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STIMULATION OF THE ANTIGEN-INDUCED CONTRACTION OF GUINEA-PIG TRACHEA AND IMMUNOLOGICAL RELEASE OF HISTAMINE AND SRS-A FROM SENSITIZED GUINEA-PIG LUNG BY (2-ISOPROPYL-3-INDOLYL)-3 PYRIDYL KETONE (L8027) AND INDOMETHACIN

MARGARET HITCHCOCK

John B. Pierce Foundation Laboratory, Yale University School of Medicine, 290 Congress Avenue, New Haven, Connecticut 06519, U.S.A.

1 (2-Isopropyl-3-indolyl)-3 pyridyl ketone (L8027) and indomethacin reduced basal tension and enhanced the antigen- and histamine-induced contractions of tracheal spirals obtained from actively sensitized guinea-pigs. The stimulating effect of L8027 required the presence of the drug, while that of indomethacin persisted after its removal from the organ bath.

2 L8027 and indomethacin stimulated the immunological release of histamine and slow reacting substance of anaphylaxis (SRS-A) and inhibited the *de novo* synthesis and release of malondialdehyde from actively sensitized guinea-pig lung fragments.

3 L8027 was 2,800 times more potent than indomethacin in both *in vitro* models of anaphylaxis.

4 A selective antagonist of SRS-A (FPL 55712) inhibited contractions produced by antigen, but had no effect on contractions produced by histamine.

5 Prostaglandins E and $F_{2\alpha}$ were continuously released into the organ bath fluid by the resting trachea. Contractions induced by antigen or histamine increased the rate of prostaglandin efflux.

6 L8027 had no effect on the efflux of prostaglandins E and $F_{2\alpha}$ at rest and during contraction. Indomethacin inhibited prostaglandin efflux at rest and during contraction while present in the organ bath. Prostaglandin efflux was restored to 80% of control after removal of indomethacin.

7 The results suggest that prostaglandins E and $F_{2\alpha}$ have no role in the stimulation by L8027 and indomethacin of the contractile responses of guinea-pig trachea. The possible mechanism for the effects of these drugs is discussed.

Introduction

Drugs which modulate the synthesis and metabolism of endogenous prostaglandins also modulate the responses of a number of isolated lung tissues. Thus indomethacin enhances the response of guinea-pig airway smooth muscle to exogenous contractile stimuli such as histamine, acetylcholine and 5-hydroxytryptamine (Grodzinska, Panczenko & Gryglewski, 1975; Orehek, Douglas & Bouhuys, 1975). Similar concentrations of indomethacin inhibited the response of isolated sensitized human bronchus to antigen, but had no effect on the response to methacholine (Dunlop & Smith, 1975).

Prostaglandin E_2 (PGE_2) and $PGF_{2\alpha}$ (Piper & Vane, 1971) and thromboxanes (Dawson, Boot, Cockerill, Mallen & Osborne, 1976) are produced when isolated sensitized lungs of the guinea-pig are challenged with antigen. In a similar *in vitro* preparation, inhibitors of cyclo-oxygenase stimulate the antigen-induced release of histamine and slow reacting substance of anaphylaxis (SRS-A) (Engineer, Niederhauser, Piper & Sirois, 1978; Hitchcock, 1978a) while inhibition of overall arachidonic acid metabolism selectively inhibits the release of SRS-A (Hitchcock, 1978a).

In the present study, the effects of indomethacin, an inhibitor of cyclo-oxygenase, and of L8027, an inhibitor of thromboxane synthetase (Gryglewski, Zmuda, Korbut, Krecioch & Bieron, 1977) have been investigated in two *in vitro* models of anaphylaxis: the antigen-induced contraction of airway smooth muscle (trachea) and the release of histamine and SRS-A from chopped lung fragments. Some of the results were presented at the Seventh International Congress of Pharmacology (Hitchcock, 1978b).

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Methods

Paired tracheal spiral strips

Male albino guinea-pigs (Hartley strain, 250 g) were actively sensitized with a single injection (i.p.) of 10 mg egg albumin in 1 ml 0.9% w/v NaCl solution. Twenty-eight days later the trachea and lungs were removed and placed in Tyrode solution, pH 7.4, the composition of which was as follows (mM): NaCl 136.7, KCl 2.7, $MgCl_2$ 0.49, $NaHCO_3$ 11.9, $CaCl_2$ 1.8, NaH_2PO_4 0.36 and glucose 5.8. The trachea was spirally cut (Constantine, 1965) and separated into two halves of equal length. Each half spiral was suspended in a separate organ bath (capacity 10 ml) containing Tyrode solution at 37°C, gassed with 5% CO_2 in 95% O_2. An initial tension of 6 g was applied and the spiral was permitted to equilibrate for 90 min. At the end of the equilibration period the resting tension was between 3.5 and 4.5 g. Under these conditions responses to constrictor agents are reproducible and maximal (Stephens, 1970). Isometric contractions were recorded as changes in tension on a Heathkit recorder with a Harvard force displacement transducer (Model 373). Throughout the experiment, contractions produced by identical concentrations of either histamine or antigen (egg albumin) were recorded from each tracheal preparation simultaneously. The volume of agonist or drug added to the organ bath never exceeded 100 µl. In all experiments, a dose-response curve to histamine (bath concentration 0.11 to 5.55 µg base/ml) was obtained for each of the paired tracheal spirals. When the response had reached a plateau, the preparation was washed several times with Tyrode solution and then allowed to return passively to its resting tension. One of the spirals was then incubated with either indomethacin, L8027 or FPL 55712 (concentration and incubation time given in the text) and the response of both spirals to antigen or histamine recorded. Responses to antigen are expressed as a percentage of the maximum response to histamine determined before treatment with drug.

Determination of prostaglandins

The concentrations of PGE_2 and $PGF_{2\alpha}$ released by the trachea were determined directly by radioimmunoassay in duplicate samples of organ bath fluid. At 50% binding, the anti-$PGF_{2\alpha}$ serum cross-reacted as follows: PGE_1 and PGE_2, 0.02%; PGA_1 and PGA_2, less than 0.01%; 13,14 dihydro-15 keto-prostaglandin $F_{2\alpha}$, 0.33%; and $PGF_{1\alpha}$, 10%. Assay of the PGE content was performed by measuring the amount of PGB produced by alkaline treatment (Levine, Gutierrez-Cernosak & Van Vunakis, 1971). The antibody used did not distinguish between PGB_1 and PGB_2, therefore the data for PGE_2 content are expressed in terms of total PGE. At 50% binding, the anti PGB serum cross-reacted as follows: PGA_1 and PGA_2, 1%; PGE_1, 0.2%; PGE_2, 0.1%. Samples of the organ bath fluid were obtained during the resting state, 3 min after peak contraction had been reached, and after washing when the preparation had passively relaxed to its resting tension.

Determination of mediator release from chopped lung

The lung tissue was prepared, incubated, and mediator release determined by published techniques (Hitchcock, 1978a). Briefly, weighed aliquots (100 mg) of chopped lung fragments were incubated in Tyrode solution at 37°C in a final volume of 5 ml. Following a 10 min equilibration period, the sensitized lung was challenged with egg albumin (20 µg/ml final concentration). After 15 min, the incubates were filtered and the amount of histamine released determined in the filtrates spectrofluorimetrically (Shore, Burkhalter & Cohn, 1959). The data are expressed in terms of the percentage of the total histamine released per 100 mg chopped lung in 15 min. In some experiments, malondialdehyde concentration was determined in 2 ml of filtrate (Flower, Cheung & Cushman, 1973). The data are expressed as femtomoles (fmol) malondialdehyde equivalents formed per 100 mg chopped lung. SRS-A in the filtrate was determined by bioassay on the guinea-pig ileum in the presence of atropine sulphate (3.4 µg/ml), pyrilamine maleate (0.34 µg/ml) and methysergide bimaleate (0.1 µg/ml). FPL 55712 (0.1 µg/ml) was used to block the contractions attributed to SRS-A. The contractions were compared with a laboratory standard of crude SRS-A and are expressed in arbitrary units. The standard was obtained by incubating sensitized chopped lung (5 g) with egg albumin (5 mg) in a final volume of 25 ml at 37°C for 30 min. The incubate was filtered and aliquots of the filtrate were frozen at −70°C. One unit of SRS-A was contained in 50 µl of filtrate. The same standard was used in all of the experiments to be described.

Statistics

Student's paired *t* test was used to determine statistical significance of differences. *P* values of less than 0.05 were considered statistically significant.

Drugs

The following chemicals and drugs were used: histamine dihydrochloride (Sigma Chemical Co., St. Louis, MO, U.S.A.) and egg albumin 5 times crystallized (Nutritional Biochemicals, Cleveland, OH, U.S.A.). Indomethacin (Merck, Sharpe and Dohme, West Point, PA), (2-isopropyl-3-indolyl)-3 pyridyl

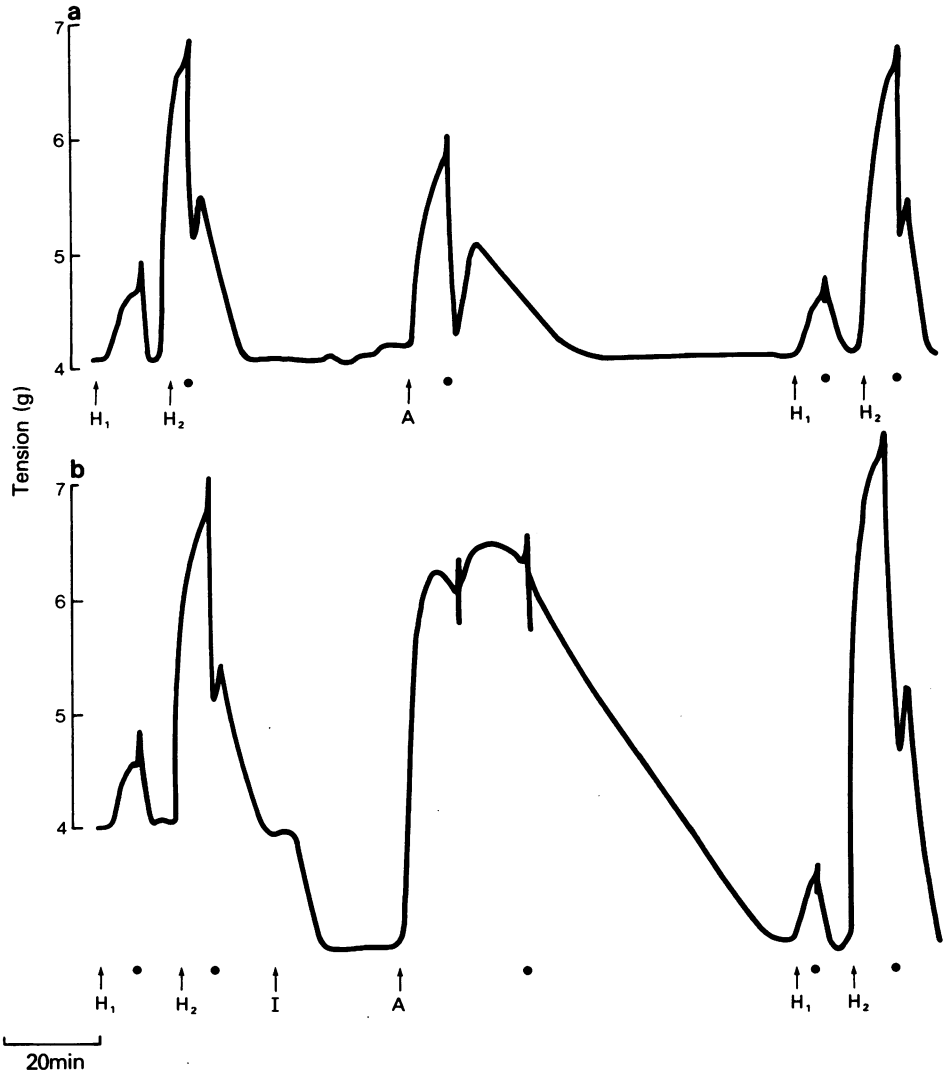

Figure 1 Enhancement by indomethacin of the guinea-pig tracheal response to antigen and histamine. Contractions of spirally cut halves of trachea to histamine (H_1 = 0.11, H_2 = 5.55 μg histamine base per ml bath) and antigen (A = 5 ng per ml bath). (a) In the absence of indomethacin; (b) response of the paired half of the trachea in the presence of indomethacin (I = 5 μg per ml bath). Black dots indicate points at which preparation was washed.

ketone (L8027, Labaz, Brussels) and sodium 7-[3-(4-acetyl-3-hydroxy-2-propyl phenoxy)-2-hydroxy propoxy]-4 oxo-8-propyl-4H-1-benzopyran-2-carboxylate (FPL 55712, Fisons, Ltd., Loughborough, Leics.) were kindly supplied by the manufacturers.

Stock solutions of all chemicals were freshly prepared each day. Indomethacin (20 mg) and L8027 (5 mg) were dissolved in ethanol (1 ml) and diluted to the required concentration with Tyrode solution. FPL 55712 was prepared in distilled water (1 mg/ml) and diluted with Tyrode solution. All other chemicals and drugs were dissolved in Tyrode solution.

Results

Experiments with paired tracheal spirals

Comparison of the dose-response curves to histamine, the ED_{50} values obtained from them and the maxi-

mum tension developed per wet wt. of tissue showed that there was no significant difference in response between the paired tracheal spirals ($P > 0.05$ for 38 pairs; mean $ED_{50} = 5.66 \pm 0.43$ μM, $n = 78$; maximum tension to histamine = 3.535 ± 0.211 g/100 mg wet wt. tissue, $n = 76$). Treatment of the paired tracheal spirals with antigen in the absence of drugs resulted in contractions that were identical in terms of percentage of the maximal response to histamine and which were concentration-dependent within the range tested (0.5 to 10 ng/ml bath concentration). In some experiments, contractions to a single concentration of antigen were recorded, while in others cumulative dose-response curves to antigen were obtained. Responses to antigen were delayed and prolonged (up to 90 min) in spite of repeated washing of the tissue. Contractions started after an average delay of 1.47 min and attained a peak in an average of 7.47 min (data from 19 pairs). Treatment of one of the paired spirals with indomethacin or L8027 for 20 min resulted in a concentration-dependent relaxation of basal tone. Indomethacin, 0.5 and 5.0 μg/ml produced relaxation of 0.32 ± 0.13 and 0.72 ± 0.25 g respectively; L8027, 0.13, 1.3 and 13.0 ng/ml produced relaxation of 0, 0.25 ± 0.08 and 0.37 ± 0.06g respectively. These drugs also ehanced the size and duration of the response of the tracheal spiral to antigen (Table 1, Figure 1). This enhancement was observed not only at the reduced basal tension, but also when the basal tension was mechanically restored to its original level. The time of onset and the time to peak contraction were not affected. The effect of indomethacin was dose-related in that the stimulation induced with 5 μg/ml drug was greater than that produced by 0.5 μg/ml at antigen concentrations of 0.5 and 5 ng/ml (Table 1). Furthermore, following treatment with 5 μg/ml indomethacin, the contraction induced by 0.5 ng/ml antigen was greater than that elicited by 5 ng/ml antigen in the absence of drug (Table 1). L8027 at a concentration of 0.13 ng/ml had no effect on the antigen-induced contraction. However, this drug at a concentration of 1.3 ng/ml (5 nM) and indomethacin at 5 μg/ml (14 μM) produced similar degrees of enhancement of the response to 5 ng/ml antigen. Thus, under these conditions L8027 was approximately 2800 times more potent than indomethacin.

Concentrations of indomethacin and L8027 that reduced the basal tone also enhanced the response of the tracheal spiral to high concentrations of histamine but not to low concentrations (Figure 2). Concentrations of L8027 below 0.26 ng/ml had no effect on the response to histamine. Enhancement of the contraction to antigen or histamine by indomethacin was irreversible in that it was not eliminated by repeated washing with Tyrode solution. In contrast, the enhancement of the responses to antigen or histamine by L8027 required the presence of the drug.

To study the effects of FPL 55712, one half of the tracheal spiral was incubated with FPL 55712 (0.1 μg/ml bath concentration) for 10 min. Contractions to histamine or antigen were then recorded from both spirals (Figures 3 and 4). FPL 55712 inhibited the antigen-induced contraction at all antigen concentrations tested (0.5 to 5 ng/ml) but had no effect on histamine-induced contractions. Similar results were obtained in indomethacin-treated tracheal spirals (Figure 4). The percentage inhibition of the antigen-induced contractions in both normal and indomethacin-treated tracheae were 70 and 60%, respectively and appeared to be the same at all antigen concentrations examined.

Table 1 Effects of indomethacin and L8027 on the contraction of guinea-pig tracheae by different concentrations of antigen

	Antigen response (% maximum response to histamine) *Antigen concentration* (ng/ml)		
Drug concentration	0.5	1.0	5.0
Control	18.7 ± 15		41.7 ± 17
Indomethacin 0.5 μg/ml	25.0 ± 9	—	69.7 ± 10
Control	13.5 ± 5		52.3 ± 25
Indomethacin 5.0 μg/ml	72.2 ± 25*	—	110.4 ± 42*
Control	14.2 ± 5	22.6 ± 11	49.0 ± 9
L8027 0.13 ng/ml	12.6 ± 6	32.7 ± 9	56.7 ± 15
Control	19.4 ± 8	46.9 ± 14	51.9 ± 4
L8027 1.3 ng/ml	30.7 ± 13	71.2 ± 12**	103.8 ± 10**
Control			46.2 ± 12
L8027 13 ng/ml	—	—	101.7 ± 25*

Figures are means ± s.e. ($n = 4$–7); * $P < 0.05$; ** $P < 0.005$.

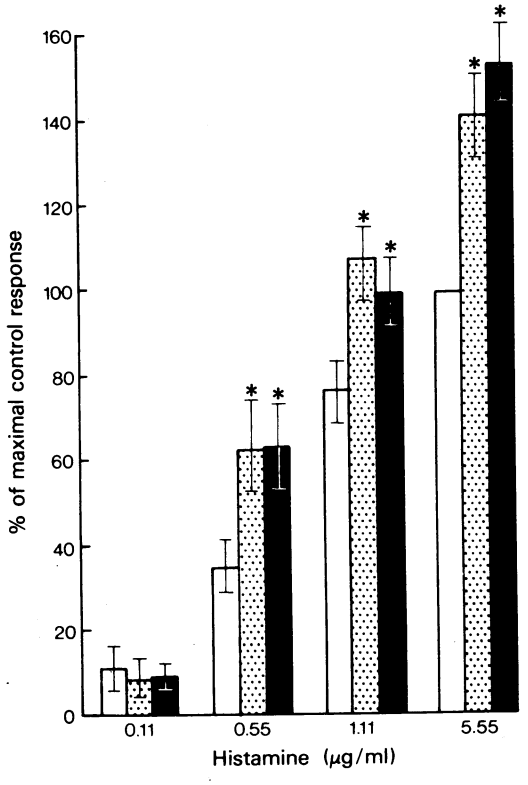

Figure 2 Effect of L8027 and indomethacin on the response of actively sensitized guinea-pig trachea to histamine. Data are presented in terms of % maximal response to histamine in the absence of drug. Open columns: Response to histamine in the absence of drug; stippled columns: response in the presence of L8027 (1.3 ng/ml); solid columns: response in the presence of indomethacin (5 μg/ml). Vertical bars are s.e. mean ($n = 8$). *Significantly different from control ($P < 0.05$).

Release of prostaglandins by guinea-pig trachea

PGE and $PGF_{2\alpha}$ were continuously released into the bath fluid while the tracheal spirals were at resting tension. Under these conditions the efflux of PGE was 0.344 ± 0.052 and of $PGF_{2\alpha}$ was 0.334 ± 0.067 ng $min^{-1} g^{-1}$ wet wt. ($n = 8$). When the spirals were maximally contracted with histamine (5.55 μg base/ml) the efflux of PGE was 1.481 ± 0.260 and of $PGF_{2\alpha}$ was 0.924 ± 0.196 ng $min^{-1} g^{-1}$ wet wt. ($n = 8$). Assay of the prostaglandins at the point of peak contraction, 3 min after peak contraction, and during the relaxation phase following washing of the tissue demonstrated that the prostaglandins were not released during development of the contraction but during the period immediately following the attainment of peak contraction. When the preparations were contracted with antigen (5 ng/ml) giving responses which averaged 40% of the response to histamine, the efflux of PGE was 0.783 ± 0.171 and of $PGF_{2\alpha}$ was 0.600 ± 0.168 ng $min^{-1} g^{-1}$ wet wt. ($n = 8$). Thus the ratios of $PGE/PGF_{2\alpha}$ in the resting, histamine-contracted and antigen-contracted tracheae were 1.03, 1.60 and 1.31, respectively. In the presence of indomethacin (5 μg/ml), resting effluxes were reduced to below detectable levels (PGE, < 0.08; $PGF_{2\alpha}$, < 0.06 ng $min^{-1} g^{-1}$ wet wt.).

The presence of indomethacin also reduced to 20% of control values the prostaglandin efflux resulting from contraction to either histamine or antigen. Removal of the indomethacin followed by washing of the tissue resulted in restoration of the prostaglandin efflux to 80% of control values at rest and after contraction to histamine or antigen. Contractions to histamine and antigen continued to be enhanced despite the removal of indomethacin and restoration of prostaglandin efflux. In contrast to the effect of indomethacin, incubation of the tracheal spirals with L8027 (13 ng/ml) had no effect on the efflux of PGE and $PGF_{2\alpha}$ at resting tension or after contraction with histamine or antigen.

Experiments with chopped lung fragments

Indomethacin increases the immunological release of histamine and SRS-A from actively sensitized guinea-pig lung (Engineer *et al.*, 1978; Hitchcock, 1978a). Since L8027 and indomethacin had similar effects on the guinea-pig trachea, it was of interest to determine whether L8027 also stimulated mediator release from chopped lung fragments. Concentrations of L8027 (2.6 to 13 ng/ml) and indomethacin (0.1 to 5 μg/ml) did not release histamine in the absence of antigen, but enhanced antigen-induced histamine release in a concentration-dependent manner. These concentrations of both drugs also stimulated the release of SRS-A. Figure 5 shows the stimulating effect of the optimum concentration of L8027 (1.3 ng/ml) and indomethacin (5 μg/ml). Also shown are the effects of L8027 and indomethacin on the *de novo* synthesis of malondialdehyde-like material in control and antigen-treated chopped lung. Malondialdehyde is a metabolite of prostaglandin endoperoxide and its formation is used as an indicator of cyclo-oxygenase activity (Flower *et al.*, 1973; Hamberg, Svensson & Samuelsson, 1974). Indomethacin (5 μg/ml) and L8027 (1.3 ng/ml) inhibited the *de novo* formation of malondialdehyde by 99% in both control and antigen-treated chopped lung (Figure 5).

Discussion

The enhancement by indomethacin of the responses to antigen of sensitized tracheae is the second *in vitro*

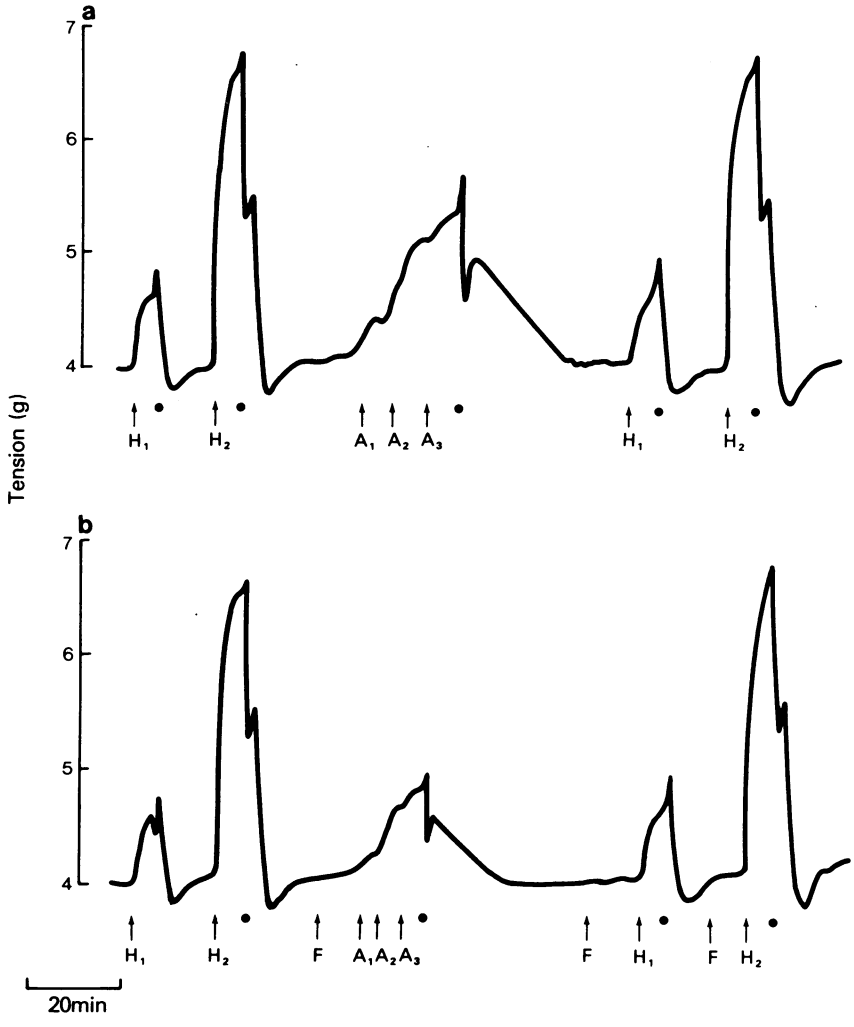

Figure 3 Inhibition by FPL 55712 of the response of the guinea-pig trachea to antigen. Contractions of spirally cut halves of trachea to histamine (H_1 = 0.11, H_2 = 5.55 μg histamine base per ml bath) and antigen (A_1 = 0.5, A_2 = 1.0, A_3 = 5.0 ng per ml cumulative concentration in bath). (a) In the absence of FPL 55712; (b) response of the paired half in the presence of FPL 55712 (F = 0.1 μg per ml bath). Black dots indicate points at which the preparation was washed.

model of anaphylaxis in which this drug has a stimulatory effect. All the effects of indomethacin on the sensitized guinea-pig trachea could be produced by L8027. L8027 and indomethacin also had similar effects on the immunological release of histamine, SRS-A and malondialdehyde from actively sensitized guinea-pig lung fragments. In this study, L8027 was about three thousand times more potent than indomethacin in both *in vitro* models of anaphylaxis.

Although indomethacin and L8027 have similar effects, there is no conclusive evidence that these drugs have the same mechanism of action in isolated sensitized lung tissues. L8027 is a specific inhibitor of thromboxane synthetase although the data have been obtained primarily with human platelets (Gryglewski *et al.*, 1977). The present evidence that it may act on thromboxane synthetase is indirect: (1) prostaglandin efflux from guinea pig trachea was unaffected; (2) the *de novo* formation of malondialdehyde from guinea-pig chopped lung was inhibited. Indomethacin acts on cyclo-oxygenase and hence should inhibit the production of prostaglandin endoperoxide and its metab-

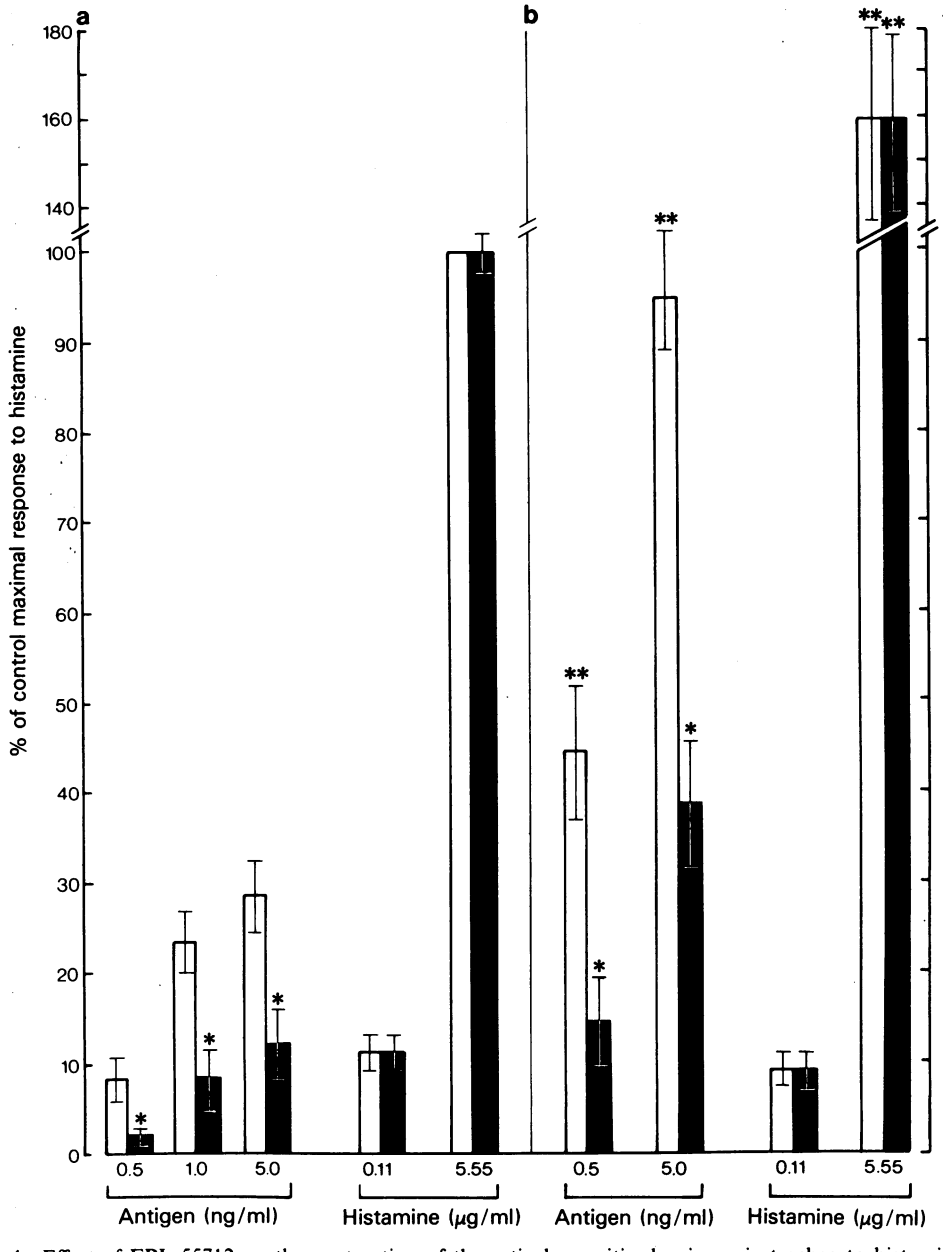

Figure 4 Effect of FPL 55712 on the contraction of the actively sensitized guinea-pig trachea to histamine and antigen in the presence and absence of indomethacin. Open columns: response of one half of the trachea in the absence of FPL 55712; solid columns: response of the paired half in the presence of FPL 55712 (0.1 μg per ml bath): (a) in the absence of indomethacin; (b) in the presence of indomethacin (5 μg per ml bath). $n = 5$ in (a) and (b). Vertical lines represent s.e. mean. *Significantly different from paired control; **significantly different from response obtained in the absence of indomethacin.

olites. The reduction in PGE and $PGF_{2\alpha}$ effluxes while indomethacin was present was evidence that cyclo-oxygenase was inhibited. The effects of indomethacin on resting tension and contractile responses persisted after the drug was removed from the organ bath and the prostaglandin efflux restored. Thus indo-

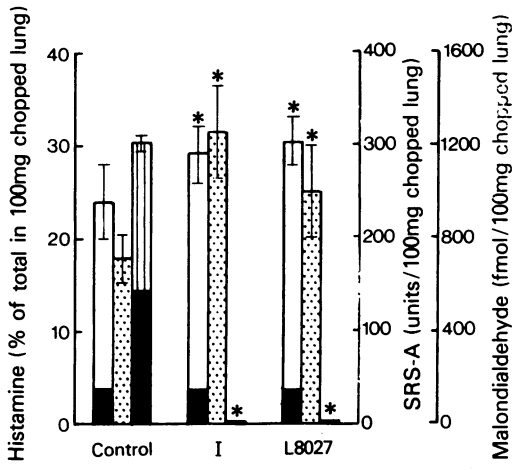

Figure 5 Effect of indomethacin and L8027 on the release of histamine (open bars), SRS-A (stippled bars) and malondialdehyde (striped bars) from actively sensitized chopped guineapig lung. Solid shading of the histogram represents release of mediators in the absence of antigen. Indomethacin (I, 5 μg/ml) and L8027 (L, 1.3 ng/ml) did not affect the release of histamine in the absence of antigen, enhanced antigen-induced release of histamine and SRS-A, and inhibited spontaneous release of malondialdehyde. Data are the mean of 5 experiments each performed on a separate lung. Vertical lines represent s.e. mean. *Significantly different from paired control.

methacin not only inhibited cyclo-oxygenase, but also acted in one or more of the following ways: (1) redirection of the metabolism of arachidonic acid in favour of the lipoxygenase pathways; (2) a persistent inhibition of an enzyme intervening in prostaglandin synthesis later than does cyclo-oxygenase; (3) modification of the production of other biologically active substances; (4) production of a metabolite of lipoxygenase which sensitizes the membrane of smooth muscle cells to the action of other mediators; (5) a direct effect on airway smooth muscle, independent of an effect on the metabolism of arachidonic acid.

Indomethacin and L8027 increase the antigen-induced synthesis of SRS-A in actively sensitized guinea-pig lung fragments. A SRS, derived from mouse mastocytoma cells has been identified as a metabolite of the 5-lipoxygenase pathway of arachidonic acid (Murphy, Hammarstrom & Samuelsson, 1979). This SRS and SRS-A have some common biological characteristics. Indomethacin and L8027 may inhibit cyclo-oxygenase and/or other prostaglandin synthesis enzymes resulting in an increase in the proportion of arachidonic acid metabolized by lipoxygenase to SRS-A. An increased intramural synthesis of SRS or SRS-A in airway smooth muscle treated with indomethacin or L8027 may be the mechanism of the enhanced contractile response. However, no SRS-A release was detected (determined by bioassay on guinea-pig ileum) either during or following an antigen-induced contraction (unpublished observations).

The selective SRS-A antagonist, FPL-55712, inhibited the contractile response to antigen at a concentration known to antagonize the contraction of guinea-pig ileum to SRS-A, but to have no effect on the response to other contractile agents (Augustein, Farmer, Lee, Sheard & Tattersall, 1973). Adams & Lichtenstein (1979) reported that FPL 55712 inhibited the response of sensitized guinea-pig tracheal rings to antigen without affecting histamine release by this tissue. A direct effect of FPL 55712 on airway smooth muscle is unlikely since this drug had no effect on the response to histamine. FPL 55712 may exert its effect by: (1) inhibition of the synthesis and/or effect of SRS-A; (2) inhibition of the immunological production of mediators other than SRS-A and histamine; (3) inhibition of the link between the antigen-antibody reaction and contraction.

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