Inhibitory actions of prostaglandin E₁ on non-adrenergic non-cholinergic contraction in guinea-pig bronchi

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We have investigated the effect of prostaglandin E₁ (PGE₁) on non-adrenergic, non-cholinergic (NANC) contraction in guinea-pig bronchial strips. PGE₁ (10 nM to 10 μ M) did not alter baseline tension but reduced NANC contractions induced by electrical field stimulation (EFS) in a concentration-dependent fashion ($-\log EC_{50}$ was 6.60 ± 0.10 M and maximum inhibition was $88.7 \pm 2.9\%$). PGE₁ (>0.3 μ M) also reduced the contraction induced by substance P (1 μ M). Removal of epithelium did not alter the effects of PGE₁ on NANC contraction. These results suggest that PGE₁ exerts both pre- and post-junctional inhibitory actions on NANC contraction.

Introduction Airway tissue from a variety of species, including man, has been found to release prostaglandins of the E series (Steel *et al.*, 1979). Release of the E series prostaglandins from airway smooth muscle was accelerated by contractions induced by a variety of mediators (Orehek *et al.*, 1975). The PGE series relaxes tracheobronchial smooth muscle (Main, 1964) and inhibits cholinergic neurotransmission (Ito & Tajima, 1981).

Tachykinins are localized to C-fibre nerve endings in the airways of many species, including man (Lundberg *et al.*, 1984). Non-adrenergic, non-cholinergic (NANC) bronchoconstriction evoked by vagal stimulation may be due to the release of neuropeptides such as tachykinins from the sensory nerves (Lundberg *et al.*, 1983). These neuropeptides have potent inflammatory effects in airways (Barnes, 1986). We have therefore studied the effects of the PGE series on the NANC bronchoconstrictor response evoked by electrical field stimulation (EFS) to determine whether the PGE series has an inhibitory action on sensory neurones.

Methods Male Hartley guinea-pigs (300–500 g) were anaesthetized with sodium pentobarbitone (50 mg kg⁻¹, i.p.). Lungs were removed rapidly and were immersed in Krebs-Henseleit solution equilibrated with 95% O₂ and 5% CO₂. The two main bronchi were dissected free of loose connective tissue, opened longitudinally along the anterior side, and cut into strips. These strips were mounted in organ baths containing 5 ml Krebs-Henseleit solution maintained at 37°C. Isometric tension was continuously measured with a force transducer (TB612-T, Nihon Kohden, Japan) connected to a pen recorder (HORIZ-8K, San-ei Co., Japan). Bronchial strips were placed between two rectangular platinum electrodes (6 × 40 mm) for EFS. The tissues were stretched initially to a tension of 1 g for 30 s and thereafter maintained for 60 min under a resting tension of 0.25 g, which was found to be optimal for measuring changes in tension. EFS (supramaximal voltage of 50 V, a frequency of 10 Hz and 1 ms duration) was applied for 20 s. The contractile responses induced under these conditions were completely abolished by tetrodotoxin (1 μ M), confirming the neural nature of the response.

Experimental protocol After the baseline response to EFS was obtained, we added increasing concentrations of PGE₁ (from 10 nM to 10 μ M) and repeated the stimulation 5 min after the administration of each concentration of PGE₁, when the

effects of PGE₁ on the response to EFS reached their maximum. PGE₁ was administered after the preceding contraction had returned to baseline. We also tested the effects of PGE₁ on contraction induced by capsaicin (1 μ M). After the baseline response to capsaicin (1 μ M) was obtained, the bath was rinsed for 15 min. Five minutes after the administration of either PGE₁ (0.3 μ M) or the vehicle for PGE₁, capsaicin (1 μ M) was administered again. We compared the second response to the first one (control).

To determine whether removal of the epithelium altered the effects of PGE₁ on the response to EFS, we performed parallel studies on paired bronchi from the same animals after removing the epithelium from one randomly chosen bronchus by rubbing the luminal surface gently with gauze held with a pair of fine forceps. Removal of the epithelium was confirmed histologically.

The effects of PGE₁ (from 10 nM to 10 μ M) on contractile responses to exogenous substance P (SP) (1 μ M, which produced a contractile response equivalent to that produced by EFS) were also evaluated. All experiments were carried out in the presence of atropine sulphate (1 μ M), propranolol (1 μ M), indomethacin (1 μ M) and phentolamine (10 μ M).

Values are expressed as mean $\pm$ s.e.mean. Statistical analysis was performed by one way analysis of variance. Significance was accepted at $P < 0.05$.

Results EFS produced a long lasting NANC contraction that was reproducible within any given tissue (Figure 1a). PGE₁ had no effect on resting tension but decreased the amplitude of both EFS- (Figure 1b) and SP-induced contractions. The inhibition was concentration-dependent ($-\log EC_{50} = 6.60 \pm 0.10$ M for EFS and 5.86 ± 0.03 M for SP) (Figure 1c). PGE₁ at 0.3 μ M inhibited EFS-induced contraction (by $47.8 \pm 8.1\%$; $P < 0.01$) but did not significantly alter the contractile response to exogenously administered SP (by $3.7 \pm 2.9\%$; $P > 0.20$). At 10 μ M, PGE₁ inhibited EFS-induced contraction (by $86.2 \pm 3.4\%$; $P < 0.001$) and SP-induced contraction (by $38.8 \pm 2.5\%$; $P < 0.001$). Inhibitory actions of PGE₁ were significantly greater on EFS-induced contractions than on SP-induced contractions in terms of both $-\log EC_{50}$ ($P < 0.001$) and maximum inhibition ($P < 0.001$). Likewise, capsaicin (1 μ M)-induced contraction was significantly inhibited by PGE₁ (0.3 μ M) ($51.5 \pm 3.0\%$ of control with PGE₁ and $86.3 \pm 10.5\%$ of control in the presence of the vehicle for PGE₁; $P < 0.05$, $n = 5$). Removal of the epithelium increased EFS-induced contractions by $20.5 \pm 6.8\%$ ($P < 0.05$, $n = 5$). However, PGE₁ inhibited EFS-induced contraction by $50.6 \pm 1.2\%$ at 0.3 μ M ($P < 0.01$, $n = 5$) and by $88.7 \pm 2.9\%$ at 10 μ M ($P < 0.01$, $n = 5$) without

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Figure 1 Typical tracing showing the contraction induced by electrical field stimulation (●) (EFS) in the presence of atropine (1 μM), propranolol (1 μM), phentolamine (10 μM) and indomethacin (1 μM) in guinea-pig bronchi *in vitro*. (a) Long-lasting contraction caused by EFS was reproducible several times, and (b) inhibited by prostaglandin E₁ (PGE₁). (c) PGE₁ inhibited EFS- (●) and substance P (1 μM)- (○) induced contraction concentration-dependently. Data are shown as mean of values from 6 guinea-pigs; s.e. mean indicated by vertical bars. Significant differences from control values are indicated by * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$.

epithelium. Thus, the inhibitory effects that PGE₁ had on the responses to EFS did not differ significantly between bronchi with and without epithelium at both concentrations ($P > 0.30$).

In contrast to PGE₁, PGE₂ increased resting tension with a threshold concentration of 10 nM, and the subthreshold concentration of PGE₂ (3 nM) did not alter EFS-induced contraction significantly ($n = 5$, $P > 0.50$).

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Discussion Our study shows that PGE₁ reduces the neurally-mediated broncho-constrictor response evoked by EFS in guinea-pig bronchi. This excitatory response resulting from nerve stimulation in the presence of atropine and propranolol is NANC in nature, and probably due to the release of neuropeptides such as SP and neurokinin A from sensory nerve endings (Lundberg *et al.*, 1983).

In order to establish whether the inhibitory effects of PGE₁ are due to its effect on neuronal terminals or on the tachykinin receptors of the airway smooth muscle, we compared the effect that PGE₁ had on the contractile response to EFS and capsaicin with the effect that it had on the contractile response to exogenous SP. PGE₁ at 0.3 μM inhibited approximately half of the NANC contraction without having an inhibitory effect on the contractile response to SP. This implies that PGE₁-induced inhibition, at concentrations of up to 0.3 μM, is not related to an alteration of smooth muscle function, but probably represents a prejunctional mechanism such as the inhibition of tachykinin release from sensory nerve endings. However, further increases in the concentrations of PGE₁ decreased the contractile response to SP, suggesting that higher concentrations of PGE₁ inhibit NANC contraction via both pre- and post-junctional mechanisms.

Removal of epithelium is reported to enhance the contractile response to tachykinins in guinea-pig trachea (Devillier *et al.*, 1988). Therefore, PGE₁ might reduce the NANC contraction via mechanisms related to epithelium. However, epithelial removal did not alter PGE₁-induced effects on the NANC contraction, suggesting that PGE₁ inhibits the NANC contraction independently of the epithelium.

Sensory nerves may play an important role in the neurogenic inflammation of the airways (Barnes, 1986). Regulation of the release of sensory neuropeptides from nerves may therefore reduce neurogenic inflammation. Our study suggests that PGE₁ has an inhibitory action on neuropeptide release from sensory nerve endings. Therefore, PGE₁ may be useful in the treatment of neurogenic inflammation in lung disease.

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