Release of epithelium-derived PGE₂ from canine trachea after antigen inhalation

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McGrogan, I., L. J. janssen, j. wattie, p. m. o’byrne, and e. e. daniel. Release of epithelium-derived PGE₂ from canine trachea after antigen inhalation. Am. J. Physiol. 274 (Lung Cell. Mol. Physiol. 18): L220–L225, 1998.—To investigate the role of prostaglandin (PG) E₂ in allergen-induced hyperresponsiveness, dogs inhaled either the allergen Ascaris suum or saline (Sham). Twenty-four hours after inhalation, some animals exposed to allergen demonstrated an increased responsiveness to acetylcholine challenge in vivo (Hyp-Resp), whereas others did not (Non-Resp). Strips of tracheal smooth muscle, either epithelium intact or epithelium denuded, were suspended on stimulating electrodes, and a concentration-response curve to carbachol (10⁻⁶ to 10⁻⁵ M) was generated. Tissues received electrical field stimulation, and organ bath fluid was collected to determine PGE₂ content. With the epithelium present, all three groups contracted similarly to 10⁻⁵ M carbachol, whereas epithelium-denuded tissues from animals that inhaled allergen contracted more than tissues from Sham dogs. In response to electrical field stimulation, Hyp-Resp tissues contracted less than Sham tissues in the presence of epithelium and more than Sham tissues in the absence of epithelium. PGE₂ release in the muscle bath was greater in Non-Resp tissues than in Sham or Hyp-Resp tissues when the epithelium was present. Removal of the epithelium greatly inhibited PGE₂ release. We conclude that tracheal smooth muscle is hyperresponsive in vitro after in vivo allergen exposure only when the modulatory effect of the epithelium, largely through PGE₂ release, is removed.

Allergen; smooth muscle; prostaglandin E₂

Several Studies have suggested a role for the inhibitory prostaglandin [prostaglandin (PG) E₂] in the regulation of airway hyperresponsiveness and asthma. Responses of canine tracheal and bronchial tissues to stimulation of cholinergic nerves or agonists are reduced by PGE₂ (1, 6, 20). Janssen et al. (12) reported that exposure to ozone produced airway hyperresponsiveness in vitro and that this was due to decreased prejunctional and postjunctional inhibition, potentially mediated by PGE₂. Pavord et al. (18) found that PGE₂ inhibited the early and late responses to allergen and the allergen-induced bronchial reactivity in human allergen-induced asthma. Moreover, Mellilo et al. (15) showed that inhaled PGE₂ decreased exercise-induced bronchoconstriction in asthmatic subjects. Thus PGE₂ plays a protective role in the airway.

In patients with asthma, inhalation of an allergen may result in a biphasic reaction consisting of an early asthmatic response (EAR) and a late asthmatic response (LAR) (23). The EAR is a period of airflow obstruction that usually begins ~10 min after inhalation of the allergen. The EAR is caused primarily by smooth muscle contraction, likely due to the combination of antigens with immunoglobulin E antibodies and a resultant release of mediators such as histamine and leukotrienes from mast cells (23). The LAR is a subsequent period of airflow obstruction and airway inflammation that occurs in ~50% of asthmatic patients and begins 3–4 h after the allergen inhalation (23). The LAR is usually associated with an increase in airway responsiveness to bronchoconstrictors such as methacholine or histamine. This hyperresponsiveness may last for several days or even weeks after exposure to the antigen and is likely a consequence of allergen-induced inflammation (23).

In the present study, we determined the role of epithelium-derived PGE₂ in an animal model of LAR induced by inhalation of Ascaris suum allergen in dogs (5, 16, 25). We examined in vitro the contractions of the trachea and release of PGE₂ by the excised tissue in animals that did or did not develop antigen-induced airway hyperresponsiveness. We found that the epithelium is a major determinant of allergen-induced changes in tracheal smooth muscle responsiveness.

METHODS

Antigen exposure. The methods of antigen exposure have been described previously (26–28). Briefly, 15 healthy adult dogs of either sex were anesthetized with pentobarbital sodium (10 mg/kg body weight). The animals were intubated and attached to a ventilator. The animals inhaled acetylcholine (ACh), and a control concentration-response curve was generated (0.7–80 mg/ml, doubling concentrations) (28). The concentration of ACh that raised the pulmonary resistance 5 cmH₂O·l⁻¹·s above baseline was termed the provocative concentration (PC₅). Ten animals subsequently inhaled the antigen dissolved in saline, and five dogs inhaled saline. The concentration of allergen was increased until the pulmonary resistance was raised to 10 cmH₂O·l⁻¹·s above baseline, after which the animals were ventilated until the pulmonary resistance returned to baseline. The animals were then allowed to recover from the anesthesia.

Twenty-four hours after exposure to the antigen, the dogs were once again anesthetized, and a second ACh concentration-response curve was generated. The animals were then euthanized with pentobarbital sodium (100 mg/kg body weight). The procedures were approved by the University Animal Care Committee following the guidelines of The Canadian Council for Animal Care.

Organ bath studies. Segments of the trachea were removed and placed in Krebs solution that was constantly bubbled with 95% O₂-5% CO₂ to achieve a pH of 7.3–7.4. The composition of the Krebs solution was (in mM) 115.5 NaCl, 4.6 KCl, 2.5 CaCl₂, 1.6 NaH₂PO₄, 1.16 MgSO₄, 21.9 NaHCO₃, and 11.1 glucose. In both epithelium-intact and -denuded tissues, the serosal side was cleaned of connective tissue. In epithelium-intact tissues, the epithelium was left unaltered. For epithelium-denuded tissues, the epithelium was cut away from the smooth muscle and the underlying connective tissue.
was removed. Dissection was performed under a dissecting microscope to prevent damage to the underlying smooth muscle fibers. The tracheal muscle was cut into strips 1–2 mm wide and ~1 cm long parallel to the direction of the smooth muscle fibers. The strips were tied with 4-0 silk thread and mounted for electrical field stimulation (EFS) and recording of contraction in 10-ml organ baths containing the same Krebs solution and bubbled with the same gas mixture as mentioned above. The lower ends of the strips were attached to a hook on the bottom of a plastic holder that also held the electrodes for EFS, and the top ends of the tissue were connected to a Grass FT-03C mechanotransducer. Isometric tension was recorded continuously on a Gould 2800 chart recorder. A preload tension of 1.5 g (previously shown to allow maximum active tension) was applied to each strip. For EFS of tracheal strips, two platinum rings were placed 1 cm apart, and the tissue was suspended through the center of the rings. The tissues were equilibrated for 1 h in the organ baths before the experiments were begun and were kept submerged in the Krebs solution and bubbled at 37°C throughout the experiment.

In each experiment, eight segments of trachea per animal were mounted in the organ baths: four strips of trachea were epithelium intact and four were epithelium denuded. Within each of the two groups of four, two tissues were electrically field stimulated and two served as equivalent time controls.

To evaluate the viability of the tissues, KCl (60 mM) was added to the organ bath to contract the tissues. Fifteen minutes later, the KCl was washed out. This procedure was repeated three times or until consistent, reproducible contractions were generated in each tissue. A cumulative concentration-response (CR) curve was generated to carbachol (CCh; 10⁻⁹ to 10⁻⁵ M, half-log steps) (14). For EFS, the tissues were stimulated at 40 V/cm, 0.5-ms duration pulses for 10 s, at frequencies of 1.0, 3.0, 10.0, and 30.0 pulses/s.

After each experiment, the tissues were removed from the organ bath, and the epithelium was removed from those tracheal strips that were epithelium intact. All tissues were air-dried for at least 48 h, and the dry weight was then determined. There were no significant differences in the dry weight of the tracheal smooth muscle strips among any of the three experimental groups (group that inhaled vehicle (Sham), 5.35 ± 0.28 mg; group that inhaled allergen and did not demonstrate an increased responsiveness to ACh in vivo (nonresponders; Non-Resp), 4.97 ± 0.56 mg; group that inhaled allergen and demonstrated an increased responsiveness to ACh in vivo (hyperresponders; Hyp-Resp), 5.40 ± 0.57 mg).

ACh and CCh were obtained from Sigma Chemical (St. Louis, MO) and were dissolved in distilled water.

Measurement of PGE₂. Two samples from tracheal strips were collected for analysis of PGE₂ content by radioimmunoassay (RIA; Advanced Magnetics, Cambridge, MA) immediately after the tissues were washed of KCl and again as soon after the EFS protocol (or the equivalent time control in parallel tissues from the same animal). The samples were collected with plastic syringes and stored at −70°C before assay, and the average of the two measurements is reported as nanomoles of PGE₂ per liter in the organ bath fluid.

Data analysis. All results are expressed as means ± SE. Significant differences were reported if P < 0.05, with the Bonferroni correction for multiple comparisons performed after analysis of variance. The maximum responses to CCh are reported as grams of tension per milligram of dry tissue. CCh responses are expressed as a percentage of the response to 10⁻⁵ M CCh.

RIA results are expressed as the increment when pre-EFS measurements were subtracted from post-EFS measurements (or time-control values). This was done to normalize for any difference in basal release of PGE₂ between tissues.

RESULTS

Figure 1 shows the degree of airway responsiveness in the animals 24 h after exposure to either vehicle or allergen inhalation expressed as a percentage of the PC₅ before inhalation. Inhalation of saline did not change the airway responsiveness to ACh (Sham group). After exposure to the antigen, five animals demonstrated at least a twofold decrease in the PC₅ (Hyp-Resp group), and five animals exhibited a less than twofold decrease in PC₅ (Non-Resp group). There was a significant increase in airway responsiveness after allergen exposure in Hyp-Resp animals compared with either Sham or Non-Resp animals.

In Fig. 2, the mean tension generated in tracheal strips by 10⁻⁵ M CCh is displayed. In epithelium-intact
tissues, there was no significant difference among the three groups. Removal of the epithelium resulted in significantly greater contraction in all experimental groups, with greater contraction observed in tissues from Hyp-Resp group than from the Sham group. Thus the presence of the epithelium attenuated the contraction to the maximum dose of CCh in all tissues, and this effect was greatest in tissues from Hyp-Resp animals.

The CR curves to CCh (10^{-9} to 10^{-5} M) appear in Fig. 3, where the responses are normalized to the maximum (100%) in each tissue. The half-maximal effective concentration (EC_{50}) values to these curves appear in Table 1. The EC_{50} values reported are the means of the EC_{50} values derived from individual curves and thus may not appear exactly as in Fig. 3. Figure 3A displays the CR curves generated in response to CCh in tissues with intact epithelium; EC_{50} values from Hyp-Resp tissues were significantly greater than those in Sham tissues, indicating that the Hyp-Resp tissues were less sensitive to CCh. Figure 3B displays the curves generated by tissues in which the epithelium had been removed. Removal of the epithelium resulted in a significant leftward shift in the CR curves in all three tissue groups. With the epithelium removed, no significant differences were seen among the EC_{50} values from the Sham, Hyp-Resp, and Non-Resp animals. Table 1 shows that the presence of the epithelium decreased the sensitivity of all the tissues to CCh and that this effect was more pronounced in tissues from Sham animals than in those from Hyp-Resp animals.

The mean contractile responses to EFS of epithelium-intact and -denuded tissues are shown in Fig. 4. Removal of the epithelium resulted in an increased contraction at 10 pulses/s for Sham tissues, at 10 and 30 pulses/s for Hyp-Resp tissues, and only at 30 pulses/s for Non-Resp tissues. Moreover, at 30 pulses/s, removal of the epithelium resulted in significantly greater contraction in all experimental groups, with greater contraction observed in tissues from the Hyp-Resp group than from the Sham group. Thus the presence of the epithelium attenuated the contraction to the maximum dose of CCh in all tissues, and this effect was greatest in tissues from Hyp-Resp animals.

![Fig. 2. Contractile responses of in vitro tracheal strips from Sham, Non-Resp, and Hyp-Resp animals to carbachol (CCh; 10^{-5} M) in presence (+) and absence (−) of epithelium. Values are means ± SE; n = 5 animals/group. Removal of epithelium significantly increased contraction of strips from animals in all 3 groups vs. matched epithelium-intact tissues: **P < 0.01; ***P < 0.001. Tissues from Hyp-Resp animals contracted more than tissues from Sham animals in epithelium-denuded tissues, *P < 0.05.

![Fig. 3. Cumulative concentration-response curves generated in muscle bath to CCh (10^{-9} to 10^{-5} M) in epithelium-intact (A) and epithelium-denuded (B) tissues. Max, maximal. Values are means ± SE; n = 5 animals/group. In A, half-maximal effective concentration of CCh to tissues from Hyp-Resp animals was significantly greater than that from Sham animals, P < 0.05.

<table>
<thead>
<tr>
<th>Table 1. Carbachol EC_{50} values</th>
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<td></td>
<td>n</td>
<td>EC_{50}, ×10^{-7} M</td>
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<tr>
<td>Sham</td>
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<tr>
<td>+Epithelium</td>
<td>5</td>
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<td>−Epithelium</td>
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<td>5</td>
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<tr>
<td>Hyp-Resp</td>
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<td>+Epithelium</td>
<td>5</td>
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<tr>
<td>−Epithelium</td>
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Values are means ± SE; n, no. of animals. EC_{50}, half-maximal effective concentration; Sham, animals inhaling vehicle; Non-Resp, animals that inhaled allergen and did not demonstrate increased responsiveness to acetylcholine challenge in vivo; Hyp-Resp, animals that inhaled allergen and demonstrated increased responsiveness to acetylcholine challenge in vivo; +, with; −, without. EC_{50} values were derived from concentration-response curves to 10^{-9} to 10^{-5} M carbachol in muscle bath.
a greater concentration of PGE$\textsubscript{2}$ was found in the baths from Non-Resp animals than from Sham or Hyp-Resp animals after EFS or a corresponding time control. When the epithelium was removed, PGE$\textsubscript{2}$ release from all tissues was virtually abolished, with the exception of tissues from Hyp-Resp dogs, which, when electrically field stimulated, released small, but significant, amounts of the prostanoid.

**DISCUSSION**

The major findings of the present study are that 1) the demonstration of tracheal smooth muscle hyperresponsiveness in vitro (correlated with that determined in vivo by airway resistance) depends on the absence of the airway epithelium and 2) the tracheal epithelium released more PGE$\textsubscript{2}$ from Non-Resp animals than from Hyp-Resp or Sham animals. Thus epithelium-derived PGE$\textsubscript{2}$ is a major determinant of allergen-induced...
changes in tracheal smooth muscle responsiveness in vitro.

In epithelium-intact tissues, there were no differences in contractile responses to the maximum concentration (10^{-5} M) of CCh among any of the groups of animals. The EC_{50} to CCh of Hyp-Resp tissues was greater than that of Sham tissues (i.e., they were less sensitive to CCh). However, when the epithelium was removed, the maximum contractile response of the tracheal smooth muscle strips to 10^{-5} M CCh was significantly greater in tissues from Hyp-Resp animals compared with tissues from Sham animals.

Similarly, epithelium-intact Hyp-Resp tissues contracted less in response to EFS than did epithelium-intact Sham tissues. The response to EFS in the absence of the epithelium was likewise greater in Hyp-Resp tissues compared with Sham tissues.

Removal of the epithelium is associated with the removal of a barrier. This barrier may be either a physical barrier to diffusion or an enzymatic barrier that degrades contractile agents before they reach the underlying smooth muscle (3, 4, 22). The removal of the barrier properties of the epithelium is not sufficient to explain the data presented in this study, however, for the following reasons. First, these experiments were performed with smooth muscle strips, not tubes, which allowed for simultaneous access of CCh to all exposed surfaces. Second, contractions were assessed after the response to each concentration of CCh reached a plateau. Furthermore, CCh is a nonhydrolyzable muscarinic agonist that is not degraded by enzymes found in the epithelium. Moreover, differences were also seen in response to EFS after removal of the epithelium. EFS induces the release of endogenous ACh from nerves near the muscle inside the epithelial barrier such that differences could not be attributed to the removal of a diffusional barrier.

Consistent with our evidence that removal of the barrier function cannot account for all the inhibitory properties of the epithelium, various studies (1, 8, 9, 24) suggest that the epithelium releases one or more inhibitory factors that can modulate the contraction of the underlying smooth muscle. McGrogan and Daniel (14) previously demonstrated that the inhibitory prostaglandins PGI_2 and PGE_2 are released from canine tracheal and bronchial epithelia and relax airway smooth muscle in vitro. Concentrations of PGE_2 as low as 1 nM can cause inhibition of airway smooth muscle contraction, and at 10 nM PGE_2, this effect is significantly increased (1, 6, 14, 21). Because PGI_2 is much less effective in inhibiting canine tracheal smooth muscle, we did not analyze for PGI_2 release.

Measurements of PGE_2 released into the muscle bath fluid in the presence of the epithelium demonstrated that there was no significant difference between tissues from Sham and Hyp-Resp dogs. In contrast, a significantly greater amount of PGE_2 was released from tissues of Non-Resp animals. When the epithelium was removed, release of PGE_2 was virtually abolished. Thus PGE_2 released from the tracheal epithelium in sufficient concentrations to inhibit smooth muscle contraction (1, 6), appears to contribute to the prevention of airway hyperresponsiveness in animals exposed to antigen.

There have recently been several studies that demonstrated that PGE_2 may play a protective role in airway disease. Gray et al. (10) reported that bronchial PGE_2 was reduced in horses with heaves. Furthermore, inhalation of PGE_2 inhibited the allergen-induced increase in airway hyperresponsiveness seen in asthmatic patients (18) and inhibited exercised-induced bronchial constriction in mild asthmatic patients (15). The results in the present study confirm the protective role of epithelium-derived PGE_2 in allergen-induced airway hyperresponsiveness.

The mechanism through which PGE_2 exerts its protective role may be multifaceted. PGE_2 has been demonstrated to directly relax canine airway smooth muscle and to inhibit ACh release from nerve endings (1, 6). PGE_2 may also act indirectly through effects on inflammatory cells. PGE_2 has been reported to inhibit mediator release from lung mast cells (17) as well as to inhibit eosinophil chemotaxis and survival (2, 7, 13, 17).

A question that arises from this study is why were tissues from Non-Resp animals capable of producing and releasing more PGE_2 than tissues from Hyp-Resp animals. The amount of PGE_2 released from Sham and Hyp-Resp tissues was similar to the amount reported in an earlier study by McGrogan and Daniel (14) in which the animals did not inhale any allergen or vehicle. Thus it appears that, in Non-Resp animals, some protective mechanism of increased epithelial PGE_2 release is activated.

Using the model described in this study, Wooley et al. (28) demonstrated that neutrophils are found in bronchoalveolar lavage (BAL) fluid from Hyp-Resp animals but are not found in BAL fluid from Non-Resp animals. Similarly, Wooley et al. (26) reported a significant increase in eosinophils in BAL fluid and biopsy samples in human asthmatic subjects after allergen challenge. It has also been shown that the number of eosinophils and neutrophils is elevated in the BAL fluid from asthmatic patients after allergen challenge (19). Thus it is possible that there exists a difference in the extent to which inflammatory cells are recruited in Non-Resp versus Hyp-Resp animals. Preliminary ultrastructural studies suggest that tracheal tissues from Non-Resp animals contain large numbers of inflammatory cells (eosinophils and neutrophils) located just under the epithelium. Further studies need to be conducted to determine whether different inflammatory cells are recruited into the Non-Resp and Hyp-Resp animals and whether this can account for differences seen in PGE_2 production after allergen exposure.

The observed increased epithelial PGE_2 release from tissues from Non-Resp animals may also be due to a difference in epithelial PGE_2 synthesis after allergen inhalation, and this may be independent of inflammatory cell recruitment.

Tissues from Non-Resp and Sham animals were equally sensitive to CCh, even though there was an increased release of PGE_2 from Non-Resp tissues when.
the epithelium was present. This may have been due to the release of an excitatory substance from Non-Resp tissues that was not present in either Sham or Hyp-Resp tissues, potentially thromboxane A2. This may also explain why Non-Resp tissues released more PGE2 than did Sham tissues during EFS, yet the Non-Resp tissues contracted more in response to EFS.

A recent study in our laboratory (11) examined the effects of antigen inhalation on the contractile responses of canine bronchi to CCh. It was found that bronchial tissues from Hyp-Resp animals exposed to Ascaris suum were less responsive to CCh administration or EFS than were tissues from Sham or Non-Resp animals (11). This finding is in accord with our present observation that epithelium-intact tracheal tissues from Hyp-Resp animals were less responsive to CCh. When the epithelium was removed from the tracheal tissues, however, the differences in the EC50 values to CCh were eliminated and the maximum contraction to CCh became greater in Hyp-Resp tissues than in Sham tissues. The experiments on bronchial tissues were all performed on epithelium-intact bronchial tissues. Thus it is not known whether removal of the epithelium would have uncovered hyperresponsiveness of the underlying smooth muscle in the bronchi.

The present study examined the role of PGE2 in antigen-induced airway hyperresponsiveness. It was demonstrated that removal of the epithelium uncovered an in vitro hyperresponsiveness in animals that demonstrated hyperresponsiveness in vivo. Measurements of epithelium-derived PGE2 suggested that this mediator played a central role in the masking of tracheal smooth muscle hyperresponsiveness in vitro.

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