Pharmacological Examination of the Neurokinin-1 Receptor Mediating Relaxation of Human Intralobar Pulmonary Artery

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ABSTRACT

The effect of selective tachykinin receptor agonists and antagonists on human isolated intralobar pulmonary arterial rings was investigated. Neither Substance P (SP) nor neurokinin A (NKA) contracted the arteries. Both of these agonists, however, were potent and efficacious at relaxing the arteries that were precontracted with phenylephrine. The negative log (M) EC50 values for SP and NKA were 9.0 and 8.3, respectively. The neurokinin (NK)-3 selective agonist, senktide-analog, and the NK-2 receptor selective agonist, [β-Ala6]NKA(4–10), caused neither contractions nor relaxations of the arteries, whereas the NK-1 receptor agonist Ac-[Arg6, Sar9, Met(O2)11]SP(6–11) (ASM-SP) relaxed the tissue with a potency similar to SP. The relaxations to SP, NKA, and ASM-SP were competitively antagonized by the selective NK-1 receptor antagonist CP 99994, with a pKb in the nanomolar range. Antagonism of the ASM-SP-induced relaxations was also noted with FK 888, RP 67580, and L 732,138, although these antagonists were much less potent than CP 99994 in this regard. Another NK-1 receptor selective antagonist, SR 140333, caused an insurmountable antagonism of the SP-induced relaxations. The NK-1 receptor-mediated relaxations could be blocked by removing the endothelium, or by a combination of N-nitro-L-arginine and indomethacin. Measurement of prostanoid generation revealed that in endothelial-intact but not endothelial-denuded tissue, ASM-SP caused a selective increase in the production of 6-keto-PGF1α, the stable metabolite of prostacyclin. The results indicate that stimulation of NK-1 receptors leads to relaxation of human intralobar pulmonary arteries, which is mediated largely by nitric oxide and prostacyclin released from the endothelium of these vessels.

Tachykinins such as Substance P (SP) and neurokinin A (NKA) can influence vascular tone, although the effect of these peptides may depend on the species and vessel studied. SP has been shown to cause relaxation in various human (Bodelsson and Stjernquist, 1992; Onoue et al., 1994; Wallerstedt and Bodelsson, 1997) and animal (D’Orléans-Juste et al., 1985; Bolton and Clapp, 1986; Bodelsson and Stjernquist, 1992; Onoue et al., 1994), although it has been suggested that in the dog endothelial-independent mechanisms may contribute as well (Enokibori et al., 1994). Several endothelial-dependent relaxant pathways have been implicated in mediating vascular relaxation produced by SP. These include the production of nitric oxide (Rosenblum et al., 1993), the production of relaxant prostanoids (Bodelsson and Stjernquist, 1994), and an endothelial-dependent hyperpolarization of the smooth muscle membranes (Petersson et al., 1995; Wallerstedt and Bodelsson, 1997). Again, involvement of specific relaxant mechanisms in SP-induced vasodilatation may depend on the particular species and vascular bed studied, so that different endothelium-dependent mechanisms may account for SP-induced relaxation in different vessels. Moreover, there is evidence that multiple relaxant pathways can be involved in the relaxation response to SP within a single vessel type (Enokibori et al., 1994; Petersson et al., 1995; Wallerstedt and Bodelsson, 1997).

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ABBREVIATIONS: SP, Substance P; NK, neurokinin; NKA, neurokinin A; ASM-SP, Ac-[Arg6, Sar9, Met(O2)11]SP(6−11); L-NNA, N-nitro-L-arginine.
SP has recently been reported to cause relaxation of human isolated pulmonary arteries via stimulation of NK-1 receptors (Corboz et al., 1998). The mechanism of this effect and pharmacological characteristics of the receptor, however, have not been thoroughly characterized. The present study was undertaken to investigate the mechanisms involved in tachykinin-mediated relaxation of human pulmonary arterial rings, and to provide additional pharmacological information on the characteristics of the receptor mediating the response.

Materials and Methods

Macroscopically normal human lung tissue was obtained from 42 organ donors (supplied by the International Institute for the Advancement of Medicine, Exton, PA or the Anatomical Gift Foundation, Woodbine, GA). Organ donor specimens were mainly obtained from victims of head trauma or cerebral vascular accidents. The donors were male (24) and female (18) with an average age of 33 ± 2 years (range 13 to 61 years). Lungs from donors with documented pulmonary pathology were not used in these studies. Organ donor specimens were placed in cooled (4°C) Eagle’s minimum essential medium and transferred to the laboratory within 24 h. On reaching the laboratory, tissues were immediately placed in 4 liters of modified Krebs’ bicarbonate solution of the following composition: NaCl, 118 mM; KCl, 5.4 mM; NaH2PO4, 1.0 mM; MgSO4, 1.2 mM; CaCl2, 1.9 mM; NaHCO3, 25 mM; and D-glucose, 11.1 mM, and gassed with 95% oxygen and 5% carbon dioxide at 37°C.

Tissue Bath Studies. Intralobar pulmonary arteries (i.d. ~5 mm) were dissected free of surrounding parenchymal lung tissue and prepared as rings 8 to 10 mm in length. The rings were placed over stirrups made of tungsten wire and inserted over straight tungsten pins suspended in water-jacketed (37°C) tissue baths containing 10 ml of Krebs’ solution. Preparations were gassed continuously with 95% oxygen and 5% carbon dioxide. Propranolol (10−6 M) was added to the buffer at the beginning of experiments to block receptor activity. Adjacent human pulmonary artery ring segments were used as control or treated tissues. In each experiment at least two tissues served as control, one ring obtained from either side of the treated tissues.

The arteries were connected to a Grass FT03C force-displacement transducer for the measurement of isometric tension, which was recorded on a Grass model 7 polygraph (Grass Instruments, Quincy, MA). Arterial preparations were suspended under an initial tension of 5g and maintained at the same throughout a 60-min equilibration period, during which tissues were washed every 15 min with fresh buffer. After the 60-min equilibration period, all tissues were exposed to phenylephrine (1 μM) to inhibit neutral endopeptidase and thus reduce tachykinin metabolism. Thiorphan was added at least 60 min before obtaining the cumulative concentration-response effects of neurokinin receptor agonists.

To examine the ability of selective neurokinin receptor agonists to relax the human pulmonary artery, the tissues were precontracted with phenylephrine (3 μM) after which cumulative concentration-response curves were generated for Ac-Lys6, Sar9, Met(Or2)11bSP(6–11) (ASM-SP), the NK-2 selective agonist b-Ala8 NKA(4–10), or the NK-3 selective receptor agonist senktide analog. In preliminary experiments it was found that 3 μM phenylephrine causes a stable contraction that is equivalent to approximately 70% of the maximal obtainable contraction with barium chloride. Only one cumulative concentration-effect curve was obtained for each ring preparation and after obtaining the concentration-effect curve, tissues were exposed to papaverine (1 mM) to elicit maximum relaxation. The effects of various receptor antagonists were examined by adding the compound to the tissue bath before the beginning of the agonist concentration-response curve. In preliminary studies (n = 4) using CP 99994 we found no difference between a 30- and 60-min incubation time regarding the magnitude of the rightward shift in the agonists’ concentration-response curves. We chose 60 min as the period of incubation for all subsequent studies with the antagonists to allow adequate time for equilibration to form between the antagonists and receptors. A number of experiments were also conducted in which the ability of neurokinins to contract this tissue was examined. In these studies, 15 min after the addition of thiorphan, human pulmonary arterial rings were exposed to cumulative concentrations of the agonist. At the end of the concentration-effect curve, tissues were exposed to barium chloride (30 mM) to elicit maximum contraction.

Determination of the involvement of nitric oxide and cyclooxygenase in the vascular relaxation produced by ASM-SP or SP in human pulmonary artery was achieved by examining the effect of the nitric oxide synthase inhibitor N-nitro-l-arginine (l-NNA) and the cyclooxygenase inhibitor indomethacin on the relaxation response to these agonists. In these experiments l-NNA (100 μM), indomethacin (3 μM), or a combination of the two was added to tissue baths 60 min before ASM-SP or SP addition. At 30 min, tissues were contracted with phenylephrine (3 μM) after the contraction reached a steady state; cumulative concentration-effect curves were generated for the agonists as described above. Control tissues received vehicle instead of l-NNA or indomethacin and only one cumulative concentration-effect curve was obtained for each ring preparation. Neither indomethacin nor l-NNA had a significant effect on the magnitude of the contraction (measured in g of tension) induced by phenylephrine.

Endothelial dependence of ASM-SP-induced relaxation of human pulmonary artery was assessed in experiments in which the endothelium of one of a pair of adjacent pulmonary arterial rings was removed by gentle rubbing of the luminal surface with a cotton swab. The second ring of the pair, in which the endothelium was left intact, served as the control. Cumulative concentration-effect curves for ASM-SP were generated in endothelial-denuded and -intact preparations as detailed above.

Measurement of Mediator Release. Specimens of human intralobar arteries were dissected free from lung tissue, cleared of surrounding parenchyma, and prepared as rings 10 to 20 mm in length. These were pooled, divided into four samples (each containing approximately 100 mg of tissue), and placed into 2 ml of Krebs’ bicarbonate buffer containing propranolol (1 μM). Tissues were incubated at 37°C in a water bath and were gassed continuously with 95% oxygen and 5% carbon dioxide. Arterial specimens underwent a 60-min equilibration period during which tissues were washed every 15 min with fresh buffer. At the end of the 60-min equilibration period, tissues were placed in 1 ml of buffer and all tissues received thiorphan (1 μM). After an additional 15 min, the buffer was removed and 1 ml of fresh buffer containing thiorphan (1 μM) was added to all tissues. Arterial specimens were then exposed to ASM-SP (0.1 μM) or vehicle control for 15 min at 37°C after which the supernatant was collected. Tissues were then gently blotted with a cotton swab to remove surface moisture, and weighed.

Prostanoid release was assayed using combined gas chromatography (negative ion chemical ionization)-mass spectroscopy as described previously (Hubbard et al., 1986). A 100-μl aliquot of the supernatant was added to 300 μl of acetone in a silanized vial. A mixture containing a known quantity (about 1 ng) of 3,3,4,4-tetradecuterated PGE2, PGD2, PGF2α, TXB2, and 6-keto-PGF1α was added to each sample to provide internal standards for the identification and quantification of these prostanoids. In addition, the identification of 9α,11β-PGF2α was based on its retention times in relation to the tetradecuterated PGF2α.

Samples were dried under a stream of nitrogen and the residue was treated with 2% methoxymine hydrochloride dissolved in pyridine. Excess pyridine was evaporated under nitrogen and the residue was subjected to sequential procedures for the synthesis of pentafluorobenzyl ester and trimethylsilyl ether derivatives as described previously (Hubbard et al., 1986). Gas chromatography/mass spectrometry analysis of the derivatized samples (1 μl) was performed.
with a Finnigan MAT 4610B electron impact chemical ionization mass spectrophotometer supplied with a Superincos data system (San Jose, CA). The sensitivity (>2× blank) of this technique is <0.1 fmol/injection for each of the six prostanoids assayed.

**Analysis of Results.** All numerical data are expressed as arithmetic mean ± S.E. The n values represent the number of separate experiments carried out with vascular tissue obtained from different donors. In studies of isolated human pulmonary artery −log (M) EC₅₀ values were determined as the −log M concentration of the agonist that produced 50% of the maximum relaxation in each concentration-effect curve. The apparent dissociation constant (Kᵦ) was calculated for antagonists that caused a parallel rightward shift in the agonists concentration-response curves, using the standard equation, [agonist]/(dose ratio − 1), converted to the negative logarithm and expressed as −log molar Kᵦ (pKᵦ). Differences between means were determined using ANOVA or Student’s t test; probability values (P) < .05 were considered significant.

**Drugs and Solutions.** SP, NKA, ASM-SP, [β-Ala⁸]NKA(4–10), and senktide analog were obtained from Cambridge Biochemical (Wilmington, DE). Thiorphan, CP 99994, FK 888, RP67580, and L732138 were synthesized at Zeneca, Inc. (Wilmington, DE). SIN-1 was a gift from Casella AG, (Frankfurt, Germany). Phenylephrine, propranolol, indomethacin, i-NNA, papaverine, and barium chloride were obtained from Sigma Chemical Co. (St. Louis, MO). Indomethacin and thiorphan were dissolved in ethanol at a stock solution of 10 mM. Stock solutions (10 mM) of the NK-receptor antagonists and agonists were prepared in dimethyl sulfoxide and diluted to final concentration in Kreb’s buffer solution. Papaverine, phenylephrine, and barium chloride were dissolved in distilled water.

**Results**

**Neurokinin Receptor Agonists.** Neither NKA nor SP, at concentrations up to 1 μM, contracted the human isolated arterial rings with or without endothelium (n = 3, data not shown). Both SP and NKA were potent and effective in relaxing arteries precontracted with phenylephrine (Fig. 1). There was no significant difference in the maximum response between the two agonists; however, SP was 5-fold more potent than NKA, with respective −log EC₅₀ values of 9.0 ± 0.1 and 8.3 ± 0.3 (n = 42 and 8, Fig. 1).

The selective NK-1 receptor agonist ASM-SP was equipotent with SP in relaxing the precontracted artery (−log M EC₅₀ of 9.0 ± 0.1). The maximum effect of ASM-SP was also similar to SP and NKA (Fig. 1). Neither the NK-2 selective agonist, [β-Ala⁸]NKA(4–10), nor the NK-3 receptor selective agonist, senktide-analog, caused relaxation of the arteries at concentrations up to 0.1 μM (n = 2, data not shown).

**Neurokinin Receptor Antagonists.** The selective NK-1 receptor antagonists CP 99994 and FK 888 shifted the ASM-SP concentration-response curves in a parallel rightward fashion (Fig. 2). The calculated pKᵦ values obtained with two different concentrations of CP99994 were the same, averaging about 9.0 (Table 1). The calculated pKᵦ values of FK 888 were also independent of antagonist concentration, averaging about 7.8 (Table 1). Two additional NK-1 selective receptor antagonists, RP 67580 and L732138, also caused rightward shifts in the ASM-SP concentration response curves, although they were considerably less potent than CP 99994. The calculated pKᵦ for L732138 was dependent on the antagonist concentration, suggesting effects of this compound in addition to simple competitive antagonism (Table 1). In three experiments, CP99994 also shifted the concentration-response curves for NKA in a parallel rightward fashion without affecting the maximum response (data not shown).

![Fig. 1. Log concentration-response curves for SP (●), NKA (○), and ASM-SP (△) in causing relaxations of human intralobar pulmonary arteries precontracted with phenylephrine. The data are expressed as a percentage of maximum relaxation obtained with papaverine (1 mM) added at the end of the experiment. Vertical lines represent the S.E.M. of 42 (●), 8 (○), and 11 (△) experiments.](https://jpet.aspetjournals.org/article/S0022-3514(99)77513-7/FIGURES/fig1.jpg)
shown). In these experiments the estimated $pK_b$ values were 9.5, 9.2, and 9.5, which is similar to that observed when ASM-SP was the agonist.

Another selective NK-1 receptor antagonist, SR140333, was a potent inhibitor of the ASM-SP-induced relaxations of the human pulmonary arterial rings, with inhibitory activity noted in the subnanomolar range. In contrast to the other compounds studied, however, the inhibition by SR was insurmountable (Fig. 2). To see if the insurmountable nature of the antagonism by SR was selective for ASM-SP, the effect of the antagonists was also evaluated against SP-induced relaxations. The effect of the antagonist on SP-induced relaxations was indistinguishable from its effects on ASM-SP-induced relaxations ($n = 6$, data not shown). For example, the maximum relaxation to SP averaged 61 ± 5 and 34 ± 7%, in the absence and presence of 1 nM SR140333, respectively. In two additional experiments, the effect of SR140333 on the ability of the nitric oxide donor, SIN-1, to relax the arterial rings was examined. The SIN-1 concentration response curves obtained in the absence and presence of SR140333 (10 nM) were superimposable. The maximum response to SIN-1 was 92 and 96% in the absence of SR140333, and 92 and 94% in the presence of the drug (data not shown).

**Relaxation Mechanism.** The relaxations to the ASM-SP were dependent on an intact endothelium. In six of seven experiments, there was no relaxant response to ASM-SP in endothelial denuded tissues, whereas the maximum response to ASM-SP averaged 47 ± 9% in the adjacent segment in which the endothelium was intact. In one of seven experiments ASM-SP caused a 34% relaxation in the tissue in which the endothelium was putatively removed.

A portion of the endothelium-dependent relaxation was due to a mechanism inhibited by L-NNA (Fig. 3). Indomethacin also inhibited the relaxation to ASM-SP (Fig. 3). The
treated with the combination of indomethacin and L-NNA the maximum 
in the vehicle-treated tissue. In five of eight experiments, the artery 
resulting in a near complete relaxation. The relaxation caused by 1 mM papaverine added at the end of the experiment was 54 ± 0.2%.

**Fig. 4.** The release of prostanoids from human intralobar pulmonary arteries in the presence of vehicle (open columns) and ASM-SP, 0.1 μM (filled columns). The prostanoids were quantified using negative ion chemical ionization gas chromatography/mass spectrometry. Values are expressed as nanograms per gram wet weight of artery. Vertical lines represent the S.E.M., n = 6. * indicates statistical significance (P < .05) difference in the amount of prostanooid released from the vehicle and ASM-SP-treated tissues. In some tissues the endothelium was removed (endothelium-) at the beginning of the experiment.

**Fig. 3.** Log concentration-response curves for ASM-SP in causing relaxation of human intralobar pulmonary arteries precontracted with phenylephrine. Sixty minutes before the addition of the first concentration of ASM-SP, the tissues were treated with vehicle ( ), indomethacin, 3 μM ( ), L-NNA, 100 μM ( ), or indomethacin and L-NNA ( ). The data are expressed as a percentage of maximum relaxation obtained with papaverine added at the end of the experiment. The vertical lines represent the S.E.M., n = 8 experiments. There was no significant effect (ANOVA) on the log M EC50 values between tissues treated with vehicle (8.9 ± 0.2), indomethacin (9.0 ± 0.2), and L-NNA (8.5 ± 0.4). The maximum response in the treated tissues was significantly (P < .05) less than that obtained in the vehicle-treated tissue. In five of eight experiments, the artery treated with the combination of indomethacin and L-NNA the maximum response to ASM-SP was less than 1%.

The antagonist was added to the tissue bath 60 min before beginning the cumulative concentration-response curve for relaxation of human isolated pulmonary arterial rings. All data represent the mean ± S.E. Some of the data were obtained from concentration-response curves illustrated in Fig. 2.

The maximum response is the maximum relaxation induced by ASM-SP expressed as a percentage of the relaxation caused by 1 mM papaverine added at the end of the experiment.

The apparent dissociation constant (Kp) was calculated using the standard equation, [antagonist]/(dose ratio – 1), converted to the negative logarithm and expressed as log molar Kp (pKp).

n = the number of paired experiments.

*, denotes statistically significant (P < .05) difference between the values in the control and treated column.

**Discussion**

This study demonstrates that the human isolated pulmonary artery is a useful functional assay for the study of human NK-1 receptor pharmacology. The NK-1 receptor-mediated relaxations are relatively consistent among tissues, and are not influenced by NK-2 or NK-3 receptor mediated effects of L-NNA and indomethacin were additive such that the combination of the two compounds nearly abolished the ASM-SP-induced relaxations (Fig. 3). Similar results were obtained when SP was used as the agonist. The maximum relaxation to SP was 54 ± 15, 24 ± 12, 33 ± 15, and 0 ± 0% in tissues treated with vehicle, indomethacin, L-NNA, and the combination of indomethacin and L-NNA, respectively (n = 3).

To gain a greater understanding of the involvement of cyclooxygenase metabolites in NK-1 mediated relaxation of human pulmonary artery, the production of PGD2, PGF2α, TxB2 (the stable metabolite of TXA2), 6-keto-PGF1α (the stable metabolite of prostacyclin), and 9α,11β-PGF2α (a metabolite of PGD2) was assessed in endothelial-intact and -denuded segments of human pulmonary artery, both spontaneously and after addition of a maximally effective concentration of ASM-SP (Fig. 4). The predominant prostanooid produced by the human isolated pulmonary artery was prostacyclin (assayed as its metabolite, 6-keto PGF1α). ASM-SP caused a significant elevation in the prostacyclin production, without affecting the production of any of the other prostanoids. Most of the spontaneous, and all of the ASM-SP-induced, prostacyclin production was dependent on an intact endothelium (Fig. 4).
responses. Moreover, the NK-1 dependent relaxations are not affected by direct contractile effects of neurokinins at the level of the smooth muscle.

The pharmacological characterization of human NK-1 receptors is based primarily on binding studies typically using cell lines expressing the human cloned receptor (Fong et al., 1992a,b; McLean et al., 1993; Cascieri et al., 1994; Walpole et al., 1998). Functional studies on human NK-1 receptors have been based mainly on rabbit and guinea pig isolated tissues. There are relatively few studies that have investigated the pharmacology of NK-1 receptors in human isolated tissues. The importance of developing a functional assay using isolated tissues for the human NK-1 receptors is underscored by the known species differences in the pharmacology of this receptor (Fong et al., 1992b; Aramori et al., 1994). The species differences in the receptor pharmacology can be noted with CP 99994. Supporting previous observations by Corboz et al. (1998), we found that CP 99994 antagonizes the NK-1 receptor-mediated relaxations with a dissociation constant in the nanomolar range. This reflects a potency that is approximately 100 times greater than that noted in functional studies using the mouse stomach (Nsa Allogho et al., 1997), and about 5 to 10 times greater than its potency in functional studies using the guinea pig ileum (Hosoki et al., 1998). By contrast, FK888 was considerably less potent in inhibiting NK-1 responses in the human pulmonary artery (present study) than in the guinea pig ileum (Walpole et al., 1998). Likewise, RP 67580 was much less potent in inhibiting the NK-1-mediated responses in the human pulmonary artery (present study) than in the mouse stomach strip (Nsa Allogho et al., 1997). The potency of RP 67580 observed in the present study was similar, however, to that observed in the guinea pig ileum and rabbit iris preparations (Carruette et al., 1992; Hall et al., 1994).

Differences that arise between functional analysis and binding studies on human cloned receptors expressed in various cell systems also serve to point out the utility of including functional data in pharmacological characterizations of the human NK-1 receptor. All of the NK-1 receptor antagonists evaluated in the present study have estimated affinity constants or IC₅₀ values, obtained from receptor bindings studies, in the subnanomolar to nanomolar range (Fong et al., 1992a; McLean et al., 1993; Cascieri et al., 1994; Walpole et al., 1998). Thus the binding affinity categorically overestimated the functional potency of the compound on the human NK-1 receptors as evaluated using the human pulmonary artery. In some cases the difference was relatively minor (e.g., CP 99994 and SR 140333). In other cases, however, a major discrepancy was noted. For example, L 732,138 inhibits SP binding to human NK-1 receptors stably expressed in Chinese hamster ovary cells with an IC₅₀ of about 2 nM (Cascieri et al., 1994). This concentration is two to three orders of magnitude off its estimated dissociation constant in our functional studies with the human pulmonary artery.

Each of the NK-1 receptor antagonists studied in the present study are competitive antagonists in binding assays. Consistent with this, with the exception of SR 140333, the antagonism with these compounds could be surmounted by increasing agonist concentrations. The basis of the insurmountable antagonism afforded by SR 140333 is not clearly understood. It is unlikely to be explained by a nonselective effect of SR 140333 insasmuch as the compound had no effect on the relaxations caused by the nitric oxide donor molecule SIN-1. It is worth noting that even within a single species both surmountable and insurmountable antagonism of NK-1 receptor agonist-induced responses have been noted with SR 140333. In the guinea pig isolated trachea SR 140333 is an apparent competitive antagonist of ASM-SP-induced contractions causing a parallel rightward shift without affecting the maximum agonist response (Canning et al., 1998). In the guinea pig ileum, however, consistent with the observations presented here, SR 140333 inhibits NK-1 receptor mediated contractions in an insurmountable fashion (Croci et al., 1995).

NKA is often considered to be a selective NK-2 receptor agonist. In receptor binding studies, SP has an affinity that is 50 times greater than NKA for the wild-type NK-1 receptor (Fong et al., 1992a). NKA, however, was nearly as potent as SP in relaxing the human pulmonary artery. That NKA was acting on the NK-1 receptor to relax the artery is supported by the competitive antagonism observed with CP99994. There has been speculation that the NK-1 receptor may exist in two conformers. One conformer shows selectivity for SP whereas the other conformer can be stimulated by NKA and other sepiptide-like agonists. The similarity in the potency of SP and NKA in relaxing the artery is consistent with the hypothesis that the NK-1 receptor involved in this response is the "general-tachykinin conformer" (Maggi and Schwartz, 1997). The average EC₅₀ value observed for NKA in the present study (8 nM) was virtually the same as the EC₅₀ value we observed for NKA in the NK-2-receptor-mediated contraction of human isolated bronchi (5 nM) (Ellis et al., 1997). Therefore, NKA is not an NK-2 selective agonist in human pulmonary tissues.

Our observation that in human pulmonary artery, ASM-SP-induced relaxation was endothelial dependent is in agreement with numerous prior studies in other types of human blood vessels (Onoue et al., 1994; Petersson et al., 1995; Wallerstedt and Bodelsson, 1997). The conclusion can thus be drawn from the data obtained with indomethacin and l-NNa (Fig. 3) that in human pulmonary artery it is a combination of the activities of both nitric oxide and cyclooxygenase that predominantly mediates the endothelium-dependent vascular relaxation evoked by NK-1 receptor stimulation.

The mass spectroscopy data presented in Fig. 4, considered with data from the tissue bath studies, leads to the conclusion that prostacyclin is most likely the cyclooxygenase metabolite, derived from endothelial cells, responsible for mediating the indomethacin-sensitive portion of the NK-1 receptor relaxation in human pulmonary artery. This is in contrast to the rabbit pulmonary artery where NK-1 receptor activation leads to endothelium-dependent thromboxane production and consequent contraction of the smooth muscle (Shirahase et al., 1995). It is possible that nitric oxide released from endothelial cells may itself be the stimulus for prostaglandin production. Several reports have shown that nitric oxide stimulates prostaglandin biosynthesis (Salvemini et al., 1993; Sautebin et al., 1995; Landino et al., 1996). It is thus possible that NO may contribute to the NK-1 receptor-mediated prostacyclin production in the pulmonary artery. It is unlikely, however, that NO is the major stimulus of prostacyclin in our studies, because the inhibitory effects of indomethacin and l-NNa were additive.
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References


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