

Release of slow-reacting substance of anaphylaxis from dispersed pig lung cells: effect of cyclo-oxygenase and lipoxygenase inhibitors

N. A. M. Paterson, M.B., F.R.C.P.(C), J. F. Burka, Ph.D., and I. D. Craig, M.B.

London, Ontario

Study of the release of slow-reacting substance of anaphylaxis (SRS-A) from lung cells has been hampered by the lack of a suitable animal model. Using both immunologic and pharmacologic stimuli, we have obtained histamine and SRS-A release from dispersed pig lung cells containing 6% mast cells (with a histamine content of 1.9 pg/cell). Lung cells dispersed from actively sensitized (with intratracheal Ascaris antigen) but not unsensitized pigs released both histamine (mean net release 33%) and SRS-A (mean release, 47 units/10⁷ cells) when challenged with Ascaris antigen. Greater release of histamine (mean net release 52%) and of slow-reacting substance (SRS) (mean release 701 units/10⁷ cells) was induced by challenge with the calcium ionophore A23187. The pharmacologic and physicochemical characteristics of the SRS together with its profile of enzymatic inactivation resembled those described for SRS-A released from human lung. Both antigen-induced and A23187-induced SRS(-A) release were enhanced by indomethacin, a cyclo-oxygenase inhibitor, but inhibited by both phenidone (IC₅₀ 35 µM) and eicosatetraenoic acid (IC₅₀ 15 µM), inhibitors of both cyclo-oxygenase and lipoxygenase, confirming that generation of SRS(-A) by either stimulus required an intact lipoxygenase pathway of arachidonic acid metabolism.

Slow-reacting substance of anaphylaxis (SRS-A)* is released following challenge with antigen or anti-IgE of passively sensitized human lung fragments¹ or

dispersed human lung cells.² Partially purified SRS-A constricts human airway smooth muscle,^{3, 4} and indirect evidence obtained in dogs⁵ suggests that SRS-A causes the reduction in mucociliary clearance induced in atopic asthmatics by aerosolized antigen.⁶ Thus SRS-A may be an important mediator of the pulmonary response to inhaled antigen in allergic asthmatics. In addition, however, an incompletely characterized slow-reacting substance (SRS) has been detected in the airway secretions of nonallergic adult asthmatics^{7, 8} and of a majority⁹ or a minority⁸ of patients with chronic bronchitis. SRS has also been detected in the airway secretions of dogs subjected to acute lung injury by exposure to an acid aerosol.¹⁰ Thus it is likely that SRS(s), which may or may not be identical to SRS-A, contributes to the pathogenesis of nonallergic asthma and possibly of other lung diseases.

Studies of the pathobiology of SRS release from lung have been hampered by the lack of a suitable in

From the Departments of Medicine, Pharmacology and Pathology, Victoria Hospital Corporation and the University of Western Ontario.

Supported by Medical Research Council Grant MA5971 and by Ontario Thoracic Society Grant MO21A8.

Received for publication April 29, 1980.

Accepted for publication Feb. 19, 1981.

Reprint requests to: Dr. Nigel A. M. Paterson, Victoria Hospital Corporation, Westminster Campus, 777 Baseline Road East, London, Ontario, Canada N6A 4S2.

*In this paper the term SRS-A is used if the activity has been generated by an immunologic stimulus (in this study specific antigen) with or without corroborative data obtained using a pharmacologic stimulus, and the term SRS if the material has been generated solely by a pharmacologic stimulus (in this study the calcium ionophore A23187).

vitro model. The use of human lung cells² is attractive, but suitable specimens are obtained only when surgical resections are performed. Furthermore the surgical population is skewed in terms of age and concomitant lung disease. Hence we wanted to develop a reliable animal model of SRS release from lung, using dispersed cells rather than fragments or vascular perfusion, to minimize the contribution of diffusion or nonspecific binding of pharmacologic agents and released mediators. We were pleased to find that dispersed pig lung cells released large amounts of SRS when stimulated pharmacologically (with the calcium ionophore A23187) and that cells from sensitized pigs also released SRS-A when incubated with specific antigen.

Recently considerable evidence has accrued that SRS(-A) is generated by the lipoxygenase pathway of arachidonate metabolism.^{11, 12} Depending on the particular cell source and activating stimulus, the SRS has been shown to be either leukotriene D,^{13, 14} leukotriene C,¹⁵ or a mixture of leukotrienes C and D.^{16, 17} Using indomethacin, a cyclo-oxygenase inhibitor,¹⁸ and phenidone¹⁹ and eicosatetraenoic acid,²⁰ inhibitors of both cyclo-oxygenase and lipoxygenase, we found that the release of SRS-A by either antigen or A23187 required an intact lipoxygenase pathway of arachidonate metabolism.

MATERIALS AND METHODS

Reagents

Amberlite XAD-8 (BDH Chemicals, Toronto, Ontario), silicic acid (Biosil A, 200-400 mesh, Bio-Rad Laboratories, Richmond, Calif.), gelatin (Difco Laboratories, Detroit) trypan blue (Matheson, Coleman and Bell, Norwood, Ohio), limpet arylsulfatase type V, atropine sulfate, deoxyribonuclease type I, HEPES, soybean lipoxidase type IV, 4-nitrocatechol, nitrocatechol sulfate, 1-phenyl,3-pyrazolidone (phenidone), and thermolysin (Sigma Chemical Co., St. Louis) were purchased from the manufacturers. All solvents were of nanograde quality. Eicosatetraenoic acid (ETYA) (Roche), indomethacin, and mepyramine maleate (Merck-Frosst Laboratories, Dorval, Quebec) were gifts. The calcium ionophore A23187 was kindly donated by Dr. R. Dolman (Eli Lilly & Co., Toronto) and Fisons compound FPL55712 by Mr. P. Sheard (Fisons Limited, Loughborough, Leicestershire). Dr. G. Strejan (Department of Microbiology and Immunology, University of Western Ontario) generously provided the concentrated *Ascaris* antigen (36 µg/ml), and Dr. J. Rokach (Merck-Frosst Laboratories) the reference leukotriene D₄.

Sensitization of pigs

Outbred pigs (3 mo old, weighing approximately 20 kg) were anesthetized and intubated, and *Ascaris* antigen (300 µg in 10 ml sterile saline) was instilled into the lungs via a catheter passed down the endotracheal tube, the exposure to

TABLE I. Constituents of the LC population

Cell type	Mean (%)*	SEM (%)	Range (%)
Mast cells	6.2	0.7	3 to 11
Neutrophils	7.4	1.5	1 to 20
Alveolar type II cells	5.8	0.4	2 to 8
Macrophages	25.7	1.6	15 to 36
Lymphocytes	8.3	0.6	5 to 12
Eosinophils	7.5	0.7	0 to 9
Plasma cells	1.4	0.3	0 to 4
Other cells†	5.3	0.5	2 to 9
Unidentified	32.4	2.0	20 to 43

*Data are the means of 12 experiments in which morphologic assessment was made.

†Other cell types recognized included goblet cells, ciliated cells, and Clara cells.

antigen being maximized by frequent repositioning of the catheter and by postural changes during the instillation. The pigs were killed 14 to 21 days after sensitization.

Lung cell dispersion

The pig was anesthetized and killed by exsanguination and the lungs perfused in situ via a pulmonary artery cannula until the effluent was blood free. The lungs were then removed; dissected free of pleura, major airways, and blood vessels; sliced (McIlwain tissue chopper, Mickle Laboratory Engineering Co., Ganshell, Surrey) into cubic millimeter fragments; and washed in Tyrode's buffer (containing per liter NaCl 8 gm, KCl 0.2 gm, MgCl₂ 0.1 gm, NaH₂PO₄ 0.5 gm, NaHCO₃ 1 gm, and glucose 1 gm) with calcium (normally 0.2 gm/l) omitted. In a modification of the method of Frazier et al.²¹ the washed fragments were then incubated for 3 hr at 25° C in Tyrode's solution buffered to pH 7.4 with 2.5 mM HEPES and containing thermolysin (50 µg/ml) and DNase (30 µg/ml). Cells were collected by sieving through nylon cloth (B. & S. H. Thompson Co. Ltd., Scarborough, Ontario) at 1-hr intervals. After resuspension in Tyrode's solution containing gelatin (1 mg/ml) and DNase (30 µg/ml) but not calcium (TGD), the cells were washed twice in TGD. They were then counted in a Newbauer hemocytometer chamber (American Optical Co., Buffalo, N. Y.) by phase contrast microscopy, with viability assessed by their appearance in phase contrast, by exclusion of 0.5% trypan blue dye, and in the case of mast cells by spontaneous histamine release. In general phase contrast microscopy gave lower estimates of viability than did trypan blue exclusion.

Morphologic assessment

Smears of the cell suspension were fixed with Aquacyte (Maynard Diagnostics, Mississauga, Ontario), and replicate smears were stained with hematoxylin and eosin, toluidine blue, and Papanicolaou's stain. Aliquots of the cell suspension were prepared for electron microscopy by exposure to 3% glutaraldehyde in Sorensen's phosphate buffer at room temperature for 30 min, then centrifuged into a pellet, held

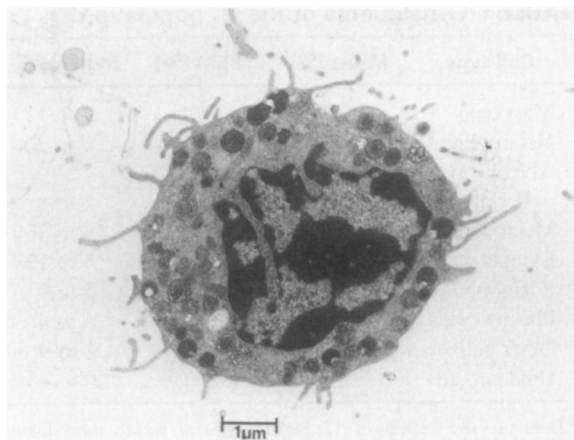


FIG. 1. A pig lung mast cell. The reticular and crystalline cytoplasmic granules are seen.

overnight at 4° C, rinsed in buffer, postfixed for 1 hr with osmium tetroxide in Sorensen's phosphate buffer, and embedded in Epon. Differential counts were performed on a minimum of 200 cells.

Mediator release by A23187 and antigen

Replicate cell suspensions in TGD containing 1 mM calcium (TGD-Ca⁺⁺) were distributed into polypropylene tubes in concentrations of 10⁷ to 10⁸ cells/ml (this range of cell concentrations having been found in preliminary experiments to give maximal total SRS release) and incubated for 5 min at 37° C. Each experimental condition was performed in duplicate. An aliquot of a solution in TGD-Ca⁺⁺ of *Ascaris* antigen or of the calcium ionophore A23187 (freshly prepared from a stock solution [1 mg/ml] in ethanol by dilution into TGD-Ca⁺⁺ spinning in a Vortex mixer) was then added. The final concentration of ethanol never exceeded 0.5% and had no effect on mediator release. After incubation at 37° C for the desired time the reaction was stopped by the addition of 2 ml chilled TGD. The cells were sedimented by centrifugation and the supernatant solutions decanted. The cell pellets were then resuspended in TGD and disrupted by repeated freeze-thawing.

Assays

Histamine was measured as previously described,² except the SRS-A antagonist FPL55712²² was added in a concentration (400 nM) that reduced SRS contractions by over 80% but did not alter the ileal response to histamine. The net percentage release, corrected for spontaneous release, was then calculated according to the standard formula.² For SRS bioassay FPL55712 was omitted and 1 μM mepyramine maleate added.² The amount of SRS was determined by interpolation from a dose-response curve constructed over a fourfold range by dilutions of a house standard. A concentration of 1 unit/ml standard caused an ileal contraction equivalent to that induced by a histamine concentration of 5 ng/ml and was equivalent to 5 units/ml standard preparation of rat SRS-A (kindly donated by the

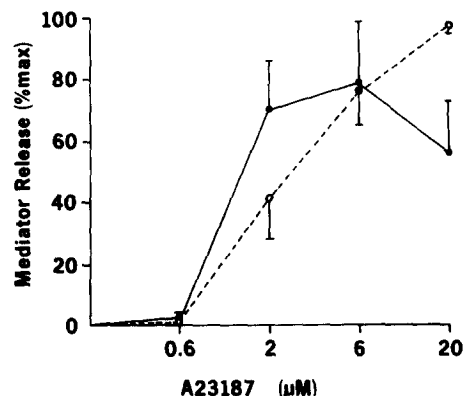


FIG. 2. Dose-response relationship of mediator release by A23187. LC were incubated for 30 min at 37° C with varied A23187 concentrations and the release of SRS (●—●) and histamine (○---○) measured. Mean results (±SEM) of six experiments are shown. Mean maximum SRS release was 3770 units and of (net) histamine release 69%.

late Dr. R. P. Orange). After completion of the experiments presented in this study, the house standard was calibrated against synthetic leukotriene D₄ (generously supplied by Dr. J. Rokach, Merck-Frosst Laboratories). The ileal contraction induced by 1 unit/ml was equivalent to that induced by 250 pg/ml leukotriene D₄. Arylsulfatase activity was assayed at pH 5.7 as previously described.²

Partial purification of SRS

SRS activity contained in the supernatant solutions of challenged cells was partially purified by a combination of previously described methods.^{1, 23} After extraction into 80% ethanol and incubation with 0.1 N NaOH (30 min, 37° C) the SRS was desalted by chromatography on Amberlite XAD-8 (1 × 15 cm column). After washing with water (30 ml) the SRS was eluted with 80% ethanol (30 ml). The eluate was evaporated to dryness, resuspended in methanol, added to a slurry of silicic acid (100 mg) in methanol, and the mixture evaporated to dryness. The dried silicic acid was then applied to a column (0.7 × 6 cm) of silicic acid that had been activated (100° C, 20 hr) and washed (with excess hexane). The column was then eluted with successive 15-ml volumes of hexane, ether, ethyl acetate, chloroform, chloroform-methanol (in ratios of 9:1, 8:2, 7:3, 6:4, 5:5 and 4:6), and methanol.

RESULTS

Lung cell dispersion

The yield of viable nucleated lung cells (LC) following enzymatic dispersion of parenchymal fragments was $5.7 \times 10^7 \pm 0.3 \times 10^7$ LC/gm (mean ± SEM, n = 22). The viability was $92\% \pm 2\%$ (mean ± SEM, n = 12, range 83% to 100%), and contamination by red cells varied from 25% to 64% (average 51%). As shown in Table I, the LC population included mast cells, neutrophils, alveolar type II epi-

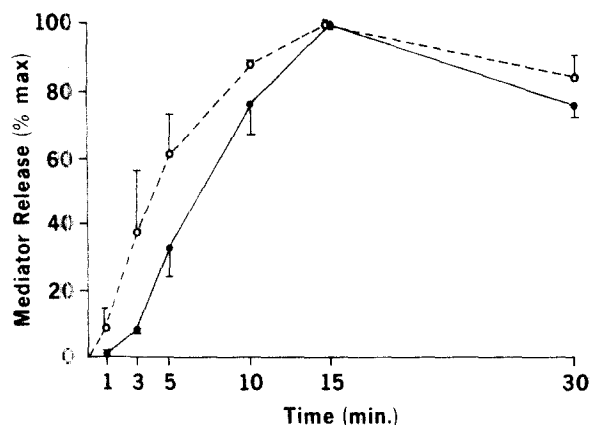


FIG. 3. Time course of mediator release by A23187. LC were incubated at 37° C for the periods shown with 6 μ M A23187 and the release of SRS (●—●) and histamine (○—○) measured. Mean results ($\pm$ SEM) of five experiments are shown. Mean maximum SRS release was 7920 units and of (net) histamine release 73%.

thelial cells, macrophages, lymphocytes, and eosinophils, with smaller numbers of other cell types. An average of 32.4% of the cells remained unidentified. The histamine content of the mast cells, which constituted $6.2\% \pm 0.7\%$ (mean $\pm$ SEM, $n = 12$, range 3% to 11%) of the population (and thus 3.5×10^6 mast cells/gm lung) was 1.9 ± 0.4 pg/cell (mean $\pm$ SEM, $n = 9$, range 0.7 to 5.5 pg/cell), representing $38.5\% \pm 5.3\%$ (mean $\pm$ SEM, $n = 23$) of the initial lung histamine. Spontaneous histamine release from the mast cells was $7.0\% \pm 0.5\%$ (mean $\pm$ SEM, $n = 26$). Transmission electron microscopy (Fig. 1) revealed that the mast cells resembled human lung mast cells more than rat peritoneal mast cells in that surface ridges were pronounced and the cytoplasmic granules, which were largely intact, were pleomorphic, with crystalline and reticular forms. The content of some granules was organized into concentric rings, reminiscent of the characteristic scrolls and whorls reported only in human mast cells.^{2, 24, 25}

Mediator release from LC

When incubated with A23187 LC exhibited a concentration-dependent release of histamine and SRS (Fig. 2). The threshold A23187 concentration for SRS release was usually 2 μ M, and maximal release, 701 ± 109 units/ 10^7 LC (mean $\pm$ SEM, $n = 19$), equivalent to 175 ng leukotriene D₄/ 10^7 LC and to 4000 units $\pm$ 620 units/gram original lung tissue, was obtained with 6 μ M A23187 with less release at 20 μ M A23187. In contrast net histamine release, which was $52\% \pm 8\%$ at 6 μ M A23187, increased to $64\% \pm 5\%$ at 20 μ M A23187. The net release of the intracellular marker enzyme arylsulfatase rose from

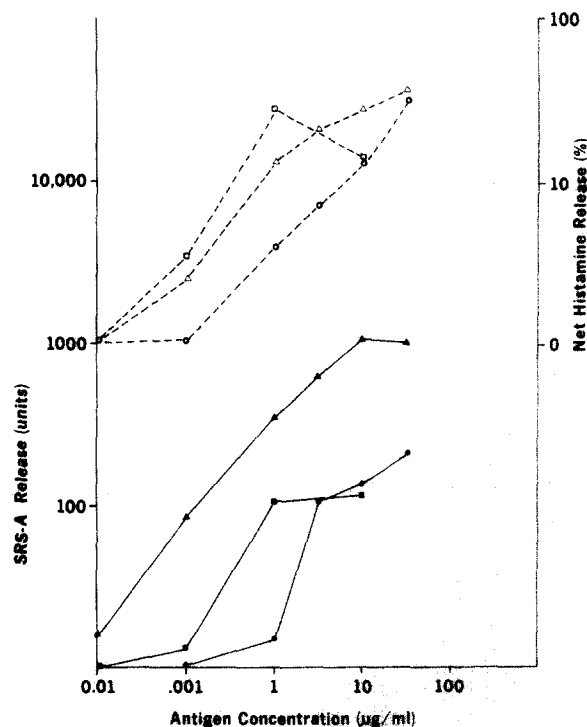


FIG. 4. Dose-response relationship of mediator release by *Ascaris* antigen from sensitized LC. In three experiments LC were incubated with varied *Ascaris* concentrations for 30 min at 37° C, and the release of SRS-A (●—●) and (net) histamine (Δ — Δ) was measured. Each experimental condition was performed in duplicate.

$12\% \pm 4\%$ (mean $\pm$ SEM, $n = 3$) at 6 μ M to $21\% \pm 6\%$ at 20 μ M.

In kinetic experiments with 6 μ M A23187, histamine release ($73\% \pm 8\%$, mean maximum $\pm$ SEM, $n = 5$) began after 1 min and was essentially complete by 10 min (Fig. 3), whereas SRS release (792 units $\pm$ 300 units/ 10^7 LC) was minimal until 5 min and peaked at 15 min, the subsequent slight decline presumably reflecting predominant inactivation.²⁶

When LC dispersed from sensitized pigs were incubated with *Ascaris* antigen, concentration-dependent release of histamine and SRS-A was observed (Fig. 4), the mean maximum release in three experiments being 33% and 47 units/ 10^7 LC (equivalent to 12 ng leukotriene D₄/ 10^7 LC), respectively. Incubation of cells from unsensitized pigs with *Ascaris* antigen over the same range of concentrations (0.01 to 30 μ g/ml) did not cause mediator release. Similarly there was no mediator release when cells from sensitized pigs were incubated with nonspecific antigen (ovalbumin). Kinetic analysis of the release (Fig. 5) revealed a rapid release of histamine, reaching 48% of maximum release at 1 min and 90% of maximum release at 3 min, with a more delayed release of SRS-A,

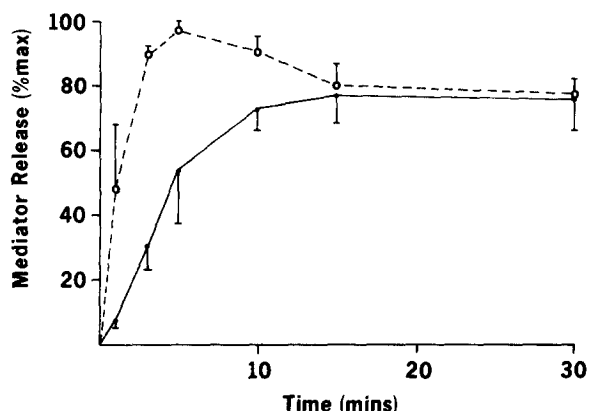


FIG. 5. Time course of mediator release by *Ascaris* antigen. Sensitized LC were incubated at 37° C with *Ascaris* antigen (10 µg/ml) for the periods shown and the release of SRS-A (●—●) and (net) histamine (○—○) measured. Mean data (±SEM, n = 4) are shown. The mean maximum release of SRS-A was 66 units/10⁷ LC and of histamine 22%.

maximal at 15 min. Both histamine and SRS-A were released more rapidly by antigen than by A23187.

Characterization of SRS

After extraction into 80% ethanol and treatment with alkali, SRS was applied to an amberlite XAD-8 column and eluted with 80% ethanol (Table II). The SRS was then applied to a silicic acid column with the bulk of the recovered SRS appearing in the 6:4 and 5:5 chloroform:methanol fractions (Table II). In five experiments, three of which are shown in Table III, SRS was inactivated by arylsulfatase (mean rate, 17 units/aryl sulfatase unit/hr) and lipoxidase (mean rate 3.4 units/µg lipoxidase/hr) but not by thermolysin. SRS was also characterized pharmacologically: the average time required for a half maximal ileal contraction of both SRS and SRS-A was 24 sec, similar to that described by Orning et al.¹⁴ for leukotriene D. The ileal contractions induced by crude SRS, SRS eluted from silicic acid, and SRS-A were susceptible to inhibition by FPL55712, the IC₅₀ for relaxation of the SRS-contracted ileum being 34 ± 10 nM with greater than 75% inhibition achieved by 400 nM, a concentration that had no effect on histamine contractions.

Effect of manipulations of arachidonic acid metabolism

When LC were pretreated with indomethacin, an inhibitor of cyclo-oxygenase,¹⁸ there was no effect on histamine release, but a bell-shaped enhancement of SRS release was observed (Fig. 6) with the maximum enhancement (79% ± 10%) at 3-µM indomethacin concentrations. In contrast the cyclo-oxygenase and lipoxygenase inhibitors phenidone¹⁹ and ETYA²⁰

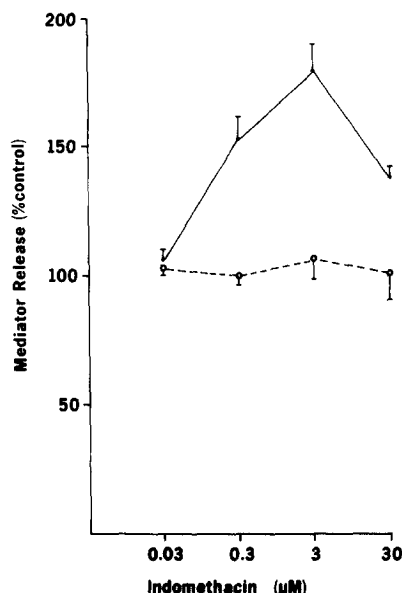


FIG. 6. Effect of indomethacin on mediator release from LC. After incubation with indomethacin for 5 min, LC were challenged with A23187 (6 µM, 30 min, 37° C) and the release of SRS (●—●) and histamine (○—○) measured. Mean data (±SEM, n = 6) are shown. Mean control release of SRS was 4090 units and of (net) histamine 71%.

caused a concentration-dependent reduction in SRS release (Figs. 7 and 8), with significant inhibition of histamine release only at the highest doses of phenidone (200 µM) and ETYA (100 µM). In terms of SRS release the IC₅₀ of phenidone was 35 µM and ETYA was 15 µM. When the pharmacologic (A23187) and immunologic (*Ascaris* antigen) stimuli for mediator release were compared (Table IV), comparable enhancement of SRS(-A) release by indomethacin and inhibition by phenidone and ETYA were observed.

DISCUSSION

The dispersion procedure gave very satisfactory yields of viable lung cells; the mean yield, 5.7 × 10⁷ LC/gm, is to be compared with 1.5 × 10⁷ LC/gm obtained from rat lung.²⁷ Based on estimates of 250,000 cells/mg lung,²¹ the LC represented 23% of the initial lung cell population. The constituent cells of the LC population (Table I) were similar to those previously observed in dispersed human lung cell populations.² The appreciable percentage (32%) of cells remaining unidentified even after electron microscopy reflects at least in part the lack of criteria for identifying in dispersed cell populations lung cell types such as alveolar type I cells, vascular endothelial cells, and fibroblasts, which together constitute over 50% of the parenchymal lung cell population.²⁸

TABLE II. Physicochemical characterization of SRS*

		Units	Percent recovery
Base hydrolysis and desalting*	Starting material	810	
	XAD ₈	800	99%
	Water	0	
	Ethanol 80%	790	
Silicic acid chromatography*	Starting material	15,000	99%
	Hexane, ether, ethyl acetate	0	
	Chloroform	0	
	Chloroform-methanol	5,380	36%
	9:1	0	
	8:2	20	
	7:3	115	
	6:4	3,980	
	5:5	900	
	4:6	280	
	Methanol	85	

*See text for methods.

The yield of mast cells, 3.5×10^6 cells/gm, is similar to the number (0.5×10^6 to 6.8×10^6 /gm) dispersed from human lung² and exceeds the 1.3×10^6 mast cells/gm found in monkey lung.²⁹ This, together with the finding that mast cells constituted 6.2% of the dispersed population, suggests a selective concentration of mast cells by the dispersion procedure and presumably reflects the lack of intercellular attachments of the interstitially located mast cells in comparison with epithelial and endothelial lung cells. The mast cell histamine content, 1.9 pg/cell, while less than indirect estimates of the content of the monkey lung mast cells,²⁹ is similar to the observed content, 3.5 pg/cell, of monkey bronchial lumen mast cells,³⁰ the 1 to 5.5 pg/cell previously found in dispersed human lung mast cells² and the 2.7 pg/cell found in dispersed guinea pig lung mast cells.³¹ The histamine content of the mast cells, the acceptable spontaneous histamine release (7%), the recovery of 38% of the initial lung histamine in the LC, and the morphologic evidence (Fig. 1) all indicate that the mast cells were largely intact. The cytoplasmic granules were of interest: their heterogeneity and crystalline ultrastructure resembled the granules seen in human lung mast cells^{2, 24, 25} rather than the homogeneous electron-dense granules characteristic of rat peritoneal mast cells.³²

In view of the previous difficulty in obtaining SRS release from dispersed rat lung cells²⁷ and guinea pig lung cells,* it was gratifying to find that the amount of SRS released from LC by A23187, 701 units/ 10^7 LC (equivalent to 175 ng leukotriene D₄/ 10^7 LC and to

TABLE III. Inactivation of SRS

Enzyme*	Number of experiments	SRS inactivation (%) (mean $\pm$ SEM)
Arylsulfatase	4	93.0% $\pm$ 1.5%
Lipoxygenase	4	93.6% $\pm$ 3.5%
Thermolysin	1	0%

*SRS was incubated (37° C, 60 min) in 2 ml buffer containing arylsulfatase (10 U/ml), lipoxygenase (50 μ g/ml), or thermolysin (50 μ g/ml) and the amount of SRS remaining after 60 min compared with that of SRS incubated in buffer alone. For arylsulfatase the buffer was 0.1 M sodium acetate, pH 5.7, and for lipoxygenase and thermolysin TGD-Ca⁺⁺. The mean (n = 4) amount of added SRS was 360 ± 45 units.

4000 units/gm lung tissue) was greater than that previously obtained from dispersed human lung cells.² The concentration of A23187 required for optimum SRS release is comparable to that reported for other systems,³³⁻³⁵ as is the time course.³⁵

Incubation with antigen of LC dispersed from actively sensitized animals caused a concentration-dependent release of histamine and SRS-A (Fig. 4), the time course of which (Fig. 5) was very similar to the antigen-induced release of mediators from human lung cells³⁶ and more rapid than the release induced by A23187 (Fig. 3). The smaller amounts (in comparison with A23187) of SRS-A released immunologically may reflect one or more of the following considerations: incomplete sensitization of (mast) cells by the immunization procedure, loss of cytophilic antibody or alteration of the antigen-binding domain of the antibody during the dispersion procedure, or failure to recruit the additional lung cell types or metabolic pathways activated by the pharmaco-

*Paterson NAM, unpublished observations.

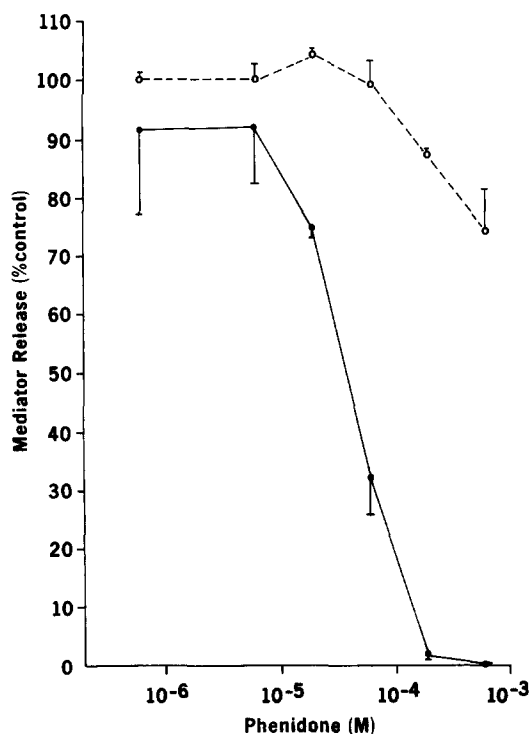


FIG. 7. Effect of phenidone on mediator release from LC. The experimental design and expression of results are the same as in Fig. 6, and mean data ($\pm$ SEM, $n = 7$) are shown. Mean control release of SRS was 5070 units and of (net) histamine 75%.

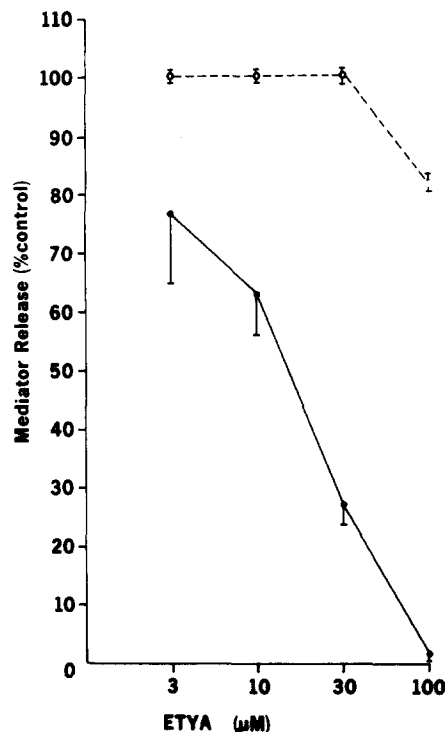


FIG. 8. Effect of ETYA on mediator release from LC. The experimental design and expression of results are the same as in Fig. 6, and mean data ($\pm$ SEM, $n = 7$) are shown. Mean control release of SRS was 5070 units and of (net) histamine 75%.

TABLE IV. Effect of cyclo-oxygenase and lipoxygenase inhibitors on mediator release*

Inhibitor	Concentration (μ M)	SRS-A (% control)†		Histamine (% control)‡	
		Antigen	A23187	Antigen	A23187
Indomethacin	3	186 $\pm$ 34	209 $\pm$ 8	87 $\pm$ 7	108 $\pm$ 4
Phenidone	60	13 $\pm$ 4	34 $\pm$ 7	95 $\pm$ 8	101 $\pm$ 1
ETYA	30	31 $\pm$ 7	32 $\pm$ 3	29 $\pm$ 5	92 $\pm$ 3

*LC from sensitized animals were incubated (30 min, 37° C) with either *Ascaris* antigen (10 μ g/ml) or A23187 (6 μ M). The effect of the inhibitors on mediator release is expressed as a percentage (mean $\pm$ SEM, $n = 4$) of control release.

†Mean control release of SRS-A was 600 units with antigen and 6080 units with A23187.

‡Mean control release of histamine was 21% with antigen and 67% with A23187.

logic stimulus. On the other hand, the ability of the washed lung cells from sensitized animals but not from unsensitized controls to respond to antigen exposure with mediator release indicates that sensitization led to the production of cytophilic antibody, at least some of which remained intact after the dispersion procedure.

To compare porcine lung SRS with that generated in other systems, the SRS was characterized pharmacologically, and its inactivation by enzymes and behavior during standard purification procedures was examined. A close similarity to human lung SRS-A, which is predominantly composed of leukotriene

D₄,¹⁷ is indicated by the following findings: (1) a characteristic contraction of the antihistamine-treated guinea pig ileum, reaching 50% of the peak in 24 sec,³⁷ (2) inhibition of ileal contraction by FPL55712,²² (3) inactivation by arylsulfatase³⁸ and lipoxidase³⁹ but not by thermolysin, and (4) polarity on silicic acid chromatography.²³

The development of a reliable lung cell model for SRS-A release allowed us to examine the relationship of SRS-A release to arachidonic acid metabolism. Indomethacin enhances SRS-A release from bovine⁴⁰ and guinea pig⁴¹ lung, and we similarly observed an enhancement of SRS release at a concentration of in-

domethacin (300 nM) that inhibits cyclo-oxygenase.¹⁸ Enhancement of SRS-A release by indomethacin may reflect either removal of the inhibitory influence of a cyclo-oxygenase product or the diversion of arachidonic acid into the lipoxygenase pathway. The demonstration (Figs. 7 and 8, Table IV) that both phenidone and ETYA inhibited SRS and SRS-A release over a concentration range corresponding to that required for lipoxygenase inhibition^{19, 20} supports the latter explanation.

We wish to thank Mr. L. Goela and Mr. K. Hutchison for their expert technical assistance and Mrs. S. Hughes for typing the manuscript.

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