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Tachykinin NK_1 receptor-mediated vasorelaxation in human pulmonary arteries

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Abstract

Tachykinin NK_1 receptors are present on human pulmonary arteries. Addition of the specific tachykinin NK_1 receptor agonist, [Met-OMe^{11}]substance P produced a concentration-dependent relaxation (0.1 nM to 100 nM) in pulmonary arteries preconstricted with phenylephrine (30 μM). The EC_{50} (agonist concentration needed to produce 50% of the maximal relaxation) value for [Met-OMe^{11}]substance P was 3.7 ± 0.7 nM. The relaxation induced by [Met-OMe^{11}]substance P was selectively inhibited by the tachykinin NK_1 receptor antagonist CP 99994 (1 nM), with a pK_b of 9.9 ± 0.3. Treatment with the tachykinin NK_2 receptor antagonist SR 48968 (100 nM) did not significantly affect the vasorelaxation due to [Met-OMe^{11}]substance P ($P > 0.05$, one-way analysis of variance; ANOVA).

Keywords: Pulmonary artery, human; Tachykinin NK_1 receptor; [Met-OMe^{11}]substance P

The tachykinins substance P, neurokinin A, and neurokinin B are a family of chemically-related peptides that act through the three tachykinin receptors, designated NK_1, NK_2, and NK_3, respectively. These neuropeptides are colocalized in afferent unmyelinated nerves ending in the trachea, bronchi and lung. Activation of these sensory nerves by a variety of physical and chemical stimulants results in the release of these peptides that in turn reproduces many of the pulmonary alterations of asthma. It is generally agreed that airway microvascular leakage, mucus secretion (Maggi et al., 1995), increased blood flow (Piedimonte et al., 1992) are mediated by the tachykinin NK_1 receptors. However, the localization of these receptors in human airways is unclear. Walsh et al. (1994) reported specific binding of ^{125}I-labeled substance P on human bronchial microvessels but not on the smooth muscle of bronchus, trachea and pulmonary artery, whereas Knight et al. (1996) demonstrated that tachykinin NK_1 receptors mRNA was expressed in bronchial smooth muscle and epithelial cells of human lung. Therefore, using fresh human pulmonary arteries, this study was undertaken to determine whether the tachykinin NK_1 (substance P-preferring) receptors exert a significant role in the pulmonary circulation.

Human lungs were procured by the International Institute for the Advancement of Medicine (IIAM, Exton, PA) and by the Anatomic Gift Foundation (AGF, Woodbine, GA) and were obtained from eight organ donors (five men and three women, mean age 45.1 ± 5.5 years). Lungs were shipped in a buffered physiological salt solution on wet ice. Small pulmonary arteries were dissected free of connective tissue and lung parenchyma. Each arterial segment was cut into rings (2.0–11.0 mm internal diameter) and attached to isometric force transducers. The rings were placed in chambers filled with Krebs–Ringer solution maintained at 37°C and aerated with 95% O_2–5% CO_2. Pulmonary artery segments were allowed to equilibrate for 60 min. To optimize the contractile response, a resting force of 1 g was applied to each segment. The tension was readjusted as needed every 15 min during the equilibration period.

Because isolated human pulmonary arteries do not exhibit intrinsic tone, the pulmonary artery rings were contracted with the selective α_1-adrenoreceptor agonist phenylephrine (30 μM) to observe the relaxation responses. Time-control experiments, identical in time duration to the protocol used to examine the effect of the

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specific tachykinin NK_1 receptor agonist, were done for eight pulmonary arteries from five patients to insure that vessel tension did not change appreciably with time. Contractile response was studied by exposing the vessels to phenylephrine (30 μM).

To determine whether functional tachykinin NK_1 receptors are present in human pulmonary arteries, the specific tachykinin NK_1 receptor agonist, [Met-OMe^{11}]substance P was added to the chambers. After the contraction with phenylephrine (30 μM) had stabilized (approximately 10 min), a cumulative response to increasing doses of [Met-OMe^{11}]substance P (0.1 nM to 100 nM) was then studied in 18 pulmonary arteries from eight patients. The [Met-OMe^{11}]substance P concentration was increased by log increments every 5 min up to the maximum concentration of 100 nM. Relaxant responses were measured at the end of each period. At the end of the experiment, the relaxation to acetylcholine (100 nM) was assessed to normalize the relaxant response to [Met-OMe^{11}]substance P.

The effect of the specific tachykinin NK_1 receptor antagonist, CP 99994 was evaluated on 10 pulmonary arteries from five patients to determine whether the relaxation induced by [Met-OMe^{11}]substance P was mediated by the tachykinin NK_1 receptors. CP 99994 (1 nM) was added at the end of the equilibration period, 10 min before the addition of phenylephrine. To evaluate the potential role of tachykinin NK_2 receptors, the effect of a high concentration (100 nM) of the specific tachykinin NK_2 receptor antagonist, SR 48968 on the response to [Met-OMe^{11}]substance P was examined in six pulmonary arteries from five patients. This concentration was selected to ensure effective blockade of tachykinin NK_2 receptors. It has been shown that the effective antagonist concentration is about 1 nM in human bronchus (Ellis et al., 1993) and urinary bladder (Zeng et al., 1995). The relaxation to acetylcholine (100 nM) was also assessed in both groups with the antagonists to normalize the relaxant response to [Met-OMe^{11}]substance P.

In parallel time-matched control preparations, phenylephrine caused significant steady state constriction (99% of initial tension after 20 min). [Met-OMe^{11}]substance P produced a concentration-dependent relaxation (0.1 nM to 100 nM) in phenylephrine-preconstricted arteries with an EC_{50} of 3.7 ± 0.7 nM. [Met-OMe^{11}]substance P (100 nM) produced 84% relaxation of the response to acetylcholine (Fig. 1). The relaxation induced by [Met-OMe^{11}] substance P was selectively inhibited by the tachykinin NK_1 receptor antagonist CP 99994 (1 nM) (Fig. 1), with a pK_b of 9.9 ± 0.3. The pK_b value found in the human pulmonary artery is comparable with pK_b values of 8.9 in rabbit vena cava (Regoli et al., 1994), 9.7 and 10.1 in guinea pig ileum (Moore et al., 1997), respectively. The EC_{50} value for [Met-OMe^{11}]substance P in the presence of 1 nM CP 99994 (23.4 ± 7.3 nM) was significantly different than its absence (3.7 ± 0.7 nM) ($P < 0.05$, one-way analysis of variance, ANOVA). The dose-dependent relaxation due to

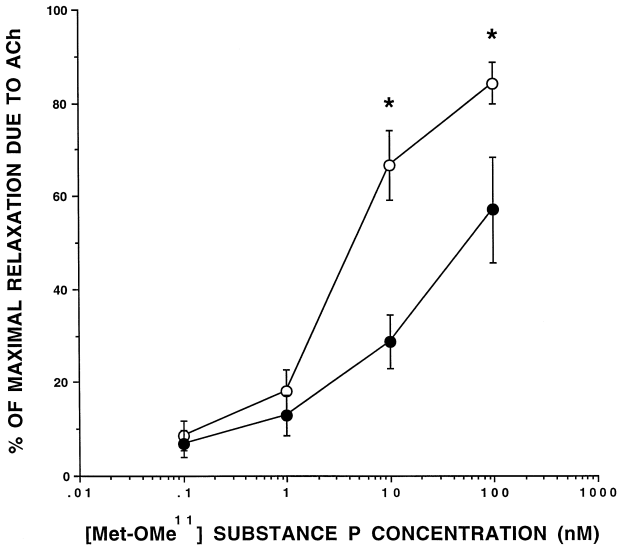

Fig. 1. Relaxant responses of [Met-OMe^{11}]substance P on human pulmonary arteries preconstricted with phenylephrine (30 μM) (expressed as a percentage of the maximal relaxation induced by 100 nM acetylcholine (ACh)). Open circles represent arteries treated with 30 μM phenylephrine to preconstrict the vessels and subsequently increasing [Met-OMe^{11}]substance P concentrations (0.1 nM to 100 nM; $n = 8$). Closed circles show arteries treated with 30 μM phenylephrine in the presence of 1 nM CP 99994 and subsequently increasing [Met-OMe^{11}]substance P concentrations (0.1 nM to 100 nM; $n = 5$). The relaxation to acetylcholine (100 nM) was assessed to normalize the relaxant response to [Met-OMe^{11}]substance P. The data are presented as means $\pm$ S.E.M. and were compared using ANOVA. Asterisks indicate significant difference ($P < 0.05$) from pulmonary arteries treated with increasing [Met-OMe^{11}]substance P concentrations in presence of 1 nM CP 99994.

[Met-OMe^{11}]substance P was not significantly affected by 100 nM SR 48968, a tachykinin NK_2 receptor antagonist. At the highest concentration (100 nM), [Met-OMe^{11}] substance P in presence of 100 nM SR 48968 produced 70% relaxation of the response to acetylcholine.

This study demonstrates that functional tachykinin NK_1 receptors are present on human pulmonary arteries and the stimulation of the tachykinin NK_1 receptors induces a vasorelaxation.

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