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Evidence that the histamine sensitivity and responsiveness of guinea-pig isolated trachea are modulated by epithelial prostaglandin E_2 production

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1 Guinea-pig isolated tracheal preparations in which the epithelium had been removed exhibited a greater contractile response to histamine (intact: 1.91 ± 0.12 g; $n = 6$ and rubbed: 2.76 ± 0.15 g; $n = 11$; $P < 0.001$). The histamine sensitivity (pD_2 value) of these preparations was also significantly greater (intact: 4.80 ± 0.04 and rubbed: 5.40 ± 0.08; $P < 0.01$).

2 Indomethacin suppressed the basal tone of both intact and rubbed preparations but was more effective in the former tissues (intact: -0.70 ± 0.14 g; $n = 22$ and rubbed: -0.17 ± 0.05 g; $n = 12$; $P < 0.02$).

3 Arachidonic acid (AA; 10 μM) suppressed the basal tone of intact tissues but contracted such preparations following indomethacin treatment (1.7 μM; 30 min). However, in rubbed tissues AA (10 μM) induced a contraction which was attenuated following indomethacin treatment.

4 Prostaglandin E_2 (PGE_2; 0.01 and 0.1 μM) suppressed the basal tone of intact preparations and always evoked contraction of rubbed tissues. Following indomethacin treatment PGE_2 (0.01 and 0.1 μM) generally evoked spasm of intact and rubbed tissues while at higher concentrations (1 μM) relaxant effects were observed.

5 Removal of the epithelium did not alter the relaxant effect of PGE_2 (pD_2 value) on histamine (50 μM)-contracted tissues (intact: 6.86 ± 0.08 and rubbed: 7.10 ± 0.3; $n = 4$; $P > 0.1$).

6 In rubbed preparations treated with indomethacin, PGE_2 (0.01 and 0.1 μM) evoked spasm. However, when added to preparations contracted with 5 μM histamine, PGE_2 always caused relaxation.

7 The release of immunoreactive PGE_2 by rubbed preparations during histamine and/or AA stimulation was significantly less than that produced by intact stimulated tissues.

8 Exogenous PGE_2 (0.01–1 μM) decreased the maximal response and sensitivity of rubbed tracheal preparations to histamine.

9. These results suggest that release of an epithelial derived cyclo-oxygenase product, namely PGE_2, may regulate basal tone, histamine response and sensitivity of the guinea-pig isolated trachea.

Introduction

The magnitude of the contractile response to histamine in isolated guinea-pig tracheal preparations is related to the inherent basal tone and to the capacity of the tissue to release products of the cyclo-oxygenase pathway (Farmer *et al.*, 1972; Orehek *et al.*, 1973; Brink *et al.*, 1981). Recently, modification of the response to various contractile agents in tracheal preparations has been associated with the mechanical removal of the epithelium (Flavahan *et*

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al., 1985; Hay *et al.*, 1985; Barnes *et al.*, 1985). Together these observations suggest that a major component in airway muscle reactivity may be the interaction of epithelial-derived products with the underlying muscle. Several investigators (Hay *et al.*, 1986; Ilhan & Sahin, 1986; Nijkamp & Folkerts, 1987; Tschirhart *et al.*, 1987) have suggested that the degree of muscle responsiveness is dependent on the production of a relaxant product from the tracheal epithelium.

It is well known that prostaglandin E_2 (PGE_2) is liberated from guinea-pig airway muscle preparations at resting tone and during muscle contaction (Orehek *et al.*, 1975; Grodzinska *et al.*, 1975). The potency of PGE_2 as a relaxant cyclo-oxygenase product is well established (Brink *et al.*, 1981). However, other workers (Coleman & Kennedy, 1985; Gardiner, 1986) have shown that this agonist also produces contractions in guinea-pig airway muscle preparations. The excitatory and inhibitory effects of PGE_2 are similar to those observed when arachidonic acid (AA) is added to intact (Orehek *et al.*, 1975) or epithelium-denuded tracheal preparations (Nijkamp & Folkerts, 1987).

This study was undertaken to examine the capacity of PGE_2 to mimic the physiological effects of AA in both intact and epithelium-denuded tracheal preparations of the guinea-pig. It was anticipated that a comparison of the effects of PGE_2 and AA might elucidate whether an epithelium-derived product of the cyclo-oxygenase pathway influences the underlying smooth muscle layer and whether PGE_2 could be that product.

Methods

Preparation of tissues

Adult male guinea pigs (360 ± 8 g; Hartley strain, CEFDA, Lyon, France) were killed by a blow to the head and exanguinated. Tracheae were removed, cut as spirals and divided into two groups: intact tissues (epithelium present) and rubbed preparations in which the epithelium had been removed by vigorously rubbing the luminal surface with filter paper.

The spirals were mounted in 10 ml organ baths under an initial load of 5–8 g. This high initial load was not mechanically altered during the experimental protocols and ensured maximal responses. These loads were determined from preliminary experiments where the effects of histamine were examined on preparations set up at different initial loads (1–10 g). The tissues were allowed to equilibrate for 90 min in Tyrode solution (composition in mM): NaCl 139.2, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, NaH_2PO_4 0.4, glucose 5.5, pH 7.4 and gassed with 5% CO_2 and 95% O_2 at 37°C. During the equilibration period the bath fluid was exchanged every 10 min with fresh Tyrode solution. All protocols were applied to both intact and rubbed preparations.

Protocols

Initial load and histamine contraction Guinea-pig tracheal preparations (intact and rubbed) were set up at specific initial loads (1–10 g). Subsequent to a 90 min equilibration period a cumulative concentration-effect curve for histamine was generated (see below). Only one intact or rubbed tracheal preparation was used for each specific initial load. These experiments were performed in order to establish those pre-loads which would ensure that agonist-induced effects would be maximal.

Histamine concentration-effect curves After the equilibration period, the tissues were contracted with histamine (50 μM). When the response reached a plateau, the bath fluid was exchanged at 10 min intervals until the preparations returned passively to their initial resting tone. A second contraction was induced with histamine (50 μM) and the tissues were again washed with Tyrode solution. When the basal tone was re-established, histamine concentrations were added to the tissue baths in a cumulative manner, beginning with the lowest concentration.

Effects of indomethacin Each preparation was contracted twice with a maximally effective concentration of histamine (50 μM), that is, the tissues were primed. Subsequent to these challenges, and, when the resting tone of the preparations was stable, indomethacin (1.7 μM) was added to the organ baths for 30 min. In some preparations at the end of this incubation period the tissues were challenged with forskolin (10 μM).

In another series of experiments, the contractile response to a fixed concentration of histamine (50 μM) was evaluated before and after treatment of the tissues with indomethacin. In these experiments, the preparations were contracted with histamine (50 μM; control contraction). When the effect reached a plateau, the bath fluid was exchanged and the tissues were washed periodically until the preparation returned passively to the resting tone. Indomethacin (1.7 μM) was then added to the organ baths and after a 30 min incubation, the tissues were washed and challenged with histamine (50 μM).

Effects of arachidonic acid and prostaglandin E_2 on basal and induced tone Data from preliminary experiments using intact tissues and several concen-

trations of AA (0.1–10 μM) showed that AA (10 μM) always induced a relaxant effect in these preparations. This concentration was then used in subsequent experiments on both intact and rubbed preparations. In one series of experiments the effects of AA (10 μM) were evaluated on basal tone before and after indomethacin (1.7 μM; 30 min). A second protocol in which tone was induced with histamine (50 μM) was also performed. When the contraction reached a plateau AA (10 μM) was added to the tissue bath.

The effects of graded concentrations of PGE_2 (0.01–1 μM) were likewise studied while the tissues were at resting tone. In these experiments PGE_2 was added to the organ bath in a cumulative fashion. When the maximal response was achieved, the bath fluid was exchanged every ten minutes until the preparations returned passively to their initial baseline. Indomethacin (1.7 μM; 30 min) was then added to the bath and, following drug equilibration, the tissues were washed and challenged with the same concentrations of PGE_2. In another group of experiments the effects of PGE_2 were assessed on intact and rubbed preparations where tone had been induced with a maximally effective concentration of histamine (50 μM).

Finally, the effects of PGE_2 (0.01 and 0.1 μM) on rubbed tissues treated with indomethacin (1.7 μM; 30 min) were also examined. A concentration of histamine (5 μM) was added to the organ bath and when the contraction reached a plateau, PGE_2 (at the concentrations indicated above) was added to the preparations in a cumulative fashion. When the effect of PGE_2 reached a plateau the preparations were washed until the tone returned to the initial level. The tissues were then challenged with the same concentrations of PGE_2. The histamine concentration (5 μM) used in these experiments was the EC_{50} determined from the cumulative concentration-effect curves of rubbed preparations.

Effect of prostaglandin E_2 on histamine concentration-effect curves in rubbed preparations We evaluated the effects of PGE_2 on histamine cumulative concentration-effect curves by incubating (20 min) the tissues with different concentrations of PGE_2 (0.01, 0.1 or 1 μM) or with vehicle. When the resting tone was stable, the histamine curves were produced by adding graded concentrations of the agonist to the tissue baths in a cumulative fashion (see above).

Radioimmunoassay of prostaglandin E_2

The measurement of PGE_2 was performed on samples of bath fluid derived from preparations at resting tone and after challenge with (1) histamine (50 μM), (2) AA (10 μM) and (3) histamine combined with AA. The following protocols were used. A bath fluid sample was collected at the end of a 25 min period at basal tone. Subsequent to this sample collection the preparations were re-equilibrated for 5 min and challenged with histamine (50 μM). A second sample was collected 20 min after the contractile agent had been added to the organ bath. The tissues were then washed until the preparation returned to baseline. A third sample was obtained after another 25 min period. The tissues were then re-equilibrated for 5 min and stimulated with AA (10 μM; 20 min). At the end of this stimulation the fourth sample was collected. In some experiments, preparations were challenged with histamine (50 μM; 10 min) and subsequently stimulated with AA (10 μM; 10 min) and then the fourth sample was collected. The samples of bath fluid were frozen immediately and kept at -20°C. At the end of the experiment the wet weight of the trachea was measured. The detection of PGE_2 in the samples was determined by use of iodinated radioimmunoassay kits. Aliquots of the sample (100 μl) were assayed without extraction according to previously published techniques (Haye-Legrand *et al.*, 1986). The sensitivity of this assay was 0.5 pg and the percentage cross-reactivity characteristics against PGE_2 for anti-PGE_2 were: PGE_1, 6.5; 6-keto-$PGF_{1\alpha}$, 0.01 and thromboxane B_2, 0.01. The amounts of the immunoreactive prostanoid PGE_2 in the samples were expressed as pg mg^{-1} tissue.

Calculations

Changes in force (g) were measured directly with Narco isometric force-transducers (F60) and Narco physiographs (MK-IV). The results obtained when the effect of a single agonist concentration was evaluated on the resting tone of tracheal preparations were expressed in g. Relaxations are designated by a negative symbol (−) while contractions are indicated by a positive sign (+). Contractile agonist concentration-effect curves are presented in g and relaxation curves are expressed as % reversal of the histamine-induced contraction. Concentration-effect curves to histamine and PGE_2 were fitted visually through the data points. The EC_{50} values (the concentration of agonist yielding 50% of maximal contraction) were interpolated from the individual concentration-effect curves. These values were transformed into negative logarithms (pD_2 values). All results are expressed as means ± s.e.mean. Statistical analysis was performed by use of Student's *t* test for paired or unpaired variables as appropriate. A probability value of less than 0.05 was considered to be statistically significant.

Drugs

The drugs and their sources were: histamine dihydrochloride, prostaglandin E_2, arachidonic acid (sodium salt) and indomethacin (each from Sigma Chemical Co., Saint Louis, MO., U.S.A.); forskolin (Calbiochem, Meudon, France). Iodinated PGE_2 ($0.1\,\mu Ci\,mmol^{-1}$) and rabbit anti-PGE_2 were obtained from the Pasteur Institute (Marnes-la-Coquette, France).

Results

Initial load and histamine contraction

The response and sensitivity to histamine of tracheal preparations (intact or rubbed) when set up under initial loads of 1–10 g are presented in Figure 1. These data suggest that high initial loads are necessary in both types of muscle preparations in order to obtain maximal responses to histamine of the greatest absolute size.

Histamine concentration-effect curves

The data presented in Figure 2 show that the rubbed preparations exhibited an increased maximal response and sensitivity to histamine. The maximal response of intact preparations (1.91 ± 0.12 g; $n = 6$) was significantly different from that obtained in rubbed tissues (2.76 ± 0.15 g; $n = 11$; $P < 0.001$). The histamine sensitivity (pD_2 value) was also significantly different in these preparations (intact: 4.80 ± 0.04 and rubbed: 5.40 ± 0.08; $P < 0.01$).

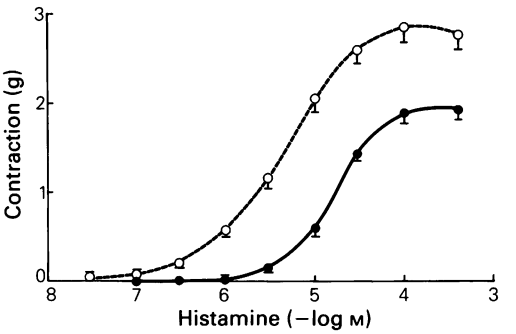

Figure 2 Histamine concentration-effect curves in intact (●; $n = 11$) and rubbed (○; $n = 6$) guinea-pig tracheal spirals under an imposed tension of 5–8 g. Results are presented in (g). The values are mean and vertical lines indicate s.e.mean.

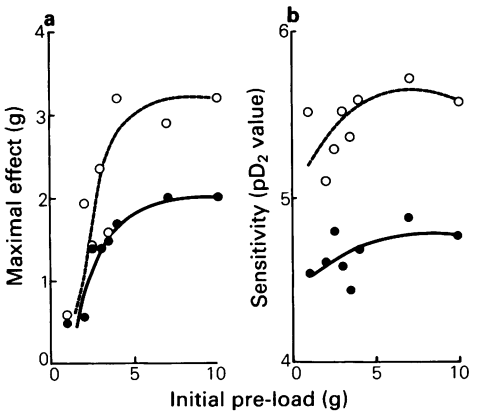

Figure 1 Effects of preload on maximal effect and pD_2 values of histamine in guinea-pig tracheal spirals. Intact (●) and rubbed (○) preparations were set up at specific initial pre-loads (1–10 g). After the equilibration period a histamine concentration-effect curve was generated and the maximal response (g: a) induced was recorded. The sensitivities (pD_2 values: b) were interpolated from the concentration-effect curves. One intact or rubbed preparation was used for each specific load.

Effects of indomethacin

Histamine ($50\,\mu M$) produced a spasmogenic response which was significantly increased following indomethacin treatment of intact preparations (before: 1.97 ± 0.16 g and after: 3.16 ± 0.21 g; $n = 22$; $P < 0.001$). In these tissues, this increased histamine maximal response (difference between the two contractions: 1.19 ± 0.16 g; $n = 22$) and the relaxation induced by indomethacin treatment (-0.70 ± 0.14 g; $n = 22$) were correlated (Figure 3, $r^2 = 0.53$; $P < 0.001$). In contrast, rubbed preparations exhibited no significant increase in the maximal response to histamine (before: 2.52 ± 0.18 g and after: 2.66 ± 0.23 g; $n = 12$; $P < 0.10$) and the difference between the two contractions was 0.14 ± 0.12 g ($n = 12$). The relaxant effect of indomethacin in these preparations (-0.17 ± 0.05 g; $n = 12$) was also correlated with the histamine response after the indomethacin treatment ($r^2 = 0.53$; $P < 0.001$). A comparison of results obtained between the two groups of preparations indicated that the initial contractions were significantly different ($P < 0.05$) while the histamine responses subsequent to the indomethacin treatment were not statistically different ($P > 0.10$). The indomethacin relaxant effect in intact tissues was significantly different from that observed in rubbed preparations ($P < 0.02$). However, we observed a residual tone in both types of preparations since forskolin ($10\,\mu M$) administered following the indomethacin treatment further reduced

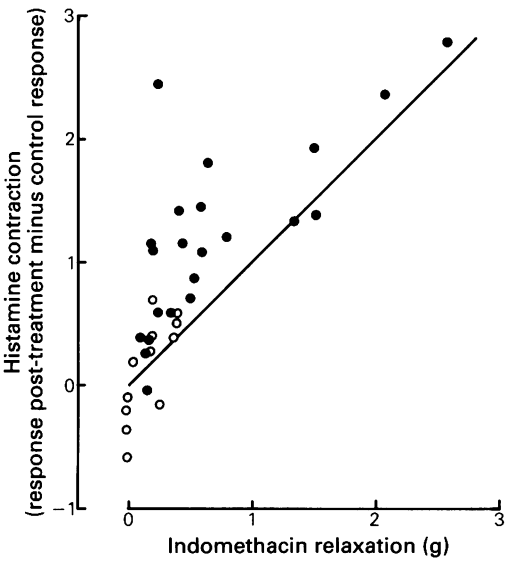

Figure 3 Guinea-pig tracheal spirals: the relationship between the relaxant effect (g) of 1.7 μM indomethacin on basal tone and the histamine contraction (g) observed after indomethacin treatment (difference between the contraction after indomethacin treatment and the control contraction). The solid line represents the line of identity. Data from intact (●; $n = 22$) and rubbed (○; $n = 12$) preparations are shown.

basal tone (intact: -0.08 ± 0.03 g; $n = 6$ rubbed: -0.05 ± 0.02 g; $n = 6$; $P > 0.3$).

Effects of arachidonic acid and prostaglandin E_2 on basal and induced tone

AA (10 μM) always relaxed the basal tone of intact guinea-pig tracheal preparations. After removal of the epithelium, this effect was converted into a contractile response (Table 1 and Figure 4). Subsequent to an incubation period with indomethacin (1.7 μM; 30 min), the relaxant response to AA (10 μM) in intact tissues was likewise changed to a contractile response. However, in rubbed preparations the contractile response to AA (10 μM) was abolished by the indomethacin treatment.

The results obtained with PGE_2 are also presented in Figure 4 and are representative of data obtained in a number of experiments ($n = 4$–5). At low concentrations (0.1 μM) PGE_2 always relaxed intact preparations but contracted either rubbed or preparations (intact or rubbed) treated with indomethacin. In contrast, a higher concentration (1 μM) always relaxed the tissues. The effects of an intermediate concentration of PGE_2 (0.1 μM) were variable in that a contraction or relaxation was observed and

Table 1 Effects of arachidonic acid (1 μM) on the basal tone of tracheal spirals

	Response (g)	
	Control	*Indomethacin*
Intact	-0.44 ± 0.10 ($n = 8$)	$+0.52 \pm 0.22$* ($n = 6$)
Rubbed	$+0.43 \pm 0.12$* ($n = 8$)	0.00 ± 0.00** ($n = 6$)

The symbols (+) and (−) indicate a contractile and relaxant response, respectively. Values are mean ± s.e.mean. n is the number of preparations used. * Indicates values significantly different from control preparations. * Indicates values significantly different from intact preparations.

frequently a contraction followed by a relaxation. These data indicate that the effects of AA and PGE_2 were similar.

When tone was induced with histamine, AA (10 μM) caused a relaxation of intact preparations

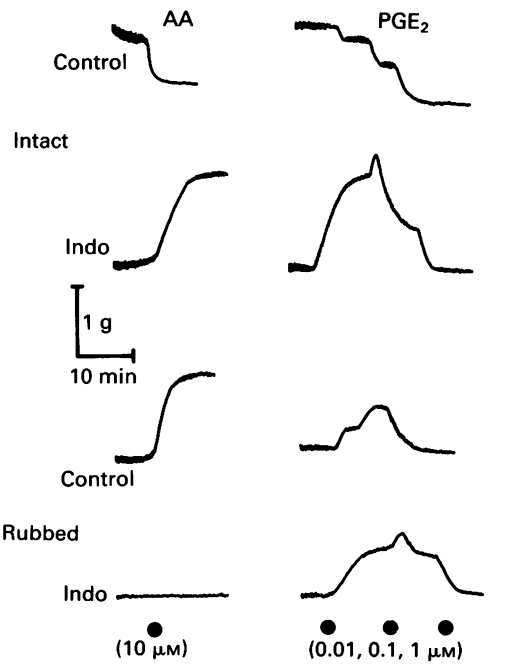

Figure 4 The effects of arachidonic acid (AA; 10 μM) and prostaglandin E_2 (PGE_2; 0.01–1 μM) on the basal tone of tracheal spirals. A representative experiment of the effects of PGE_2 ($n = 4$–5) and arachidonic acid ($n = 6$–8) on resting tone of intact (top) and rubbed (bottom) tracheal preparations. Data from control and indomethacin (Indo, 1.7 μM)-treated tissues are presented. Both force (g) and time (min) scales are shown.

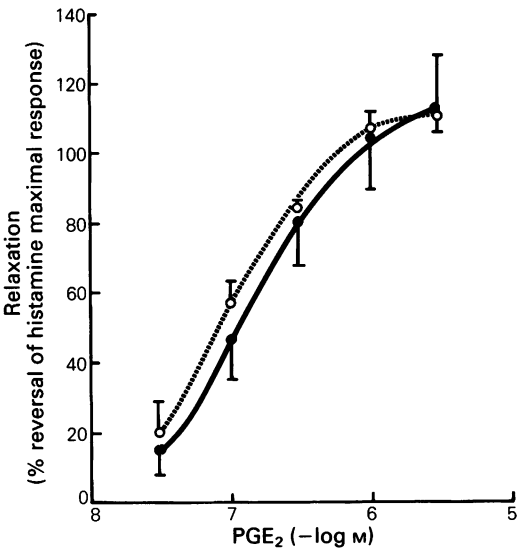

Figure 5 Log concentration-relaxation curves produced by prostaglandin E_2 (PGE_2)in histamine (50 μM)-contracted intact (●; $n = 4$) and rubbed (○; $n = 4$) guinea-pig tracheal spirals. Results are expressed as % reversal of the histamine-induced maximal response. The values are mean and vertical lines show s.e.mean.

(70 ± 7%, $n = 11$) while on rubbed preparations the relaxation was significantly attenuated (17 ± 6%; $n = 7$; $P < 0.05$). The effects of PGE_2 on histamine-contracted preparations (intact and rubbed) are shown in Figure 5. The PGE_2 sensitivities (pD_2 values) were the same (intact: 6.86 ± 0.08 and rubbed: 7.10 ± 0.13; $n = 4$; $P > 0.1$).

In another series of experiments using rubbed, indomethacin-treated preparations ($n = 3$), the same concentrations of PGE_2 induced either a relaxation or contraction, an effect which was dependent on the tone of the preparation. In these protocols histamine (5 μM; the EC_{50} obtained in rubbed preparations) was used to elevate the tone. A representative recording from these experiments is shown in Figure 6.

Effect of prostaglandin E_2 on histamine concentration-effect curves in rubbed preparations

The histamine maximal response and sensitivity of rubbed preparations were significantly reduced in the presence of exogenously added PGE_2 (Figure 7). The histamine maximal responses were: 2.90 ± 0.33 g; 2.15 ± 0.47 g and 1.32 ± 0.15 g ($n = 5$) for the different concentrations of PGE_2 (0.01, 0.1 and 1 μM, respectively). The pD_2 values under the same conditions were: 5.17 ± 0.04; 5.06 ± 0.07 and 4.75 ± 0.04 ($n = 5$, respectively). The pD_2 data

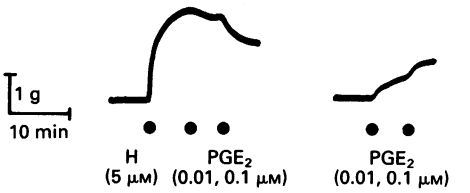

Figure 6 The effect of prostaglandin E_2 (PGE_2; 0.01 and 0.1 μM) on rubbed tracheal spirals treated with 1.7 μM indomethacin. A representative of 3 experiments is presented. Force calibration and time scales are shown. The histamine (H) concentration (5 μM) used was sufficient to induce a tone similar to the basal tone of intact preparations.

demonstrate that there was a dextral displacement of the histamine concentration-effect curve in rubbed preparations which was dependent on the concentration of PGE_2.

Determination of prostaglandin E_2 by radioimmunoassay

The basal production of immunoreactive PGE_2 did not differ between intact and rubbed isolated tracheal preparations of the guinea-pig. The quantities of PGE_2 released by both types of preparations when stimulated with either histamine (50 μM), arachidonic acid (10 μM) or a combination of these agents are shown in Figure 8. The increased PGE_2 release observed during stimulation of both types of preparations was significantly less in rubbed than that observed for intact tissues.

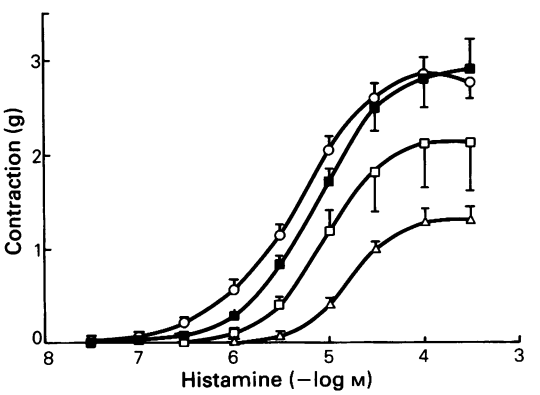

Figure 7 Cumulative concentration-effect curves to histamine in rubbed tracheal spirals in the presence of exogenous prostaglandin E_2 at three different concentrations: 0.01 μM (■; $n = 5$), 0.1 μM (□; $n = 5$), 1 μM (△; $n = 5$) and under control conditions (○; $n = 11$). Results are presented in g and are mean with vertical lines indicating s.e.mean.

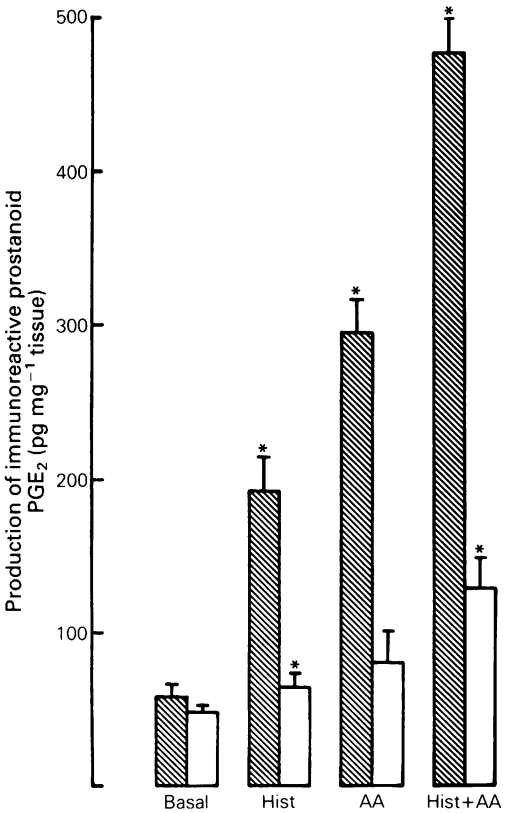

Figure 8 The production of immunoreactive prostaglandin E_2 (PGE_2) by intact (hatched columns) and rubbed (open columns) tracheal preparations. Basal production of PGE_2 and release from preparations stimulated with either histamine (Hist; 50 μM), arachidonic acid (AA; 10 μM) or the combination of these agents were determined. Values are mean with vertical lines showing s.e.mean. * Indicates values significantly different from results obtained in appropriate control experiments.

Discussion

The observation that there was an increase in sensitivity to several contractile agents in guinea-pig isolated tracheal ring preparations subsequent to the removal of the epithelial lining has been made by a number of investigators (Goldie *et al.*, 1986; Farmer *et al.*, 1986; Holroyde, 1986). The results obtained with histamine on guinea-pig tracheal spiral preparations (this paper) are in agreement with those initial observations and demonstrate that this phenomenon was independent of the types of tissue preparations used (spirals or rings). In addition to the contractile agonist hypersensitivity, Hay & co-workers (1986) demonstrated that epithelium-denuded airway muscle preparations exhibited an increased maximal response to several agonists including histamine. In contrast, other investigators (Goldie *et al.*, 1986; Farmer *et al.*, 1986; Holroyde, 1986) failed to demonstrate an increase in maximal response to this contractile agent. However, these differences may be related to the low initial pre-loads used by these investigators when setting up the airway muscle preparations. Data presented in this paper suggest that high initial loads are necessary to ensure maximal responses of the greatest absolute size in intact and rubbed guinea-pig tracheal spirals.

It is at present not clear whether the alteration in maximal response and/or the increased sensitivity of the tissue to the contractile agent after epithelium denudation result from a common mechanism or from differing mechanisms. Therefore, it may be important to distinguish between these two pharmacological parameters.

The capacity of the epithelial lining of guinea-pig isolated airway muscle preparations to produce a factor which regulates muscle responsiveness has already been suggested by Orehek & co-workers (1973). In addition, Orehek *et al.* (1975) demonstrated the conversion of the arachidonic acid relaxant effect to a contractile response in the presence of indomethacin. This observation has been confirmed by several other investigators (Adcock & Garland, 1980; Mitchell & Denborough, 1980; this paper). These results suggest that AA-induced relaxations are caused by a cyclo-oxygenase product. The conversion of an AA-induced relaxation to a contraction has also been demonstrated in epithelium-denuded preparations (Nijkamp & Folkerts, 1987; this paper). We observed (this study) that the contraction of rubbed preparations with AA (10 μM) was attenuated by treatment of these tissues with indomethacin. Previous investigators (Nijkamp & Folkerts, 1987; Farmer *et al.*, 1987) were unable to prevent the AA contractile effect in rubbed preparations with indomethacin. The discrepancy between these observations and the results presented here may be due to the high concentrations of AA (100 μM) used in those initial studies. The relaxant effects of indomethacin were greater in intact preparations compared with the relaxant effects in rubbed tissues, results which are similar to those found in other studies (Hay *et al.*, 1986). Furthermore, the maximal response to histamine was greater in denuded as compared to intact preparations (this paper), results similar to those observed after indomethacin treatment (Orehek *et al.*, 1975; Brink *et al.*, 1981) in intact tissues. These data, together with the results that indomethacin treatment of denuded airway muscle preparations does not modify the contraction induced by histamine (Figure 3), suggest that a product of the cyclo-oxygenase pathway may be responsible for the

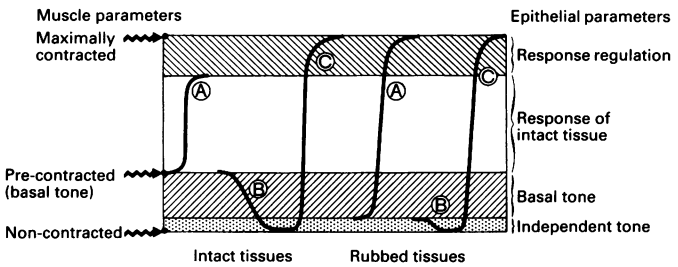

Figure 9 Epithelium-dependent airway muscle regulation. The muscle can be characterized as non-contracted. Intact airway preparations will contract upon appropriate stimulation (A). The magnitude of the response will depend on the initial basal tone (pre-contracted). Indomethacin treatment will reduce the basal tone (B) so that the tissue will exist in a reduced pre-contracted state. Stimulation with a contractile agent now induces a significant increase in response (C). In rubbed airway muscle preparations the pre-contracted level is reduced (A) and, therefore, the response (C) will be similar to intact tissues after indomethacin treatment. The epithelial layer functions principally as a regulator of basal tone in airway muscle preparations by releasing a product that is contractile and elevates the tone of the airways.

spontaneous tone of guinea-pig isolated tracheal preparations. The similar effects produced by AA and PGE_2 after indomethacin or subsequent to removal of the epithelium suggests that denudation, like indomethacin, reduced the tone of the muscle preparation by suppressing a contractile cyclo-oxygenase-dependent input at the airway muscle level (Figure 9). These results suggest that the tone of the muscle preparations can be reduced by removal of the epithelial lining and that this tone is dependent on the cyclo-oxygenase pathway. Indeed, the detection of immunoreactive PGE_2 in rubbed preparations which were stimulated with either histamine and/or AA was always less compared with results obtained in appropriate control tissues (intact). These data are similar to those results obtained in the rabbit bronchial explants (Butler *et al.*, 1987).

Gardiner & Collier (1980) originally proposed that PGE_2 could act at two receptors on the airway muscle, one involved in the relaxation and the other linked to contraction. However, our results in rubbed preparations treated with indomethacin (Figure 6) demonstrated relaxation and contraction using the same concentration of PGE_2. These data indicate the importance of muscle tone in determining the effect of PGE_2 on airway muscle preparations and offer another possible explanation for the dual action of this agent.

It has been known for some time that PGE_2 can restore basal tone and reverse the enhanced contractility to agonists in tracheal preparations treated with indomethacin (Orehek *et al.*, 1975). In rubbed preparations (this paper) PGE_2 caused a dextral displacement of the histamine concentration-effect curve. This functional antagonism further suggests a regulatory role for PGE_2 in guinea-pig airway muscle. preparations. It is difficult to understand why indomethacin treatment does not alter histamine pD_2 values while removal of the epithelium does. However, there may be other modulatory mechanisms involved in epithelium removal which result in an alteration of tissue sensitivity.

These data clearly link the epithelial cyclo-oxygenase pathway with the reactivity of the underlying airway muscle. This interaction is, in part, intimately regulated by PGE_2.

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