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Antigen-induced contraction of guinea-pig isolated trachea: studies with novel inhibitors and antagonists of arachidonic acid metabolites

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1 Responses of antigen-challenged isolated trachea from sensitized guinea-pigs were pharmacologically characterized by use of some novel inhibitors and antagonists of arachidonic acid metabolites.

2 The cyclo-oxygenase inhibitor, indomethacin, prolonged without altering the maximum response to antigen in the absence of the anti-muscarinic agent, atropine, and/or the H_1-receptor blocker, mepyramine. In the presence of mepyramine, indomethacin both prolonged and increased the magnitude of the response. The selective (SQ-29548) and non-selective (L-640,035) thromboxane A_2 (TXA_2) antagonist and the TXA_2 synthetase inhibitor, OKY-046, were essentially inactive.

3 Two novel inhibitors of 5-lipoxygenase product formation, AA-861 and L-651,896 produced complete inhibition of the response to antigen on tissues treated with atropine and mepyramine, with or without indomethacin.

4 Equimolar concentrations of the leukotriene D_4 (LTD_4)receptor antagonists LY-171883 > L-649,923 ≧ L-648,051 ≧ FPL-55712 blocked part of the response to antigen on tissues treated with atropine, mepyramine and indomethacin. All compounds tended to block a larger component of the response in the absence of indomethacin. A similar tendency was observed with the potent phosphodiesterase inhibitor, isobutylmethylxanthine (IBMX) but not the less potent phosphodiesterase inhibitor theophylline.

5 These results are consistent with the hypothesis that 5-lipoxygenase products acting on LTD_4 receptors play only a minor role in the mediation of the contraction of guinea-pig trachea to antigen challenge. The nature of the residual contractile mediator is unknown; however, it can be completely blocked by the 5-lipoxygenase inhibitors AA-861 and L-651,896 and non-selectively blocked by the phosphodiesterase inhibitor, IBMX and non-selective LTD_4 receptor antagonists, such as LY-171883.

Introduction

Antigen-induced contraction of isolated trachealis from actively or passively sensitized guinea-pigs has been used by many investigators as a model of immediate hypersensitivity (Chand & Eyre, 1978; Adams & Lichtenstein, 1979; Hand & Buckner, 1979; Burka & Paterson, 1980; 1981a,b). Similarly, contraction to antigen can be elicited in passively (Dunlop & Smith, 1975; Dunlop *et al.*, 1977; Adams & Lichtenstein, 1979; Davis *et al.*, 1982) and intrinsically sensitized human tracheobronchial smooth muscle (Davis *et al.*, 1982; Dahlén *et al.*, 1983). The nature of the mediators and mechanisms involved in this response have been the subject of extensive biochemical and pharmacological investigation over the years (Chand & Eyre, 1978; Adams & Lichtenstein, 1979; Hand & Buckner, 1979; Burka & Paterson, 1980; 1981a,b; Saad *et al.*, 1983; De Nucci *et al.*, 1986). Conclusions about the precise contribution of various mediators to the contractile response vary depending on the species studied (Dunlop & Smith, 1975; Dunlop *et al.*, 1977; Dahlén *et al.*, 1983; Jones *et al.*, 1987), the type of antibody mediating the response (Andersson, 1980; Regal, 1984; Undem *et al.*, 1985) the nature of the experiment (biochemical or functional response) and the experimental conditions such as the presence or absence of additional blocking drugs (Hand *et al.*, 1986).

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Most studies concur that contractile responses are the result of the collective actions and interactions of released histamine and arachidonic acid metabolites generated via the 5-lipoxygenase (leukotrienes C_4, D_4 and E_4) and cyclo-oxygenase (prostaglandins, endoperoxide and thromboxane) enzyme systems (Adams & Lichtenstein, 1979; Hand & Buckner, 1979; Burka & Paterson, 1980; Burka & Paterson, 1981b; Hand *et al.*, 1986). A major role for 5-lipoxygenase products in the mediation of the response has been postulated based on pharmacological studies with FPL-55712 and some relatively weak 5-lipoxygenase inhibitors (Adams & Lichtenstein, 1979; Hand & Buckner, 1979; Burka & Paterson, 1980; Hand *et al.*, 1986). Moreover, this component of the response in central airway preparations is potentiated by cyclo-oxygenase inhibitors, suggesting an interaction between prostanoids and leukotriene production and/or actions.

The present study was designed to characterize pharmacologically the actions and interactions of arachidonate metabolites in the tracheal chain preparation from actively sensitized guinea pigs. In order to achieve this, controlled studies were carried out on trachea from actively sensitized guinea-pigs (predominantly IgG_1 response) in the presence of the H_1-receptor antagonist, mepyramine, and the anti-muscarinic agent, atropine. A number of novel leukotriene antagonists, two recently described 5-lipoxygenase inhibitors, a thromboxane A_2 (TxA_2) synthetase inhibitor and a selective and non-selective TxA_2 receptor antagonist were tested in the absence and presence of the cyclo-oxygenase inhibitor, indomethacin. It has been recently reported that FPL-55712 (Chasin & Scott, 1978) and some related acetophenone compounds have phosphodiesterase inhibiting properties in certain biochemical assays which may contribute to their activity in airway smooth muscle (Fleisch *et al.*, 1985). In light of these reports additional studies were carried out with two known phosphodiesterase inhibitors, theophylline and isobutylmethylxanthine (IBMX).

Methods

Male Hartley strain guinea-pigs weighing 300–500 g were sensitized by injecting intraperitoneally, 0.5 ml of a solution containing 10 μg of egg albumin (Ovalbumin, Grade V, Sigma Chemical Co.) and 0.1 g aluminium hydroxide dissolved in saline. A minimum of two weeks was permitted for sensitization to occur and the animals were used 15–30 days post sensitization. Tracheal chains were prepared and mounted in 10 ml tissue baths containing a modified Krebs solution as described previously (Jones *et al.*, 1983; 1986b). Experiments were carried out on tonal (no-indomethacin) or non-tonal (1.4×10^{-6} M indomethacin) preparations in the presence of 1×10^{-7} M atropine and 7×10^{-6} M mepyramine.

For non-tonal experiments, indomethacin was added to the Krebs buffer at the beginning of the experiment so that the tissues were continuously bathed with this drug throughout the experiment. All tissues were primed 2–3 times with a maximum concentration of histamine ($10\,\mu g\,ml^{-1}$) and responses to antigen were expressed as a percentage of the maximum contraction to histamine.

In the absence of indomethacin, the tissues spontaneously developed intrinsic tone following the initial priming procedure with histamine ($10\,\mu g\,ml^{-1}$). Isoprenaline ($0.5\,\mu g\,ml^{-1}$) was administered following the priming histamine dose in order to determine maximum relaxation. Some of the compounds tested in the absence of indomethacin (tonal preparation) produced variable decreases in intrinsic tracheal tone. This alteration in baseline tone was taken into account and responses to antigen challenge were expressed as a percentage of the new histamine maximum (histamine response plus the decrease in baseline tone).

Ovalbumin was administered as a single dose only $0.1\,\mu g\,ml^{-1}$ or $0.01\,\mu g\,ml^{-1}$ final bath concentration). Previous experimnets in which dose-response curves were constructed indicated that a response equivalent to 90–100% of the maximum antigen response could be consistently produced with $0.1\,\mu g\,ml^{-1}$ antigen. Lower concentrations of antigen produced smaller and somewhat more variable responses whereas higher concentrations of antigen ($1\,\mu g\,ml^{-1}$) did not offer any advantage. The effects of various blocking drugs alone or in combination (30 min pretreatment) or drug vehicle (dimethylsulphoxide (DMSO) or H_2O)) were determined against a standard single dose challenge to $0.1\,\mu g\,ml^{-1}$ ovalbumin. The responses were expressed as a percentage of the maximal contraction that could be produced by histamine ($10\,\mu g\,ml^{-1}$) before addition of the various blocking drugs. Peak percent maximal contractile responses to antigen were recorded at various time intervals (0–60 min) after addition of antigen. The peak mean % s.e.mean responses (maximum contractions) and the peak mean % s.e.mean responses at 60 min have been summarized in tabular form. The mean responses obtained in some test and control tissues are also displayed in graphic form.

Analysis

Statistical analysis was carried out on the data obtained at maximum contraction and at 60 min post antigen challenge. Differences between control and drug-treated tissues were considered significant

at $P < 0.05$ as determined by a Student's t test for non-paired data or by a Mann-Whitney test when required (non-parametric).

Materials

The following drugs were used: histamine phosphate, eicosatetraynoic acid (ETYA), chicken ovalbumin (grade V); atropine sulphate, mepyramine maleate, isoprenaline HCl (buffered in saline, pH 3), (Sigma Chemical Co.); BW-755C (3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline), (Burroughs Welcome); indomethacin (dissolved in 100% ethanol). The following compounds were synthesized in the Medicinal Chemistry Department, Merck Frosst Canada Inc.: L-651,896 (2,3-dihydro-6-[3-(2-hydroxymethyl)phenyl-2-propenyl]-5-benzofuranol), L-648,051 ([sodium 4-(3-(4-acetyl-3-hydroxy-2-propylphenoxy)propylsulphonyl)-γ-oxo-benzenebutanoate]), L-649,823 ([sodium (βS*, γR*)-4-(3-(4-acetyl-3-hydroxy-2-propylphenoxy)propylthio)-q-hydroxy-β-methylbenzene-butanoate] and FPL-55712 (7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-1-benzopyran-2-carboxylic acid), dissolved in twice distilled H_2O) and AA-861 (2,3,5-trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone). Dazoxiben was a gift to Dr R. Hall from Squibb, OKY-046 ((E)-3-[4-1-imidazolylmethyl)phenyl]-2-propenoic acid HCl monohydrate) was synthesized by Dr J. Gillard, Merck Frosst Canada, Inc. and SQ-29548 ([1S-[1α,2β(5z),3β,4α]]-7-[3-[[2-[(phenylamino)-carbonyl] hydrazino] methyl]-7-oxabicyclo [2.2.1]-hept-2-yl]-5-heptenoic acid) was a gift to Dr G. Letts from Squibb. Histamine, ovalbumin, atropine, and mepyramine were dissolved in double distilled H_2O or saline while stock solutions of all other drugs, unless indicated otherwise, were dissolved in DMSO.

Results

Results obtained from a series of historical control experiments with and without our standard blocking agents are displayed in Figures 1 and 2. Mepyramine (Figure 1) reduced the magnitude of the peak contractile response and increased the time to peak contraction in the absence of indomethacin but did not significantly alter the slowly declining contractile phase of the response. Similar results were obtained when atropine and mepyramine (Figure 1) were tested together indicating that atropine did not significantly affect the overall response. Indomethacin treatment delayed the time until peak response (from < 1 min to 5–10 min) and markedly prolonged the secondary declining phase but did not alter the magnitude of the peak response (Figure 2). In the presence of indomethacin, mepyramine in combination

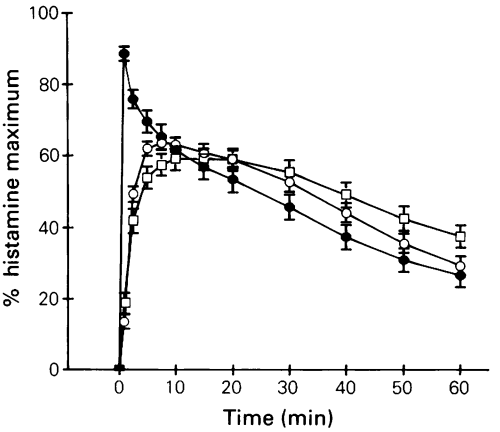

Figure 1 Effect of 7×10^{-6} M mepyramine alone (□; $n = 39$) and in combination with 1×10^{-7} M atropine (○; $n = 30$) on the contractile responses of isolated trachea from sensitized guinea-pigs to 0.1 μg ml^{-1} ovalbumin compared to untreated control responses to antigen (●; $n = 39$). Symbols represent mean responses expressed as a percentage of the maximum contraction to histamine; vertical lines show s.e.mean. Note that mepyramine alone or in combination with atropine produced a similar reduction and delay in the peak response to antigen.

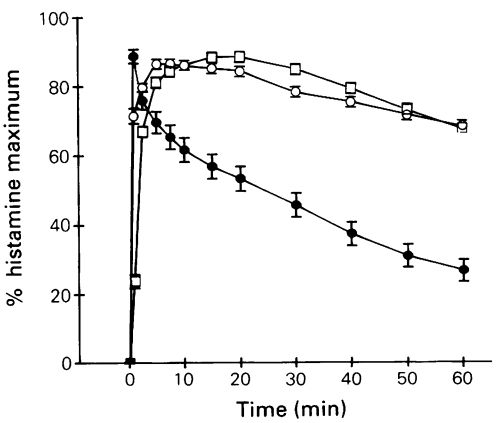

Figure 2 Effect of 1.4×10^{-6} M indomethacin alone (○; $n = 6$) and in combination with 7×10^{-6} mepyramine and 1×10^{-7} M atropine (□; $n = 67$) on contractile responses of isolated trachea from sensitized guinea-pigs to 0.1 μg ml^{-1} ovalbumin compared to untreated control responses to antigen (●; $n = 39$). Symbols represent mean responses expressed as a percentage of the maximum contraction of histamine; vertical lines show s.e.mean. Note that, in the presence of indomethacin or indomethacin with atropine and mepyramine, the response is potentiated over the 5–60 min time period.

Table 1 Effects of indomethacin, thromboxane A_2 (TXA_2)/prostaglandin receptor antagonists and TXA_2 synthetase inhibitors on contractile responses of trachea from sensitized guinea-pigs to ovalbumin (0.1 $\mu g\,ml^{-1}$)

Treatment	Conc (μM)	(n)	Response (% histamine maximum) Peak mean (± s.e.mean)	at 60 min (± s.e.mean)
A *Atropine* (1×10^{-7} M) *and mepyramine* (7×10^{-6} M)				
Control		30	66.6 ± 2.4	30.6 ± 3.3
Indomethacin	1.4	67	88.8 ± 0.9*	67.8 ± 1.2*
Control		5	70.0 ± 8.1	23.0 ± 9.9
L-640,035	37	4	66.0 ± 5.1	40.3 ± 11.5
Control		5	69.6 ± 2.9	31.8 ± 4.4
SQ-29548	26	4	59.3 ± 4.0	34.0 ± 8.8
Control		3	52.8 ± 5.1	17.6 ± 7.6
OKY-046	10	3	49.6 ± 7.5	14.6 ± 7.6
B *Indo* (1.4×10^{-6} M), *atropine* (1×10^{-7} M) *and mepyramine* (7×10^{-6} M)				
Control		3	99.0 ± 3.6	72.0 ± 5.1
L-640,035	37	3	96.3 ± 2.3	72.7 ± 5.2
Control		3	95.0 ± 8.0	74.5 ± 7.5
SQ-29548	26	3	101.0 ± 2.7	79.7 ± 7.2
Control		3	84.3 ± 0.9	50.0 ± 2.6
OKY-046	10	3	80.3 ± 2.2	43.0 ± 6.0
C *No additional blockers*				
Control		5	84.8 ± 2.9	18.0 ± 8.6
SQ-29548	26	3	84.0 ± 6.6	13.3 ± 9.6
Control		4	94.5 ± 9.9	29.8 ± 8.0
Indomethacin	28	3	93.7 ± 2.4	58.0 ± 16.0

* Significantly different from control: $P < 0.05$.

with atropine (Figure 2) further delayed the time to peak response (to 15–20 min) but did not significantly alter the magnitude of the peak response or the declining contractile phase.

Effects of a cyclo-oxygenase inhibitor, prostaglandin/TXA_2 receptor antagonists and a TXA_2 synthetase inhibitor on responses to antigen

As shown above, the cyclo-oxygenase inhibitor indomethacin markedly potentiated the contractile response to antigen in terms of prolonging the contractile response and increasing the area under the time-contraction curve without affecting the maximum response. This effect could only be resolved when studies were performed in the presence of mepyramine (Figure 1 and 2; Table 1) which effectively antagonized the histamine component of the overall response. Two recently described prostaglandin/TXA_2 receptor antagonists, L-640,035 and SQ-29548 were tested in atropine- and mepyramine-treated tissues and were found to be essentially inactive both in the absence and presence of indomethacin (Table 1). Although these agents reduced intrinsic tracheal tone (L-640,035; 57.2 ± 4.5% reduction; SQ-29548: 27.0 ± 10.4% reduction) in the absence of indomethacin, they produced only modest and non-significant reductions in the overall response to antigen. In contrast to indomethacin these agents did not produce significant potentiation of the antigen-induced contraction in tonal preparations (Table 1). In four experiments, the TXA_2 synthetase inhibitor, OKY-046 (10 $mg\,ml^{-1}$), decreased intrinsic tone slightly (9.4 ± 4.0%) but did not significantly alter the peak or 60 min responses to antigen in tonal preparations. In non-tonal preparations (bathed in indomethacin), OKY-046 ($n = 3$) did not produce significant alterations of the response to antigen (Table 1).

Effects of leukotriene antagonists on reponses to antigen

Some recently reported selective and non-selective leukotriene D_4 receptor antagonists were compared to the prototype SRS-antagonist, FPL-55712, both in the absence and presence of various blocking drugs. The results are summarized in Table 2 and displayed in graphic form in Figures 3, 4, 5 and 6 for

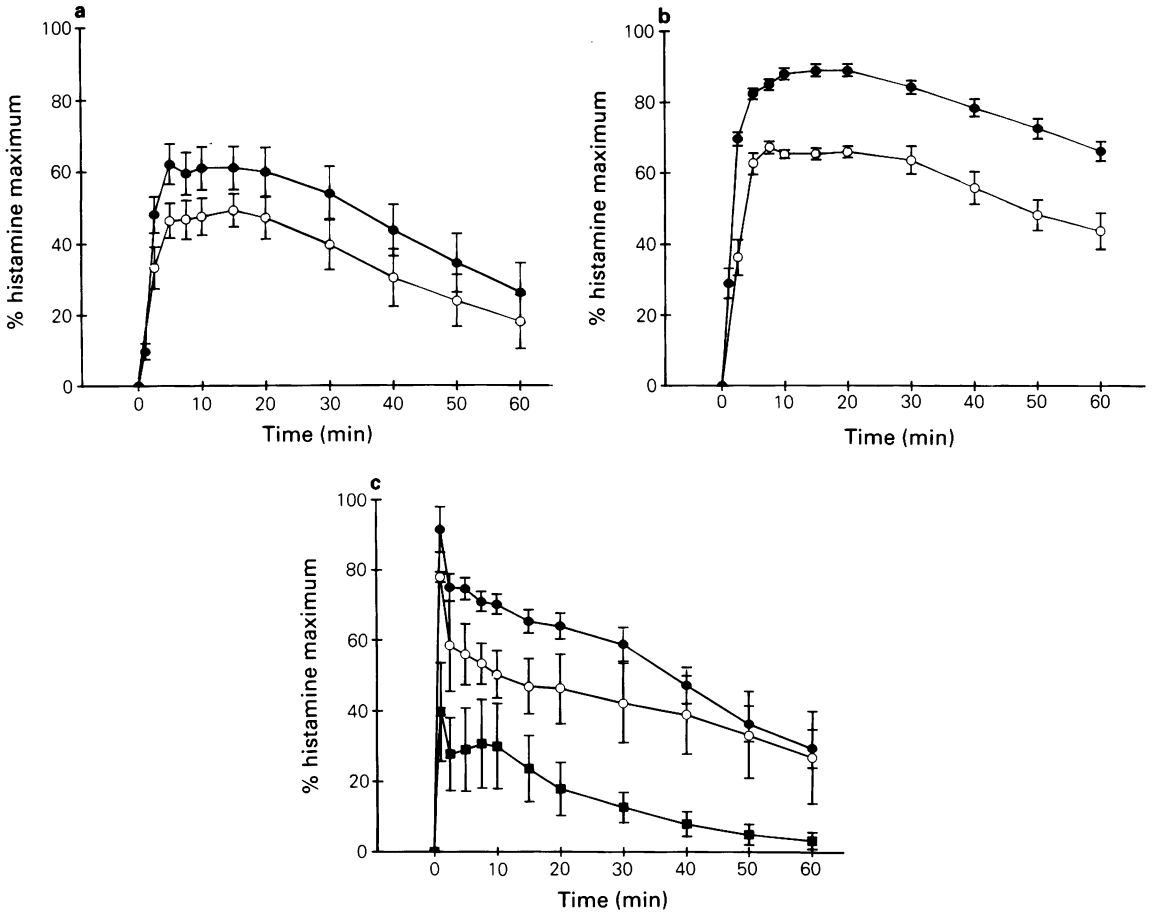

Figure 3 Effect of 2×10^{-5} M FPL-55712 (○; $n = 4$ in a; $n = 5$ in b; $n = 3$ in c) on control contractile responses (●; $n = 4$ in a; $n = 5$ in b; $n = 6$ in c) of antigen-challenged isolated trachea from sensitized guinea-pigs treated with 1×10^{-7} M atropine and 7×10^{-6} M mepyramine in (a), 1×10^{-7} M atropine, 7×10^{-6} M mepyramine and 1.4×10^{-6} M indomethacin in (b) and no additional blocking drugs in (c). Also included in (c) are results with 6×10^{-5} M FPL-55712 (■; $n = 3$). Symbols represent mean responses expressed as a % of the maximum histamine contraction; vertical lines show s.e.mean. Note that FPL-55712 blocked only a component of the response in (a), (b) and (c).

FPL-55712, L-649,923, L-648,051 and LY-171883, respectively. In the presence of mepyramine and atropine, all leukotriene antagonists tested produced a significant inhibition of the peak response to antigen challenge (Table 2 and Figures 3a, 4a, 5a, 6a), and facilitated recovery to baseline (compare 60 min responses). In terms of magnitude of inhibition of the peak response the rank order of activity was LY-171883 > L-649,923 > L-648,051 > FPL-55712. In the presence of indomethacin these antagonists, still produced significant inhibition of the peak and 60 min responses to antigen, however, apart from FPL-55712 (Figure 3a and 3b), these antagonists displayed less activity (blocked a smaller component) than that observed in the absence of indomethacin (Table 2 and Figures 3b, 4b, 5b and 6b).

In order to assess purported non-specific activity of these acetophenone compounds, experiments were carried out in the absence of additional blocking drugs (Table 2 and Figures 3c, 4c, 5c and 6c). At 2×10^{-5} M, LY-171883 = L-649,923 > L-648,051 > FPL-55712 produced a small but significant ($P < 0.05$) reduction in the peak contraction with a rank order of activity similar to that observed on tonal preparations in the presence of atropine and mepyramine. There was a non-significant tendency for LY-171883 to be more active than the other

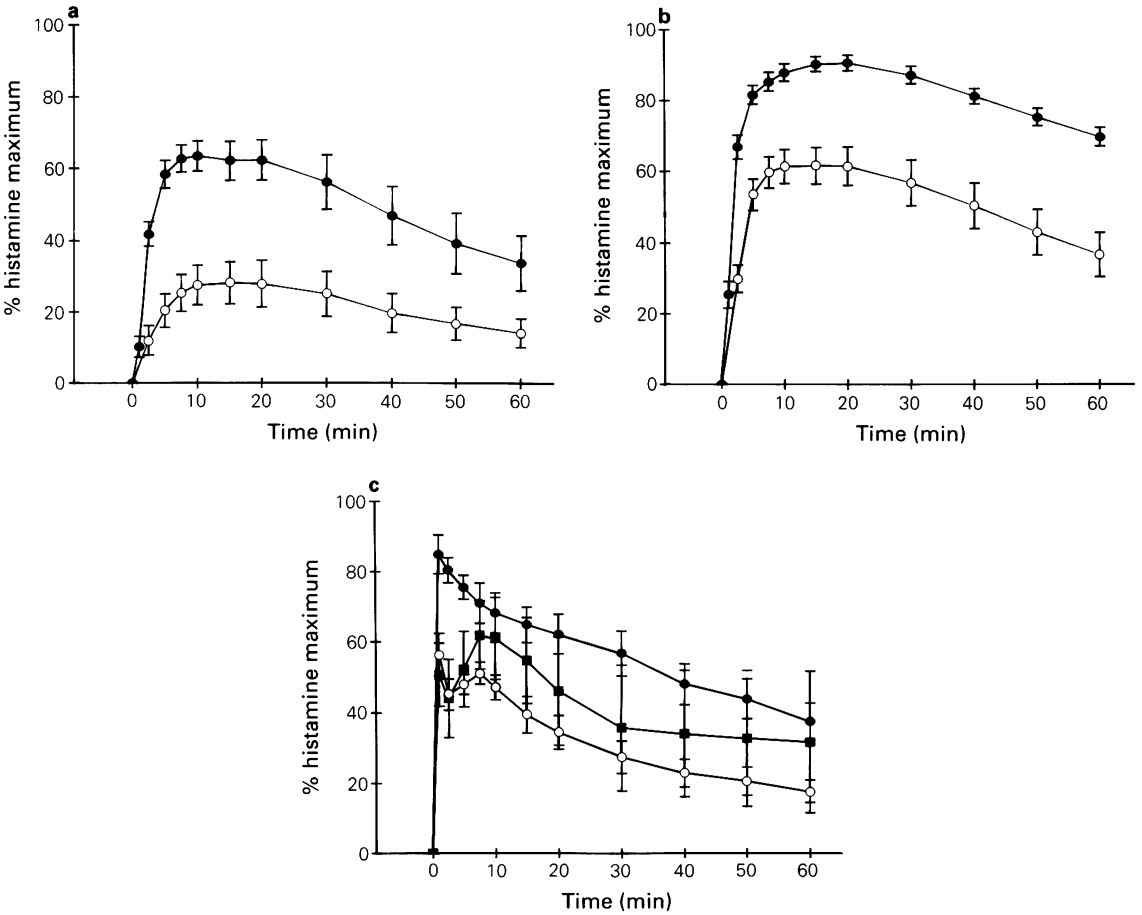

Figure 4 Effect of 2×10^{-5} M L-649,923 (○; $n = 8$ in a; $n = 10$ in b; $n = 6$ in c) on control contractile responses (●; $n = 6$ in a; $n = 7$ in b; $n = 6$ in c) of antigen-challenged isolated trachea from sensitized guinea-pigs treated with 1×10^{-7} M atropine and 7×10^{-6} M mepyramine in (a), 1×10^{-7} M atropine, 7×10^{-6} M mepyramine and 1.4×10^{-6} M indomethacin in (b) and no additional blocking drugs in (c). Also included in (c) are results with 6×10^{-5} M L-649,923 (■; $n = 3$). Symbols represent mean responses expressed as a % of the maximum histamine contraction; vertical lines show s.e.mean. Note that L-649,923 blocked only a component of the response in (a), (b) and (c).

antagonists on tonal preparations, however, this agent also produced a greater reduction in intrinsic tone in these preparations. Mean percentage ± s.e.mean reductions in intrinsic tracheal tone obtained with LY-171883, L-649,923 and L-648,051 in the absence of additional blocking drugs were 98.7 ± 1.3%, 46.0 ± 11.9% and 46.6 ± 11.2%, respectively. An additional series of experiments was carried out with 3 fold higher concentrations (6×10^{-5} M) of these antagonists. Under these conditions LY-171883 (Figure 6c) and FPL-55712 (Figure 3c) produced a further inhibition of the response to antigen (Table 2) in contrast to both L-649,923 (Figure 4c) and L-648,051 (Figure 5c).

5-Lipoxygenase inhibitors and dual inhibitors

A purported 5-lipoxygenase inhibitor (AA-861) and a dual inhibitor of both cyclo-oxygenase and 5-lipoxygenase product formation (L-651,896) were examined in the absence and presence of various blocking drugs. Results obtained are summarized in Table 3. AA-861 and L-651,896 were effective inhibitors both in the absence and presence of indomethacin. Both compounds produced complete inhibition of the antigen-induced contraction when tested at concentrations greater than or equal to 30 mM. With 3 fold lower concentrations AA-861 > L-651,896 still produced almost complete inhibition of the peak

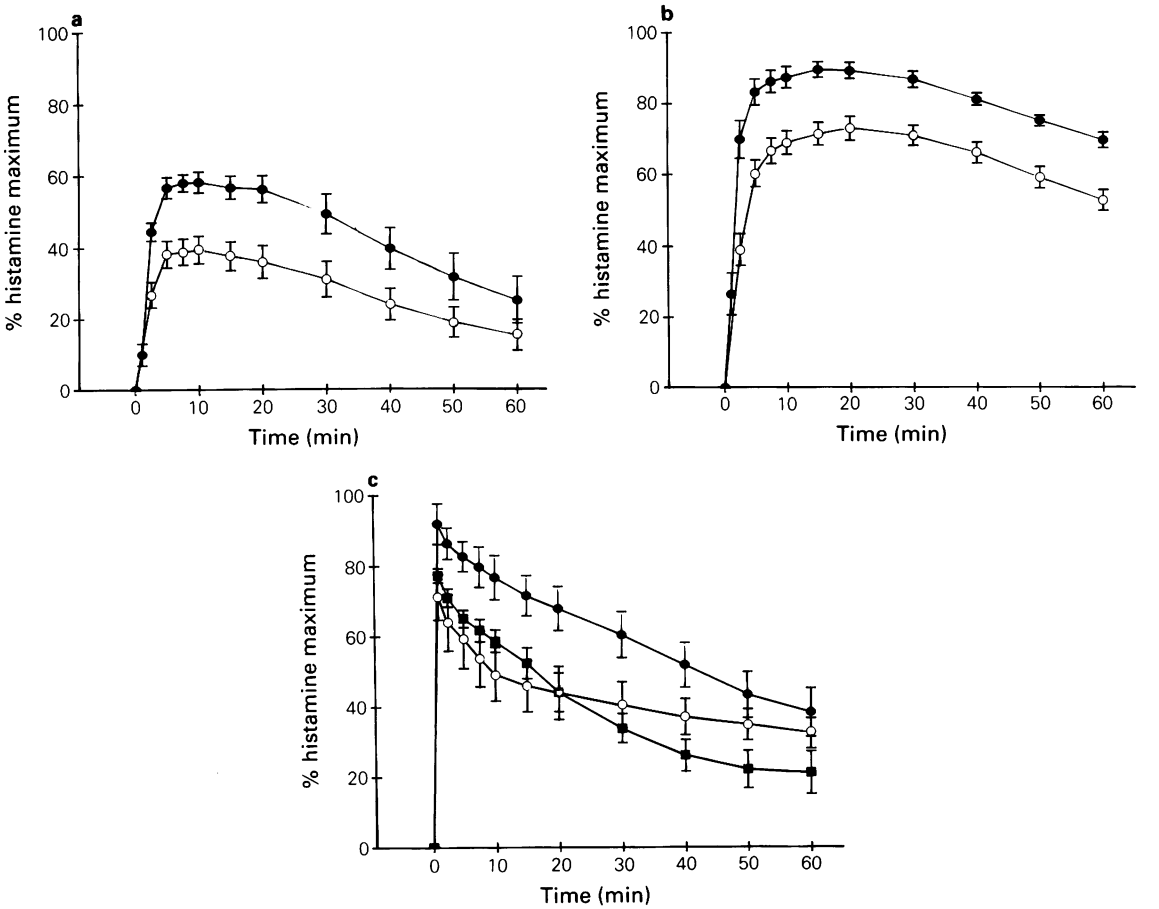

Figure 5 Effect of 2×10^{-5} M L-648,051 (○; $n = 8$ in a; $n = 7$ in b; $n = 8$ in c) on control contractile responses (●; $n = 7$ in a; $n = 6$ in b; $n = 6$ in c) of antigen-challenged isolated trachea from sensitized guinea-pigs treated with 1×10^{-7} M atropine and 7×10^{-6} M mepyramine in (a), 1×10^{-7} M atropine, 7×10^{-6} M mepyramine and 1.4×10^{-6} M indomethacin in (b) and no additional blocking drugs in (c). Also included in (c) are results with 6×10^{-5} M L-648,051 (■; $n = 3$). Symbols represent mean responses expressed as a % of the maximum histamine contraction; vertical lines show s.e.mean. Note that L-648,051 blocked only a component of the response in (a), (b) and (c).

contractile response and the secondary plateau phase (60 min). Other less potent inhibitors of leukotriene synthesis BW 755C ($n = 5$) and ETYA ($n = 5$) reduced the peak response in the presence of indomethacin to $37.5 \pm 4.7\%$ and $29.8 \pm 11.7\%$, respectively, when tested at 100 mM. These inhibitors were not tested in the absence of indomethacin.

In another series of experiments both AA-861 and L-651,896 were examined against antigen-induced contraction in the absence of other pharmacological blocking drugs (Table 3). Under these conditions the initial peak response was slightly reduced by L-651, 896 and unaffected by AA-861. These findings demonstrate that these compounds are not potent antagonists of the effects of histamine or inhibitors of histamine release in this model.

Effect of known phosphodiesterase inhibitors

Theophylline (55 mM) was essentially inactive versus antigen under the various treatment conditions tested (i.e. alone or in the presence of additional blockers). The more potent phosphodiesterase inhibitor, IBMX, however, produced significant inhibition of the overall response under all experimental conditions but was significantly more active (blocked a larger component of the response) when tested on tonal preparations (no indomethacin). The results

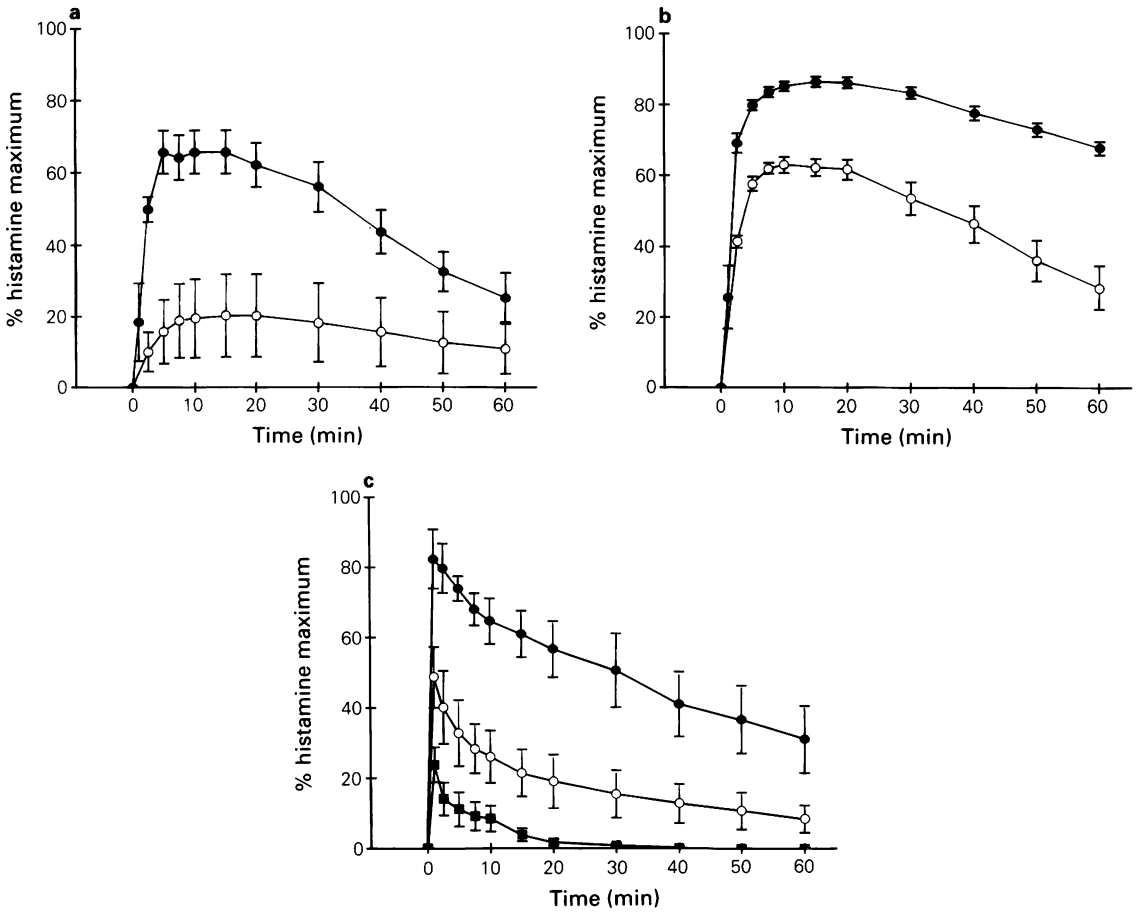

Figure 6 Effect of 2×10^{-5} M LY-171883 (○; $n = 3$ in a; $n = 3$ in b; $n = 3$ in c) on control contractile responses (●; $n = 3$ in a; $n = 3$ in b; $n = 3$ in c) of antigen-challenged isolated trachea from sensitized guinea-pigs treated with 1×10^{-7} M atropine and 7×10^{-6} M mepyramine in (a), 1×10^{-7} M atropine, 7×10^{-6} M mepyramine and 1.4×10^{-6} M indomethacin in (b) and no additional blocking drugs in (c). Also included in (c) are results with 6×10^{-5} M LY-171883 (■; $n = 3$). Symbols represent mean responses expressed as a % of the maximum histamine contraction; vertical lines show s.e.mean. Note that LY-171883 blocked a larger component of the response in (a), (b) and (c) than the other leukotriene D_4 receptor antagonists.

with these phosphodiesterase inhibitors are shown in Table 4.

Discussion

The present results are consistent with previous findings that antigen-induced contractions of guinea-pig isolated trachea are mediated primarily by endogenously released histamine and 5-lipoxygenase products. It is known from previous studies that multiple mediators are released following antigen challenge of this tissue (Adams & Lichtenstein, 1979; Hand & Buckner, 1979). Moreover, the method of sensitization (Andersson, 1980), the type of immunological response (Regal, 1984), the dose of antigen (Hand *et al.*, 1986), the presence or absence of additional blocking drugs (Hand *et al.*, 1986) can influence the relative contributions made by these various mediators. Our assay conditions were standardized in terms of the sensitization procedure, the dose of antigen *in vivo* and *in vitro* and the inclusion of additional pharmacological blocking drugs. Our sensitization procedure would favour stimulation of IgG_1 so we have assumed an immunological response to antigen similar to that described by other investigators (Andersson, 1980; Undem *et al.*, 1985). This type of response to antigen can be dramatically influenced

Table 2 Effect of leukotriene receptor antagonists on contractile responses of trachea from sensitized guinea-pigs to ovalbumin (0.1 $\mu g\,ml^{-1}$)

Treatment	*Conc.* (μM)	(n)	*Response (% histamine maximum)* *peak mean* (± s.e.mean)	*at 60 min* (± s.e.mean)
A *Atropine* (1×10^{-7} M) *and mepyramine* (7×10^{-6} M)				
Control		4	62.0 ± 5.6	26.0 ± 8.4
FPL-55712	20	4	49.0 ± 4.6	18.0 ± 7.6
Control		6	63.2 ± 4.3	33.7 ± 7.8
L-649,923	20	8	27.9 ± 5.9**	14.1 ± 4.1
Control		7	58.0 ± 2.9	25.0 ± 6.6
L-648,051	20	8	39.2 ± 3.8*	15.2 ± 4.3
Control		3	62.3 ± 4.4	32.0 ± 15.1
LY-171883	20	3	20.0 ± 11.6**	10.7 ± 7.1
B *Indo* (1.4×10^{-6} M) *atropine* (1×10^{-7} M) *and mepyramine* (7×10^{-6} M)				
Control		5	89.0 ± 1.8	66.2 ± 2.8
FPL-55712	20	5	67.2 ± 1.7**	43.6 ± 5.2**
Control		7	90.6 ± 2.2	69.7 ± 2.7
L-649,923	20	10	61.5 ± 5.2**	36.6 ± 6.3**
Control		6	89.5 ± 2.3	69.5 ± 2.2
L-648,051	20	7	73.0 ± 3.4**	52.7 ± 3.0**
Control		3	84.6 ± 2.6	68.0 ± 3.6
LY-171883	20	3	63.0 ± 2.3**	28.3 ± 6.2**
C *No additional blockers*				
Control		6	91.5 ± 6.4	29.5 ± 5.4
FPL-55712	20	3	78.0 ± 1.5	27.0 ± 13.1
Control		6	84.8 ± 5.5	37.5 ± 5.3
L-649,923	20	6	56.3 ± 6.1*	22.5 ± 5.5*
Control		6	91.8 ± 5.7	38.2 ± 6.9
L-648,051	20	8	71.1 ± 6.5*	32.3 ± 4.3
Control		3	82.3 ± 8.4	31.0 ± 9.5
LY-171883	20	3	48.7 ± 8.7*	8.3 ± 3.8*
Control		6	91.5 ± 6.4	29.5 ± 5.4
FPL-55712	60	3	39.7 ± 14.0*	3.3 ± 2.4*
L-649,923	60	3	50.7 ± 9.0*	31.7 ± 20.1
L-648,051	60	3	77.3 ± 2.0*	21.0 ± 6.1
LY-171883	60	3	23.7 ± 5.0**	0.0 ± 0.0*

* Significantly different from control: $P < 0.05$.
** Significantly different from control: $P < 0.01$.

by released histamine and inhibitory cyclo-oxygenase products. In particular, treatment with an antihistamine in combination with a cyclo-oxygenase inhibitor changes the time course and magnitude of the response (present study), confirming previous reports (Burka & Paterson, 1980; 1981b). It follows that assessment of the pharmacological activity of various leukotriene receptor antagonists and leukotriene synthesis inhibitors may be influenced by the absence or presence of endogenously released prostanoids and histamine. Previous studies have demonstrated that cyclo-oxygenase inhibitors enhance the contractile response of guinea-pig trachea to exogenously applied mediators (Orehek *et al.*, 1973) and antigen (Hand & Buckner, 1979; Burka & Paterson, 1980). Similar results were obtained in the present study. The lack of significant effect with agents that block the synthesis or effects of contractile prostanoids supports the hypothesis that the potentiation by cyclo-oxygenase inhibitors is the result of removal of inhibitory prostanoids. As a consequence the amount and/or effects of other released mediators would be increased. Moreover, these findings support the widely held view that contractile prostanoids play only a minor role in the overall response to antigen in this preparation (Burka & Paterson, 1981b; Hand *et al.*, 1986).

5-Lipoxygenase products have been considered the major anaphylatic mediators of contraction in this model. Support for this contention comes from our results with two novel, structurally different, inhibitors of leukotriene production AA-861 (Ishihara *et al.*, 1983) and L-651,896 (Bonney *et al.*, 1987). These agents abolished the contraction to

Table 3 Effect of leukotriene synthesis inhibitors on contractile responses of trachea from sensitized guinea-pigs to ovalbumin 0.1 $\mu g\,ml^{-1}$

Treatment	Conc. (μM)	(n)	Response (% histamine maximum) peak mean (± s.e.mean)	Response (% histamine maximum) at 60 min (± s.e.mean)
A *Atropine* (1×10^{-7} M) *and mepyramine* (7×10^{-6} M)				
Control		4	73.8 ± 9.4	21.0 ± 12.4
AA-861	10	5	14.4 ± 5.1**	2.6 ± 2.6
	30	4	0.0 ± 0.0**	0.0 ± 0.0**
Control		4	63.0 ± 5.7	29.5 ± 8.3
L-651,896	10.5	3	49.0 ± 1.2	4.7 ± 2.4**
	35	5	5.8 ± 1.2**	0.0 ± 0.0**
B *Indo* (1.4×10^{-6} M), *atropine* (1×10^{-7} M) *and mepyramine* (7×10^{-6} M)				
Control		6	90.3 ± 1.4	70.8 ± 1.2
AA-861	3	3	71.0 ± 5.9*	43.7 ± 6.1*
	10	6	3.0 ± 0.0**	0.3 ± 0.3**
Control		7	85.0 ± 2.9	65.3 ± 3.0
L-651,896	3.5	3	71.0 ± 2.3*	29.7 ± 4.9**
	10.5	4	26.8 ± 8.1**	4.8 ± 2.3**
	35	6	0.0 ± 0.0**	0.0 ± 0.0**
C *No additional blockers*				
Control		4	86.8 ± 10.5	22.3 ± 9.1
AA-861	30	4	75.8 ± 15.1	20.8 ± 7.4
Control		5	85.0 ± 4.3	20.4 ± 7.0
L-651,896	35	4	65.5 ± 3.3*	15.3 ± 6.2

* Significantly different from control: $P < 0.05$.
** Significantly different from control: $P < 0.01$.

Table 4 Effect of known phosphodiesterase inhibitors on contractile responses of trachea from sensitized guinea-pigs to ovalbumin (0.1 $\mu g\,ml^{-1}$)

Treatment	Conc (μM)	(n)	Response (% histamine maximum) peak mean (± s.e.mean)	Response (% histamine maximum) at 60 min (± s.e.mean)
A *Atropine* (1×10^{-7} M) *and mepyramine* (7×10^{-6} M)				
Control		2	48.5	33.0
Theophylline	55	3	52.7 ± 4.9	36.0 ± 2.5
Control		4	69.8 ± 9.9	25.5 ± 7.8
IBMX	30	4	4.0 ± 2.6**	0 ± 0**
B *Indo* (1.4×10^{-6} M), *atropine* (1×10^{-7} M) *and mepyramine* (7×10^{-6} M)				
Control		2	86.0	77
Theophylline	55	3	87.0 ± 0.6	75.3 ± 0.7
Control		9	94.3 ± 1.9	71.6 ± 1.7
IBMX	30	10	45.6 ± 4.9**	12.1 ± 3.1**
C *No additional blockers*				
Control		6	91.0 ± 6.8	9.8 ± 3.5
Theophylline	50*	3	76.7 ± 5.7*	7.7 ± 3.9
Control		10	93.3 ± 4.5	10.3 ± 2.7
IBMX	10	3	46.3 ± 9.0**	1 ± 1**
IBMX	30	3	9.0 ± 3.8**	0 ± 0**

* Significantly different from control: $P < 0.05$.
** Significantly different from control: $P < 0.01$.
* Tested as aminophylline (25 μM).

antigen in the presence of atropine and mepyramine in both tonal and non-tonal preparations. These effects were observed with concentrations of these agents that had previously been shown to inhibit leukotriene biosynthesis in rat neutrophils and guinea-pig lungs (Yoshimoto *et al.*, 1982; Ishihara *et al.*, 1983; Bonney *et al.*, 1987). The activity of these two inhibitors was not significantly altered by indomethacin, indicating that their ability to block contraction (i.e. to inhibit the synthesis and/or release of leukotrienes) was not influenced by newly formed cyclo-oxygenase products. Furthermore, the specificity of these compounds was further illustrated by the fact that in the absence of mepyramine both compounds failed to block the histaminergic response to antigen which is thought to occur independent of 5-lipoxygenase product formation (Hand *et al.*, 1986). Biochemical measurements of leukotriene formation in this tissue are needed to substantiate the pharmacological evidence for a major role for 5-lipoxygenase products.

Results somewhat inconsistent with the above findings were obtained when a number of novel leukotriene D_4 receptor antagonists were compared to FPL-55712 in this model. The present findings have been interpreted to indicate that only a small component of the response to antigen is mediated by leukotrienes acting on LTD_4 receptors. A possible explanation is that LTC_4 receptors may be involved in the mediation of a large part of the response to antigen. This interpretation assumes that L-651,896 and AA-861 effects are solely due to the inhibition of 5-lipoxygenase product formation. FPL-55712 (Snyder & Krell, 1984), LY-171883 (Fleisch *et al.*, 1985) and L-649,923 (Jones *et al.*, 1986b) and L-648, 051 (Jones *et al.*, 1986a) are ineffective antagonists of LTC_4-mediated contractions in this tissue, when LTC_4 metabolism to LTD_4 is inhibited by L-serine borate (Charette & Jones, 1987). Following antigen challenge, endogenous leukotriene C_4 which is the first product formed from the interaction of glutathione S-transferase with LTA_4, may preferentially interact with LTC_4 receptors to produce contraction. Conversion to LTD_4 may proceed slower with endogenously generated compared to exogenously applied LTC_4 thereby increasing the likelihood of LTC_4 receptor activation. Previous studies by Saad *et al.* (1983) support this since they found that LTC_4 was the major product released from immunologically challenged guinea-pig trachea. LTC_4 is also released from human lung tissues (Dahlén *et al.*, 1983) but contractions to leukotriene C_4, D_4 and antigen in human airway are mediated by LTD_4 receptor mechanisms (Buckner *et al.*, 1986; Jones *et al.*, 1987).

Another interesting aspect of the present study was that some differences were observed in the activity of the various LTD_4 antagonists. LY-171883, in general, blocked a larger component of the response to antigen than the other LTD_4 antagonists studied. This was particularly evident when 60 μM of LY-171883 was tested in the absence of indomethacin. This activity did not correlate with LTD_4 blocking properties on guinea-pig trachea since the $-\log K_B$ values for L-648,051, FPL-55712, L-649,923 and LY-171883 are not significantly different from each other (see, Krell *et al.*, 1983; Jones *et al.*, 1983; 1986a,b; Fleisch *et al.*, 1985). One interpretation is that LY-171883 which is known to have some non-specific relaxant activity (Fleisch *et al.*, 1985) may display greater potency versus antigen due to more than one pharmacological action. This compound is reported to inhibit cyclic AMP-dependent photodiesterase and such compounds are known to produce tracheal relaxation, to antagonize functionally contractions to LTD_4 and histamine (Armour *et al.*, 1982) and to inhibit mediator release (Ennis *et al.*, 1983). In the present study moderately high concentrations of LY-171883 produced almost complete relaxation of intrinsic tracheal tone and almost completely blocked the response to antigen on tonal preparations in contrast to the other LTD_4 antagonists. IBMX, a phosphodiesterase inhibitor of comparable potency to LY-171883 in biochemical assays (Fleisch *et al.*, 1985), displayed a similar profile of activity to that observed with LY-171883 on tonal preparations. It follows that some LTD_4 receptor antagonists may interact with endogenous inhibitory prostanoids in our tonal preparations to relax tracheal tone and antagonize release and/or effects of contractile mediators through cyclic AMP-dependent mechanisms. Such an interaction is possible with 20 and 60 μM LY-171883 (Fleisch *et al.*, 1985) and FPL-55712 (Chasin & Scott, 1978) but less likely with similar concentrations of more functionally specific compounds in this structural class.

In conclusion, the present study supports previous pharmacological evidence that anaphylactic contractions of guinea-pig trachea are preferentially mediated by 5-lipoxygenase products and histamine. The inability of reasonably potent and selective LTD_4 receptor antagonists to block completely the response to antigen in the presence of indomethacin, atropine and mepyramine is consistent with the hypothesis that part of the response to antigen is mediated by LTC_4 receptors. However, pharmacological studies with specific LTC_4 receptor antagonists devoid of effects on LTD_4 receptors, as well as biochemical measurements of leukotriene production are still needed to support this hypothesis. The presence of a functional cyclo-oxygenase enzyme pathway did not influence the activity of the 5-lipoxygenase inhibitors but did improve the activity of some LTD_4 antagonists, particularly the less selective antagonist, LY-171883 and IBMX, a known

phosphodiesterase inhibitor. Pharmacological interactions of such agents with prostaglandin E_2 or other inhibitory mechanisms to affect the actions and/or release of mediators is likely in this model and could explain the different activities observed in the absence and presence of indomethacin. The antigen-challenged guinea-pig trachea is of limited value for identifying selective LTD_4 receptor antagonists but with additional biochemical characterization could be a useful functional model to study inhibitors of 5-lipoxygenase product formation, inhibitors of mediator actions and release and possibly LTC_4 receptor antagonists.

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