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Inherent tone of human bronchus: role of eicosanoids and the epithelium

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1 Airway preparations of different species possess varying degrees of inherent tone which is the result of different metabolites of arachidonic acid in different species. In human bronchial smooth muscle *in vitro* we have investigated the effects of 5-lipoxygenase inhibition (zileuton, 10 μM), cyclo-oxygenase inhibition (indomethacin, 1 μM) and mechanical epithelium removal on inherent tone. The shunting of arachidonic acid by inhibition of one or other of these enzymes, as a possible explanation for the effects observed, has also been investigated.

2 Zileuton caused a significant fall in tone either alone (-107 ± 33 mg) or after cyclo-oxygenase inhibition (-203 ± 48 mg) and this effect was not significantly altered by epithelial removal (-191 ± 43 mg alone; -333 ± 88 mg after indomethacin). Indomethacin increased tone when applied alone (160 ± 94 mg), but this effect only reached statistical significance after 5-lipoxygenase inhibition, (210 ± 81 mg; $P<0.05$). Epithelial removal did not alter the effect of indomethacin when applied alone (213 ± 97 mg), but significantly reduced the effect of indomethacin after 5-lipoxygenase inhibition (34 ± 23 mg; $P<0.05$).

3 These data suggest that inherent tone in human bronchus is largely the result of contractile 5-lipoxygenase products. However, the involvement of cyclo-oxygenase products cannot entirely be discounted, since in the presence of 5-lipoxygenase inhibition contractile and relaxant eicosanoids originating from the bronchial epithelium appear to influence significantly inherent tone.

Keywords: 5-Lipoxygenase inhibition; cyclo-oxygenase inhibition; bronchial epithelium; human bronchial smooth muscle

Introduction

Human airways possess inherent tone (Rabe *et al*., 1993; Ellis & Undem, 1994) which *in vitro* is, for the most part, the result of cysteinyl-leukotriene production (Ellis & Undem, 1994). The source of the contractile leukotrienes is unclear, but airway smooth muscle cells, bronchial epithelium or inflammatory cells associated with the smooth muscle and epithelium are potential candidates. The enzyme responsible for the production of cysteinyl-leukotrienes, 5-lipoxygenase, and its activating protein, 5-lipoxygenase activating protein (FLAP), have been identified by immunolocalization in epithelial cells, macrophages and sub-epithelial inflammatory cells, including eosinophils and mast cells (Haley *et al*., 1995). The role of cyclo-oxygenase metabolites in inherent tone of human bronchial smooth muscle is unclear. Indomethacin has been shown to enhance (Hutás *et al*., 1981; Ito *et al*., 1985; Coleman *et al*., 1996), reduce (Ito *et al*., 1989) or have no effect (Brink *et al*., 1980; Ellis & Undem, 1994) on tone in human airways *in vitro*. However, there are patients with bronchial asthma in whom cyclo-oxygenase inhibition precipitates a dramatic bronchoconstriction (Szczeklik, 1990). The mechanisms underlying this condition are unclear, but this observation suggests that cyclo-oxygenase metabolites may under certain circumstances significantly influence bronchial smooth muscle tone.

It is evident from studies in animal tissue that the bronchial epithelium plays an important role in regulating airway smooth muscle function (Flavahan *et al*., 1985; Butler *et al*., 1987; Gao & Vanhoutte, 1994). An epithelium-derived product of arachidonic acid metabolism has been identified in canine airways, which inhibits bronchial smooth muscle tone (Flavahan *et al*., 1985; Stuart-Smith & Vanhoutte, 1988; Gao & Vanhoutte, 1994). Human epithelial cells have the potential for generating both cyclo-oxygenase and lipoxygenase products of arachidonic acid metabolism (Churchill *et al*., 1989; Salari & Chan-Yeung, 1989; Kumlin *et al*., 1990; Bradding *et al*., 1995), but the involvement of these products in human bronchial smooth muscle tone and responsiveness is unclear. The relevance of inherent tone to bronchial smooth muscle responsiveness in human airways is largely uninvestigated. The effects of 5-lipoxygenase inhibition on responsiveness to spasmogens has not been addressed, but cyclo-oxygenase inhibition by indomethacin, while having no effect on basal tone *in vitro*, significantly depressed maximal responses to histamine and acetylcholine (Brink *et al*., 1980).

The primary aim of the present study was to investigate the role of 5-lipoxygenase products, cyclo-oxygenase products and the bronchial epithelium in production of inherent tone of human airways *in vitro*. The shunting of arachidonic acid by inhibition of one or other of these enzymes, as a possible explanation for the effects observed, has also been investigated. This has been achieved by studying the effects of the 5-lipoxygenase inhibitor, zileuton (10 μM), and the cyclo-oxygenase inhibitor, indomethacin (1 μM), alone and in combination, in intact and epithelium denuded preparations of human airways. In addition the effects of indomethacin and zileuton were investigated on carbachol responsiveness, to determine whether the routine inclusion of either of these agents in human airway preparations is warranted.

A preliminary account of this work has been presented to the European Respiratory Society (Watson *et al*., 1995).

Methods

Macroscopically normal bronchial tissue was obtained from 25 patients undergoing surgery for lung cancer (20 male, 5 female; mean age 60 range: 36–75 years). One patient was taking oral steroids (10 mg) and another was taking an inhaled anticholinomimetic agent and β-adrenoceptor agonists, as required. Therapy was withdrawn 24 h before surgery and no apparent difference in responsiveness was observed in tissues from these two patients. Pre-operative lung function parameters were

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generally normal with mean vital capacity (VC) of 3.9 ± 0.1 l, forced expiratory volume in 1 s (FEV_1) of 2.6 ± 0.1 l (equivalent to 86% of the lower predicted value) and airway resistance of 2.7 ± 0.2 cmH_2O l^{-1} s^{-1}. One patient was a non-smoker, six were ex-smokers, having not smoked for at least the past two years, and the remaining eighteen were active smokers. Individual data are shown in Table 1.

Small bronchi with an internal diameter of 2–4 mm were dissected from the tissue and placed in oxygenated (95% O_2: 5% CO_2) modified Krebs buffer (composition (mM): NaCl 118.4, KCl 4.7, $MgSO_4$ 0.6, $CaCl_2$ 1.3, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.1) at 4°C and were used within 24 h. Ring segments, 2–4 mm in length, were prepared and mounted in 10 ml organ baths containing oxygenated modified Krebs (pH 7.4, 37°C) at a resting tension of 250–300 mg. Tissues were equilibrated for at least 60 min before beginning experimental protocols, during which time a stable baseline tension was achieved. Concentration-effect curves to carbachol were performed in a cumulative manner, by incremental concentrations, spaced at half $\log_{10}$ intervals.

Effect of zileuton and indomethacin on inherent tone in epithelium intact bronchi

After the initial 60 min equilibration period, zileuton (10 μM) or indomethacin (1 μM) was added and equilibrated for 60 min. After this time tissues which had previously received indomethacin were given zileuton (10 μM) in the continued presence of indomethacin (1 μM) for a further 60 min. Those which had previously received zileuton were given indomethacin (1 μM) in the continued presence of zileuton (10 μM). Experiments were then terminated by the addition of isoprenaline (1 μM) in order to achieve a maximal relaxation. Time control tissues were run in parallel to determine the change in inherent tone associated with time.

Effect of epithelial removal

In four preparations taken from tissue from the same individual, the epithelium was removed from three preparations by rubbing the luminal surface gently over a dampened pipe cleaner. The protocol described above was repeated such that one rubbed preparation acted as a time control, another rubbed preparation received indomethacin at the first exposure followed by zileuton at the second and the remaining two tissues (one rubbed and one unrubbed) received zileuton at the first exposure followed by indomethacin at the second. Tissues were preserved at the end of the experiment for histological evaluation of epithelial integrity.

Effects of zileuton and indomethacin on carbachol responsiveness

After the initial 60 min equilibration period tissues were relaxed with isoprenaline (1 μM) to determine the level of inherent tone associated with each preparation. Tissues were then washed and re-equilibrated for a further 60 min, after which time cumulative concentration-effect curves to carbachol (10 nM–30 μM) were performed. Tissues were then washed repeatedly for a further 60 min until a stable baseline was again established and at this time zileuton (10 μM) or indomethacin (1 μM) was added. After equilibration in the presence of these agents for 60 min concentration-effect curves to carbachol were repeated.

Histology

At the end of studies involving epithelial removal, all tissues were fixed by immersion in buffered formaldehyde (2%)/picric acid (15%) for 12–18 h and then rinsed in 0.1 M phosphate buffer (pH 7.4). Specimens were then mounted in paraffin wax for sectioning and staining. Epithelial integrity was verified microscopically. Tissues were evaluated to assess the integrity of the smooth muscle, basement membrane and epithelial layer in rubbed and unrubbed preparations. Unrubbed tissues demonstrated greater than 90% epithelial integrity while in rubbed preparations more than 90% of the epithelium had been removed. Where epithelium could be detected in rubbed preparations this was restricted to small folded areas of the mucosal layer.

Table 1 Individual data of tissue donors

Patient	*Sex*	*Age*	*S.S.*	*VC* (l)	FEV_1 (l)	FEV_1 (% pred.)	R_{AW} (cmH_2O l^{-1} s^{-1})	*Histo.*
H.H.	M	52	Ex	4.1	2.1	65	2.4	SqCa
E.J.	M	64	Yes	4.4	2.7	86	1.7	AdCa
H.S.	M	74	Ex	3.4	2.6	83	1.0	AdCa
H.H.	M	68	Yes	3.8	2.0	62	2.5	SqCa
R.S.	M	44	Yes	4.5	2.1	53	2.3	SqCa
H.-T.S.	M	54	Yes	4.5	4.1	100	1.6	SqCa
G.W.	F	60	Ex	4.0	2.9	122	2.5	SqCa
G.S.	M	64	Ex	3.7	2.7	72	2.6	SqCa
W.B.	M	47	Yes	5.3	3.8	87	2.0	Cond
H.-H.H.	M	65	Yes	3.4	2.1	67	5.3	SqCa
L.D.	M	56	Yes	5.2	3.4	88	1.7	S.Cell
H.-G.S.	M	61	Ex	4.1	3.1	103	2.2	AdCa
W.G.	M	62	Yes	4.4	2.9	93	2.3	SqCa
R.P.	F	67	Yes	2.4	1.8	93	2.9	SqCa
G.N.	M	57	Yes	3.2	2.0	59	4.6	SqCa
D.W.	M	36	Yes	5.0	3.8	98	2.3	AdCa
W.N.	M	55	Yes	3.7	2.4	74	3.0	SqCa
T.B.	F	75	No	2.3	1.9	165	3.6	AdCa
R.K.	F	53	Ex	3.6	2.5	109	3.1	AdCa
G.S.	M	68	Yes	3.3	1.9	68	4.5	SqCa
M.B.	F	64	Yes	3.2	2.4	108	2.7	AdCa
H.B.	M	69	Yes	3.8	2.2	76	2.3	SqCa
K.-H.S.	M	55	Yes	4.0	2.1	59	4.0	SqCa
G.K.	M	72	Yes	4.1	2.6	88	2.2	SqCa
W.P.	M	66	Yes	3.5	2.4	80	1.9	AdCa

Initials of individual patients are shown. S.S. – smoking status, VC – vital capacity, FEV_1 – forced expiratory volume in 1 second, R_{AW} – airway resistance, AdCa – adenocarinoma, SqCa – squamous cell carcinoma, Cond – Chondromatous Hamartoma, S.Cell – Small cell carcinoma.

Data analysis

All responses were recorded as changes in isometric tension (mg). The effects of zileuton, indomethacin and epithelial removal on tone were expressed as absolute changes in tension in mg. Contractions were normalized to the maximum contraction in each tissue during the first exposure to carbachol. Individual concentration-effect curve data were analysed by a non-linear iterative curve fitting procedure (KaleidaGraph, Synergy Software, Reading, PA) from which maximal response and potency (pD_2; $-\log_{10}$ of the concentration of agonist producing half-maximal effect) were determined.

Statistical analysis of the data was performed by paired and unpaired Student's *t* test where appropriate. Repeated measure analysis of variance was performed to determine if differences between treatments were significant. When significance was found, a Student-Newman-Keuls multiple comparisons test was performed to identify the difference. $P<0.05$ was considered significant and all values quoted are the mean $\pm$ s.e. mean of at least 5 experiments with tissues derived from different individuals.

Materials

Carbachol, isoprenaline and indomethacin were obtained from Sigma Chemical Company (Deisenhofen, Germany). Zileuton (N-1(1benzo[b]thien-2-ylethyl)-N-hydroxyurea, A64077) was a generous gift from Abbott Laboratories (Abbott Park, Illinois, U.S.A.). All drugs were prepared in distilled water, with the exception of zileuton (10 mM stock solution in equal volumes of 0.1 M NaOH and distilled water) and indomethacin (10 mM stock solution in 1% $NaHCO_3$). Dilutions of these stock solutions were made in distilled water and final bath concentrations of NaOH and $NaHCO_3$ were 50 μM and 0.0001%, respectively, which had no effect on inherent tone on their own.

Results

Effect of zileuton and indomethacin on inherent tone in epithelium-intact bronchi

During the 120 min of this protocol the tone of control tissues remained stable (Figure 1, shaded columns) and at the end of the experiment isoprenaline relaxed tissues by $62\pm10\%$ of the available tension. Zileuton caused a significant reduction in tone (Figure 2, shaded columns), while indomethacin tended to increase tone, but this did not reach statistical significance (Figure 3, shaded columns). When these agents were applied in combination, zileuton still produced a significant reduction in tone in the presence of cyclo-oxygenase inhibition by indomethacin (Figure 3, shaded columns), but in the presence of 5-lipoxygenase inhibition by zileuton, the addition of indomethacin caused a significant increase in tone (Figure 2, shaded columns). Isoprenaline relaxed tissues treated with indomethacin and then zileuton by $77\pm6\%$ of the available tension and tissues treated with zileuton and then indomethacin by $71\pm9\%$ of the available tension. These effects were not significantly different between the treatment groups.

Effect of epithelial removal

In rubbed tissues, tone increased over the 120 min time period, but this did not quite reach statistical significance ($P=0.09$) compared to unrubbed preparations (Figure 1, open columns). At the end of the experiment, isoprenaline relaxed tissues by $67\pm10\%$ of the available tension which was not significantly different from unrubbed preparations. Zileuton caused a sig-

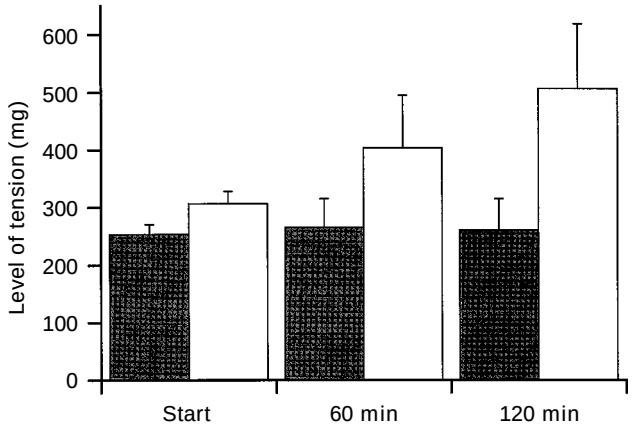

Figure 1 Level of tension of unrubbed (shaded columns) and rubbed (open columns) ring preparations of human bronchial smooth muscle over time. Data are the mean $\pm$ s.e.mean of 6–7 experiments, with tissue from 6–7 individuals.

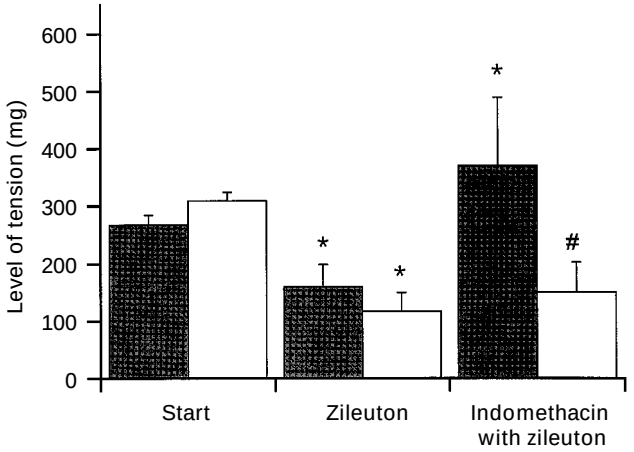

Figure 2 Level of tension of unrubbed (shaded columns) and rubbed (open columns) ring preparations of human bronchial smooth muscle before (start) and after 60 min exposure to 10 μM zileuton (middle columns) then after 60 min exposure to 1 μM indomethacin in the continued presence of 10 μM zileuton. Data are the mean $\pm$ s.e.mean of 6–7 experiments, with tissue from 6–7 individuals. $*P<0.05$ paired comparison of level of tension before and after treatment. # $P<0.05$ unpaired comparison of the effect of indomethacin after zileuton treatment between unrubbed and rubbed preparations.

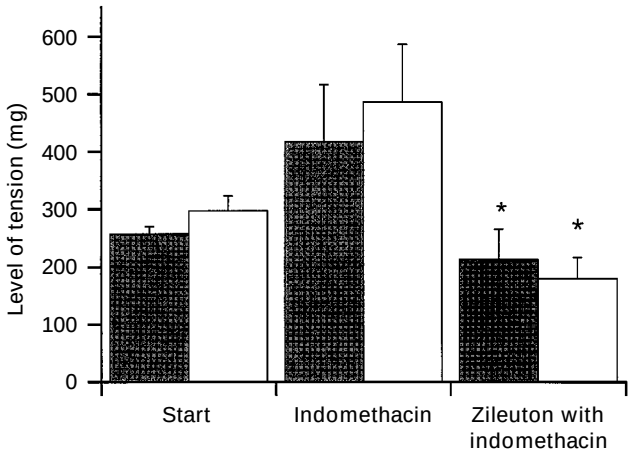

Figure 3 Level of tension of unrubbed (shaded columns) and rubbed (open columns) ring preparations of human bronchial smooth muscle before (start) and after 60 min exposure to 1 μM indomethacin (middle columns) then after 60 min exposure to 10 μM zileuton in the continued presence of 1 μM indomethacin. Data are the mean $\pm$ s.e.mean of 6–7 experiments, with tissue from 6–7 individuals. $*P<0.05$ paired comparison of level of tension before and after treatment.

nificant fall in tone in rubbed preparations, whether applied alone (Figure 2, open columns) or after indomethacin pretreatment (Figure 3, open columns), consistent with observations in unrubbed preparations. Indomethacin did not cause a significant increase in tone in rubbed preparations, either alone (Figure 3, open columns) or after pretreatment with zileuton (Figure 2, open columns). The lack of effect of indomethacin after zileuton treatment in rubbed preparations was significantly different from unrubbed preparations. Isoprenaline relaxed rubbed tissues treated with indomethacin and then zileuton by $83 \pm 7\%$ of the available tension and tissues treated with zileuton and then indomethacin by $44 \pm 12\%$ of the available tension. These effects were not significantly different between the treatment groups or between rubbed and unrubbed preparations within these groups.

In the unrubbed tissues, which were included in this series of experiments to provide a positive control for the effects of indomethacin, zileuton significantly reduced tone and subsequent addition of indomethacin significantly increased tone, reproducing the findings in the epithelium-intact tissues.

Effect of zileuton and indomethacin on carbachol responsiveness

All tissues relaxed in response to 1 μM isoprenaline ($57 \pm 6\%$ of the available tone, 280 ± 114 mg, $n = 6$). There was no significant difference in the level of tension before the first carbachol concentration-effect curves (286 ± 35 mg) and after washing and re-equilibration after carbachol (268 ± 46 mg).

Zileuton, applied after re-equilibration following the first carbachol concentration-effect curve, caused a significant reduction in tone (336 ± 80 mg decrease; $89 \pm 9\%$ of available tone) when compared to control tissues (23 ± 33 mg increase), while indomethacin caused an increase (76 ± 51 mg) which was once again not significant when compared to control tissues.

By comparison of first and second concentration-effect curves with paired Student's t tests, no significant change over time was observed, in either the maximum response to carbachol (Curve $1 = 700 \pm 84$, Curve $2 = 572 \pm 55$) or its potency (pD_2: Curve $1 = 6.48 \pm 0.13$, Curve $2 = 6.47 \pm 0.11$), or in tissues treated with zileuton (Curve 1 max. $= 774 \pm 107$, $pD_2 = 6.63 \pm 0.19$ and Curve 2 max $= 996 \pm 140$, $pD_2 = 6.42 \pm 0.10$). In tissues treated with indomethacin there was a significant fall in the maximum contraction to carbachol (Curve $1 = 628 \pm 160$, Curve $2 = 494 \pm 138$), but no significant change in potency (Curve $1 = 6.56 \pm 0.16$, Curve $2 = 6.46 \pm 0.06$). When changes in maxima were compared throughout the three treatment groups by use of repeated measures analysis of variance and Student-Newman-Keuls multiple comparisons test, there was no significant difference in the magnitude of the maximum responses to carbachol in control tissues and those treated with indomethacin, but the increase in the maximum response in tissues treated with zileuton was significant. Overall, therefore, potency was unaffected by any of these treatments while changes in maximum contractile responses were observed, the significance of which was related to whether the small ($16 \pm 8\%$) drop in maximum response in time control tissues was taken into account or not.

Discussion

The aim of the present study was to determine the role of 5-lipoxygenase and cyclo-oxygenase products of arachidonic acid metabolism and bronchial epithelium in inherent tone of human airways. The results suggest that inherent tone in human bronchial smooth muscle is largely the result of contractile 5-lipoxygenase products, the source of which is unlikely to be the bronchial epithelium, since epithelial removal did not alter the effects of 5-lipoxygenase inhibition. Cyclo-oxygenase products appear to have little involvement in inherent tone under resting conditions. However, in the presence of 5-lipoxygenase inhibition there was a significant effect of relaxant cyclo-oxygenase products and/or contractile lipoxygenase products, one or both of which appear to originate from the bronchial epithelium. The potency of the muscarinic agonist carbachol was unaltered by either 5-lipoxygenase or cyclo-oxygenase inhibition. Changes in maximal responses to this agonist appear to be a reflection of changes in baseline tension associated with either 5-lipoxygenase or cyclo-oxygenase inhibition. Therefore, routine inclusion of zileuton would inhibit inherent tone in human airway preparations without influencing subsequent tissue responsiveness to carbachol.

In unrubbed ring preparations of human bronchial smooth muscle the tone remained stable over the 120 min period of experimental protocols, suggesting a balance in the forces which maintain inherent tone. In rubbed preparations, tone increased gradually with time, suggesting that this balance may have been upset, possibly by the loss of an inhibitory factor. However, the increase in tone seen in rubbed preparations was not statistically significant, suggesting that the inhibitory influence of the epithelium plays only a minor role in maintaining tone under these conditions. Relaxant responses to isoprenaline were also not significantly different between rubbed and unrubbed preparations, an observation which is consistent with previous findings from our laboratory, with rubbed and unrubbed superfused human bronchial strip preparations (Rabe *et al*., 1995). Removal of the bronchial epithelium, therefore, did not significantly influence inherent tone.

It has been shown previously that inherent tone in human bronchial smooth muscle is largely the result of contractile 5-lipoxygenase products (Ellis & Undem, 1994). In the present study, the fact that epithelial removal did not lead to either a fall in inherent tone or an alteration in the relaxant effect of zileuton, suggests that the bronchial epithelium is not the source of the contractile 5-lipoxygenase products. Therefore, despite the fact that this enzyme has been localized to the epithelium (Haley *et al*., 1995), these cells do not appear to be the source of cysteinyl-leukotrienes involved in the generation of inherent tone, under the present experimental conditions.

The role of cyclo-oxygenase products in inherent tone of human airways is unclear; indomethacin has been shown to enhance (Hutás *et al*., 1981; Ito *et al*., 1985; Coleman *et al*., 1996), reduce (Ito *et al*., 1989) or have no effect (Brink *et al*., 1980; Ellis & Undem, 1994) on inherent tone of human airways. Therefore, the tendency for indomethacin to increase tone in the present study is consistent with some findings, but at odds with others. This tendency of indomethacin to increase tone was not altered by epithelial removal in the present study. In fact, the effects of epithelial removal and indomethacin were very similar; both caused a gradual increase in tone with time, but neither was significant when compared to control tissues. Given the similarity in the effects of indomethacin and epithelial removal in this study it is tempting to suggest that indomethacin mediates its effects via the epithelium.

It has been suggested that shunting of arachidonic acid between the lipoxygenase and cyclo-oxygenase pathways may occur when one of these enzymes is inhibited (Ito *et al*., 1985; Szczeklik, 1990). The present study investigated the effects of 5-lipoxygenase inhibition after first inhibiting cyclo-oxygenase and *vice versa*. Zileuton caused a significant reduction in tone independent of cyclo-oxygenase inhibition and this effect was also unaltered by epithelial removal. In the present study, cyclo-oxygenase inhibition resulted in a small, but not statistically significant increase in tone. Where indomethacin has previously been shown to cause a significant increase in tension in human bronchial smooth muscle, increased production of contractile cysteinyl-leukotrienes has been suggested to account for this effect, through shunting of arachidonate caused by cyclo-oxygenase inhibition (Ito *et al*., 1985). Our findings do not support this hypothesis, since the increase in tension with indomethacin was more marked in the presence of 5-lipoxygenase inhibition by zileuton, reaching statistical

significance under these conditions. Additionally, this effect of indomethacin was lost after epithelial removal, once again implicating the epithelium as the site of action of indomethacin.

An alternative explanation for these observations may be that in the presence of 5-lipoxygenase inhibition there may be diversion of arachidonate to the production of some other contractile factor, possibly a 15-lipoxygenase product, which is balanced by the production of a prostanoid relaxant factor, possibly PGE_2. Addition of indomethacin then inhibits the production of this relaxant factor resulting in an increase in tone. The fact that epithelial removal significantly inhibited the effects of indomethacin after zileuton treatment suggests a number of possibilities; (i) that the bronchial epithelium is the source of the prostanoid relaxant factor, (ii) that the bronchial epithelium is the source of the non-5-lipoxygenase contractile factor, or (iii) that the bronchial epithelium is the source of both factors.

There is evidence from animal studies that the bronchial epithelium is capable of producing both relaxant and contractile factors (Flavahan *et al*., 1985; Butler *et al*., 1987; Stuart-Smith & Vanhoutte, 1988; Gao & Vanhoutte, 1994). Indeed, studies in human cultured tracheal epithelial cells have shown that these cells are capable of producing prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ under basal conditions and that release of both of these prostanoids is strongly inhibited by 1 μM indomethacin (Churchill *et al*., 1989). PGE_2 is a bronchodilator and inhibits leukotriene synthesis (Kuehl *et al*., 1984) and, hence, is a candidate for the indomethacin-sensitive, epithelium-derived relaxant factor detected in the present study. Immunoreactivity for the enzyme 15-lipoxygenase, which is not inhibited by zileuton, has been demonstrated in the bronchial epithelium (Bradding *et al*., 1995). 15(**S**)-hydroxyeicosatetraenoic acid (15-HETE) is a major product of the 15-lipoxygenase metabolic pathway in human epithelial cells (Kumlin *et al*., 1994) and is capable of contracting bronchial smooth muscle (Salari & Shellenberg, 1991). Hence, it may be responsible for the increase in tone after cyclo-oxygenase and 5-lipoxygenase inhibition. Interestingly, in the absence of exogenous arachidonic acid no lipoxygenase products are produced by human tracheal epithelial cells in suspension, but when incubated with arachidonic acid 15-lipoxygenase, but not 5-lipoxygenase products, are detected (Hunter *et al*., 1985). In fact, the introduction of exogenous arachidonic acid was found to be the only stimulus which lead to the production of significant amounts of 15-HETE in epithelium-intact human bronchus (Kumlin *et al*., 1994). This suggests that 15-HETE may only be produced in significant amounts when arachidonate is available and it may be speculated that 5-lipoxygenase inhibition makes arachidonate available for the production of this contractile eicosanoid.

Therefore, these data suggest that under resting conditions the tone of human airways is largely the result of cysteinyl-leukotrienes, with little influence of the bronchial epithelium or involvement of cyclo-oxygenase products. However, inhibition of 5-lipoxygenase, while largely eliminating inherent tone, appears to uncover a regulatory role of the bronchial epithelium and an influence of relaxant cyclo-oxygenase products. Under these conditions epithelial cells appear to produce eicosanoids, which are not derived from 5-lipoxygenase metabolism, but which cause both relaxation and contraction of human bronchial smooth muscle. Further studies are required in order to identify these arachidonic acid metabolites.

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