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An epithelial-derived factor inhibits contraction in canine perfused bronchial segments

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Abstract

We have previously reported, using a novel preparation of canine airway segments, that the sensitivity of acetylcholine was greater when applied to the adventitial (outside) surface than the epithelial (inside) surface. The present study investigated if this "barrier-effect" was partly the result of pharmacological modulation by the epithelium. As previously demonstrated, canine airway segments were less sensitive to inside than outside application of acetylcholine (pD_2 3.0 ± 0.4 and 4.5 ± 0.4, respectively, $P < 0.001$, $n = 5$). The addition of donor bronchi significantly decreased the sensitivity of the airway segment to outside application of acetylcholine (pD_2 4.3 ± 0.2 and 3.6 ± 0.2, respectively, $P < 0.002$, $n = 4$). Indomethacin (2.5 μM) treatment of both the donor bronchi and the airway segment and removal of donor epithelium abolished the rightward shift in the acetylcholine–response curves. In addition, inhibition of cyclooxygenase within the airway segments themselves, but not the donor bronchi, also inhibited the rightward shift in the curves. These results indicate that the donor epithelium is capable of pharmacologically modulating responses of the airway segment to outside applied acetylcholine by producing an epithelial-derived factor, which in turn causes the release of a downstream cyclooxygenase product from within the airway segment.

Keywords: Acetylcholine; Airway epithelium; Smooth muscle, airway; Nitric oxide (NO); Indomethacin; Airway segment

1. Introduction

Damage of the airway epithelial layer may be involved in the pathophysiology of asthma (Dunnill, 1960; Naylor, 1962; Laitinen et al., 1985; Jeffery et al., 1989; Ollerenshaw and Woolcock, 1992) and may be a contributing factor to non-specific airway hyperresponsiveness to inhaled histamine or methacholine (Laitinen et al., 1985; Jeffery et al., 1989). In vivo, inhaled agonists gain access to the smooth muscle by diffusing from the epithelial surface. Similarly, agonists applied to the epithelial surface of perfused airway segment preparations also gain access to the smooth muscle by diffusing from the epithelial surface.

Removal of epithelium from airway segments results in an increased sensitivity, approximately 100-fold, to bronchoconstrictors applied to the epithelial/inside surface (Mitchell et al., 1993; Gao and Vanhoutte, 1994; McParland et al., 1998) and this increase is comparable to the range of airway sensitivities to the inhaled agonists histamine or methacholine observed in vivo (Woolcock et al., 1991). Several mechanisms could account for this increase in airway sensitivity. The epithelium may provide a diffusion barrier (Gao and Vanhoutte, 1994; Hulsmann et al., 1996), a metabolic barrier (Small et al., 1990; Koga et al., 1992; Ohrui et al., 1992; Tamaoki et al., 1994; Taisne et al., 1997) and a pharmacological barrier (Flavahan et al., 1985; Barnes et al., 1985; Aizawa et al., 1988; Gao and Vanhoutte, 1994) to bronchoconstrictors applied to the epithelial surface.

We have previously reported that by selective application of acetylcholine to either the epithelial (inside) or the adventitial (outside) surface, a 10-fold difference in sensitivity can be demonstrated (McParland et al., 1998). If this difference is in part a pharmacological barrier, then the airway epithelium must release an inhibitory factor, which attenuates airway smooth muscle contraction and which

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can be detected in a biological assay system that consists of two tissues in close proximity; one the source of the inhibitory factor and the other used to detect the effect of the factor.

The aim of this study was to use our novel perfused bronchial segment preparation to bioassay a pharmacological mediator released from a donor airway preparation.

2. Materials and methods

2.1. Lung collection and preparation of canine airway segments

Adult mongrel dogs were killed with an overdose of barbiturate (thiopentone sodium, 160 $mg \cdot kg^{-1}$). The left lung was resected and placed into Krebs–Henseleit buffered solution (Krebs) at 4°C. Airway segments were prepared as previously reported (McParland et al., 1998). Briefly, airways were dissected free from the surrounding parenchyma of the cranial portion of the cranial lobe to produce a 2-cm-long airway segment (4 to 6 mm mean internal diameter (ID) at 7 $cm \cdot H_2O$) that was "fluid tight" throughout its length. To examine the effect of the addition of donor airway to the organ bath, two large airway segments (ID > 4 mm) were dissected from the lower left lobe and the cranial portion of the cranial lobe. These donor airways were cut down their longitudinal axis and, with the epithelial surface facing outwards, were pinned to cork boards. The donor bronchi were equilibrated at 37°C in carbogenated (95% O_2, 5% CO_2) Krebs and remained in a separate organ bath until they were added to the bath with the bioassay bronchial segment in place. The bathing and luminal fluid for these tissues was replaced at approximately 20-min intervals.

This method has been previously described in detail (McParland et al., 1998). Briefly, bioassay bronchial segments were attached to adapters and placed into a 50-ml horizontal organ bath (Fig. 1). The transmural pressure inside the bronchial segment was set at 7 $cm \cdot H_2O$, since this was within the range of optimal pressure for canine airway segment responsiveness. Following a 90-min equilibration in Krebs, acetylcholine was added at 100 μM to the outside of the bronchial segment to test for tissue viability. When the response to acetylcholine had reached a plateau, a complete washout of acetylcholine was achieved by exchange of the bathing and luminal fluid three to four times. In order to make it easier to measure responses of the bronchial segment to inside application of agonist, the method published previously was slightly altered (McParland et al., 1998). In this present study, luminal/transmural pressure within the segment remained relatively constant during inside injections of agonist, because the excess effluent was allowed to overflow out of a secondary column (Fig. 1g). When the effect of the agonist had reached a plateau, a positive pressure was applied to the outside of the bronchial segment. This temporarily collapsed the bronchial segment and displaced the remaining luminal fluid to the pipette (Fig. 1h). The positive pressure was created by temporarily removing the bath fluid and sealing the organ bath while pumping air into the bath at a pressure of 50 $cm \cdot H_2O$. This pressure was sufficient to maximally close the segment. The difference between pre-stimulated luminal volume and post-stimulated volume was the change in luminal volume. The use of this method also meant that cumulative, rather than

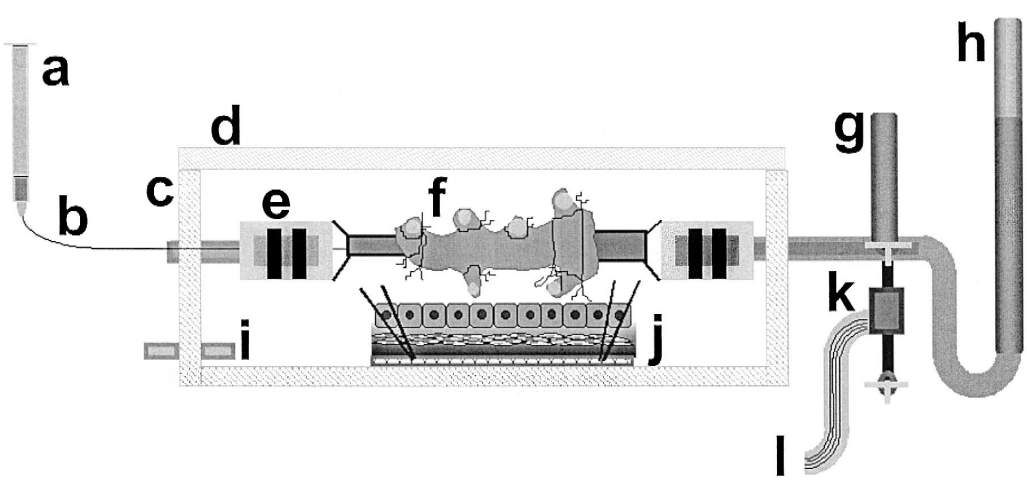

Fig. 1. Schematic illustration of the volumetric apparatus. (a) Syringe to inject drugs to the inside of the bronchial segment, (b) injection port which has a narrow internal diameter to minimise fluid dead-space, (c) organ bath, (d) removable lid, which is attached to seal the organ bath chamber before pumping air into the chamber to temporarily close the bronchial segment, (e) adapters to cannulate bronchial segment, (f) cannulated bronchial segment, (g) effluent column, set to give a pressure of 7 $cm \cdot H_2O$ during internal washes, (h) detecting column (7 $cm \cdot H_2O$) before contraction, (i) air inlet (h) pinned out, donor bronchi, and a (h) Cobe® pressure transducer provided the signal which was processed by (j) a MacLab® hardware/software package integrated with an apple Macintosh computer.

bolus dose–response curves to acetylcholine on the inside of the bronchial segment could be performed.

2.2. Outside and inside cumulative concentration–response curves to acetylcholine

To establish that differences between inside and outside responses to acetylcholine (McParland et al., 1998) could be reproduced in this study, concentration–response curves to acetylcholine applied to the outside and inside of bronchial segments at increasing log concentrations (10 nM to 10 mM, $n = 5$ dogs) were performed.

2.3. Addition of donor bronchi

For the control cumulative concentration–response curves, acetylcholine was added to the outside of the bronchial segment ("the bioassay bronchial segment") from four dogs at increasing log concentrations (10 nM to 10 mM). The preparation was then washed until no further changes in tone were observed. Once the tone of the bioassay bronchial segment had returned to baseline values, two donor bronchi were placed in parallel and in close proximity (< 5 mm) to both sides of the bioassay bronchial segment. A final wash to the inside and outside of the segment was performed after a 10-min equilibration, and the second cumulative concentration–response curve was performed in the presence of the donor bronchi.

2.4. Addition of donor bronchi denuded of epithelium

After the control cumulative concentration–response curve, a second cumulative concentration–response curve to acetylcholine added to the outside of the bioassay bronchial segment was performed in the presence of two donor bronchi with the epithelium removed by gentle rubbing with a cotton swab. When using a similar procedure, approximately 80% of the epithelium was removed (Black et al., 1994) and in the present study, light microscopy revealed that greater than 95% of the basement membrane was devoid of epithelium (data not shown).

2.5. Addition of donor bronchi not treated with indomethacin to bronchial segments pre-treated with indomethacin

After completion of a control cumulative concentration–response curve generated to acetylcholine added to the outside of the bioassay bronchial segment (from five dogs), indomethacin was added to the outside and inside of the bioassay bronchial segment at a concentration of 2.5 μM for 30 min before generating the second cumulative concentration–response curve to acetylcholine added to the outside of the bronchial segment. On completion of the second cumulative concentration–response curve, indomethacin and acetylcholine were washed out of the bronchial segment by exchanging the bath fluid and the luminal fluid four to five times over a period of 40 to 60 min. This washing process should have been sufficiently stringent to remove any free indomethacin from the bioassay bronchial segment. After the washout period, two donor bronchi, not treated with indomethacin, were placed in parallel with the bronchial segment and then the third cumulative concentration–response curve to acetylcholine applied to the outside of the bronchial segment was performed.

2.6. Addition of donor bronchi in the presence of indomethacin

Following a control cumulative concentration–response curve generated to acetylcholine added to the outside of the bioassay bronchial segment (from four dogs), indomethacin was added to the outside and inside of the bioassay bronchial segment at a concentration of 2.5 μM for 30 min before generating the second cumulative concentration–response curve to acetylcholine added to the outside of the bronchial segment. At the same time, indomethacin was also added to the organ bath that contained the donor bronchi. Upon completion of the cumulative concentration–response curve to acetylcholine on the outside of the bioassay bronchial segment, acetylcholine was washed out with Krebs containing indomethacin (2.5 μM). Then the two indomethacin-treated donor bronchi were placed in parallel as described above and, 5 min later in the presence of indomethacin (2.5 μM), a third cumulative concentration–response curve was generated to acetylcholine added to the outside of the bronchial segment.

2.7. The effect of N^g-monomethyl L-arginine (L-NMMA) on inside responses to acetylcholine

After control inside and outside acetylcholine responses, inside responses in the presence of L-NMMA (100 or 300 μM; (Nijkamp et al., 1993)) were obtained from eight dogs.

2.8. Solutions, chemicals and equipment

2.8.1. Solutions and chemicals

Krebs–Henseleit solution, pH 7.35 (composition in mM: NaCl, 118; KCl, 4.7: $CaCl_2$, 2.5; $MgSO_4$, 1.2; NaH_2PO_4, 1.2; $NaHCO_3$, 25.5; D-glucose, 11.1). Krebs was continually gassed with 95% O_2 and 5% $C0_2$. Acetylcholine chloride, indomethacin (in 5% $NaHCO_3$) and N^g-monomethyl L-arginine were purchased from Sigma, MO, USA. Agonists were dissolved in deionised water and serially diluted with Krebs solution on the day of the experiment. Acetylcholine chloride solutions were kept on ice throughout the experiment.

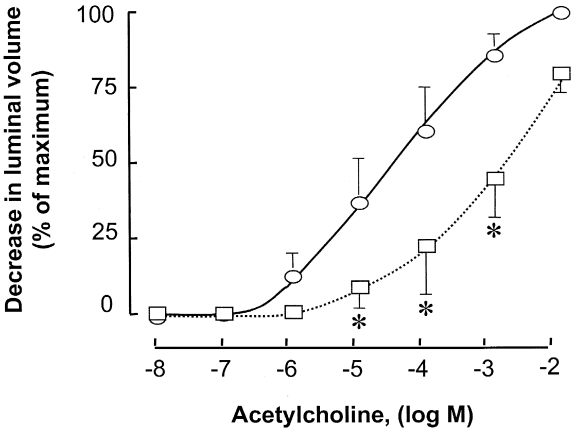

Fig. 2. Cumulative concentration–response curves to acetylcholine applied to the outside (○) and inside (□) surface of bronchial segments. Data points are means ± S.E.M., $n = 5$ dogs. (* significantly different from outside responses).

2.8.2. Equipment

Silk suture (Cyanamid Australia); CDX3 pressure transducer (Cobe® Laboratories Australia); organ bath (Department of Pharmacology, University of Sydney, Australia); MacLab® 2e, MacLab® bridge amplifier and MacLab®-Chart software (ADInstruments, Australia).

2.9. Statistical analysis

Results were expressed as means ± standard error of the mean (S.E.M.). Contraction (decrease in luminal volume) was expressed as a percentage of the maximum obtainable contraction produced by 10 mM acetylcholine added to the outside surface. Values for pD_2 ($-\log EC_{50}$, negative log concentration which gives a 50% of maximum decrease in luminal volume) obtained from cumulative concentration–response curves were expressed as means ± S.E.M. Analysis of variance (ANOVA) and Fishers protected least-squares difference test were used to detect differences between curves and pD_2 values were compared by Student's *t*-test. A P value less than 0.05 was considered significant in all cases.

2.10. Ethical approval

The Animal Experimentation Ethics Committee (AEEC) of the University of Sydney approved our request to obtain airway tissue from dogs.

3. Results

3.1. Outside and inside cumulative concentration–response curves to acetylcholine

As before (McParland et al., 1998), perfused bronchial segments were less sensitive to inside than outside application of acetylcholine (mean pD_2, 3.0 ± 0.4 and 4.5 ± 0.4, respectively, $P < 0.05$, $n = 5$, Fig. 2). There was no significant difference between the baseline luminal volume of the bronchial segments before outside application of acetylcholine (mean, 226 μl, 95% CI, 160–303 μl) and inside application of acetylcholine (mean, 223 μl, 95% CI, 155–303 μl). Maximal airway narrowing in response to 10 mM acetylcholine applied to the outside surface of airway segments was 92.5% of the baseline luminal volume (95% CI, 90.3–94.7%).

3.2. Addition of donor bronchi

In the presence of donor bronchi, cumulative concentration–response curves generated in response to acetylcholine applied to the outside surface of bronchial segments were significantly shifted to the right (Fig. 3a, $P < 0.05$, $n = 4$). The mean pD_2 in the absence and presence of donor bronchi was 4.3 ± 0.4 and 3.6 ± 0.2, respectively ($P < 0.002$, $n = 4$). The baseline luminal vol-

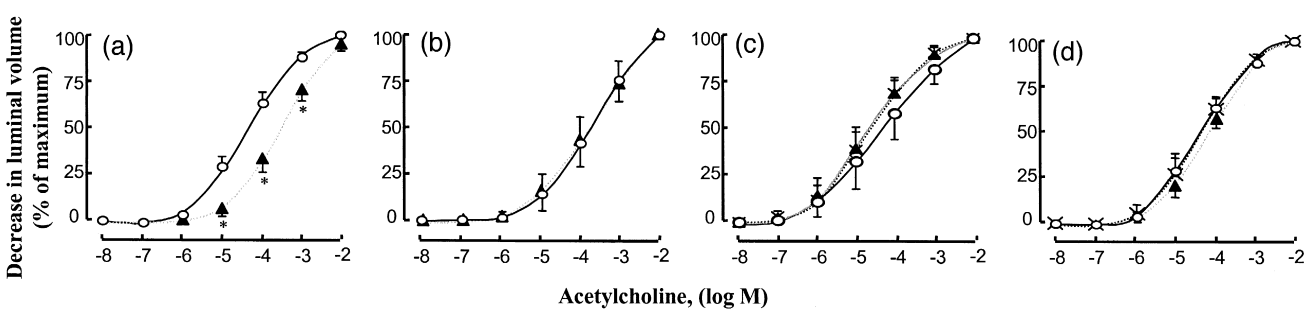

Fig. 3. (a) Cumulative concentration–response curves to acetylcholine applied to the outside surface of bronchial segments in the absence (○) and in the presence of donor bronchi (▲). Data points are means ± S.E.M., $n = 4$ dogs. (* significantly different from control). (b) Cumulative concentration–response curves to acetylcholine applied to the outside surface of bronchial segments in the absence (○) and in the presence of donor bronchi denuded of epithelium (▲), $n = 4$ dogs. (c) The effect of selectively inhibiting cyclooxygenase within the bioassay bronchial segment and not the donor bronchi. Cumulative concentration–response curves to acetylcholine applied to the outside surface of bronchial segments, control (○), in the presence of indomethacin (2.5 μM) (▲) and in the presence of donor bronchi that were not inhibited by indomethacin (×), $n = 4$ dogs. (d) The effect of inhibiting cyclooxygenase within both the bioassay bronchial segment and the donor bronchi. Cumulative concentration–response curves to acetylcholine applied to the outside surface of bronchial segments, control (○), in the presence of indomethacin (2.5 μM) (▲) and in the presence of indomethacin and donor bronchi (×), $n = 4$ dogs.

ume of the bronchial segments in the presence of the donor bronchi (mean, 187 μl, 95% CI, 126–259 μl) was not significantly different from the control baseline volume (mean, 190 μl, 95% CI, 128–264 μl).

3.3. Addition of donor bronchi denuded of epithelium

When donor bronchi were denuded of epithelium, the rightward shift of the cumulative concentration–response curve generated in response to acetylcholine applied to the outside of bronchial segments was abolished (mean pD_2 of the control and in the presence of denuded donor bronchi, 3.8 ± 0.4 and 3.8 ± 0.4, respectively, $P > 0.05$, $n = 4$, Fig. 3b). The baseline luminal volume of bronchial segments in the presence of donor bronchi denuded of epithelium (mean, 189 μl, 95% CI, 105–298 μl) was not significantly different from the control baseline volume (mean, 191 μl, 95% CI, 104–305 μl).

3.4. Addition of donor bronchi not treated with indomethacin to bronchial segments pre-treated with indomethacin

Pre-treatment of the bioassay bronchial segment with indomethacin (mean pD_2 4.6 ± 0.5) also abolished the rightward shift in the cumulative concentration-response curve observed in the presence of donor bronchi (mean pD_2 4.7 ± 0.5, $P > 0.05$, $n = 4$, Fig. 3c). The addition of indomethacin had no effect on control outside cumulative concentration–response curves generated in response to acetylcholine applied to the outside of bronchial segments (control pD_2 4.4 ± 0.5). The baseline luminal volume of bronchial segments pre-treated with indomethacin or in the presence of donor bronchi not treated with indomethacin (mean, 218 μl, 95% CI, 135–322 μl and mean, 214 μl, 95% CI, 133–315 μl, respectively) was not significantly different from control baseline volume (mean, 226 μl, 95% CI, 143–327 μl).

3.5. Addition of donor bronchi in the presence of indomethacin

The cumulative concentration–response curves generated in response to acetylcholine applied to the outside of bronchial segments were not altered by either indomethacin or the presence of donor bronchi plus indomethacin (mean pD_2 of the control, indomethacin alone and indomethacin plus donor bronchi, 4.3 ± 0.2, 4.2 ± 0.2, and 4.3 ± 0.2, respectively, $P > 0.05$, $n = 4$, Fig. 3d). The baseline luminal volume of bronchial segments treated with indomethacin or in the presence of donor bronchi plus indomethacin (mean, 208 μl, 95% CI, 156–268 μl and mean, 209 μl, 95% CI, 150–278 μl, respectively) was not significantly different from control baseline volume (mean, 215 μl, 95% CI, 163–274 μl).

3.6. The effect of L-NMMA on inside responses to acetylcholine

L-NMMA, a nitric oxide synthase inhibitor, caused a rightward shift in the cumulative concentration–response curves generated in response to acetylcholine applied to the inside of bronchial segments (mean pD_2 for the control and L-NMMA cumulative concentration–response curves were 3.3 ± 0.2 and 3.7 ± 0.2, respectively, $P < 0.05$, $n = 8$). The baseline luminal volume of bronchial segments treated with L-NMMA (mean, 233 μl, 95% CI, 136–356 μl) was not significantly different from control baseline volume (mean, 224 μl, 95% CI, 133–338 μl).

4. Discussion

This study used a method that has been previously described (McParland et al., 1998) to measure responsiveness of bronchial segments in vitro, and to bioassay a product(s) released from the airway epithelium (Fig. 1). As was found previously (McParland et al., 1998), canine bronchial segments were less sensitive to acetylcholine applied to the epithelial/inside surface than the outside surface (Fig. 2). This difference in sensitivity of acetylcholine may be partly the result of the release of an epithelial-derived inhibitory factor that attenuates responses to exogenous acetylcholine. Two possible inhibitory factors are products of the cyclooxygenase pathway, namely prostaglandin E_2 and prostacyclin. However, if these inhibitory cyclooxygenase metabolites were released from the donor epithelium to cause attenuation of acetylcholine responses (Fig. 2a), then attenuation should not have been abolished by inhibiting cyclooxygenase within the bioassay bronchial segment and not the donor bronchi (Fig 2c). This raised the possibility that an upstream epithelial-derived substance from the donor tissue mediated its effect on the bioassay bronchial segment by stimulating the release of a downstream cyclooxygenase-dependent product.

Although there are many reports of the release of a transferable relaxant factor for airway epithelium, few have used airway tissue to bioassay this factor. The results of the present study are consistent with those of Manning et al. (1990), who demonstrated a donor airway epithelium-dependent attenuation of responses to acetylcholine and also histamine in bioassay tracheal strips from dogs. Guc et al. (1988a) also used airway tissue to detect the release of a relaxant factor and found that indomethacin did not affect epithelial-dependent attenuation of contraction to carbachol, a cholinesterase resistant cholinergic agonist, for guinea-pig tracheal smooth muscle. This effect of indomethacin differed from the observed effect in the present study and may have resulted from using different species, since Guc et al. (1988a) used guinea-pigs and the

Table 1
Summary of all pD_2 values obtained for acetylcholine for all experiments performed on canine bronchial segments using the volumetric method (McParland et al., 1998)

Experiment performed	CCRC 1	CCRC 2	CCRC 3	*n*
Outside and inside CCRCs to acetylcholine	4.5 ± 0.4	3.0 ± 0.4[a]		5
+ Donor bronchi	4.3 ± 0.4	3.6 ± 0.2[a]		4
+ Donor bronchi − epithelium	3.8 ± 0.4	3.8 ± 0.4		4
+ Donor bronchi (bronchial segment + indomethacin)	4.4 ± 0.5	4.6 ± 0.5[a]	4.7 ± 0.5[a]	4
+ Donor bronchi + indomethacin	4.3 ± 0.2	4.2 ± 0.2	4.3 ± 0.2	4
Effect of L-NMMA on inside responses to acetylcholine	4.7 ± 0.2	3.7 ± 0.2[a]	3.3 ± 0.1[b]	8

The values shown are means ± S.E.M. The pD_2 values were derived from each individual cumulative concentration–response curve (CCRC). The *n* value refers to the number of bronchial segments used, all of which were from different dogs.
[a]Significantly different from CCRC 1.
[b]Significantly different from CCRC 2.

present study used dogs. Manning et al. (1990) did not pursue the possible mechanisms that caused attenuation of responses to acetylcholine.

There is a possibility that the donor airway epithelium-dependent relaxant responses were caused by an increased metabolism of acetylcholine by epithelial cholinesterases. However, if this were the case, then the donor airway epithelium-dependent relaxant effect would not have been abolished by the presence of indomethacin, unless indomethacin decreased acetylcholinesterase activity. Taisne et al. (1997) have shown that indomethacin in fact increases rather than decreases cholinesterase activity, which would result in greater metabolism of acetylcholine and thus, a greater donor airway epithelium-dependent relaxant effect.

The responses between bronchial segments from different dogs were more variable than those that usually occur when isometric or even isotonic responses for airway ring, strip or spiral preparations are measured. This variability has been attributed to the variability in outer wall thickness of the bronchial segment (Gray and Mitchell, 1996), which is consistent with the observation that permeability to a hydrophilic tracer was negatively correlated with bronchial segment internal perimeter (Hulsmann et al., 1996). This variability should not have affected the interpretation of the present results, since comparisons were always made within the one segment.

This study revealed that removal of the donor epithelium (Fig. 3b) or inhibition of cyclooxygenase (Fig. 3d)

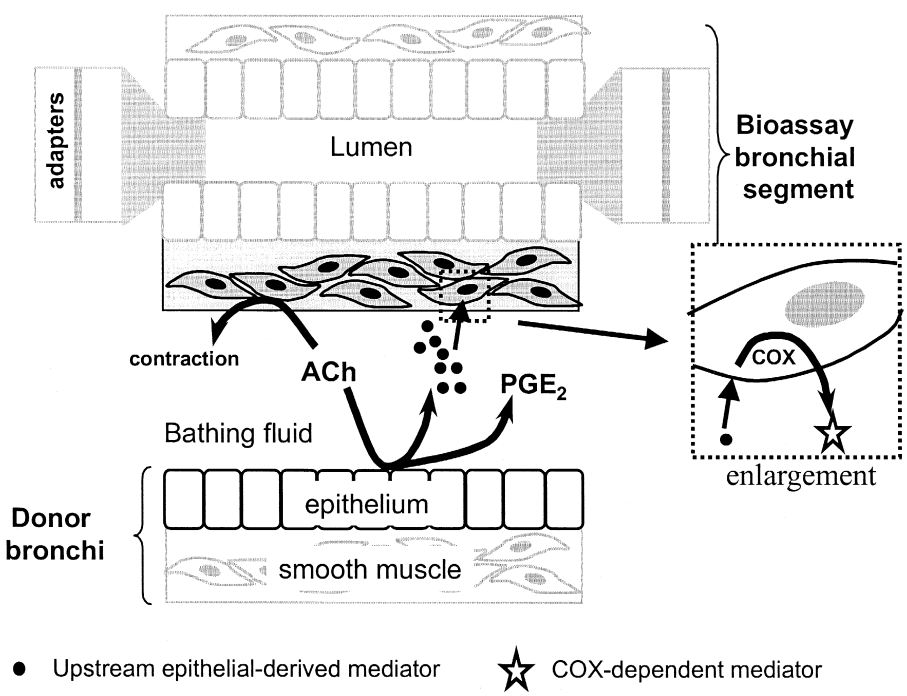

Fig. 4. A schematic diagram showing a hypothetical mechanism by which the donor epithelium may mediate its effect on the bioassay bronchial segment. Acetylcholine added to the bathing fluid gains access to the smooth muscle of both tissues. However, only the epithelium from the donor bronchi is directly stimulated by acetylcholine and as a consequence, an upstream epithelial-derived mediator is released from the donor epithelium. This uncharacterised mediator appears to stimulate either the smooth muscle or some other cell within the adventitia of the bioassay bronchial segment to produce a downstream cyclooxygenase-dependent (inhibited by indomethacin 2.5 μM) mediator(s) that inhibits the contractile effect of exogenous acetylcholine (an enlargement caption of the smooth muscle cell and cellular pathway is also shown).

prevented an "inhibitory substance" attenuating the contractile effect induced by acetylcholine. These findings are consistent with the hypothesis that an epithelial-derived inhibitory prostaglandin attenuates the contractile effect induced by acetylcholine applied to the outside of a bronchial segment. In the present study (data not shown), a single application of prostaglandin E_2 (1 μM) reduced the acetylcholine contraction to almost 50% — an effect similar to that caused by the donor tissue. Numerous studies support the role of an inhibitory prostaglandin in the attenuation of responses to acetylcholine in airway preparations since indomethacin, which inhibits cyclooxygenase, increased airway responsiveness in guinea-pigs (Akbar and Sharma, 1992; Burgaud et al., 1993), rabbits (Butler et al., 1987), humans (Knight et al., 1995) and dogs (Flavahan et al., 1985; McGrogan and Daniel, 1996), but not in cattle (Barnes et al., 1985) or horses (Tessier et al., 1991; Yu et al., 1993). In addition, canine epithelial cells in culture release several prostaglandins of which prostaglandin E_2 predominates (Barnett et al., 1988; Yu et al., 1992; Matsumoto et al., 1994, 1996). Furthermore, in vitro studies using whole airway preparations indicate that the epithelium not only spontaneously releases prostaglandin E_2, but its release is increased by electrical field stimulation, which would lead to the release of endogenous acetylcholine (McGrogan and Daniel, 1996). Prostaglandin E_2 relaxes canine airways pre-constricted to histamine (Yu et al., 1992), 5-hydroxytryptamine (McLarty et al., 1993) or carbachol (Krell, 1978) and when concentration–response curves are generated in response to histamine (Madison et al., 1989) or acetylcholine (Madison et al., 1989; Abela and Daniel, 1995) in the presence of prostaglandin E_2, they are displaced rightward of control curves.

The current study (Fig. 3c) and those of others (Ilhan and Sahin, 1986; Guc et al., 1988b; Fernandes and Goldie, 1990; Spina and Page, 1991; Hay et al., 1992; Cakici et al., 1993; Tunctan et al., 1998) suggest that the attenuating factor released from the donor epithelium is unlikely to be a prostanoid. Fernandes and Goldie (1990) showed that the mediator released from the guinea-pig epithelium (epithelial-derived relaxant factor, EpDRF) was not a cytochrome *P*-450 metabolite, nitric oxide or platelet-activating factor (PAF). One possible candidate is 5,6-epoxyeicosatrienoic acid, which mediates its renal vasodilator activity in the rabbit through prostacyclin (Carroll et al., 1993) and prostacyclin is the predominant prostaglandin released from the airway smooth muscle (Shore et al., 1985).

Nitric oxide could have been a possible candidate for an upstream epithelial mediator from the donor tissue, however, nitric oxide has little relaxing effect on canine airway smooth muscle, pre-contracted to an agonist (McLarty et al., 1993; Brown et al., 1994; Gwyn et al., 1996). In addition, in the present study, inhibition of nitric oxide using L-NMMA, led to a decrease in bronchial segment responsiveness to acetylcholine applied to the inside surface (Table 1), therefore indicating that nitric oxide in canine airways, if anything, increases airway responsiveness. Nitric oxide is therefore not likely to be a candidate for the upstream mediator released from the donor epithelium.

Of recent interest as an another possible candidate is trypsin(ogen). Trypsin(ogen) is produced by human bronchiolar epithelial cells and is capable of activating protease-activated receptors, subtype 2 (PAR-2) (Cocks et al., 1999). Mouse airways precontracted to acetylcholine are relaxed by the addition of PAR-2 activators and the effect is cyclooxygenase and epithelial-dependent (Cocks et al., 1999). This finding appears to be consistent with the present study, except Cocks et al. (1999) propose that the effect is due to the release of prostaglandin E_2 from the epithelium that in turn causes relaxation. This proposed mechanism differs from that of the present study, which shows that the cyclooxygenase-dependent effect is localised to the bioassay bronchial segment and not the donor epithelium.

In summary, our results clearly demonstrate that the epithelium in canine bronchial segments reproducibly provides a "barrier-effect" to inside application of acetylcholine, which has a pharmacological component that is due to the release of an epithelial-derived upstream mediator that exerts its effect through a cyclooxygenase-dependent product (Fig. 4).

Acknowledgements

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References

Abela, A.P., Daniel, E.E., 1995. Neural and myogenic effects of cyclooxygenase products on canine bronchial smooth muscle. Am. J. Physiol. 268, L47–L55.

Aizawa, H., Miyazaki, N., Shigematsu, N., Tomooka, M., 1988. A possible role of airway epithelium in modulating hyperresponsiveness. Br. J. Pharmacol. 93, 139–145.

Akbar, A., Sharma, J.N., 1992. Influence of indomethacin on histamine- and acetylcholine-induced responses in the intact and denuded epithelium of guinea pig tracheal smooth muscle. Pharmacol. Res. 25, 279–286.

Barnes, P.J., Cuss, F.M., Palmer, J.B., 1985. The effect of airway epithelium on smooth muscle contractility in bovine trachea. Br. J. Pharmacol. 86, 685–691.

Barnett, K., Jacoby, D.B., Nadel, J.A., Lazarus, S.C., 1988. The effects of epithelial cell supernatant on contractions of isolated canine tracheal smooth muscle. Am. Rev. Respir. Dis. 138, 780–783.

Black, J.L., Johnson, P.R.A., McKay, K.O., Carey, D., Armour, C.L., 1994. Levcromakalim and isoprenaline-induced relaxation of human isolated airways — role of the epithelium and of K+ channel activation. Pulm. Pharmacol. 7, 195–203.

Brown, R.H., Zerhouni, E.A., Hirshman, C.A., 1994. Reversal of bronchoconstriction by inhaled nitric oxide. Histamine versus methacholine. Am. J. Respir. Crit. Care. Med. 150, 233–237.

Burgaud, J.L., Javellaud, J., Oudart, N., 1993. Do perfused small caliber airways of guinea-pig release an epithelium-dependent relaxing factor?. Pulm. Pharmacol. 6, 217–224.

Butler, G.B., Adler, K.B., Evans, J.N., Morgan, D.W., Szarek, J.L., 1987. Modulation of rabbit airway smooth muscle responsiveness by respiratory epithelium. Involvement of an inhibitory metabolite of arachidonic acid. Am. Rev. Respir. Dis. 135, 1099–1104.

Cakici, I., Tunctan, B., Abacioglu, N., Kanzik, I., 1993. Epithelium-dependent responses of serotonin in a co-axial bioassay system. Eur. J. Pharmacol. 236, 97–105.

Carroll, M.A., Balazy, M., Margiotta, P., Falck, J.R., McGiff, J.C., 1993. Renal vasodilator activity of 5,6-epoxyeicosatrienoic acid depends upon conversion by cyclooxygenase and release of prostaglandins. J. Biol. Chem. 268, 12260–12266.

Cocks, T.M., Fong, B., Chow, J.M., Anderson, G.P., Frauman, A.G., Goldie, R.G., Henry, P.J., Carr, M.J., Hamilton, J.R., Moffatt, J.D., 1999. A protective role for protease-activated receptors in the airways. Nature 398, 156–160.

Dunnill, M.S., 1960. The pathology of asthma with special reference to the change in the bronchial mucosa. J. Clin. Pathol. 13, 27–33.

Fernandes, L.B., Goldie, R.G., 1990. Pharmacological evaluation of a guinea-pig tracheal epithelium-derived inhibitory factor (EpDIF). Br. J. Pharmacol. 100, 614–618.

Flavahan, N.A., Aarhus, L.L., Rimele, T.J., Vanhoutte, P.M., 1985. Respiratory epithelium inhibits bronchial smooth muscle tone. J. Appl. Physiol. 58, 834–838.

Gao, Y., Vanhoutte, P.M., 1994. Epithelium acts as a modulator and a diffusion barrier in the responses of canine airway smooth muscle. J. Appl. Physiol. 76, 1843–1847.

Gray, P.R., Mitchell, H.W., 1996. Effect of diameter on force generation and responsiveness of bronchial segments and rings. Eur. Respir. J. 9, 500–505.

Guc, M.O., Ilhan, M., Kayaalp, S.O., 1988a. Epithelium-dependent relaxation of guinea-pig tracheal smooth muscle by carbachol. Arch. Int. Pharmacodyn. Ther. 294, 241–247.

Guc, M.O., Ilhan, M., Kayaalp, S.O., 1988b. The rat anococcygeus muscle is a convenient bioassay organ for the airway epithelium-derived relaxant factor. Eur. J. Pharmacol. 148, 405–409.

Gwyn, D.R., Lindeman, K.S., Hirshman, C.A., 1996. Inhaled nitric oxide attenuates bronchoconstriction in canine peripheral airways. Am. J. Respir. Crit. Care. Med. 153, 604–609.

Hay, D.W., Muccitelli, R.M., Page, C.P., Spina, D., 1992. Correlation between airway epithelium-induced relaxation of rat aorta in the co-axial bioassay and cyclic nucleotide levels. Br. J. Pharmacol. 105, 954–958.

Hulsmann, A.R., Raatgeep, H.R., Den Hollander, J.C., Bakker, W.H., Saxena, P.R., De Jongste, J.C., 1996. Permeability of human isolated airways increases after hydrogen peroxide and poly-L-arginine. Am. J. Respir. Crit. Care. Med. 153, 841–846.

Ilhan, M., Sahin, I., 1986. Tracheal epithelium releases a vascular smooth muscle relaxant factor: demonstration by bioassay [published erratum appears in Eur J Pharmacol 1987 Apr 7; 136(1):140]. Eur. J. Pharmacol. 131, 293–296.

Jeffery, P.K., Wardlaw, A.J., Nelson, F.C., Collins, J.V., Kay, A.B., 1989. Bronchial biopsies in asthma. An ultrastructural, quantitative study and correlation with hyperreactivity. Am. Rev. Respir. Dis. 140, 1745–1753.

Knight, D.A., Stewart, G.A., Lai, M.L., Thompson, P.J., 1995. Epithelium-derived inhibitory prostaglandins modulate human bronchial smooth muscle responses to histamine. Eur. J. Pharmacol. 272, 1–11.

Koga, Y., Satoh, S., Sodeyama, N., Hashimoto, Y., Yanagisawa, T., Hirshman, C.A., 1992. Role of acetylcholinesterase in airway epithelium-mediated inhibition of acetylcholine-induced contraction of guinea-pig isolated trachea. Eur. J. Pharmacol. 220, 141–146.

Krell, R.D., 1978. Pharmacologic characterization of isolated canine bronchial smooth muscle. Eur. J. Pharmacol. 49, 151–155.

Laitinen, L.A., Heino, M., Laitinen, A., Kava, T., Haahtela, T., 1985. Damage of the airway epithelium and bronchial reactivity in patients with asthma. Am. Rev. Respir. Dis. 131, 599–606.

Madison, J.M., Jones, C.A., Sankary, R.M., Brown, J.K., 1989. Differential effects of prostaglandin E2 on contractions of airway smooth muscle. J. Appl. Physiol. 66, 1397–1407.

Manning, P.J., Jones, G.L., Otis, J., Daniel, E.E., O'Byrne, P.M., 1990. The inhibitory influence of tracheal mucosa mounted in close proximity to canine trachealis. Eur. J. Pharmacol. 178, 85–89.

Matsumoto, K., Aizawa, H., Inoue, R., Hamano, S., Ikeda, S., Xie, Z., Hirata, M., Hara, N., Ito, Y., 1994. Effects of epithelial cell supernatant on membrane potential and contraction of dog airway smooth muscles. Am. J. Respir. Cell Mol. Biol. 10, 322–330.

Matsumoto, K., Aizawa, H., Takata, S., Koto, H., Inoue, H., Hara, N., 1996. Cultured epithelial cells release cyclooxygenase-dependent and cyclooxygenase-independent factors that inhibit cholinergic contraction of canine airway smooth muscles. Respiration 63, 205–212.

McGrogan, I., Daniel, E.E., 1996. Release and actions of inhibitory prostaglandins from canine tracheal epithelium. Can. J. Physiol. Pharmacol. 74, 1055–1060.

McLarty, A.J., McGregor, C.G., Miller, V.M., 1993. Endothelium-derived factors modulate contraction of bronchial smooth muscle. Am. J. Physiol. 264, R999–R1003.

McParland, B.E., Johnson, P.R., Armour, C.L., Black, J.L., 1998. Novel adaptation of a method to assess responsiveness of bronchial segments in vitro. Eur. Respir. J. 11, 1248–1256.

Mitchell, H.W., Fisher, J.T., Sparrow, M.P., 1993. The integrity of the epithelium is a major determinant of the responsiveness of the dog bronchial segment to mucosal provocation. Pulm. Pharmacol. 6, 263–268.

Naylor, B., 1962. The shedding of the mucosa of the bronchial tree in asthma. Thorax 17, 69–72.

Nijkamp, F.P., Van der Linde, H.J., Folkerts, G., 1993. Nitric oxide synthesis inhibitors induce airway hyperresponsiveness in the guinea pig in vivo and in vitro. Role of the epithelium. Am. Rev. Respir. Dis. 148, 727–734.

Ohrui, T., Yamauchi, K., Sekizawa, K., Ohkawara, Y., Maeyama, K., Sasaki, M., Takemura, M., Wada, H., Watanabe, T., Sasaki, H., 1992. Histamine *N*-methyltransferase controls the contractile response of guinea pig trachea to histamine. J. Pharmacol. Exp. Ther. 261, 1268–1272.

Ollerenshaw, S.L., Woolcock, A.J., 1992. Characteristics of the inflammation in biopsies from large airways of subjects with asthma and subjects with chronic airflow limitation. Am. Rev. Respir. Dis. 145, 922–927.

Shore, S.A., Powell, W.S., Martin, J.G., 1985. Endogenous prostaglandins modulate histamine-induced contraction in canine tracheal smooth muscle. J. Appl. Physiol. 58, 859–868.

Small, R.C., Good, D.M., Dixon, J.S., Kennedy, I., 1990. The effects of epithelium removal on the actions of cholinomimetic drugs in opened segments and perfused tubular preparations of guinea-pig trachea. Br. J. Pharmacol. 100, 516–522.

Spina, D., Page, C.P., 1991. The release of a non-prostanoid inhibitory factor from rabbit bronchus detected by co-axial bioassay. Br. J. Pharmacol. 102, 896–903.

Taisne, C., Norel, X., Walch, L., Labat, C., Verriest, C., Mazmanian, G.M., Brink, C., 1997. Cholinesterase activity in pig airways and epithelial cells. Fundam. Clin. Pharmacol. 11, 201–205.

Tamaoki, J., Chiyotani, A., Tagaya, E., Isono, K., Konno, K., 1994. Histamine *N*-methyltransferase modulates human bronchial smooth muscle contraction. Mediators Inflammation 3, 125–129.

Tessier, G.J., Lackner, P.A., O'Grady, S.M., Kannan, M.S., 1991. Modulation of equine tracheal smooth muscle contractility by epithelial-derived and cyclooxygenase metabolites. Respir. Physiol. 84, 105–114.

Tunctan, B., Cakici, I., Abacioglu, N., Kanzik, I., 1998. Bradykinin-induced responses in a coaxial bioassay system composed of rat anococcygeus muscle and guinea pig trachea. Gen. Pharmacol. 30, 477–482.

Woolcock, A.J., Anderson, S.D., Peat, J.K., Du Toit, J.I., Zhang, Y.G., Smith, C.M., Salome, C.M., 1991. Characteristics of bronchial hyperresponsiveness in chronic obstructive pulmonary disease and in asthma. Am. Rev. Respir. Dis. 143, 1438–1443.

Yu, M., Robinson, N.E., Wang, Z., Derksen, F.J., 1993. Independent modulation of horse airway smooth muscle by epithelium and prostanoids. Respir. Physiol. 93, 279–288.

Yu, X.Y., Hubbard, W., Spannhake, E.W., 1992. Inhibition of canine tracheal smooth muscle by mediators from cultured bronchial epithelial cells. Am. J. Physiol. 262, L229–L234.