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Pharmacological modulation of responses of guinea-pig airways contracted with antigen and calcium ionophore A23187

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1 Ovalbumin (OA) and calcium ionophore A23187 were used to induce contractions of sensitized guinea-pig tracheal and lung parenchymal preparations in the presence and absence of indomethacin. This model was used to examine the properties of a series of compounds reported to inhibit 5-lipoxygenase or to antagonize lipoxygenase products at the receptor level.

2 FPL55712 and piriprost appeared to act as pharmacological antagonists because they rapidly reduced tracheal tone established by OA.

3 The prolonged phase (i.e. >10 min post-challenge) of airways contractions induced by OA is assumed to be lipoxygenase-dependent and was inhibited by nordihydroguaiaretic acid (NDGA), FPL55712, nafazatrom and benoxaprofen, in order of potency. Piriprost had similar inhibitory effects on the trachea, but not on lung parenchyma. The inhibitory effects of NDGA and FPL55712 were reduced, and those of nafazatrom increased by indomethacin. Indomethacin decreased the inhibitory effect of piriprost on the trachea, but facilitated inhibition by this agent on the parenchyma. A roughly similar pattern was seen on A23187-induced contractions, but FPL55712 did not modify these contractions and benoxaprofen enhanced the response of the trachea.

4 BW755C enhanced the overall contractile response of OA- and A23187-induced tracheal contractions (but not parenchymal contractions) in a bell-shaped manner, an effect not seen in the presence of indomethacin. This indicated that BW755C could be acting as a cyclo-oxygenase inhibitor.

5 The results suggested that, although inhibitors of the lipoxygenase pathway were partially effective in inhibiting the contractions of the airways induced by OA or A23187, other mediators in addition to those of the lipoxygenase pathway contribute to these contractions.

Introduction

Pharmacological control of the enzymes in the 5-lipoxygenase pathway of arachidonic acid (AA) metabolism theoretically should, by decreasing the synthesis of the bronchoconstrictor leukotrienes C_4 and D_4, be therapeutically useful for asthma. The present study was designed to examine the efficacy of six agents that have been shown to inhibit leukotriene synthesis in leukocytes and, in some cases, lung tissue and to inhibit the contraction of the airways induced by antigen and calcium ionophore A23187. The compounds include nafazatrom (Mardin & Busse, 1983), 3-amino-1-[m-trifluoromethyl)-phenyl]-2-pyrazoline (BW755C) (Higgs *et al.*, 1979), nordihydroguaiaretic acid (NDGA) (Tappel *et al.*, 1953), benoxaprofen (Cashin *et al.*, 1977), 6,9-deoxy-6,9-N-phenylimino- $\Delta^{6,8}$ prostaglandin I_1 (piriprost) (Bach *et al.*, 1982) and diethylcarbamazine citrate (DECC) (Harned *et al.*, 1948). In addition sodium 7-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy]-4-oxo-8-propyl-4H-benzopyran-2-carboxylate (FPL55712) (Augstein *et al.*, 1973) was examined as it blocks peptidoleukotriene receptors in the airways (Sheard *et al.*, 1977; Jones *et al.*, 1983).

Although it is known that peptidoleukotrienes are released from sensitized airways following challenge with antigen and A23187, (Morris *et al.*, 1980; Saad *et al.*, 1983) their actual contribution to airways contractility is not clear. Thus it was hoped that the use of the above substances would provide further insight into the roles of lipoxygenase products in the contraction of the airways.

Methods

Male English short-hair guinea-pigs (200–250 g) (Connaught Laboratories, Toronto, Ontario) were sensitized with ovalbumin (OA), 100 mg subcutaneously and 100 mg intraperitoneally. Lungs and trachea were removed 3–4 weeks later. The trachea was spirally cut (Constantine, 1965), divided into four segments and each suspended under 1 g tension in 10 ml organ baths containing Krebs-Henseleit solution (KHS) maintained at 37°C and aerated with 95% O_2 plus 5% CO_2. Parenchymal strips were carefully cut from the distal edges of the lung lobes (Lulich *et al.*, 1979) and suspended under 500 mg tension in 10 ml organ baths as described above.

Contractions of the tracheal spirals and parenchymal strips were measured isotonically using rotary motion transducers (Type 386 heart/smooth muscle transducers; Ealing Scientific, St Laurent, Quebec). Tissues were incubated for 1 h before use and constant maximal contractions to histamine (10^{-4}M) were obtained before modulatory drugs were administered.

Experiments were carried out in the presence and absence of indomethacin, which inhibits the cyclooxygenase pathway of AA metabolism. Indomethacin (8.4×10^{-6}M) was added to the organ baths at least 30 min before challenge with OA (1 μg ml^{-1}) or calcium ionophore A23187 (5.7×10^{-6}M). FPL55712 and the putative lipoxygenase inhibitors were also added to the organ baths 30 min before challenge except piriprost which was added 1 min before challenge. However, in one series of experiments FPL55712, piriprost, and nafazatrom were added at fixed time points following challenge.

Analysis and statistical evaluation of results

Responses to OA or A23187 were measured as a percentage of the maximum response obtained with histamine on each tissue in the absence of any modulatory agent. These responses were calculated for a period of 60 min beginning at the point of challenge. The response over this period of time constitutes the contraction curve. Two methods of comparison were used in this study: (1) the areas under the contraction curves over a fixed time period, as recorded on a linear chart recorder, were calculated on a computer programme to give an indication of the total contractile response; (2) the response of the peak contraction, or of the contraction at 60 min post-challenge of tissues, in the presence of test drugs were compared to the response of a paired tissue in the absence of drug. The response of the peak contraction and the 60 min post-challenge contraction were used only for responses induced by ovalbumin. The data are presented as the means ± s.e.mean. Data for area under the curves, peak height and height at 60 min were analysed for significance using Student's *t* test for paired data. A minimum of 4 animals was used for each experiment, except where otherwise indicated.

Drugs

Histamine dihydrochloride, ovalbumin (grade II for sensitization and grade V for challenge), nordihydroguaiaretic acid, diethylcarbamazine citrate, calcium ionophore A23187 and arachidonic acid (99% pure) were purchased from Sigma (St Louis, MO). I am grateful for the kind gifts of the following agents: piriprost (Dr M.K. Bach, Upjohn, Kalamazoo, MI), nafazatrom (Dr A. Scriabine, Miles, New Haven, CN), BW755C (Dr P.J. McHale, Wellcome, Beckenham, Kent), FPL55712 (Mr P. Sheard, Fisons, Loughborough, Leics.), benoxaprofen (Dr R. Thompson, Dept. of Medicine, Rheumatology Div., University of Alberta) and indomethacin (Dr W. Dorian, Merck-Frosst, Pointe Claire-Dorval, Quebec).

Indomethacin (1 mg ml^{-1}) and piriprost (10^{-2}M)

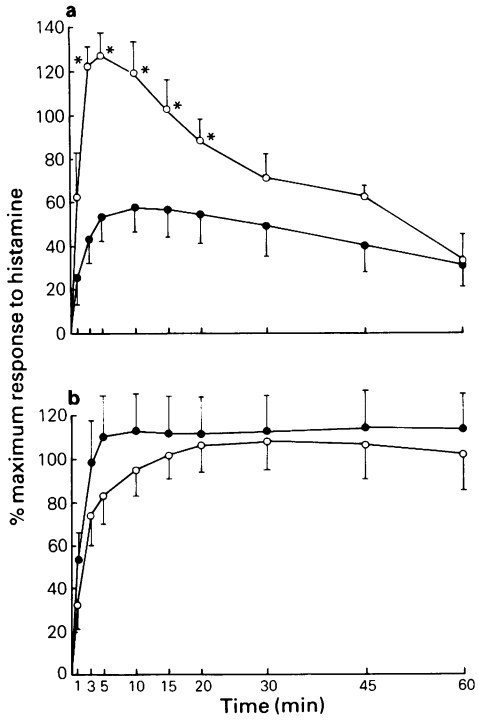

Figure 1 The contractile responses of sensitized (a) tracheal spirals and (b) parenchymal strips when challenged with ovalbumin (1 μg ml^{-1}) in the presence (○) and absence (●) of indomethacin (8.4 μM). Values show the mean response, and vertical lines s.e.mean, of tissues from 4 animals. * $P < 0.05$, significantly different from contractions of paired tissues in the absence of indomethacin.

were dissolved in 1 M Tris buffer (pH 8.5), diluted in KHS, and kept on ice. A23187 (1 mg ml^{-1}) was dissolved in ethanol and stored in the dark at − 5°C. NDGA (10^{-2}M) was dissolved in distilled water and a few drops of NaOH (1N) were added to make a salt. Nafazatrom (10 mg ml^{-1}) and benoxaprofen (10^{-1}M) were dissolved in dimethyl sulphoxide (DMSO). FPL55712 (10^{-2}M) and BW755C (10^{-1}M) were dissolved in distilled water and diluted with KHS. Diethylcarbamazine citrate (10^{-1}M) was dissolved in KHS. The vehicles (ethanol, Tris buffer, or DMSO) did not affect tissue responses at the concentrations used.

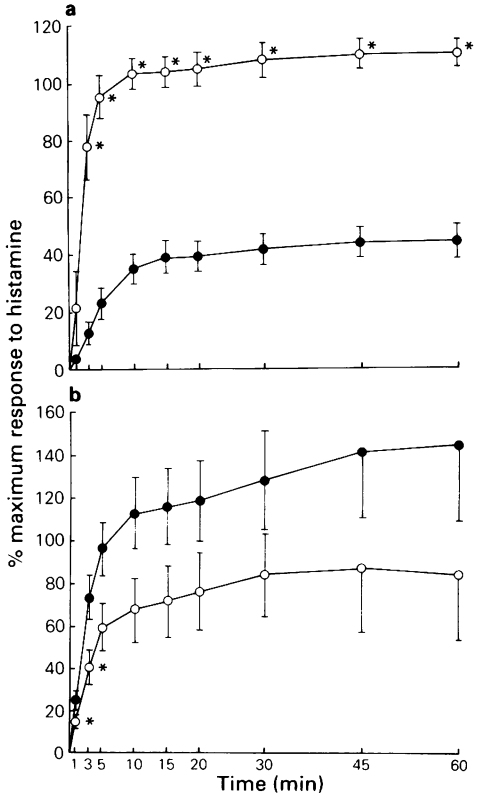

Figure 2 The contractile responses of sensitized (a) tracheal spirals and (b) parenchymal strips when challenged with calcium ionophore A23187 (5.7 μM) in the presence (○) and absence (●) of indomethacin (8.4 μM). Values show the mean response, and vertical lines s.e.mean, of tissues from 5 animals. * $P < 0.05$, significantly different from contractions of paired tissues in the absence of indomethacin.

Results

With the exception of DECC, the agents examined caused varying degrees of modulation of the responses of the airways induced by OA or A23187. DECC (10^{-5}–10^{-3}M) was only examined in the presence of indomethacin (8.4×10^{-6}M) and had no effect on tissue responses.

Effects of indomethacin on contractions induced by ovalbumin and A23187

Indomethacin enhanced the peak height of OA- and A23187-induced contractions of trachea but had no significant effects on parenchymal contractions at the concentrations of challenging agent used (Figures 1 and 2), confirming previous results (Burka, 1983). However, several interesting points regarding the time course of OA-induced contractions in the presence and absence of indomethacin were noted. Indomethacin enhanced the peak height of OA-induced contractions of trachea, but not the height at 60 min (Figure 1a). Secondly, although in the parenchymal strip indomethacin did not significantly affect the peak height, area under the contraction curve, or the height at 60 min, the time to reach the peak height in this tissue was in all cases increased from 5–10 min to 20–30 min (Figure 1b).

The effects of drugs on tracheal contractions induced by ovalbumin

The effects of potential inhibitors of the lipoxygenase pathway and of FPL55712 on OA-induced contractions were analysed by studying their effects on the area under the curve, the peak height, and the height at 60 min (Table 1). In the absence of indomethacin, BW755C differed from all the other compounds by enhancing tracheal contractions in a bell-shaped manner, peaking at 10^{-5}M with a 2.8 fold enhancement of the total contraction and of the peak height. It is interesting that the height of the contraction at 60 min was no longer significantly enhanced ($P > 0.05$) and with a higher concentration of BW755C (10^{-4}M) no contraction was observed at 60 min, although the peak contraction was still enhanced by 142.9% above control. Enhancement of peak and total tracheal contractions was no longer observed in the presence of indomethacin, but the inhibition of the contraction at 60 min was still observed with the highest concentration of BW755C (10^{-4}M). Lower concentrations of BW755C had no effect.

Nafazatrom, NDGA, piriprost, benoxaprofen and FPL55712 all had some inhibitory effects on OA-induced contractions of trachea in the absence of indomethacin (Table 1). When the area under the curve is used as an index of the contraction induced by

Table 1 The effects of putative inhibitors of the lipoxygenase pathway and peptidoleukotriene receptor antagonists on contractions of trachea induced by ovalbumin

	− Indomethacin			*+ Indomethacin*		
	Area	Peak height	Height at 60 min	Area	Peak height	Height at 60 min
Nafazatrom						
3.7×10^{-5}M	61.1 ± 11.6*	72.5 ± 14.8	44.0 ± 22.1*	72.8 ± 14.4*	91.0 ± 7.9	63.0 ± 24.3*
1.2×10^{-4}M	58.8 ± 21.7*	65.7 ± 21.4	41.5 ± 17.8*	27.7 ± 9.5*	54.1 ± 18.1*	10.3 ± 7.3*
3.7×10^{-4}M	18.5 ± 11.4*	41.3 ± 17.2*	0*	0*	0*	0*
NDGA						
10^{-6}M	—	—	—	81.5 ± 8.1	93.7 ± 9.4	86.3 ± 8.7
10^{-5}M	25.8 ± 5.8*	56.6 ± 17.1*	7.1 ± 7.1*	70.0 ± 18.0	79.1 ± 22.9	82.1 ± 6.7
3×10^{-5}M	3.4 ± 1.5*	26.7 ± 10.9*	0*	—	—	—
10^{-4}M	0.2 ± 0.2*	2.4 ± 2.4*	0*	9.8 ± 5.5*	22.2 ± 10.3*	1.7 ± 1.7*
Piriprost						
10^{-6}M	91.0 ± 21.1	89.5 ± 5.0	96.6 ± 27.7	90.7 ± 8.9	101.8 ± 7.6	85.8 ± 7.2
10^{-5}M	83.3 ± 34.8	104.5 ± 10.5	78.1 ± 45.5	89.5 ± 5.0*	98.3 ± 3.8	76.5 ± 11.3*
10^{-4}M	36.1 ± 14.6*	89.6 ± 4.3*	12.7 ± 12.7*	61.1 ± 10.5*	97.7 ± 6.9	31.5 ± 15.9*
Benoxaprofen						
10^{-6}M	154.1 ± 38.2	133.3 ± 23.9	134.0 ± 26.2	128.1 ± 35.1	114.8 ± 21.8	156.0 ± 37.0
10^{-5}M	229.6 ± 90.9	211.9 ± 53.2*	146.9 ± 74.8	93.2 ± 24.9	102.1 ± 13.7	73.9 ± 15.7
10^{-4}M	33.6 ± 14.4*	92.3 ± 32.9	6.8 ± 29.9*	0.1 ± 0.1*	0.5 ± 0.5*	0*
BW755C						
10^{-6}M	220.5 ± 45.6*	203.0 ± 30.3*	310.2 ± 106.9	75.9 ± 15.3	84.8 ± 7.6	53.0 ± 60.3
10^{-5}M	380.2 ± 102.5*	372.0 ± 99.6*	213.9 ± 84.4	93.1 ± 34.1	83.9 ± 12.3	32.7 ± 63.9
10^{-4}M	139.2 ± 54.4	242.9 ± 66.3*	0*	65.7 ± 29.5	104.4 ± 22.2	0*
FPL55712						
10^{-6}M	75.8 ± 14.4	83.8 ± 15.4	79.4 ± 15.5	85.5 ± 10.4	95.7 ± 9.3	79.3 ± 8.9*
3×10^{-6}M	53.2 ± 10.7*	79.7 ± 14.9	55.4 ± 12.8*	82.7 ± 7.8*	97.7 ± 6.2	79.7 ± 4.7*
10^{-5}M	46.7 ± 12.3*	85.2 ± 14.9	14.5 ± 14.5*	63.1 ± 8.4*	80.3 ± 4.9*	54.9 ± 8.4*

Values are mean % (± s.e.mean) of control.
NDGA = nordihydroguaiaretic acid.
* $P < 0.05$.

OA, FPL55712 and NDGA were the most potent inhibitors (IC_{50}s: 7×10^{-6}M and $< 10^{-5}$M respectively) and nafazatrom the least potent (IC_{50}: 1.5×10^{-4}M). However, when the contraction was dissected to examine both the inhibition of the peak height and of the contraction at 60 min it could be seen that the agents were unequivocally more potent in inhibiting the latter part of the contraction than the peak (Table 1). The order of potency also changed and the IC_{50}s were all shifted to the left. FPL55712 (IC_{50} for contraction at 60 min: 3.5×10^{-6}M) and NDGA (IC_{50}: $< 10^{-5}$M) were still the most potent substances but nafazatrom was no longer the least potent inhibitor. The IC_{50}s for both piriprost and benoxaprofen were 2.5×10^{-5}M and 7×10^{-5}M respectively. It was also noted that benoxaprofen (10^{-5}M) enhanced the peak height of OA-induced contractions of trachea by 111.9%.

In the presence of indomethacin none of the compounds tested enhanced OA-induced contractions of trachea. Both benoxaprofen and nafazatrom were more potent in reducing the peak height of the contraction. Whereas benoxaprofen (10^{-4}M) had no effect on the peak height in the absence of indomethacin, total reduction was seen in the presence of indomethacin. Similarly, nafazatrom (3.7×10^{-4}M) inhibited the peak height by 58.7 ± 17.2% in the absence, and totally in the presence, of indomethacin.

Table 2 The effects of putative inhibitors of the lipoxygenase pathway and peptidoleukotriene receptor antagonists on contractions of lung parenchyma induced by ovalbumin

	− Indomethacin			*+ Indomethacin*		
	Area	Peak height	Height at 60 min	Area	Peak height	Height at 60 min
Nafazatrom						
3.7×10^{-5}M	61.8 ± 12.7*	86.0 ± 7.0	68.3 ± 29.6	74.3 ± 8.3*	76.9 ± 8.3*	70.4 ± 7.4*
1.2×10^{-4}M	90.3 ± 27.7	97.4 ± 18.4	60.0 ± 24.0	54.5 ± 18.3*	58.2 ± 17.6*	49.4 ± 20.6*
3.7×10^{-4}M	1.2 ± 0.6*	3.1 ± 1.1*	0*	0.5 ± 0.3*	1.4 ± 1.3*	0*
NDGA						
10^{-6}M	—	—	—	92.0 ± 6.1	96.3 ± 6.3	85.6 ± 11.0
10^{-5}M	19.2 ± 9.1*	27.5 ± 8.1*	19.2 ± 15.8*	85.7 ± 18.2	88.2 ± 19.1	85.5 ± 19.9
3×10^{-5}M	9.2 ± 8.4*	13.1 ± 6.6*	4.9 ± 4.9*	—	—	—
10^{-4}M	3.9 ± 2.2*	12.7 ± 9.1*	0*	57.8 ± 21.9*	62.0 ± 22.9	53.8 ± 22.8*
Piriprost						
10^{-6}M	128.3 ± 8.1*	126.4 ± 8.7*	126.2 ± 8.6*	115.4 ± 6.3*	122.3 ± 8.7*	123.9 ± 8.2*
10^{-5}M	125.7 ± 12.2*	127.9 ± 14.8*	126.1 ± 14.9	117.7 ± 21.6	130.4 ± 25.0	132.5 ± 25.7
10^{-4}M	116.4 ± 7.8*	118.3 ± 11.2	103.4 ± 4.6	73.0 ± 16.0	79.1 ± 13.8	65.3 ± 17.4*
Benoxaprofen						
10^{-6}M	90.4 ± 22.3	95.5 ± 21.6	86.9 ± 27.8	95.9 ± 19.5	96.5 ± 18.7	81.6 ± 8.5
10^{-5}M	99.8 ± 14.9	118.1 ± 9.1	92.2 ± 23.2	92.3 ± 8.5	94.3 ± 10.0	87.3 ± 5.0
10^{-4}M	15.3 ± 10.3*	18.4 ± 10.6*	6.3 ± 12.7*	1.5 ± 0.7*	2.2 ± 1.3*	0*
BW755C						
10^{-6}M	111.4 ± 19.6	114.7 ± 18.5	105.3 ± 14.3	109.5 ± 12.0	110.4 ± 13.0	97.0 ± 11.0
10^{-5}M	95.5 ± 10.6	103.2 ± 8.7	89.9 ± 10.5	93.5 ± 9.0	93.8 ± 9.7	87.2 ± 3.2
10^{-4}M	39.0 ± 5.5*	55.7 ± 10.4*	24.5 ± 4.8*	76.0 ± 11.0*	78.5 ± 12.8	65.1 ± 7.2*
FPL55712						
10^{-6}M	58.6 ± 4.7*	66.6 ± 7.7*	57.8 ± 5.2*	133.3 ± 22.7	127.0 ± 22.3	121.8 ± 24.5
3×10^{-6}M	62.4 ± 5.8*	75.1 ± 9.5*	52.7 ± 4.1*	111.9 ± 6.9	115.6 ± 2.8*	97.2 ± 14.4
10^{-5}M	57.9 ± 21.3*	77.3 ± 22.2	50.9 ± 29.0*	85.5 ± 13.8	92.3 ± 8.1	53.9 ± 8.1*

Values are mean % (± s.e.mean) of control.
* $P < 0.05$.

In contrast, the inhibitory effects of NDGA and FPL55712 were reduced by indomethacin. Whereas the peak height and the height at 60 min were reduced by 43.4 ± 17.1% and 92.9 ± 7.1% respectively by NDGA (10^{-5}M) in the absence of indomethacin, the inhibitions were only 20.9 ± 22.9% ($P > 0.05$) and 17.9 ± 6.7% ($P < 0.05$) respectively in the presence of indomethacin. FPL55712 (3×10^{-6} and 10^{-5}M) had no major modulatory effects on the peak height of OA-induced tracheal contractions but did inhibit the height at 60 min. In the absence of indomethacin the reductions with 3×10^{-6}M and 10^{-5}M were 44.6 ± 12.8% and 85.5 ± 14.5% respectively and in the presence of indomethacin these values were halved to 20.3 ± 4.7% and 45.1 ± 8.4%, respectively.

The effects of drugs on parenchymal contractions induced by ovalbumin

The potency of nafazatrom, NDGA, benoxaprofen and FPL55712 in inhibiting OA-induced contractions of lung parenchymal strips in the absence of indomethacin (Table 2) was, in general, similar to that seen on trachea (Table 1). Again the inhibition of the height of the contraction at 60 min was greater than that of the peak height, but the difference was not as pronounced as on trachea. As on the trachea, indomethacin increased the inhibitory effects of nafazatrom and benoxaprofen and reduced those of NDGA and FPL55712. The inhibition of the height of the contraction at 60 min by nafazatrom (1.2×10^{-4}M) was

increased from 40.0 ± 24.0% ($P > 0.05$) to 50.6 ± 20.6% ($P < 0.05$) by indomethacin. The inhibitions by NDGA (10^{-5}M) and FPL55712 (3×10^{-6}M) were reduced from 80.8 ± 15.8% ($P < 0.05$) and 47.3 ± 4.1% ($P < 0.05$) respectively to 14.5 ± 19.9% ($P > 0.05$) and 2.8 ± 14.4% ($P > 0.05$).

Piriprost (10^{-6}–10^{-4}M) did not inhibit OA-induced contractions of parenchyma, but the lower concentrations (10^{-6}–10^{-5}M) actually enhanced contractions by 20–30%. This enhancement was also seen in the presence of indomethacin, but in this case, the higher concentration of piriprost (10^{-4}M) caused some inhibition (34.7 ± 17.4%) of the height of the contraction at 60 min post-challenge.

BW755C (10^{-4}M) inhibited OA-induced contractions. The inhibition of the peak height and of the height at 60 min was considerably less in the presence of indomethacin. The contractions at peak and at 60 min were inhibited by 44.3 ± 10.4% ($P < 0.05$) and 77.5 ± 4.8% ($P < 0.05$) respectively in the absence, and 21.5 ± 12.8% ($P > 0.05$) and 34.9 ± 7.2% ($P < 0.05$) in the presence, of indomethacin.

The effects of drugs on tracheal contractions induced by A23187

Only NDGA (10^{-4}M) and piriprost (10^{-4}M) were effective inhibitors of A23187-induced contractions in the absence of indomethacin (Table 3). FPL55712 did not modify A23187-induced airways contractions of trachea in the presence or absence of indomethacin. However, the lower concentrations of piriprost

Table 3 The effects of putative inhibitors of the lipoxygenase pathway and peptidoleukotriene receptor antagonists on airways contractions induced by calcium ionophore A23187

	Trachea		*Parenchyma*	
	− Indomethacin	*+ Indomethacin*	*− Indomethacin*	*+ Indomethacin*
Nafazatrom				
3.7×10^{-5}M	95.4 ± 11.8	88.4 ± 6.6	87.0 ± 12.1	72.3 ± 9.9*
1.2×10^{-4}M	146.8 ± 13.9*	44.4 ± 6.6*	96.0 ± 8.8	64.7 ± 12.3*
3.7×10^{-4}M	98.5 ± 28.7	0.7 ± 0.3*	52.8 ± 19.3*	18.9 ± 3.4*
NDGA				
10^{-6}M	—	94.5 ± 13.2	—	90.2 ± 2.5
10^{-5}M	150.1 ± 35.0	90.1 ± 20.7	66.0 ± 12.9*	86.0 ± 12.0
3×10^{-5}M	96.6 ± 36.8	—	36.1 ± 13.3*	—
10^{-4}M	1.4 ± 0.9*	49.5 ± 11.5*	10.3 ± 4.2*	75.9 ± 12.8*
Piriprost				
10^{-6}M	125.8 ± 1.6*	84.7 ± 12.7	123.0 ± 18.4	163.1 ± 43.0
10^{-5}M	136.4 ± 17.6*	74.8 ± 10.0*	110.6 ± 9.7	129.8 ± 6.1*
10^{-4}M	0*	36.7 ± 13.0*	68.0 ± 15.7*	71.2 ± 23.5
Benoxaprofen				
10^{-6}M	99.2 ± 10.3	104.6 ± 9.5	100.3 ± 8.6	105.5 ± 9.6
10^{-5}M	171.7 ± 27.3*	100.3 ± 14.5	102.2 ± 5.7	120.8 ± 8.2*
10^{-4}M	319.0 ± 68.3*	72.1 ± 15.6*	103.4 ± 22.0	76.6 ± 4.5*
BW755C				
10^{-6}M	143.5 ± 12.7*	126.6 ± 19.7*	110.0 ± 9.7	115.7 ± 19.6
10^{-5}M	271.6 ± 31.1*	116.1 ± 15.2	99.9 ± 5.7	87.1 ± 28.9
10^{-4}M	225.5 ± 35.9*	111.9 ± 19.7	86.7 ± 7.4	83.9 ± 10.6
FPL55712				
10^{-6}M	87.0 ± 12.7	123.3 ± 22.2	101.6 ± 1.8	95.9 ± 13.9
3×10^{-6}M	113.8 ± 30.3	119.9 ± 19.3	121.9 ± 10.7	103.2 ± 24.4
10^{-5}M	91.6 ± 30.3	105.5 ± 17.9	106.1 ± 11.6	102.0 ± 15.8

Values are mean % (± s.e.mean) of control of the areas under the contraction curves.
* $P < 0.05$.

(10^{-6}–10^{-5}M) enhanced tracheal contractions by 25.8% and 36.4% respectively. Nafazatrom (1.2×10^{-4}M), benoxaprofen (10^{-5}–10^{-4}M) and BW755C (10^{-6}–10^{-4}M) all enhanced tracheal contractions as well. The enhancements were all bell-shaped with the exception of benoxaprofen where the maximal enhancement obtained was with the highest concentration used (10^{-4}M).

In the presence of indomethacin, none of the agents enhanced tracheal contractions. In contrast, all the agents, with the exceptions of BW755C and FPL55712, caused concentration-dependent inhibitions of tracheal contraction. The IC_{50}s for nafazatrom, NDGA, and piriprost were in the range of 3×10^{-5}–10^{-4}M. Benoxaprofen was the least potent and the highest concentration used (10^{-4}M) only caused an inhibition of 27.9 ± 15.6%. As in the case of OA-induced contractions, the inhibitory effects of NDGA and piriprost on A23187-induced contractions of the trachea were reduced in the presence of indomethacin. Whereas NDGA (10^{-4}M) and piriprost (10^{-4}M) both totally inhibited A23187-induced contractions in the absence of indomethacin, inhibition was only 50.5 ± 11.5% and 43.3 ± 13.0% respectively in the presence of indomethacin.

The effects of drugs on parenchymal contractions induced by A23187

A23187-induced contractions of parenchyma were inhibited by nafazatrom (3.7×10^{-4}M), NDGA (10^{-5} – 10^{-4}M) and piriprost (10^{-4}M) in the absence of indomethacin and by nafazatrom (3.7×10^{-5} – 3.7×10^{-4}M), NDGA (10^{-4}M) and benoxaprofen (10^{-4}M) in the presence of indomethacin (Table 3). Again the inhibition by NDGA was greatly reduced by indomethacin. Whereas NDGA (10^{-4}M) reduced A23187-induced contractions of parenchyma by 89.7 ± 4.2% ($P < 0.05$) in the absence of indomethacin, the inhibition was only 24.1 ± 12.8% ($P > 0.05$) in the presence of indomethacin.

Lower concentrations of piriprost (10^{-6} and 10^{-5}M) enhanced A23187-induced contractions of parenchyma by 63.1% ($P > 0.05$) and 29.8% ($P < 0.05$) respectively in the presence of indomethacin, and benoxaprofen (10^{-5}M) also enhanced contractions by 20.8% ($P < 0.05$) in the presence of indomethacin. BW755C and FPL55712 had no effect on parenchymal contractions in the presence or absence of indomethacin.

The effects of drug addition following challenge

Drugs were administered to the organ baths 5, 10 or 20 min following challenge of indomethacin-treated tissues with OA. The areas under the contractile curves following addition of drugs were compared to paired

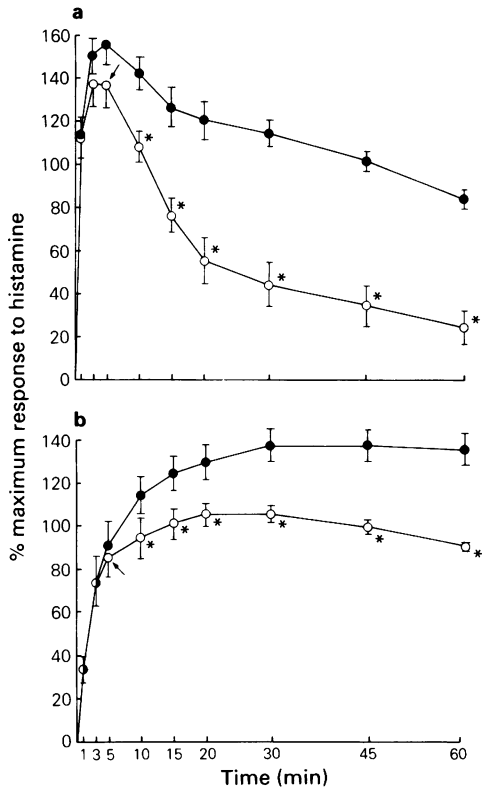

Figure 3 The contractile responses of sensitized (a) tracheal spirals and (b) parenchymal strips when challenged with ovalbumin (1 μg ml^{-1}) in the presence of indomethacin (8.4 μM). FPL55712 (10^{-5}M) was added to a paired tissue at 5 min (shown with the arrow) following administration of antigen to the organ bath. Values show the mean response, and vertical lines s.e.mean, of tissues from 4 animals. * $P < 0.05$, significantly different from contractions of paired tissues which were not treated with FPL55712. (○) FPL55712 treated tissues; (●) untreated tissues.

control tissues to which no drugs were administered. FPL55712 (10^{-5}M) (Figure 3a) and piriprost (10^{-4}M) caused an immediate reduction of tracheal tone induced by OA, by 35–50% ($n = 4$) and 55–80% ($n = 2$) respectively. In contrast, nafazatrom (1.2×10^{-4}M) did not significantly inhibit OA-induced contractions of trachea when added post-challenge ($n = 4$).

Neither FPL55712 (Figure 3b), piriprost nor nafazatrom caused a clear relaxation of parenchymal contractions induced by OA. This was in contrast to the rapid relaxation observed with FPL55712 and piriprost on trachea. Rather, further contraction did not occur once the drugs were added and a gradual

decline in tone was observed. OA-induced contractions were reduced by 20–40% ($n = 4$) after FPL55712 (10^{-5}M) was added to the organ baths. Piriprost did not cause a clear inhibition of parenchymal contractions although there appeared to be some inhibition (38.4 ± 8.2%; $n = 2$) when the drug was added 10 min post-OA challenge. Nafazatrom only inhibited OA-induced parenchymal contractions when added 5 min after challenge (42.3 ± 6.8%; $n = 4$).

Discussion

Although peptidoleukotrienes are released when sensitized guinea-pig lung and tracheal tissues are challenged with specific antigen (Morris *et al.*, 1980; Saad *et al.*, 1983), the extent of their contribution in antigen-induced contractions of lung parenchymal strips and tracheal spirals is still not clear. Adams & Lichtenstein (1979) suggested that histamine was responsible for the early part of the tracheal contraction, whereas leukotrienes were responsible for the latter part. Our own results showed that H_1-antihistamine compounds only blocked antigen-induced contractions of trachea in the first minute post-challenge, but not the peak height, whereas the leukotriene receptor antagonist, FPL55712, only blocked the prolonged phase of the response (i.e. after the peak which occurs at about 10 min), and then only at fairly high concentrations (5×10^{-6}M) (Burka & Paterson, 1981). It was interesting that the peak height of the tracheal contraction was not easily inhibited by FPL55712 or anti-oxidant lipoxygenase inhibitors, such as phenidone and NDGA, whereas the prolonged phase was readily inhibited (see Burka, 1983 and results presented here). What was also confusing was that indomethacin, a cyclo-oxygenase inhibitor, enhanced the peak height of the tracheal contraction, but not the prolonged phase (Figure 1a). If the enhancement was due to increased synthesis of leukotrienes by diversion of endogenous substrate AA from the cyclo-oxygenase to the lipoxygenase pathway, one would expect particular enhancement of the prolonged phase. However, this was not observed.

In the case of the parenchyma, enhancement by indomethacin of OA-induced contractions of this tissue was not seen (Figure 1b). In fact, with this tissue there was an increase in the time required to reach maximal contraction. Since leukotrienes are known to induce some thromboxane A_2 (TXA_2) release in lung tissue (Piper & Samhoun, 1981; 1982; Schiantarelli *et al.*, 1981; Zijlstra *et al.*, 1984), it is possible that TXA_2 contributes to the early phase (1–10 min) of the OA-induced contraction of parenchyma and that this contribution, although small, is eliminated by indomethacin. Although cyclo-oxygenase inhibitors enhance leukotriene release from chopped lung challenged with antigen or A23187 (Piper & Seale, 1979), enhancement of contraction is never seen when lung is examined as a parenchymal strip preparation (Burka, 1983).

Because of the shape of OA-induced contractions of trachea and parenchyma, analysis was carried out not only of the peak height but also of the height at 60 min, as it was thought that the latter might represent the lipoxygenase-dependent stage of the contraction (Adams & Lichtenstein, 1979; Burka, 1983). In addition, the area under the contraction curve was analysed to give an indication of the entire contractile response.

It had previously been shown that A23187-induced contractions of trachea were enhanced by indomethacin whereas those of parenchyma were not modified (Burka, 1983). Since A23187-induced contractions do not have a peak phase, but rather are represented by a gradual rise which plateaus by about 30 min, it seemed reasonable to analyse A23187-induced contractions using the area under the curve.

A23187 is believed to result in the release of AA from multiple phospholipid sites, whereas antigen activation is more specific (Siraganian, 1983). Thus the differences in the potencies of the drugs tested need not be surprising. For example, NDGA is considerably more potent in inhibiting OA-induced airways contractions than those induced by A23187 or AA (Burka, 1985). There is also the possibility that other substances in addition to AA metabolites are involved in OA-induced contractions and NDGA may be a particularly potent inhibitor of the synthesis of these compounds. We know that AA metabolites are synthesized as a result of OA and A23187 challenge of airways (Burka *et al.*, 1981; Saad *et al.*, 1983) and assume that they are partly responsible for the contractions of the airways. From the present results it appears that lipoxygenase products of AA metabolism may not contribute to the peak phase of OA-induced airways contractions, but probably do contribute to the prolonged phase. Thus, if antigen-induced contraction is taken as a screen for lipoxygenase inhibitors, the height at 60 min might be a better measure than the peak height. The only agents observed to inhibit the peak height of OA-induced tracheal contractions were NDGA, which is a fairly non-specific anti-oxidant, and nafazatrom, but then only at fairly high concentrations (3.7×10^{-4}M). Piriprost (10^{-4}M) also caused a slight (10.4 ± 4.3%), but significant, inhibition of the peak height in the absence of indomethacin. The effects of these agents on the peak height of OA-induced contractions of parenchyma were also somewhat equivocal. NDGA (10^{-5}–10^{-4}M), BW755C (10^{-4}M) and FPL55712 (10^{-6}–3×10^{-6}M) inhibited the peak height in the absence, but not in the presence, of indomethacin.

Interestingly, a higher concentration of FPL55712 (10^{-5}M) had no effect on the peak height. In contrast to the above agents, nafazatrom (3.7×10^{-5}–3.7×10^{-4}M) dose-dependently inhibited the peak height in the presence of indomethacin, but only the highest concentration (3.7×10^{-4}M) was inhibitory in the absence of indomethacin. Benoxaprofen (10^{-4}M) inhibited the peak height in the presence and absence of indomethacin.

It is interesting that BW755C (10^{-4}M) inhibited OA-induced contractions of trachea 60 min post-challenge, but enhanced the height of the peak contraction. The enhancement is also observed with lower concentrations and is maximal at 10^{-5}M. Bell-shaped enhancement of antigen- and A23187-induced contractions of trachea is typically observed with cyclo-oxygenase inhibitors such as indomethacin (Burka, 1983). A similar enhancement by BW755C was also seen on A23187-induced contractions. No further enhancement by BW755C was observed in the presence of indomethacin. Higgs *et al.* (1979) demonstrated that BW755C blocks both the cyclo-oxygenase and lipoxygenase pathways of AA metabolism. This evidence also suggests that BW755C is inhibiting the cyclo-oxygenase pathway in the trachea at concentrations as low as 10^{-6}M and does not inhibit the lipoxygenase pathway until higher concentrations (10^{-4}M) are used. However, it is puzzling that a similar phenomenon is not seen in the parenchyma with either BW755C or indomethacin. A similar differential inhibition of cylo-oxygenase and lipoxygenase pathways may occur with benoxaprofen, since lower concentrations (10^{-5}M) enhance and higher concentrations (10^{-4}M) inhibit OA- and A23187-induced contractions of trachea. This is somewhat in contrast to the findings in several models of inflammation where benoxaprofen has been shown to be more potent as an inhibitor of the lipoxygenase than of the cyclo-oxygenase pathway (Dawson *et al.*, 1982).

Diethylcarbamazine citrate (up to 10^{-3}M) had no effect on airways contractions induced by antigen or A23187 in contrast to its reported ability to inhibit leukotriene release from chopped lung (Orange *et al.*, 1971; Burka & Eyre, 1975) and perfused lung (Engineer *et al.*, 1978). DECC also has no effect on airways contractions induced with exogenous AA (Burka, 1985). The lack of activity of DECC in the isolated airways preparation suggests that either the drug is incapable of modulating mediator release in this model, or that leukotrienes may not be involved to any significant extent in airways contraction.

Although these experiments have provided further data on the modulatory capacity of several inhibitors of leukotriene synthesis, several major questions are still unresolved. One is the role of leukotrienes in antigen- and A23187-induced contractions of the airways. Although FPL55712 is a good inhibitor of the contractions induced by AA in the presence of indomethacin and also readily reverses these contractions (Burka, 1985) and those induced by synthetic peptidoleukotrienes (Jones *et al.*, 1983), contractions induced by OA are only partly reversed or inhibited (Tables 1 and 2) and A23187-induced contractions are not affected (Table 3). This would suggest that mediators other than peptidoleukotrienes may be responsible for up to 50% of the prolonged phase of OA-induced contractions and perhaps totally for A23187-induced contractions. It is interesting that the inhibitory effects of FPL55712 were in general reduced by indomethacin. This may imply that either prostaglandins, particularly prostaglandin E_2, contribute to the inhibitory effects of FPL55712, or that more non-peptidoleukotriene mediators are produced in the presence of indomethacin. The latter possibility may also explain the decreased potency of NDGA and piriprost in the presence of indomethacin. In contrast, nafazatrom is more potent in the presence of indomethacin.

Another complicating factor is the intricate interaction of prostaglandins, thromboxanes, leukotrienes and possibly other mediators in the airways. A simple shift of the AA cascade to the synthesis of more leukotrienes does not necessarily explain the results with indomethacin. The lack of effect of indomethacin on the parenchymal response remains unexplained. A shift to leukotriene synthesis does not explain why the prolonged phase of OA-induced contractions of trachea is not enhanced and we have recently shown that there does not appear to be an increased synthesis of leukotrienes as a consequence of blocking the cyclo-oxygenase pathway (Burka & Saad, 1984). Inhibition of prostaglandin synthesis, removing a negative feedback component, probably contributes to the enhancement as well, as would increased synthesis of leukotrienes and HPETEs which could sensitize the tissue to the activity of other mediators. Further studies are obviously necessary to examine these interactions.

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