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Differential effect of cyclo-oxygenase inhibition on antigen- and ionophore-induced release of slow reacting substance from fragmented guinea-pig lung

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1 The nonsteroidal anti-inflammatory drugs (NSAID) indomethacin and mefenamic acid, at concentrations ranging from 3 μM to 18 μM, enhanced antigen-induced slow reacting substance of anaphylaxis (SRS-A) release from sensitized fragmented guinea-pig lung. In contrast, these agents had no effect on SRS-A release from nonsensitized guinea-pig lung induced by several concentrations of the calcium ionophore, A23187.

2 Neither increasing preincubation time with the NSAID nor the use of sensitized tissue resulted in an enhancement of A23187-induced SRS-A release by indomethacin.

3 NSAID did not alter histamine release by either stimulus.

4 These results suggest that antigen and A23187 induce SRS-A release from different sources or by different mechanisms in guinea-pig lung.

Introduction

The non-steroidal anti-inflammatory drug (NSAID), indomethacin has been shown to enhance antigen-induced release of slow reacting substance of anaphylaxis (SRS-A) from a variety of tissues (Walker, 1973; Bach *et al.*, 1977; Hitchcock, 1978; Krell & Chakrin, 1978; Engineer *et al.*, 1978; Laekeman & Herman, 1979). One postulate regarding the mechanism underlying this effect is that cycloxygenase products exert feedback inhibition of SRS-A production and that indomethacin inhances release by preventing synthesis of these inhibitors (Walker, 1973; Hitchcock, 1978; Engineer *et al.*, 1978).

More recently, SRS-A has been shown to consist of peptidoleukotrienes, primarily leukotriene C_4 (LTC_4) and LTD_4, products of the lipoxygenase pathway of arachidonic acid (AA) metabolism (Watanabe-Kohno & Parker, 1980; Piper & Samhoun, 1981; Samuelsson, 1982). Based on these recent developments it has been suggested that NSAID enhance SRS-A release by diverting available AA substrate into the lipoxygenase pathway (Burka & Paterson, 1980; Spannhake *et al.*, 1981).

This paper describes qualitatively different effects of NSAID on antigen- and ionophore-induced (A23187) SRS-A release from guinea-pig lung.

Methods

Male guinea pigs (Hartley) weighing 300–400 g were used. For experiments with antigen, animals were sensitized 21 to 28 days beforehand by intraperitoneal injection of 5 mg ovalbumin (OA) on day 1 and 10 mg OA on day 3 (Forsberg & Sorenby, 1981).

Preparation and challenge of fragmented lung

Animals were killed by decapitation and the lungs removed. The large airways and blood vessels were dissected away and the lung parenchyma cut with a McIlwain tissue chopper into segments of approximately 1 × 1 × 2 mm. The tissue was washed five times with Tyrode solution and divided into aliquots weighing approximately 600 mg. Each sample of lung tissue was resuspended in 3.6 ml Tyrode solution containing cysteine (10^{-2} M) with or without experimental compound. Compounds were dissolved in dimethylsulphoxide (DMSO) or polyethylene glycol 400 and diluted to final concentration with Tyrode solution. Solvents did not exceed 0.2% v/v and had no effect on mediator release or assay procedures. Samples were preincubated with compounds at 37°C for 10 min. Tyrode solution (0.4 ml) or Tyrode solution with OA or A23187, as appropriate, was added

to respective samples and incubation was continued for 45 min after which the extracellular fluid was decanted through cheesecloth. Triplicate determinations were undertaken for each sample.

The extracellular fluid was divided and prepared for assay as follows: (a) 0.9 ml were added to 0.1 ml 4N $HClO_4$ and stored at −5°C for histamine assay; (b) in some experiments 0.2 ml were stored at −70°C for prostacyclin or thromboxane B_2 assay; (c) the remainder of each sample was adjusted to pH 8 with 0.1 N NaOH, bubbled with argon gas and stored at −70°C for SRS-A bioassay. Tyrode (4 ml) was added to the remaining lung tissue in each tube. These samples were placed in a boiling water bath for 10 min to extract the residual histamine.

Assay procedures

SRS-A was quantitated by bioassay on guinea-pig isolated ileum in a manner similar to that previously described (Krell & Chakrin, 1976). Contractions were monitored by means of a Grass (FT.03C) force displacement transducer and recorded on a Beckman R-511A recorder. Synthetic LTD_4 was used as a standard (Tsai *et al.*, 1982). SRS-A is expressed as nanogram equivalents of LTD_4 released per gram wet weight lung. Piper & Samhoun (1981) have found that LTD_4 is the predominant leukotriene released by guinea-pig lung.

Histamine was assayed by an automated fluorometric procedure using a Technicon AA II as in the method described by Siraganian (1975).

Thromboxane B_2 and 6-keto-$PGF_{1\alpha}$: these metabolites (of thromboxane A_2 and prostacyclin, respectively) were determined by radioimmunoassay (New England Nuclear). Radioactivity was measured using a Packard Tri-Carb scintillation counter.

Statistics

Statistical comparisons were made using Student's *t* test or, where appropriate, Student's *t* test for paired observations.

Materials

The following drugs were used: ovalbumin, indomethacin and histamine dihydrochloride: (Sigma Chemical Co., St. Louis, MO); A23187 (Calbiochem-Behring Corp., LaJolla, CA); orthophthaldialdehyde, cysteine, dimethylsulphoxide (Gold Label) (Aldrich Chemical Co., Milwaukee, WI); polyethylene glycol 400 (Fisher Scientific, King of Prussia, PA); mefenamic acid (Parke-Davis, Detroit, MI) and ibuprofen (Upjohn, Kalamazoo, MI).

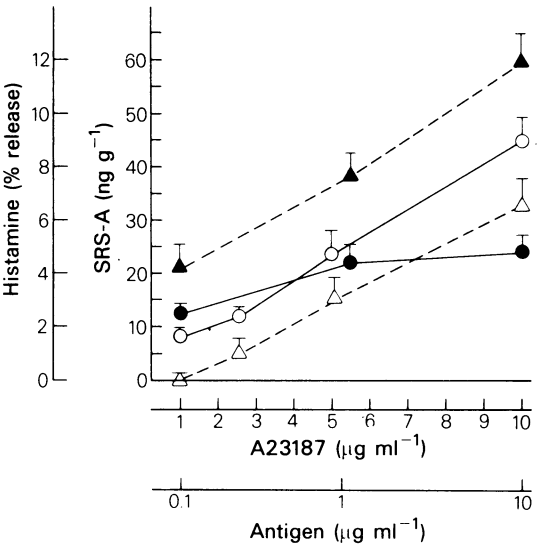

Figure 1 Mediator release from guinea-pig lung *in vitro*. Values for release of SRS-A (●) and histamine (▲) by antigen and SRS-A (○) and histamine (△) by A23187 are shown. Each point represents the mean ($n = 3$ to 5); s.e.mean shown by vertical lines.

Results

SRS-A release by antigen and by A23187

The calcium ionophore, A23187, induced a concentration-related release of both histamine and

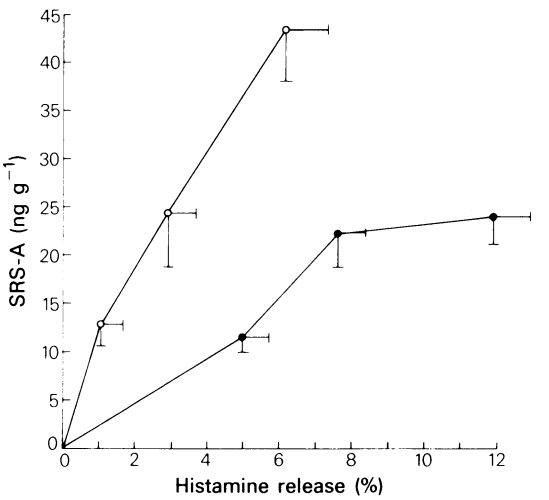

Figure 2 SRS-A release per unit of histamine release for several concentrations of A23187 (○) and antigen (●). Each point represents the mean ($n = 3$–5, determined in triplicate); s.e.mean shown by vertical and horizontal lines.

SRS-A from chopped guinea-pig lung parenchyma (Figure 1). Likewise, OA antigen produced a concentration-dependent release of histamine, while SRS-A release appeared only modestly dependent upon antigen concentration (Fig 1). Moreover, the relative amounts of histamine and SRS-A released by OA and A23187 differed markedly. A23187 caused considerably greater release of SRS-A per μg of histamine than did antigen at all concentrations (Figure 2).

The effect of indomethacin on SRS-A release by antigen and by A23187

Preincubation of lung fragments with indomethacin (3 μM) caused a significant ($P<0.05$) enhancement of SRS-A release by antigen (Figure 3a). The enhancement was apparent at all three concentrations of antigen evaluated. Increasing the concentration of indomethacin from 3 to 6 μM produced no further enhancement of SRS-A release (Figure 3a).

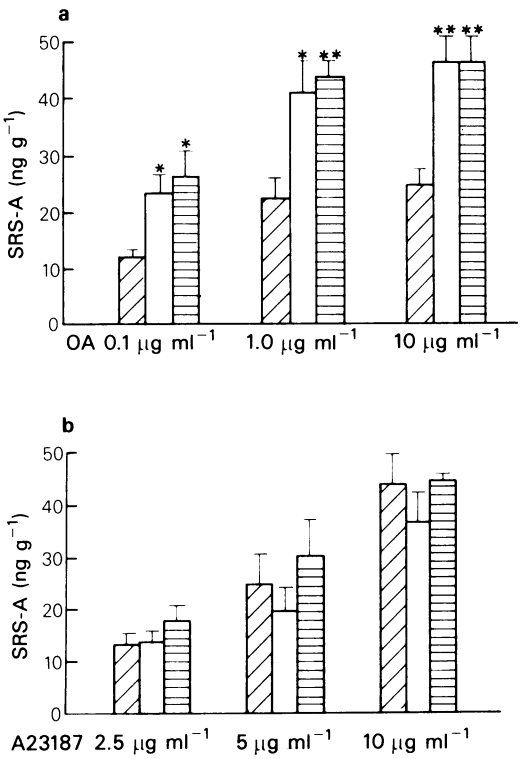

Figure 3 Effect of indomethacin on SRS-A release by antigen (a) and by A23187 (b) Diagonal shading: control release; open bars: 3 μM indomethacin; horizontal shading: 6 μM indomethacin. *$P<0.05$; **$P<0.01$ cf. 0 μM indomethacin.

Table 1 Alteration of indomethacin preincubation time and concentration on A23187-induced SRS-A release from fragmented guinea-pig lung[a]

Indomethacin (μM)	*Preincubation time*	*SRS-A* (ng g^{-1})	P[c]
0	10	34.2 ± 9.8[b]	-
6	10	33.4 ± 11.8	> 0.05
12	10	37.0 ± 11.4	> 0.05
18	10	33.2 ± 13.5	> 0.05
0	60	34.8 ± 9.9	-
6	60	34.0 ± 5.3	> 0.05

[a]The releasing agent was A23187, 5 μg ml^{-1}, in all experiments.
[b]Value for SRS-A is the mean ± s.e.mean ($n=3$).
[c]Level of significance.

In contrast, the SRS-A release evoked by various concentrations of A23187 was not enhanced by indomethacin (Figure 3b). Increasing the concentration of indomethacin from 3 μM to 6 μM (Figure 3b) or 18 μM (Table 1) did not result in enhancement of A23187-induced release. Moreover, increasing the indomethacin preincubation period from 10 to 60 min was also without effect (Table 1).

Indomethacin had no significant ($P>0.05$) effect on histamine release from lung tissue by either antigen or A23187 (Table 2).

The effect of indomethacin on thrombaxane A_2 and prostacyclin synthesis by lung tissue

To confirm that the concentration of indomethacin employed was sufficient to inhibit cyclo-oxygenase, its effect on thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) release by lung was monitored. As illustrated in Table 3, substantial spontaneous release of both cyclo-oxygenase products was observed. Challenge with antigen caused a further increase ($P<0.05$) above basal levels for both products. Pretreatment with 3 μM indomethacin reduced OA-induced increases in thromboxane B_2 and 6-keto-$PGF_{1\alpha}$ levels to below basal, indicating that cyclo-oxygenase was inhibited.

The effect of indomethacin on A23187-induced release of SRS-A from sensitized lung tissue

As illustrated in Table 4, A23187 caused concentration-dependent SRS-A release from sensitized lung tissue similar to that observed in normal lung tissue (Figure 1). Once again, indomethacin did not enhance A23187-induced SRS-A release.

Table 2 Effect of indomethacin on histamine release by antigen and A23187[a]

Stimulus	($\mu g\,ml^{-1}$)	*Histamine release (% of total tissue content)* *Control*	*Indomethacin,* (3 μM)	P[c]	*Indomethacin,* (6 μM)	P
Antigen	0.1	5.0±0.67[b]	6.0±0.91	>.05	5.9±0.80	>.05
Antigen	1.0	7.6±0.75	9.1±1.10	>.05	9.5±0.96	>.05
Antigen	10.0	11.9±1.04	11.9±1.00	>.05	12.9±1.35	>.05
A23187	2.5	1.05±0.64	0.8±0.40	>.05	1.4±0.51	>.05
A23187	5.0	2.9±0.79	2.7±0.92	>.05	4.7±0.47	>.05
A23187	10.0	6.2±1.13	5.8±0.74	>.05	8.4±0.15	>.05

[a]Lung fragments were preincubated for 10 min with various concentrations of indomethacin and then challenged with A23187 or antigen for 45 min.
[b]Each value is the mean ± s.e.mean for 3 to 5 experiments.
[c]Level of significance.

Table 3 Effect of indomethacin on spontaneous and antigen-induced release of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) from sensitized lung tissue[a]

Treatment	*Thromboxane* B_2 ($ng\,g^{-1}$)	p[c]	*6-keto-*$PGF_{1\alpha}$ ($ng\,g^{-1}$)	P
Tyrode 'spontaneous'	434.6±51.2[b]	-	70.4±21.6	-
Indomethacin (3 μM)	132.4±27.4	<0.01	29.4±14.9	<0.05
OA (10 $\mu g\,ml^{-1}$)	836.6±74.6	-	156.9±43.7	-
Indomethacin, (3 μM) and OA (10 $\mu g\,ml^{-1}$)	147.0±34.3	<0.01	64.2±21.2	<0.05

[a]Tissues were preincubated with or without indomethacin for 10 min and then challenged with antigen for 45 min.
[b]Values are the mean ± s.e.mean (n =4 to 5 experiments)
[c]Level of significance.

The effect of mefenamic acid on SRS-A release induced by antigen and by ionophore

To determine whether the lack of effect of indomethacin on A23187-induced SRS-A release was unique to this agent, the effect of another NSAID was evaluated. Mefenamic acid enhanced SRS-A release induced by antigen but had no effect on SRS-A release induced by A23187 (Table 5).

Table 4 Effect of indomethacin on A23187-induced SRS-A release from sensitized lung tissue[a]

A23187, ($\mu g\,ml^{-1}$)	*SRS-A release* ($ng\,g^{-1}$) *Control*	*Indomethacin,* (3 μM)	P[c]
2.5	10.4±0.68[b]	10.4±0.70	>0.05
5.0	17.4±0.27	19.1±1.17	>0.05
10.0	42.9±2.19	49.2±5.97	>0.05

[a]Tissues were exposed to indomethacin for 10 min and then challenged with various concentrations of A23187.
[b]Values are the mean ± s.e.mean for three experiments.
[c]Level of significance.

Discussion

Since antigen stimulation of sensitized guinea-pig lung resulted in substantial histamine release (indicating mast cell activation) it is probable that much of the SRS-A release by antigen comes from mast cells. Indeed, MacGlashen *et al.*, (1982) found that purified sensitized human lung mast cells generate SRS-A without a requirement for other cell types when exposed to antigen.

The observed enhancement of antigen-induced SRS-A release by NSAID is in agreement with similar studies conducted in human lung (Walker, 1973), rat peritoneal cells (Burka & Flower, 1979), dog lung (Krell & Chakrin, 1978) and guinea-pig lung (Engineer *et al.*, 1978; Watanabe-Kohno & Parker, 1980). The mechanism is presumably related to diversion of additional substrate, arachidonic acid, from the cyclo-oxygenase to the lipoxygenase pathway (Burka & Paterson, 1980; Spannhake *et al.*,1981).

The cellular source of SRS-A release from guinea-pig lung in response to A23187 probably involves

Table 5 Effect of mefenamic acid on SRS-A release by antigen and A23187[a]

			SRS-A release ($ng\,g^{-1}$)			
Stimulus	($ug\,ml^{-1}$)	*Control*	*Mefenamic Acid*, (10^{-6}M)	P[c]	*Mefenamic Acid*, (10^{-5}M)	P
Antigen	10	28.5 ± 3.2[b]	51.0 ± 5.0	<0.05	58.0 ± 5.3	<0.01
A23187	5	22.2 ± 6.5	26.4 ± 8.0	>0.05	22.9 ± 6.8	>0.05

[a]Sensitized lung tissue was used in the experiments with antigen. Normal lung tissue was used in the experiments with A23187. Tissues were preincubated with mefenamic acid for 10 min and then challenged with antigen or A23187 for 45 min.
[b]Values are the mean ± s.e.mean for 3 experiments.
[c]Levels of significance.

mast cells (since some histamine is also released) plus other unidentified types. Evidence for cellular sources other than mast cells is the high ratio of SRS-A to histamine release for A23187 as compared to antigen. Also, A23187 has been reported to cause SRS-A release from broncho-alveolar macrophages (Sirois, 1980) polymorphonuclear neutrophils (Jouvin-Marche *et al.*, 1983) and pulmonary artery (Fleisch & Haisch, 1982). SRS-A release from mast cells may therefore represent a small portion of the overall SRS-A release by A23187.

The principle observation of these experiments was that NSAID, which enhanced the release of SRS-A by antigen, did not enhance SRS-A release by A23187. Attempts to ascribe this difference between the two stimuli to incubation time with, or concentrations of, NSAID were unrewarded. Although sensitization has been reported to induce changes in the response of tissues to calcium ionophore (Burka *et al.*, 1981), in the present studies tissue sensitization with antibodies did not account for the differences observed. The lack of effect of NSAID on A23187-induced SRS-A release from guinea-pig lung is in apparent disagreement with the studies of Piper & Seale (1978). The reason for this discrepancy is not obvious.

One possible explanation for the differential effect of NSAID on SRS-A release by antigen or A23187 is that they enhance A23187-induced SRS-A release from mast cells but have no effect on A23187-induced SRS-A release from other cells. Since it has been hypothesized that mast cells may contribute less to the total SRS-A release by A23187 than by antigen, the enhancement is not apparent. Reasons why NSAID do not enhance A23187-induced SRS-A release from non-mast cell sources could be: (1) in these cells the lipoxygenase pathway is already saturated with arachidonic acid, or (2) the cyclo-oxygenase and lipoxygenase pathways in these cells are not in direct competition for arachidonic acid.

Although these observations suggest several cellular sources for SRS-A release by A23187, another possibility must be considered. Antigen and A23187 may affect different arachidonic acid pools within the same cell. Indeed, certain cell types have been reported to release different arachidonic acid products, depending on the stimulus used (Humes *et al.*, 1982; Hsuch & Sun, 1982). If there are different arachidonic acid pools within cells of guinea-pig lung these pools may exhibit the potential differences listed above for cells; i.e., lipoxygenase pathway saturation, degree of competition between pathways, etc. This could also account for the differential effects of NSAID on antigen and A23187-induced SRS-A release.

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References

BACH, M.K., BRASHLER, J.R. & GORMAN, R.R. (1977). On the structure of slow reacting substance of anaphylaxis: evidence of biosynthesis from arachidonic acid. *Prostaglandins*, **14**, 21–38.

BURKA, J.F., ALI, M., MCDONALD, J.W.D., & PATERSON, N.A.M. (1981). Immunological and non-immunological synthesis and release of prostaglandins and thromboxanes from isolated guinea-pig trachea. *Prostaglandins*, **22**, 683–691.

BURKA, F.F. & FLOWER, R.J. (1979). Effects of modulators of arachidonic acid metabolism on the synthesis and release of slow-reacting substance of anaphylaxis. *Br. J. Pharmac.*, **65**, 35–41.

BURKA, J.F. & PATERSON, N.A.M. (1980). Evidence for lipoxygenase pathway involvement in allergic tracheal contraction. *Prostaglandins*, **19**, 499–515.

ENGINEER, D.M., NIEDERHAUSER, U., PIPER, P.J. & SIROIS, P. (1978). Release of mediators of anaphylaxis:

inhibition of prostaglandin sythesis and the modification of release of slow reacting substance of anaphylaxis and histamine. *Br. J. Pharmac.*, **62,** 61–66.

FLEISCH, J.H. & HAISCH, K.D. (1982). Release of slow reacting substance from various tissues by A23187. *J. Pharm. Pharmac.*, **34,** 809–811.

FORSBERG, K., & SORENBY, L. (1981). The influence of a new corticosteroid, budesonide, on anaphylactic bronchoconstriction and SRS-A release in the guinea pig. *Agents and Actions*, **11,** 391–395.

HITCHCOCK, M. (1978). Effect of inhibitors of prostaglandin sythesis and prostaglandins E_2 and $F_{2\alpha}$ on the immunologic release of mediators of inflammation from actively sensitized guinea-pig lung. *J. Pharmac. Exp. Ther.*, **207,** 630–640.

HSUCH, W. & SUN, F.F. (1982) Leukotriene B_4 biosynthesis by alveolar macrophages. *Biochem. biophys. Res. Commun.*, **106,** 1085–1091.

HUMES, J.L., SADOWSKI, S., GALAVAGE, M., GOLDENBERG, M., SUBERS, E., BONNEY, R.J. & KUEHL, F.A. (1982). Evidence for two sources of arachidonic acid for oxidative metabolism by mouse peritoneal macrophages. *J. biol. Chem.*, **257,** 1591–1594.

JOUVIN-MARCHE, E., CERRINA, J., COEFFIER, E., DUROUX, P. & BENVENISTE, J. (1983). Effect of the Ca^{2+} antagonist nifedipine on the release of platelet-activation factor (PAF-ACETHER), slow-reacting substance and β-glucuronidase from human neutrophils. *Eur. J. Pharmac.*, **89,** 19–26.

KRELL, R.D. & CHAKRIN, L.W. (1976). An *in vitro* model of canine immediate-type hypersensitivity reactions. *Int. Archs Allergy appl. Immun.*, **51,** 641–655.

KRELL, R.D. & CHAKRIN, L.W. (1978). Pharmacologic regulation of antigen-induced mediator release from canine lung. *Int. Archs Allergy appl. Immun.*, **56,** 39–47.

LAEKEMAN, G.M. & HERMAN, A.G. (1979). Indomethacin increases the production of SRS-A by the ileum from sensitized guinea pigs. *Archs Int. Pharmacodyn.*, **242,** 307–309.

MACGLASHEN, D.W. SCHLEIMER, R.P., PETERS, S.P., SCHULMAN, E.S., ADAMS, G.K., NEWBALL, H.H. & LICHTENSTEIN, L.M. (1982). Generation of leukotrienes by purified human lung mast cells. *J. clin. Invest..*, **70,** 747–751.

PIPER, P.J. & SAMHOUN, M.N. (1981). The mechanism of action of leukotrienes C_4 and D_4 in guinea-pig isolated perfused lung and parenchymal strips of guinea pig, rabbit and rat. *Prostaglandins*, **21,** 793–803.

PIPER, P.J. & SEALE, J.P. (1978). Release of slow-reacting substance from guinea-pig and human lung by calcium ionophore A23187. *Br. J. Pharmac.*, **63,** 364–365P.

SAMUELSSON, B. (1982). The leukotrienes: an introduction. In *Advances in Prostaglandin, Thromboxane, and Leukotriene Research*, Vol. 9, ed. Samuelsson, B. & Paoletti, R. pp. 1–17. New York: Raven Press.

SIRAGANIAN, R.P. (1975). Refinements in the automated fluorometric histamine analysis system. *J. Immunol. Methods*, **7,** 283–290.

SIROIS, P. (1980). Release of mediators from free bronchoalveolar cells: effect of ionophore A23187 and antigen challenge. *J. Immunopharmac.*, **2,** 543–551.

SPANNHAKE, E.W., HYMAN, A.L. & KADOWITZ, P.J. (1981). Bronchoactive metabolites of arachidonic acid and their role in airway function. *Prostaglandins*, **22,** 1013–1026.

TSAI, B.S., BERNSTEIN, P., MACIA, R.A., CONATY, J. & KRELL, R.D. (1982). Comparative potency and pharmacology of isomers of leukotriene D_4 on guinea-pig trachea: requirement for a 5(S)6(R) configuration. *Prostaglandins*, **23,** 489–506.

WALKER, J.L. (1973). The regulatory function of prostaglandins in the release of histamine and SRS-A from passively sensitized human lung tissue. In *Advances in the Biosciences*, Vol. 9, ed. Bergstrom, S., & Bernhard, S. pp. 235–240. Braunschweig: Pergamon Press.

WATANABE-KOHNO, S. & PARKER, C.W. (1980). Role of arachidonic acid in the biosynthesis of slow reacting substance of anaphylaxis (SRS-A) from sensitized guinea-pig lung fragments: evidence that SRS-A is very similar or identical structurally to nonimmunologically-induced forms of SRS. *J. Immunol.*, **125,** 946–955.

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