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Eosinophils and neutrophils modify arachidonic acid-induced relaxation of guinea-pig trachea

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Abstract

The influence of inflammatory cells on airway reactivity was investigated on arachidonic acid-induced relaxations of guinea-pig trachea and on arachidonic acid metabolism in guinea-pig tracheal epithelial cells. The presence of either eosinophils or neutrophils (1.0×10^7 cells/ml), from bronchoalveolar lavage, decreased the tracheal relaxations induced by arachidonic acid (1.0–30 μM). The basal synthesis of prostaglandin E_2 was increased in epithelial cells (from 176 ± 36 to 7920 ± 898 pg/ml), eosinophils (from 360 ± 56 to 2693 ± 686 pg/ml) and neutrophils (from 352 ± 81 to 4400 ± 272 pg/ml) following incubation with arachidonic acid (10 μM). The co-incubation of either eosinophils or neutrophils with epithelial cells, in the presence of arachidonic acid, decreased the synthesis of prostaglandin E_2 (2600 ± 686 and 4400 ± 272 pg/ml respectively) but increased the synthesis of thromboxane B_2 (from 60 ± 6 to $11\,634 \pm 840$ and 9282 ± 485 pg/ml respectively). Similarly, when major basic protein-treated (100 μg/ml) epithelial cells were incubated with arachidonic acid, the prostaglandin E_2 synthesis decreased (75%) but thromboxane B_2 synthesis was unaffected. The results suggest that eosinophils and neutrophils may impair arachidonic acid metabolism in guinea-pig epithelium in favor of production of bronchoconstrictor prostanoids.

Keywords: Tracheal epithelial cell, guinea pig; Eosinophil; Neutrophil; Arachidonic acid

1. Introduction

Inflammation of the airways has a fundamental role in impairing airway reactivity. Although the mechanisms underlying the change in the airway reactivity remain largely unknown, a number of studies have suggested that eosinophils (Leff et al., 1991) rather than neutrophils (Fabbri et al., 1984) are the major inflammatory cells that contribute in part to this process. This evidence is based on a marked airway eosinophilia (Gleich, 1990) or neutrophilia (Holtzman et al., 1983) observed in association with airway dysfunction. Eosinophils contain four important cationic proteins: major basic protein, eosinophil peroxidase, eosinophil cationic protein and eosinophil-derived neurotoxin (Gleich and Adolphson, 1986; Barker et al., 1989; Ten et al., 1989). Levels of major basic protein are increased in the sputum of asthmatics (Frigas et al., 1981; Dor et al., 1984). In addition, major basic protein causes desquamation and damage to epithelial cells (Gleich et al., 1979; Frigas et al., 1980, 1981). These changes are similar to the lesions observed in the bronchial mucosa of asthmatics (Dunnill, 1960). The accumulation of eosinophils or neutrophils in the airway mucosa requires their migration through endothelial cells, the extravascular matrix and epithelial cells (Leff et al., 1991). Eosinophil-epithelial cell interactions during the inflammatory response may contribute to impair the airway reactivity (Wardlaw et al., 1988). It has been suggested that epithelial desquamation is due to the cytotoxic effect of major basic protein (Gleich et al., 1979) which in turn leads to the enhancement of airway responsiveness by removing the protective effect of an epithelium-derived relaxing factor (Morrison et al., 1990). However, other studies demonstrated that major basic protein, at subcytotoxic concentrations, could also increase the responsiveness of airway smooth muscle (Flavahan et al., 1988; Coyle et al., 1995).

The objective of this study was to assess the role of eosinophils, neutrophils and major basic protein in modulating guinea-pig tracheal relaxation induced by arachidonic acid in vitro and prostanoid synthesis by isolated epithelial cells.

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2. Materials and methods

2.1. Materials

Protease type XXIV, arachidonic acid, aspirin, lipopolysaccharide (0111: B4) and Percoll were purchased from Sigma (St. Louis, MO, USA). The culture medium DMEM (Dulbecco's modified Eagle medium)/F12, penicillin and streptomycin were purchased from Gibco Laboratories (Gaithersburg, MD, USA). Sephadex beads (G-50 Superfine) were purchased from Pharmacia (Uppsala, Sweden).

2.2. Cells and cultures

2.2.1. Tracheal epithelial cell culture

Dunkin-Hartley guinea pigs (250–300 g) of either sex were killed by cervical dislocation and the trachea was rapidly placed in a sterile Krebs buffer I (Krebs buffer supplemented with penicillin (100 IU/ml) and streptomycin (100 μg/ml)). The tracheae were trimmed of fat and connective tissue. Krebs buffer contained (mM): NaCl, 118.07; glucose, 11.1; $NaHCO_3$, 25; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4$, 10 and $CaCl_2 \cdot 2H_2O$, 2.51 (95% O_2, 5% CO_2; pH 7.4). The lumen was filled with Krebs buffer II (Krebs buffer I supplemented with protease type XXIV, 0.1%) and incubated during 40 min at 37°C. The trachea was opened longitudinally along the posterior surface and rinsed with DMEM/F12 medium containing 10% heat-inactivated fetal bovine serum (FBS). The epithelium was removed by gently rubbing the luminal surface with a rubber policeman. The cell suspension was pelleted (300 $\times g$; 10 min) and suspended in a serum-free DMEM/F12. Cell counts (3.0×10^6 cells/trachea) and viability ($\geq$ 80%) were assessed using Trypan blue dye. Cells were plated at a concentration of 0.5×10^6 viable cells/2 cm^2 onto uncoated 24-well plates. The medium (1 ml/well) was replaced by serum-free DMEM/F12 each 24 h until cells reached confluence (4–5 days).

2.2.2. Inflammatory cells

2.2.2.1. Lung eosinophilia. The technique described herein to elicit the migration of eosinophils into the lungs is a modification of a procedure previously reported by Walls and Beeson (1972) to induced airway eosinophilia in rats. Sephadex beads (G-50 Superfine) were suspended in sterile isotonic saline (56 mg/ml) and 0.43 ml/kg of this bead suspension (a non-lethal dose) was immediately administered to conscious guinea pigs. This bead suspension, corresponding to a dose of 24 mg/kg body weight, was injected in the ear vein after a local anesthesia with a solution of xylocaine (2%).

2.2.2.2. Lung neutrophilia. Polymorphonuclear neutrophils were elicited in guinea-pig lungs by intratracheal injection of lipopolysaccharide from *Escherichia coli* (2.5 μg per animal in 0.9% NaCl). The administration was performed under anesthesia, using sodium pentobarbital (50 mg/kg, intraperitoneally).

2.2.2.3. Isolation and purification of inflammatory cells. Guinea pigs were killed one day after the injection of either Sephadex or lipopolysaccharide by cervical dislocation and bronchoalveolar lavage was performed. Briefly, the trachea was canulated with a catheter joined by a three-way stopcock to a pair of 60 ml syringes. Phosphate-buffered saline (PBS, pH 7.4, 37°C) was infused into the lungs in 5-ml aliquots (total volume 50 ml) and reaspirated with the second syringe after gentle massage of the lungs. After lysis of red blood cells by hypotonic shock, the remaining cells were resuspended in PBS and purified by centrifugation on a 65% and 50% continuous Percoll gradient for eosinophils and neutrophils respectively. The appropriate dilution of Percoll was prepared by mixing a solution containing 0.9 g NaCl in 10 ml of 10-fold concentrated PBS with a commercial solution of Percoll (2.26:27.74, v/v) and pH adjusted to 7.4. This stock Percoll solution was diluted with PBS to obtain the percentage of Percoll desired. The gradients were obtained by centrifugation at $20\,000 \times g$ for 15 min at 4°C. Total bronchoalveolar lavage cells were laid on the gradient and centrifuged at $360 \times g$ for 30 min at room temperature. Cells were collected at the bottom of the gradient and washed twice with PBS to remove Percoll. Cell counts (eosinophils, 5.0×10^7; neutrophils, 6.0×10^7 cells/lung) and viability ($\geq$ 95%) were assessed by Trypan blue exclusion. Cell purity ($\geq$ 95%) was estimated using Wright-Giemsa staining.

2.2.3. Incubation conditions

Confluent monolayers of guinea-pig tracheal epithelial cells (1.0×10^6 cells/2 cm^2) were used for experiments in the absence or presence of freshly isolated inflammatory cells (1.0×10^6 cells/ml). Experiments were performed in Krebs buffer solution (pH 7.4) at 37°C in humidified 5% CO_2 atmosphere. Both untreated guinea-pig tracheal epithelial cells and inflammatory cells were preincubated either separately or together for 2 h at 37°C in 1 ml of Krebs buffer before stimulation with arachidonic acid (3 and 10 μM) for 10 min. Aspirin-treated (100 μM; 30 min) epithelial cells were preincubated alone (2 h; 37°C) whereas aspirin-treated (100 μM; 30 min) inflammatory cells were preincubated either separately or together with untreated epithelial cells for 2 h at 37°C in 1 ml of Krebs buffer before stimulation with arachidonic acid (3 and 10 μM) for 10 min. Similarly, the epithelial cells were pretreated with either sodium acetate buffer (control experiments) or major basic protein (10 and 100 μg/ml) for 2 h at 37°C in 1 ml of Krebs buffer before stimulation with arachidonic acid (3 and 10 μM) for 10 min. Aliquots of incubation media (500 μl) were kept at −80°C until assayed for

prostaglandin E_2 and thromboxane B_2 using enzyme immunoassays (EIA).

2.3. Purification of human major basic protein

Major basic protein was purified from eosinophils obtained from patients with the hypereosinophilic syndrome by cytapheresis (yield 2.0×10^{10} to 2.0×10^{11} per patient) as described previously (Slifman et al., 1986). Briefly, eosinophils were lysed with sucrose (0.25 M) and heparin (200 U/ml) and granules were isolated by centrifugation ($10\,000 \times g$; 20 min). The enriched granule fractions were stored in liquid nitrogen for up to 3 years. Eosinophil granules were lysed by exposure to 0.01 M HCl, pH 2, with brief sonication (30–60 s). Insoluble materials were removed by centrifugation ($40\,000 \times g$; 5 min) and the supernatant was fractionated on 1.2×47 cm Sephadex G-50 column (Pharmacia, Piscataway, NJ, USA), equilibrated with 0.025 M sodium acetate, 0.15 M NaCl (pH 4.3). Fractions from the third protein peak containing major basic protein were pooled (Ackerman et al., 1983). The purified protein was stored in aliquots at -70°C and samples were thawed immediately before use. Major basic protein was pure as judged by its banding pattern on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; Laemmli, 1970) after staining with Coomassie brillant blue R. Major basic protein concentrations were determined by the E_{277} value (Gleich et al., 1976).

2.4. Analysis by EIA

Prostaglandin E_2 and thromboxane B_2 were analyzed by EIA according to Pradelles et al. (1985). The assays were performed by adding 50 μl of incubation media, specific rabbit antibodies for prostaglandin E_2 or thromboxane B_2 and conjugated eicosanoid-acetylcholinesterase in 96-well microtiter plates coated with antirabbit IgG antibodies. After an overnight incubation, the plates were washed and Ellman's reagent containing the acetylcholinesterase enzymatic substrate was added to each well to monitor the activity. The absorbance was measured at 414 nm using a Flow Titertek Multiscan spectrophotometer.

2.5. Relaxation of guinea-pig trachea

After cervical dislocation, the trachea was isolated and cleaned of surrounding tissue. Each trachea was cut open helically into 0.5 cm strips and placed in a classical organ bath system under an initial tension of 1.5 g. The strips were randomly allocated for experiment in the absence or presence of inflammatory cells (1.0×10^7 cells/ml). The tissues were washed every 15 min with fresh Krebs solution (as described in guinea-pig tracheal epithelial cell culture) bubbled with 95% O_2, 5% CO_2 (37°C). After an equilibration period of 1 h, relaxant responses to cumulative concentrations of arachidonic acid (1.0–30 μM) were obtained. After washout and an equilibration period of 45 min, a second concentration-response curve to arachidonic acid was generated in the presence of untreated inflammatory cells (eosinophils or neutrophils), or aspirin-treated cells (100 μM; 30 min) which were added to the organ bath 10 min before. The pretreated eosinophils and neutrophils were washed twice with Krebs to remove the aspirin. In some experiments, a third concentration-response curve to arachidonic acid was generated after a resting period of 45 min under the same experimental conditions as the first concentration-response curves to arachidonic acid. In a few cases small increases in resting tension occurred which were restored to 1.5 g by additional washing before initiating a cumulative concentration-response curve. Relaxations were measured isometrically with Grass FT03C transducers and recorded on a Grass polygraph.

2.6. Statistical analysis

The values are means ± S.E.M. The statistical significances were determined by analysis of variance. $P \leq 0.05$ was considered to be statistically significant.

3. Results

3.1. Effect of eosinophils and neutrophils on arachidonic acid-induced guinea-pig tracheal relaxation

Arachidonic acid (1.0–30 μM) induced concentration-dependent relaxations of epithelium-intact guinea-pig trachea with an E_{max} averaging 10 μM (Fig. 1). The threshold concentration of arachidonic acid was 1.0 μM to induce the guinea-pig tracheal relaxation which reached 50% of the maximal response at a concentration of 3 μM (EC_{50}). Three successive concentration-response curves to arachidonic acid alone were carried out in some preparations to confirm the reproducibility of the experiment (data not shown).

In the presence of eosinophils (1.0×10^7 cells/ml added to the organ bath), the relaxant responses of guinea-pig trachea induced by 3, 10 and 30 μM arachidonic acid were reduced by 90, 50 and 30%, respectively (Fig. 1A) whereas in the presence of neutrophils (1.0×10^7 cells/ml), the guinea-pig tracheal relaxations were reduced by 60, 30 and 20%, respectively (Fig. 1B). The inflammatory cells decreased mainly the relaxations induced by the submaximal concentrations of arachidonic acid (1–10 μM). Eosinophils appeared to induce a more significant decrease in arachidonic acid-induced relaxations of guinea-pig trachea. Following removal of eosinophils from the organ baths, by repeated washouts, the guinea-pig tracheal relaxations induced by 3, 10 and 30 μM arachidonic acid were restored by 54, 34 and 12%, respectively, and after removal of

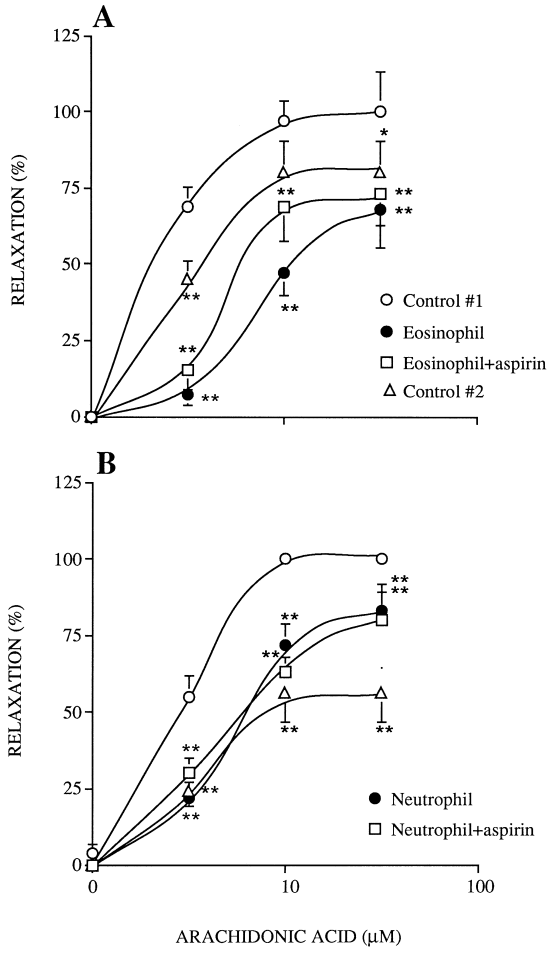

Fig. 1. Concentration-response relaxations of guinea-pig trachea induced by arachidonic acid. Guinea-pig tracheal relaxations were obtained in the absence (Control #1,2) and in the presence of untreated or aspirin-treated (100 μM) eosinophils (panel A) or neutrophils (panel B) (1.0×10^7 cells/ml). Responses are plotted as a percentage of the maximal response obtained for the first concentration-response curve (Control #1). Results are the mean ± S.E.M. of 6 experiments. * $P < 0.05$, ** $P < 0.01$, significantly different from the first concentration-response curve.

neutrophils from the organ baths, the guinea-pig tracheal relaxations induced by 10 and 30 μM arachidonic acid were similar, approximately 30% lower than in presence of these cells. The removal of eosinophils but not neutrophils from the organ baths significantly enhanced (approximately 2.0-fold) the guinea-pig tracheal relaxations induced by submaximal concentrations of arachidonic acid.

In the presence of inflammatory cells (1.0×10^7 cells/ml) which were pretreated with aspirin (100 μM), the guinea-pig tracheal relaxations induced by arachidonic acid were also decreased. Relaxations of guinea-pig trachea induced by 3, 10 and 30 μM arachidonic acid were decreased by 80, 30 and 27%, respectively, in the presence of aspirin-treated eosinophils (Fig. 1A). Similarly, aspirin-treated neutrophils decreased the guinea-pig tracheal relaxations induced by 3, 10 and 30 μM arachidonic acid by 40, 35 and 20%, respectively (Fig. 1B), which was similar to the inhibition observed in the presence of untreated inflammatory cells.

3.2. Effect of eosinophils and neutrophils on prostaglandin E_2 and thromboxane B_2 synthesis by guinea-pig tracheal epithelial cells

The basal release of prostaglandin E_2 by cultured tracheal epithelial cells was 175 pg/ml, whereas eosinophils and neutrophils released 350 pg/ml during a 2-h incubation period (Fig. 2A). The release of thromboxane B_2 from guinea-pig tracheal epithelial cells was low (20 pg/ml) whereas both eosinophils and neutrophils released large amounts (10 000 pg/ml) (Fig. 2B). Incubation of eosinophils with cultured guinea-pig tracheal epithelial cells significantly decreased the basal release of thromboxane B_2 (430 pg/ml) when compared to the amount released by eosinophils alone (10 000 pg/ml) (Fig. 2B). However, incubation of neutrophils with guinea-pig tracheal epithelial cells did not significantly affect the release of thromboxane B_2 when compared to neutrophils alone. The release of prostaglandin E_2 was significantly decreased (from 360 to 160 pg/ml) following incubation of eosinophils with guinea-pig tracheal epithelial cells when compared to eosinophils alone (Fig. 2A). In contrast, the

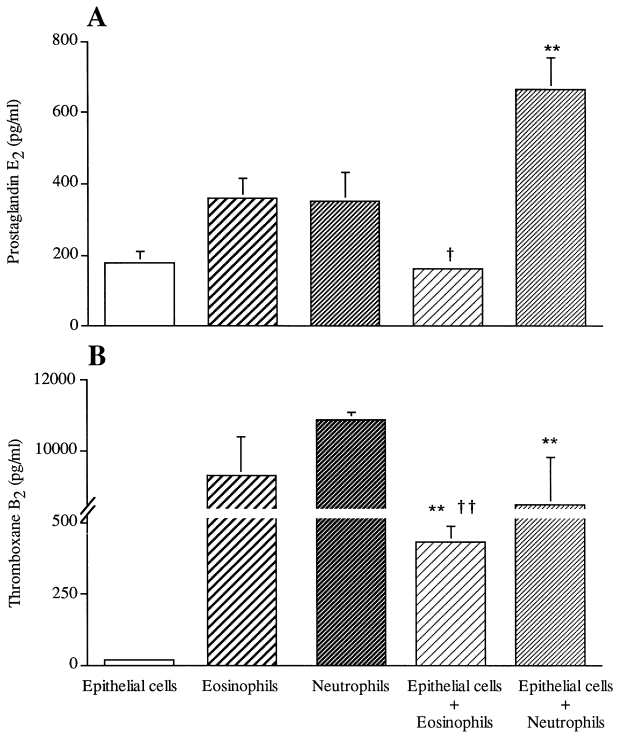

Fig. 2. Basal release of prostaglandin E_2 (panel A) and thromboxane B_2 (panel B) by cultured guinea-pig tracheal epithelial cells, eosinophils and neutrophils alone or following co-incubation (epithelial cells + eosinophils; epithelial cells + neutrophils) for 2 h. Results are expressed as pg/ml and are the mean ± S.E.M. of 3 experiments. ** $P < 0.01$, significantly different from epithelial cells. † $P < 0.05$, †† $P < 0.01$, significantly different from eosinophils.

incubation of neutrophils with guinea-pig tracheal epithelial cells did not significantly affect the synthesis of prostaglandin E_2 when compared to neutrophils alone. The increase in synthesis of prostaglandin E_2 observed following co-incubation of guinea-pig tracheal epithelial cells with neutrophils may be due to an additive effect.

When guinea-pig tracheal epithelial cells were incubated with arachidonic acid for 10 min there was a significant increase in the release of prostaglandin E_2 representing a 20-fold (3900 pg/ml) and 45-fold (7900 pg/ml) increase above basal levels at 3 and 10 μM, respectively (Fig. 3A). Similarly when eosinophils were incubated with 3 and 10 μM arachidonic acid, prostaglandin E_2 release was increased by 10 (3850 pg/ml) and 22 times (7880 pg/ml), respectively (Fig. 4A). In contrast, when neutrophils were incubated with 3 and 10 μM arachidonic acid, there was a smaller, yet significant increase in prostaglandin E_2 release (Fig. 4B). This increase was approximately 3-fold (1100 pg/ml) above basal release following stimulation with 10 μM arachidonic acid. Treatment of all three cell types with aspirin (100 μM) markely inhibited the arachidonic acid-induced release of prostaglandin E_2 (Fig. 3A, Fig. 4A and B). Following incubation of guinea-pig tracheal epithelial cells with arachidonic acid (10 μM), the release of thromboxane B_2 was significantly increased (2–3-fold; 60 pg/ml) above basal levels (Fig. 3B). This increase was inhibited following pretreatment with aspirin. Arachidonic acid did not appear to significantly enhance the release of thromboxane B_2 from either eosinophils or neutrophils (data not shown).

When cultured guinea-pig tracheal epithelial cells were co-incubated with either eosinophils or neutrophils for 2 h and then stimulated with 3 and 10 μM arachidonic acid, a significant ($P < 0.05$) decrease in the release of prostaglandin E_2 (Fig. 3A) and a significant increase in the

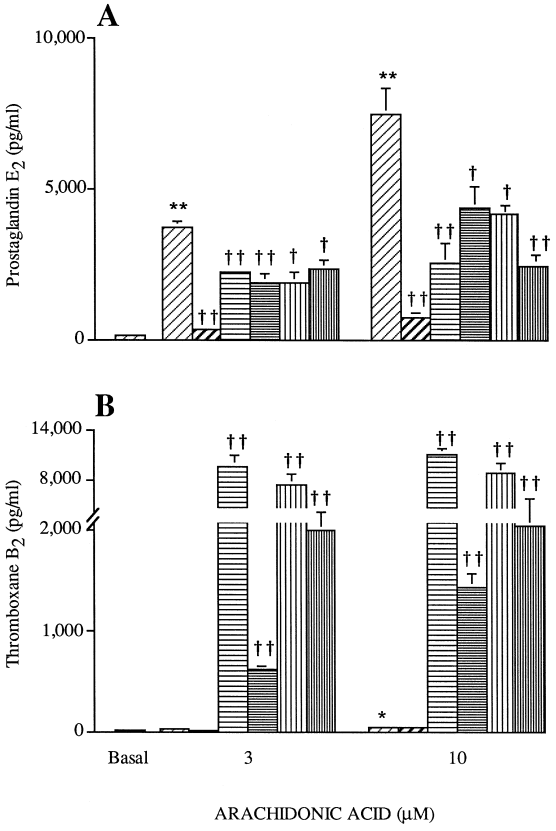

Fig. 3. Prostaglandin E_2 (panel A) and thromboxane B_2 (panel B) released by guinea-pig tracheal epithelial cells (fine-lined hatched bars), aspirin-treated (100 μM) epithelial cells (dark-lined hatched bars), epithelial cells co-incubated with untreated (fine-lined horizontally striped bars) or aspirin-treated (100 μM) (dark-lined horizontally striped bars) eosinophils and epithelial cells co-incubated with untreated (fine-lined vertically striped bars) or aspirin-treated (100 μM) (dark-lined vertically striped bars) neutrophils. Cells were preincubated (2 h) before stimulation with 3 and 10 μM arachidonic acid (10 min). Results are expressed as pg/ml and are the mean ± S.E.M. of 3 experiments. * $P < 0.05$, ** $P < 0.01$, significantly different from basal release by epithelial cells. † $P < 0.05$, †† $P < 0.01$, significantly different from epithelial cells stimulated with arachidonic acid.

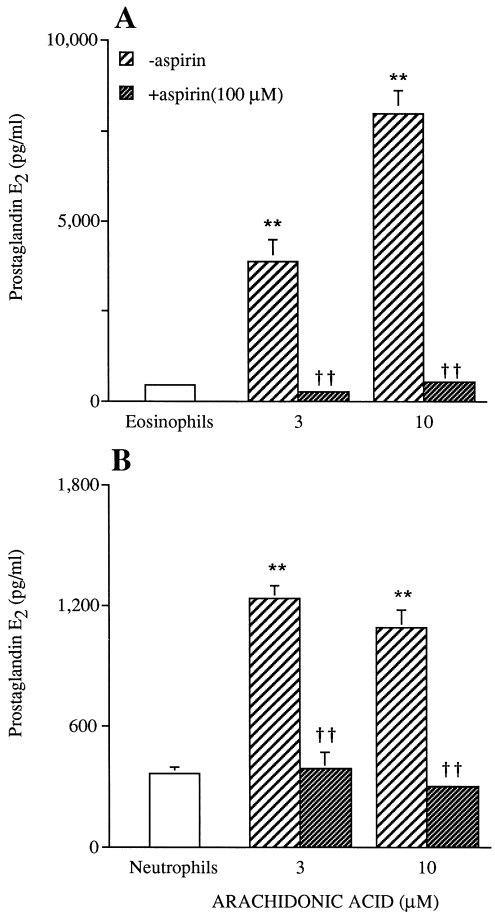

Fig. 4. Prostaglandin E_2 released by eosinophils (panel A) and neutrophils (panel B) either untreated (hatched bars) or pretreated (dark-lined hatched bars) with aspirin (100 μM). Cells were preincubated (2 h) before stimulation with 3 and 10 μM arachidonic acid (10 min). Results are expressed as pg/ml and are the mean ± S.E.M. of 3 experiments. ** $P < 0.01$, significantly different from basal release from inflammatory cells. †† $P < 0.01$, significantly different from inflammatory cells stimulated with arachidonic acid.

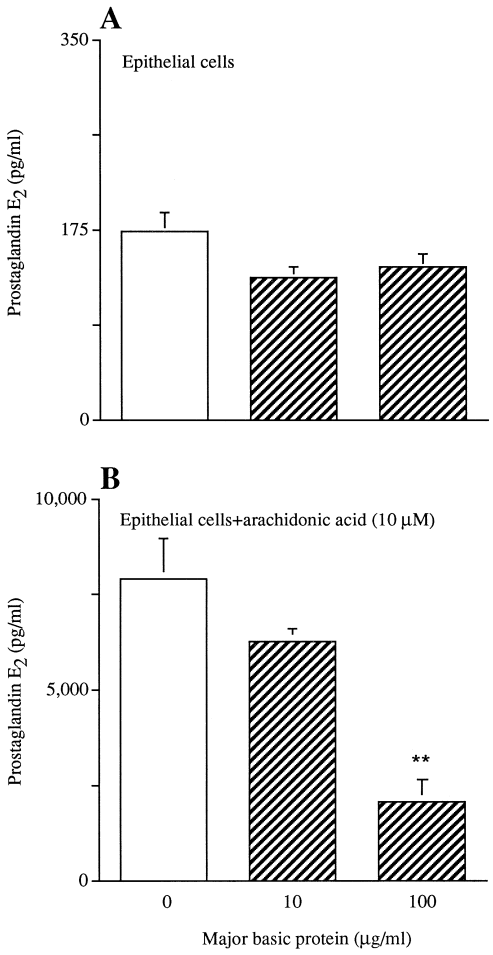

Fig. 5. Effect of major basic protein (10 and 100 μg/ml) on the basal release of prostaglandin E_2 (panel A) and the release following incubation with arachidonic acid (10 μM) (panel B) from cultured guinea-pig tracheal epithelial cells. Results are expressed as pg/ml and are the mean ± S.E.M. of 3 experiments. ** $P < 0.01$, significantly different from epithelial cells stimulated with arachidonic acid.

release of thromboxane B_2 ($P < 0.01$) were noted (Fig. 3B). Pretreatment of eosinophils or neutrophils with aspirin did not significantly influence the decrease of prostaglandin E_2 synthesis during the incubation with guinea-pig tracheal epithelial cells (Fig. 3A). However, the incubation of guinea-pig tracheal epithelial cells with aspirin-pretreated eosinophils or neutrophils decreased the synthesis of thromboxane B_2 by 15-fold (650 pg/ml) and 4-fold (2100 pg/ml) in the presence of 3 μM arachidonic acid respectively and by 8-fold (1500 pg/ml) and 4-fold (2100 pg/ml) in the presence of 10 μM arachidonic acid, respectively (Fig. 3B).

3.3. Effect of major basic protein on prostaglandin E_2 and thromboxane B_2 synthesis by guinea-pig tracheal epithelial cells

The increase in prostaglandin E_2 synthesis (45-fold) following stimulation of guinea-pig tracheal epithelial cells with arachidonic acid (10 μM), as previously shown in Fig. 3, was inhibited by 12% (6180 pg/ml) and 75% (1980 pg/ml) following incubation for 2 h with 10 and 100 μg/ml major basic protein, respectively (Fig. 5B). In contrast, major basic protein (10 and 100 μg/ml) did not significantly inhibit the arachidonic acid-induced increase in thromboxane B_2 release (data not shown). Major basic protein (10 and 100 μg/ml) did not significantly attenuate the basal release of either prostaglandin E_2 (Fig. 5A) or thromboxane B_2 (data not shown) from guinea-pig tracheal epithelial cells.

4. Discussion

Several reports suggested that the epithelium could release an inhibitory factor which modulates the airway smooth muscle responsiveness (Barnes et al., 1985; Hay et al., 1986). Inflammatory events, involving a predominant eosinophil infiltration of the epithelium, may compromise the protective function of the epithelial cells and account for the changes in airway reactivity (Leff et al., 1991).

In the present study, we investigated the influence of eosinophils and neutrophils on arachidonic acid metabolism by the guinea-pig tracheal epithelial cells. Our results demonstrate that cultured guinea-pig tracheal epithelial cells synthesized significantly greater amounts of prostaglandin E_2 (bronchorelaxant agent) than thromboxane B_2 (bronchoconstrictor agent). In contrast, eosinophils and neutrophils released mainly thromboxane B_2. In the presence of arachidonic acid, the significant increase of prostaglandin E_2 synthesis was concentration-dependent in epithelial cells and eosinophils but was not concentration-dependent in neutrophils. However, incubation with arachidonic acid did not significantly increase the release of thromboxane B_2 by the three cell types.

Prostaglandin E_2 was also reported to be the main cyclooxygenase metabolite released by cultured feline tracheal epithelial cells (Wu et al., 1995). Kroegel and Matthys (1993) demonstrated that thromboxane A_2 was the main cyclooxygenase metabolite synthesized by either unstimulated eosinophils or platelet-activating factor-stimulated eosinophils. On the other hand, it was also demonstrated that guinea-pig eosinophils release significant amounts of prostaglandin E_2 as well as thromboxane B_2 (Aizawa et al., 1990). In addition, human neutrophils have been shown to release more prostaglandin E_2 than thromboxane B_2 (Yu et al., 1992). Kurosawa (1995) reported that thromboxane synthetase inhibitors prevented increased airway reactivity after exposure to allergens and irritants. Our results suggest that inflammatory cells contribute in part to impair the bronchial reactivity by releasing more bronchoconstrictor than bronchorelaxant cyclooxygenase products. Furthermore, the release of large amounts of prostaglandin E_2 by tracheal epithelial cells is in accordance with the major role of the epithelium as modulator of guinea-pig airway smooth muscle reactivity.

The synthesis of thromboxane B_2 by guinea-pig tracheal epithelial cells was not influenced following incubation in the presence of eosinophils and neutrophils. The predominant synthesis of thromboxane B_2 during co-incubation was probably due to the release of this metabolite from these inflammatory cells. However, eosinophils and neutrophils decreased the metabolism of exogenous arachidonic acid to prostaglandin E_2 by guinea-pig tracheal epithelial cells. Alpert and Walenga (1995) suggested that the decrease of prostaglandin E_2 production by the epithelial cells contributes to ozone-induced airway dysfunction. Our results suggest that inflammatory cells could contribute in part to airway dysfunction by impairing release of prostaglandin E_2 by epithelial cells.

In the present study, the incubation of guinea-pig tracheal epithelial cells with major basic protein for 2 h did not affect the basal release of either prostaglandin E_2 or thromboxane B_2. Following the treatment of guinea-pig tracheal epithelial cells with major basic protein, the metabolism of exogenous arachidonic acid to prostaglandin E_2 was decreased without modulating the synthesis of thromboxane B_2. White et al. (1993) showed that major basic protein increased the secretion of prostaglandin E_2, but not thromboxane B_2, from unstimulated guinea-pig tracheal epithelial cells. This discrepancy could possibly be due to the culture conditions used, since in the aforementioned study the guinea-pig tracheal epithelial cells were cultured in the presence of fetal calf serum and growth factors. A correlation between the concentration of basic proteins in the bronchoalveolar lavage and the intensity of bronchial hyperreactivity (Wardlaw et al., 1988) has been demonstrated. Furthermore, major basic protein was reported to induce a bronchial hyperreactivity in primates (Gundel et al., 1991). The intratracheal administration of human major basic protein augmented the bronchoconstriction to intravenous acetylcholine and 5-hydroxytryptamine in guinea pigs (Desai et al., 1993). Hulsmann et al. (1996) suggested that the release of major basic protein by the eosinophils increased the airway permeability to bronchoactive agonists and that this may partly explain the airway dysfunction. Moreover, Flavahan et al. (1988) demonstrated that major basic protein enhanced guinea-pig tracheal responsiveness by inhibiting the function of epithelial but not smooth muscle cells. Our results support the involvement of eosinophil major basic protein as modulator of the airway reactivity by impairing the synthesis of a relaxant factor in the epithelial cells. The incubation of cultured epithelial cells with eosinophil major basic protein inhibited the arachidonic acid-induced release of prostaglandin E_2 by guinea-pig tracheal epithelial cells. However, in the present study it was not possible to evaluate the inhibitory effect of major basic protein on the thromboxane B_2 synthesis since guinea-pig tracheal epithelial cells produced this metabolite in small amounts. Therefore, our results support the hypothesis that major basic protein may impair the ability of guinea-pig respiratory epithelium to produce a relaxing factor (Flavahan et al., 1988), which could be in part related to the synthesis of prostaglandin E_2 in the epithelial cells.

In order to establish the relationship between the effect of inflammatory cells on the synthesis of prostanoids during coincubation with epithelial cells and a possible modulation of the tracheal responsiveness, we measured the epithelium-dependent relaxation of guinea-pig trachea to arachidonic acid in the presence and absence of either eosinophils or neutrophils. Our results also show that eosinophils and neutrophils significantly decreased guinea-pig tracheal relaxations induced by arachidonic acid. This inhibitory effect on tracheal relaxations was reversible after the removal of eosinophils from the organ baths but was not reversed following the removal of neutrophils. The reversibility of the inhibition of relaxation to arachidonic acid by eosinophils but not by neutrophils was probably linked to different mechanisms of action that remain to be clarified. Farmer et al. (1987) demonstrated that the arachidonic acid-induced guinea-pig tracheal relaxations were epithelium-dependent and mediated by an inhibitory product of the cyclooxygenase pathway. Prostaglandin E_2 appears as the main relaxant prostanoid synthesized by guinea-pig trachea (Brink et al., 1981). Irvin et al. (1985) demonstrated that inhalation of an unidentified substance released by the neutrophils modulated the airway reactivity. Our results suggest that inflammatory cells could decrease the synthesis of prostaglandin E_2, an arachidonic acid-derived bronchorelaxant, from guinea-pig tracheal epithelial cells.

In an attempt to establish whether eosinophils and/or neutrophils were releasing a cyclooxygenase product which could affect the arachidonic acid-induced relaxations of guinea-pig trachea, the cells were pretreated with aspirin. Aspirin-pretreated eosinophils and neutrophils caused a similar inhibition of arachidonic acid-induced relaxations to that observed in the presence of untreated cells. This suggests that the release of eicosanoids by either the eosinophils or the neutrophils following stimulation with arachidonic acid did not influence the guinea-pig tracheal relaxations. However, aspirin-treated inflammatory cells could release 5-lipoxygenase metabolites which may modulate the arachidonic acid-induced relaxations of guinea-pig trachea. Indeed, Aizawa et al. (1990) have demonstrated that leukotrienes released by eosinophils induced an increase of airway reactivity in the guinea pig.

In conclusion, the present results show that guinea-pig tracheal relaxations induced by incubation with arachidonic acid were reduced in the presence of either eosinophils or neutrophils and suggest that these cells decrease the synthesis of a bronchorelaxant prostanoid by guinea-pig tracheal epithelial cells and/or release a bronchoconstrictor prostanoid in large amounts. It is unlikely that inflammatory cells could act as a metabolic sink for arachidonic acid to explain these results. Indeed, the concentration of arachidonic acid used was optimal to provide

the maximal synthesis of prostanoids by both epithelial and inflammatory cells during an incubation period of 10 min. It appears that the release of major basic protein from the eosinophil granules could be linked to the decrease of a bronchorelaxant prostanoid synthesized by guinea-pig tracheal epithelial cells, since this polycationic protein significantly inhibited the release of prostaglandin E_2 from guinea-pig tracheal epithelial cells following stimulation with arachidonic acid.

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References

Ackerman, S.J., D.A. Loegering, P. Venge, I. Olsson, J.B. Harley, A.S. Fauci and G.J. Gleich, 1983, Distinctive cationic proteins of the human eosinophil granule; major basic protein, eosinophil cationic protein and eosinophil-derived neurotoxin, J. Immunol. 131, 2977.

Aizawa, T., K. Sekizawa, T. Aikawa, N. Maruyama, S. Itabashi, G. Tamura, H. Sasaki and T. Takishima, 1990, Eosinophil supernatant causes hyperresponsiveness of airway smooth muscle in guinea pig trachea, Am. Rev. Respir. Dis. 142, 133.

Alpert, S.E. and R.W. Walenga, 1995, Ozone exposure of human tracheal epithelial cells inactivates cyclooxygenase and increases 15-HETE production, Am. J. Physiol. 269, L734.

Barker, R.L., R.M. Ten, D.A. Loegering, K.J. Hamann, L.R. Pease and G.J. Gleich, 1989, Eosinophil cationic protein cDNA. Comparison with other toxic cationic proteins and ribonucleases, J. Immunol. 143, 952.

Barnes, P.J., F.M. Cuss and J.B. Palmer, 1985, The effect of airway epithelium on smooth muscle contractility in bovine trachea, Br. J. Pharmacol. 86, 685.

Brink, C., P.G. Duncan and J.S. Douglas, 1981, Histamine, endogenous prostaglandins and cyclic nucleotides in the regulation of airway muscle responses in the guinea-pig, Prostaglandins 22, 729.

Coyle, A.J., S.J. Ackerman, R. Burch, D. Proud and C.G. Irvin, 1995, Human eosinophil-granule major basic protein and synthetic polycations induce airway hyperresponsiveness in vivo dependent on bradykinin generation, J. Clin. Invest. 95, 1735.

Desai, S.N., G. Van, J. Robson, L.G. Letts, R.H. Gundel, G.J. Gleich, P.J. Piper and T.C. Noonan, 1993, Human eosinophil major basic protein augments bronchoconstriction induced by intravenous agonists in guinea pigs, Agents Actions 39, C132.

Dor, P.J., S.J. Ackerman and G.J. Gleich, 1984, Charcot-Leyden crystal protein and eosinophil granule major basic protein in sputum of patients with respiratory diseases, Am. Rev. Respir. Dis. 130, 1072.

Dunnill, M.S., 1960, The pathology of asthma, with special reference to changes in the bronchial mucosa, J. Clin. Pathol. 13, 27.

Fabbri, L.M., H. Aizawa, S.E. Alpert, E.H. Walters, P.M. O'Byrne, B.D. Gold, J.A. Nadel and M.J. Holtzman, 1984, Airway hyperresponsiveness and changes of cell counts in bronchoalveolar lavage after ozone exposure in dogs, Am. Rev. Respir. Dis. 129, 288.

Farmer, S.G., D.W.P. Hay, D. Raeburn and J.S. Fedan, 1987, Relaxation of guinea-pig tracheal smooth muscle to arachidonate is converted to contraction following epithelium removal, Br. J. Pharmacol. 92, 231.

Flavahan, N.A., N.R. Slifman, G.J. Gleich and P.M. Vanhoutte, 1988, Human eosinophil major basic protein causes hyperreactivity of respiratory smooth muscle, Am. Rev. Respir. Dis. 138, 685.

Frigas, E., D.A. Loegering and G.J. Gleich, 1980, Cytotoxic effects of the guinea pig eosinophil major basic protein on tracheal epithelium, Lab. Invest. 42, 35.

Frigas, E., D.A. Loegering, G.O. Solley, G.M. Farrow and G.J. Gleich, 1981, Elevated levels of the eosinophils major basic protein in the sputum of patients with bronchial asthma, Mayo Clin. Proc. 56, 345.

Gleich, G.J., 1990, The eosinophil and bronchial astma. Current understanding, J. Allergy Clin. Immunol. 85, 422.

Gleich, G.J. and C.R. Adolphson, 1986, The eosinophilic leukocyte: structure and function, Adv. Immunol. 39, 177.

Gleich, G.J., D.A. Loegering, K.G. Mann and J.E. Maldonado, 1976, Comparative properties of the Charcot-Leyden crystal protein and the major basic protein from human eosinophils, J. Clin. Invest. 57, 633.

Gleich, G.J., E. Frigas, D.A. Loegering, D.L. Wassom and D. Steinmuller, 1979, Cytotoxic properties of eosinophil major basic protein, J. Immunol. 123, 2925.

Gundel, R.H., L.G. Letts and G.J. Gleich, 1991, Human eosinophil major basic protein induces airway constriction and airway hyperresponsiveness in primates, J. Clin. Invest. 87, 1470.

Hay, D.W.P., S.G. Farmer, D. Raeburn, V.A. Robinson, W.W. Fleming and J.S. Fedan, 1986, Airway epithelium modulates the reactivity of guinea-pig respiratory smooth muscle, Eur. J. Pharmacol. 129, 11.

Holtzman, M.J., L.M. Fabbri, P.M. O'Byrne, B.D. Gold, H. Aizawa, E.H. Walters, S.E. Alpert and J.A. Nadel, 1983, Importance of airway inflammation for hyperresponsiveness induced by ozone, Am. Rev. Respir. Dis. 127, 686.

Hulsmann, A.R., H.R. Raatgeep, J.C. Den Hollander, W.H. Bakker, P.R. Saxena and J.C. De Jongste, 1996, Permeability of human isolated airways increases after hydrogen peroxide and poly-L-arginine, Am. J. Respir. Crit. Care Med. 153, 841.

Irvin, C.G., G. Baltopoulos and P. Henson, 1985, Airways hyperreactivity produced by products from phagocytozing neutrophils, Am. Rev. Respir. Dis. 131, A278.

Kroegel, C. and H. Matthys, 1993, Platelet-activating factor-induced human eosinophil activation. Generation and release of cyclooxygenase metabolites in human blood eosinophils from asthmatics, Immunology 78, 279.

Kurosawa, M., 1995, Role of thromboxane A_2 synthetase inhibitors in the treatment of patients with bronchial asthma, Clin. Ther. 17, 2.

Laemmli, U.K., 1970, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227, 680.

Leff, A.R., J.H. Kimm and D.W. Craig, 1991, Inflammation and cell-cell interactions in airway hyperresponsiveness, Am. J. Physiol. 260, L189.

Morrison, K.J., Y. Gao and P.M. Vanhoutte, 1990, Epithelial modulation of airway smooth muscle, Am. J. Physiol. 258, L254.

Pradelles, P., J. Grassi and J. Maclouf, 1985, Enzyme immunoassays of eicosanoids using acetylcholine esterase as label. An alternative to radioimmunoassay, Anal. Chem. 57, 1170.

Slifman, N.R., D.A. Loegering, D.J. McKean and G.J. Gleich, 1986, Ribonuclease activity associated with human eosinophil-derived neurotoxin and eosinophil cationic protein, J. Immunol. 137, 2913.

Ten, R.M., L.R. Pease, D.J. McKean, M.P. Bell and G.J. Gleich, 1989, Molecular cloning of the human eosinophil peroxidase. Evidence for the existence of a peroxidase multigene family, J. Exp. Med. 169, 1757.

Walls, R.S. and P.B. Beeson, 1972, Mechanism of eosinophilia. IX. Induction of eosinophilia in rats by certain forms of dextran, Proc. Soc. Exp. Biol. Med. 140, 689.

Wardlaw, A.J., S. Dunnette, G.J. Gleich, J.V. Collins and A.B. Kay, 1988, Eosinophils and mast cells in bronchoalveolar lavage in subjects with mild asthma. Relationship to bronchial hyperreactivity, Am. Rev. Respir. Dis. 137, 62.

White, S.R., K.S. Sigrist and S.M. Spaethe, 1993, Prostaglandin secretion

by guinea pig tracheal epithelial cells caused by eosinophil major basic protein, Am. J. Physiol. 265, L234.

Wu, T., R.D. Rieves, C. Logun, J.H. Shelhamer, 1995, Platelet-activating factor stimulates eicosanoid production in cultured feline tracheal epithelial cells, Lung 173, 89.

Yu, C.L., W.M. Lin, T.S. Liao, C.Y. Tsai, K.H. Sun and K.H. Chen, 1992, TammHorsfall glycoprotein (THG) purified from normal human pregnancy urine increases phagocytosis, complement receptor expressions and arachidonic acid metabolism of polymorphonuclear neutrophils, Immunopharmacology 24, 181.