled column (control) indicates the effect of aerosolized NKA in animals pretreated with the vehicle of SR 48968 (10% dimethyl sulphoxide in 0.9% saline). Open column indicates the effect of the vehicle (40 breaths, 10% dimethyl sulphoxide in 0.9% saline) on baseline value of RL. Each column is the mean ± s.e.mean of at least five experiments. *$P<0.05$ vs control.

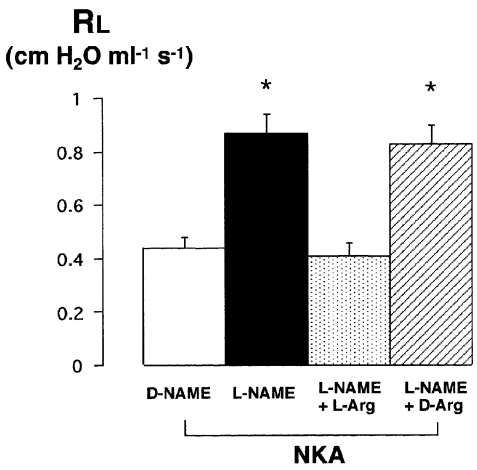

Figure 2 Effect of aerosolized L-NAME (1 mM), D-NAME, (1 mM), L-NAME plus L-arginine (L-Arg: 3 mM), and L-NAME plus D-arginine (D-Arg: 3 mM) on the increase in total pulmonary resistance (RL) induced by aerosolized NKA (40 breaths; 10 μM) in anaesthetized and artificially ventilated guinea-pigs, pretreated with phosphoramidon (4.5 μmol kg^{-1}, i.v.) and atropine (1.4 μmol kg^{-1}, i.v.). Each column is the mean ± s.e.mean of at least five experiments. *$P<0.05$ vs D-NAME.

Studies with tachykinin receptor antagonists

Animals pretreated with L-NAME (1 mM) showed no significant difference to the bronchoconstrictor response of NKA (10 μM) (0.53 ± 0.06 cmH$_2$O ml^{-1} s^{-1}, $n=6$) to those animals

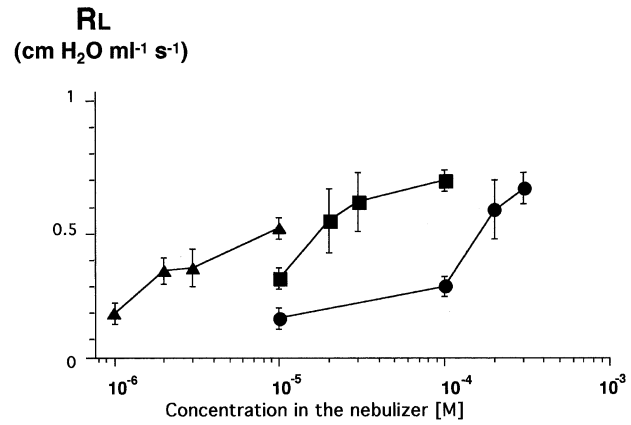

Figure 3 Dose-dependent increase in RL induced by the aerosolization (40 breaths) of the selective NK_1 receptor agonists, septide (closed triangles) or [Sar^9,$Met(O_2)^{11}$]SP (closed circles) and the selective tachykinin NK_2 receptor agonist, [βAla^8]NKA (4-10) (closed squares) in anaesthetized and artificially ventilated guinea-pigs, pretreated with phosphoramidon (4.5 μmol kg^{-1}, i.v.) and atropine (1.4 μmol kg^{-1}, i.v.). Each point represents the mean ± s.e.mean of at least five experiments.

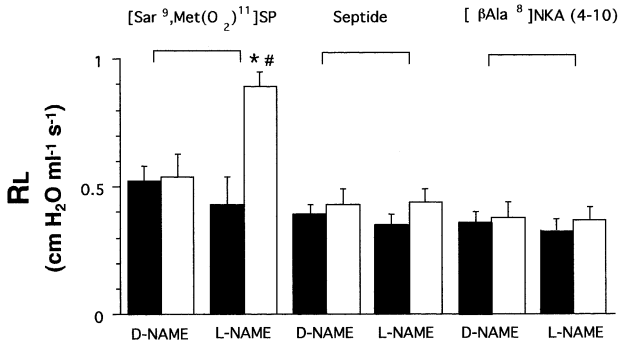

Figure 4 Effect of aerosolized 0.9% saline (filled columns), L-NAME (1 mM) or D-NAME (1 mM) (both open columns) on the increase in RL induced by 40 breaths of the aerosolized selective NK_1 receptor agonists, Sar^9,$Met(O_2)^{11}$]SP (0.2 mM) and septide (2 μM), and the selective tachykinin NK_2 receptor agonist, [βAla^8]NKA (4-10) (10 μM), in anaesthetized and artificially ventilated guinea-pigs, pretreated with phosphoramidon (4.5 μmol kg^{-1}, i.v.) and atropine (1.4 μmol kg^{-1}, i.v.). Each column represents the mean ± s.e.mean of at least five experiments. $^*P<0.05$ vs vehicle and $^{\#}P<0.05$ vs respective D-NAME.

pretreated with D-NAME (1 mM) (0.46 ± 0.07 cmH_2O ml^{-1} s^{-1}, $n=6$), in the presence of the selective NK_1 receptor antagonist, CP-99,994 (2 μmol kg^{-1}, i.v.). The amount of ethanol contained in the vehicle of SR 142801 (20 μl 100 g body $weight^{-1}$) did not affect bronchoconstriction to aerosolized NKA (not shown). Animals pretreated with L-NAME (1 mM) showed increased bronchoconstrictor response to NKA (10 μM) (0.86 ± 0.12 cmH_2O ml^{-1} s^{-1}, $n=5$, $P<0.05$) to those animals pretreated with D-NAME (1 mM) (0.42 ± 0.06 cmH_2O ml^{-1} s^{-1}, $n=5$), in the presence of the selective NK_3 receptor antagonist, SR 142801 (1 mg kg^{-1}, intraperitoneally).

Discussion

Tachykinins are powerful bronchoconstrictor agents in a large variety of mammalian species, including guinea-pig and man. Their bronchoconstrictor action is mainly due to the direct activation of NK_2 receptors in the airway smooth muscle (Emonds-Alt *et al*., 1992). However, in guinea-pigs *in vitro* (Maggi *et al*., 1991) and *in vivo* (Bertrand *et al*., 1993; Buckner *et al*., 1993; Boni *et al*., 1995) evidence suggests that direct stimulation of NK_1 receptors in the smooth muscle may also contribute. More recently, using isolated tracheal tube preparations, it has been shown that different mediators, including histamine (Nijkamp *et al*., 1993), bradykinin (Figini *et al*., 1996b) and endothelin (Emanueli *et al*., 1998), in addition to contracting tracheal smooth muscle, cause relaxation *via* activation of specific receptors in the airway epithelium. Pharmacological evidence indicates that activation of these epithelial receptors results in the release of NO that causes relaxation of the airway smooth muscle (Folkerts & Nijkamp, 1998). The guinea-pig tracheal tube preparation shows that tachykinins may limit their own bronchoconstrictor action by stimulating epithelial NK_1 receptors (Figini *et al*., 1996a; 1997). Low concentrations of SP, NKA and NKB were able to relax precontracted tracheal tube preparations (Figini *et al*., 1996a), and this relaxation was blocked by an NK_1 receptor antagonist. Thus, *in vitro*, the motor effect mediated by tachykinins results from the activation of a multiple mechanism that involves both contractile, smooth muscle NK_2 and NK_1 receptors and relaxant, epithelial NK_1 receptors.

In vitro data obtained in tracheal tube preparations with histamine (Nijkamp *et al*., 1993) and bradykinin (Figini *et al*., 1996a) have been reproduced *in vivo* in anaesthetized guinea-pigs. For instance, the mild to moderate bronchoconstriction by aerosolized bradykinin was changed in a robust bronchoconstrictor response after inhibition of the L-Arg/NOs pathway (Ricciardolo *et al*., 1994b). However, it is not known whether bronchoconstriction evoked by tachykinins in guinea-pig *in vivo* is also limited by their ability to release bronchorelaxant NO.

NK_1 receptors may be stimulated with similar potency by the three naturally occurring tachykinins, SP, NKA, and NKB. In guinea-pigs NKA-induced bronchoconstriction is inhibited completely when not only NK_2 receptors, but also NK_1 receptors are blocked (Bertrand *et al*., 1993; Buckner *et al*., 1993). Thus, we hypothesized that if aerosolized NKA stimulates smooth muscle contractile NK_1 receptors, it may also activate bronchorelaxant epithelial NK_1 receptors. The observation that NKA-induced bronchoconstriction was potentiated by pretreatment with L-NAME supports this hypothesis.

To determine which receptor(s) may be involved in the release of NO evoked by NKA, we used selective agonists of NK_1 and NK_2 receptors and a selective NK_1 receptor antagonist. Bronchoconstriction evoked by the selective NK_2 receptor agonist, [βAla^8]NKA (4-10), was not affected by inhibition of NOs. In contrast, the finding that bronchoconstriction induced by the selective NK_1 receptor agonist, [Sar^9,$Met(O_2)^{11}$]-SP, was markedly increased by L-NAME points to the role of NK_1 as the receptor subtype able to release bronchorelaxant NO, and strongly supports the hypothesis that NK_1 receptors mediate the release of bronchorelaxant NO evoked by NKA.

NKA is the preferred agonist of NK_2 receptors (Maggi & Schwartz, 1997; Regoli *et al*., 1994). However, it can also stimulate NK_3 receptors (Maggi & Schwartz, 1997; Regoli *et al*., 1994). Pharmacological evidence suggests the presence of excitatory NK_3 receptors on non-cholinergic parasympathetic ganglion neurones which probably originate in the oesophagus (Fischer *et al*., 1996; Canning *et al*., 1998) and which induce NO-dependent relaxation in the airways (Canning & Undem, 1994). NK_3 receptors may play a role in NKA induced and NO-mediated relaxation. However, L-NAME failed to increase

NKA-induced bronchoconstriction in guinea-pigs pretreated with the NK_1 receptor antagonist, CP-99,994. In contrast, after blockade of NK_3 receptors with SR 142801, L-NAME was still able to increase bronchoconstriction to NKA. These findings indicate that after NK_1 but not NK_3, receptor blockade the ability of NO to limit the bronchoconstrictor response to NKA is abolished. Thus, studies with the tachykinin receptor antagonists further strengthen the hypothesis that aerosolized NKA causes bronchorelaxation in guinea-pigs that is solely mediated by the activation of NK_1 receptors.

In tracheal tube preparations NO-mediated and epithelium-dependent tracheal relaxation was exquisitely sensitive to certain selective agonists of NK_1 receptors ([Sar^9,$Met(O_2)^{11}$]-SP-like compounds), whereas different NK_1 receptor agonists (septide-like compounds) were ineffective (Figini *et al*., 1996a; 1997). Our present data, shows that inhibition of the L-Arg/NOs pathway did not affect bronchoconstriction by septide, whereas it caused a robust potentiation of bronchoconstriction induced by [Sar^9,$Met(O_2)^{11}$]-SP *in vivo*, confirming previous *in vitro* studies (Figini *et al*., 1996a; 1997). Thus, the higher potency of septide to cause bronchoconstriction in the guinea-pig airways *in vivo* is, at least in part, due to its failure to stimulate epithelial NK_1 receptors that release bronchorelaxant NO.

The hypothesis that different selective NK_1 receptor agonists discriminate between diverse NK_1 receptor subtypes has been proposed previously (Petitet *et al*., 1992; Glowinski, 1995). However, convincing proof for the existence of a 'septide-sensitive' tachykinin receptor subtype is still lacking. A possible explanation for the different ability of [Sar^9, $Met(O_2)^{11}$]-SP and septide to activate bronchorelaxant NK_1 receptors derives from the hypothesis that has been previously proposed to explain similar data obtained *in vitro* (Figini *et al*., 1997). This hypothesis is based on the so called 'agonist-directed receptor trafficking' theory (Kenakin, 1995), which proposes that in the guinea-pig airways certain ([Sar^9, $Met(O_2)^{11}$]-SP-like) agonists may 'select or induce' the active conformer of the NK_1 receptor in both smooth muscle and epithelium, whilst other agonists (septide-like) may 'select or induce' the active conformer of the NK_1 receptor only in the smooth muscle. A similar pattern of 'selection/induction' of the active conformer of the NK_1 receptor may occur *in vivo*. If this hypothesis holds true the guinea-pig airways could represent an *in vivo* example of the 'agonist-directed receptor trafficking' theory.

Recently, it has been shown that bronchoconstriction by aerosolized bradykinin is potentiated by inhibition of the L-Arg/NOs pathway in mild asthmatics (Ricciardolo *et al*., 1996). The presence of NK_1 receptors have been found in human airway epithelium by using an antiserum that recognizes a portion of the human NK_1 receptor (A Fischer & P Geppetti, unpublished observation). The bronchomotor action of epithelial NK_1 receptor in the human airways is unknown. Aerosolized NKA is being used to test the efficacy and potency of NK_2 receptor antagonists (Joos *et al*., 1996; Van Schoor *et al*., 1998), which may eventually be used in clinical trials in asthma or other airway diseases. The present findings suggest that it may be of interest to evaluate if bronchomotor response induced by aerosolized NKA in humans is due only to NK_2-mediated bronchoconstriction or if NK_1-mediated and NO-dependent relaxation is also involved.

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