JCI The Journal of Clinical Investigation

The Effects of the Immunologic Release of Histamine upon Human Lung Cyclic Nucleotide Levels and Prostaglandin Generation

Leslie F. Platshon, Michael Kaliner

J Clin Invest. 1978;62(6):1113-1121. https://doi.org/10.1172/JCI109230.

Research Article

The effect of the antigen-induced, immunoglobulin (Ig)E-dependent release of mediators from human lung tissue was analyzed for coincident changes in the tissue levels of cyclic nucleotides. Simultaneously with the appearance of mediators, lung cyclic guanosine 3′,5′-monophosphate (GMP) increased from 0.9±0.2 to 12.63±4.5 pmol/mg protein and cyclic AMP increased threefold from the initial levels of 5.1±1.4 pmol/mg protein. The release of histamine and prostaglandin (PG)$F_{2\alpha}$, as well as the associated increases in cyclic nucleotides, peaked within 10 min of anaphylaxis. Antagonists of histamine's H-1 receptor prevented anaphylaxis-associated increases in cyclic GMP, whereas H-2 antagonists prevented the cyclic AMP response. Neither of these antagonists influenced the pattern or quantity of histamine or slow-reacting substance of anaphylaxis release. Prevention of $PGF_{2\alpha}$ synthesis with acetylsalicylic acid failed to influence histamine or slow-reacting substance of anaphylaxis release or the concomitant increases in cyclic nucleotides. Histamine, added exogenously, produced a prompt increase in the cyclic AMP and cyclic GMP levels of human lung. As was seen after anaphylaxis, H-1 anatagonists prevented the cyclic GMP response to histamine, whereas H-2 antagonists prevented the cyclic AMP response.

H-1 antagonists prevented 50% of the $PGF_{2\alpha}$ synthesis accompanying anaphylaxis; H-2 antagonists had no effect. Exogenous histamine induced $PGF_{2\alpha}$ synthesis; this synthesis was prevented by H-1 but not H-2 antagonists, and was reproduced by 2-methylhistamine (H-1 agonist) but not by dimaprit […]

Find the latest version:

https://jci.me/109230/pdf

The Effects of the Immunologic Release of Histamine upon Human Lung Cyclic Nucleotide Levels and Prostaglandin Generation

LESLIE F. PLATSHON and MICHAEL KALINER, *Allergic Diseases Section, Laboratory of Clinical Investigation, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014*

ABSTRACT The effect of the antigen-induced, immunoglobulin (Ig)E-dependent release of mediators from human lung tissue was analyzed for coincident changes in the tissue levels of cyclic nucleotides. Simultaneously with the appearance of mediators, lung cyclic guanosine 3',5'-monophosphate (GMP) increased from 0.9±0.2 to 12.63±4.5 pmol/mg protein and cyclic AMP increased threefold from the initial levels of 5.1±1.4 pmol/mg protein. The release of histamine and prostaglandin (PG)$F_{2\alpha}$, as well as the associated increases in cyclic nucleotides, peaked within 10 min of anaphylaxis. Antagonists of histamine's H-1 receptor prevented anaphylaxis-associated increases in cyclic GMP, whereas H-2 antagonists prevented the cyclic AMP response. Neither of these antagonists influenced the pattern or quantity of histamine or slow-reacting substance of anaphylaxis release. Prevention of $PGF_{2\alpha}$ synthesis with acetylsalicylic acid failed to influence histamine or slow-reacting substance of anaphylaxis release or the concomitant increases in cyclic nucleotides. Histamine, added exogenously, produced a prompt increase in the cyclic AMP and cyclic GMP levels of human lung. As was seen after anaphylaxis, H-1 anatagonists prevented the cyclic GMP response to histamine, whereas H-2 antagonists prevented the cyclic AMP response.

H-1 antagonists prevented 50% of the $PGF_{2\alpha}$ synthesis accompanying anaphylaxis; H-2 antagonists had no effect. Exogenous histamine induced $PGF_{2\alpha}$ synthesis; this synthesis was prevented by H-1 but not H-2 antagonists, and was reproduced by 2-methylhistamine (H-1 agonist) but not by dimaprit (H-2 agonist). Arachidonic acid generation of $PGF_{2\alpha}$ was not influenced by antihistamines. Therefore, histamine interactions with human lung result in the synthesis of both $PGF_{2\alpha}$ and cyclic GMP in response to H-1 stimulation, and of cyclic AMP through H-2 stimulation.

Received for publication 8 December 1977 and in revised form 14 July 1978.

INTRODUCTION

The pathophysiologic events leading to allergic bronchial asthma involve an interaction between inhaled allergens and immunoglobulin (Ig)E molecules fixed to the surface of mast cells. The resultant secretion of granules from the mast cell generates a variety of biologically active compounds (1, 2) that induce the airways obstruction. The biochemical steps in mast cell secretion have been partially sequenced (3) and a modulating role of cyclic AMP and cyclic guanosine 3',5'-monophosphate (GMP)[1] defined (4, 5). Cyclic nucleotides influence the release of secretory granules by affecting the degree of mast cell microtubule polymerization-depolymerization (6).

Of the mediators of anaphylaxis, histamine and the prostaglandins are capable of altering the cyclic nucleotide content of guinea pig (7–9), canine (10), and human (11, 12) lung and therefore might conceivably influence their own release process. Indeed, it has been suggested that histamine secreted from human peripheral basophilic leukocytes might be capable of autoinhibition through stimulation of cyclic AMP increases (13). Thus, it was of interest to investigate the cyclic nucleotide responses of human lung to the endogenous release of the mediators of anaphylaxis. Much as has been seen in the guinea pig (7–9), profound elevations of lung cyclic AMP and cyclic GMP accompanied mediator release. Histamine was responsible both for the cyclic nucleotide changes and for a portion of the prostaglandin synthesis that occurs with antigen challenge. Furthermore, the lung responses to histamine (either endogenous or exogenous) were found to be related to specific receptor interactions; stimulation of H-1 re-

[1] *Abbreviations used in this paper*: AA, arachidonic acid; ASA, acetylsalicylic acid; GMP, guanosine 3',5'-monophosphate; IR-$PGF_{2\alpha}$, immunoreactive $PGF_{2\alpha}$; RIA, radioimmunoassay; SRS-A, slow-reacting substance of anaphylaxis.

ceptors resulted in elevations of cyclic GMP and prostaglandin (PG)$F_{2\alpha}$ synthesis, whereas H-2 stimulation produced increases in cyclic AMP.

METHODS

Chemicals. Histamine diphosphate, acetylsalicylic acid, cyclic AMP, cyclic GMP, cyclic AMP-dependent protein kinase, phenol red, diphenhydramine, and trizma (Sigma Chemical Co., St. Louis, Mo.); [^{3}H]cyclic AMP (37.7 Ci/mmol), [8-^{14}C] cyclic AMP (45 mCi/mmol), [^{3}H]cyclic GMP (9.92 Ci/mmol), [^{3}H]histamine (5–10 Ci/mmol), [^{3}H]$PGF_{2\alpha}$ (100–150 Ci/mmol), and Aquasol (New England Nuclear, Boston, Mass.); [*methyl*-^{14}C]S-adenosyl-L-methionine (40–50 mCi/mmol) (Amersham/Searle Corp., Arlington Heights, Ill.); monospecific rabbit anti-cyclic GMP antisera and ^{125}I-2′-0-succinyl-cyclic GMP-tyrosine methyl ester (Collaborative Research Inc., Waltham, Mass.); lyophilized rabbit sera (ICN Pharmaceuticals Inc., Life Sciences Group, Cleveland, Ohio); goat anti-rabbit γ-globulin (N. L. Cappel Laboratories Inc., Cochranville, Pa.); rabbit anti-$PGF_{2\alpha}$ (Clinical Assays Inc., Cambridge, Mass.); silica gel 60 F-254 thin layer chromatography plates (EM Laboratories, Darmstadt, West Germany); cyclic nucleotide phosphodiesterase-beef heart (Boehringer Mannheim Biochemicals, Indianapolis, Ind.); pyrilamine maleate (Merck Sharp & Dohme, Div. Merck & Co., Inc., West Point, Pa.); ragweed antigen E (Research Resources Branch, National Institute of Allergy and Infectious Diseases, Bethesda, Md.); Agl-X8, 200–400 mesh, formate form (Bio-Rad Laboratories, Richmond, Calif.); Norit A (Fisher Scientific Co., Pittsburgh, Pa.); and dextran T-70 (Pharmacia Fine Chemicals, Uppsala, Sweden) were obtained from the manufacturers. The prostaglandins were kindly provided by Dr. John Pike (Upjohn Co., Kalamazoo, Mich.). The histamine antagonists metiamide and cimetidine as well as 2-methylhistamine, 4-methylhistamine, and dimaprit were kindly supplied by the Smith Kline & French Laboratories, Philadelphia, Pa. Histamine-N-methyl transferase was kindly provided by Dr. Michael Beaven, National Heart and Lung Institute, Bethesda, Md.

Buffers. Tyrode's buffer was employed throughout the experiments. This buffer consists of (in grams per liter): NaCl, 8.0; KCl, 0.2; NaH_2PO_4, 0.05; glucose, 1.0; $NaHCO_3$, 1.0; $CaCl_2$, 0.2; $MgCl_2$, 0.1; pH 7.8.

All agents under study were prepared in Tyrode's buffer just before use. The H-2 antagonists cimetidine and metiamide were dissolved in Tyrode's buffer containing 0.1 ml 0.1 N HCl and the pH was adjusted back to 7.8 with 0.1 N NaOH.

Preparation of human lung tissue for the antigen-induced release of mediator. Human lung tissue, obtained at the time of resection generally for cancer or bronchiectasis, was prepared for the antigen-induced release of histamine and slow-reacting substance of anaphylaxis (SRS-A) as previously described (12). Macroscopically normal areas of peripheral lung tissue were dissected free of pleura, large bronchi (>3–5 mm), and blood vessels, fragmented into ≅200-mg (wet wt) replicates, and washed extensively with Tyrode's buffer. The replicates were incubated for 2 h at 37°C in undiluted serum from a patient (F. P.) allergic to ragweed. The IgE-sensitized replicates were washed and placed in 3 ml of Tyrode's buffer at 37°C.

The experimental protocol generally employed consisted of: after an initial 10 min at 37°C, ragweed antigen E (0.5 μg/ml) was added to each sample to initiate mediator release. From 15 s to 60 min later, the lung fragments were transferred to 1 ml iced 10% perchloric acid or to 3 ml distilled water. The mediator-containing supernate was frozen in a dry ice:acetone bath and kept at −80°C until assayed for histamine, SRS-A, and prostaglandin content (usually within 24 h). In each triplicate group of samples, all three supernates were assayed for mediators, one replicate was employed for the determination of residual histamine, and two replicates were employed for cyclic nucleotide and protein content (14). The effects of pharmacologic agents were studied by incubating lung samples with the agent for an appropriate period (see text) before antigen challenge. All pharmacologic manipulations were studied in parallel with nonmanipulated (control) sets of lung samples.

In experiments not involving anaphylaxis, the tissue replicates were incubated for the appropriate interval with the antagonist or inhibitor under study and challenged with a stimulant for an appropriate time period (see text). The supernates and fragments were handled as above.

Bioassay of histamine and SRS-A. Histamine and SRS-A were quantitated by bioassay on the isolated, atropinized guinea pig ileum as described (15). Residual tissue histamine was extracted from the lung fragments into distilled H_2O by heating at 100°C for 5 min. The presence of acetylsalicylic acid (ASA) or H-2 antagonists had no effects on the bioassay. In contrast, H-1 antagonists prevented histamine's ability to contract the guinea pig ileum but did not interfere with the SRS-A assay. Preliminary analysis revealed that supernates that contained SRS-A did not lose activity through at least 7 days when rapidly frozen (−150°C) and maintained at −70°C.

Microenzyme assay of histamine. Samples collected in the presence of H-1 antagonists were analyzed by the microenzyme assay as described (16). Briefly, histamine is converted to [^{14}C]methylhistamine by incubation with [*methyl*-^{14}C]S-adenosyl methionine. The [^{14}C]methylhistamine is extracted into chloroform after alkalinization and is quantitated by liquid scintillation counting. Recovery is calculated on the basis of 10 nCi [^{3}H]histamine added to each sample at the outset. Neither of the H-1 antagonists employed in these experiments interfered with the enzyme assay of histamine. Multiple samples were quantitated by both bioassay and microenzyme assay. The results were equivalent (±10%) in all cases.

Net percent histamine released is determined by: (nanograms released − spontaneous release/nanograms released + residual) × 100. SRS-A is expressed as units released per gram lung tissue. 1 U of SRS-A causes a contraction of the guinea pig ileum equivalent in height to that induced by 5 ng/ml histamine. The average release of histamine was 16.7 ±1.5% (n = 14) and SRS-A was 2,400±901 U/g (n = 16).

Radioimmunoassay (RIA) of $PGF_{2\alpha}$. The prostaglandins were determined by RIA (17). The assay involved incubating 100 μl of unknown with 6,000 cpm [^{3}H]$PGF_{2\alpha}$ and 50 μl of rabbit anti-$PGF_{2\alpha}$ sera (diluted to permit binding of 35% of the [^{3}H]$PGF_{2\alpha}$) in a final volume of 450 μl Tris (0.012%)-NaCl (0.083%)-gelatin (0.1%) (pH 7.4) at 4°C for 12–16 h. The bound [^{3}H]$PGF_{2\alpha}$ was separated from the uncomplexed tracer after the addition of 0.5 ml iced Tris-NaCl-0.5% gelatin by adding 1.0 ml iced charcoal (0.25%)-dextran (0.025%) in Tris-NaCl buffer and incubating at 4°C for 20 min. After centrifugation (200 g; 4°C; 10 min), the supernate was decanted into scintillation vials, 10 ml Aquasol was added, and the radioactivity was determined in an LS-350 (Beckman Instruments, Inc., Fullerton, Calif.). The sensitivity of this assay is 10 pg.

The specificity of antisera employed was investigated and the amount of prostaglandin required to inhibit 50% binding of antiserum plus tritiated antigen was determined (in picograms): $PGF_{2\alpha}$, 44; $PGF_{1\alpha}$, 2,700; PGE_2, 140,000; 6-Keto $PGF_{1\alpha}$, 200,000; and PGA_2, arachidonic acid, PGE_1, and PGB_1, all >1,000,000. As an addition analysis of specificity, mediator-rich supernates were chromatographed on silica gel thin-layer chromatography plates developed in $CHCl_3$:methanol: acetic acid:H_2O (87:10:2:1) by employing a double-develop-

ment technique. The $PGF_{2\alpha}$ migrated in the same area as authentic $PGF_{2\alpha}$ and $PGF_{1\alpha}$ (R_f of $PGF_{2\alpha} = 0.386$ and $PGF_{1\alpha} = 0.401$). Deproteination, extraction, and chromatography (18) were all without significant effects upon the RIA of $PGF_{2\alpha}$. Therefore, the $PGF_{2\alpha}$ in lung supernates were determined without purification.

The data are expressed as picograms immunoreactive $PGF_{2\alpha}$ (IR-$PGF_{2\alpha}$) per milligram protein to indicate the crossreactivity with $PGF_{1\alpha}$ and to acknowledge the possibility that as yet unrecognized prostaglandins may also cross-react with the antibody employed. The IR-$PGF_{2\alpha}$ in the lung supernates was corrected for spontaneous generation in matched control samples. In kinetic experiments, spontaneous synthesis of $PGF_{2\alpha}$ is indicated in each figure and represents that quantity present in supernates of control specimens maintained for the longest time period under the experimental conditions. The mean peak quantity of IR-$PGF_{2\alpha}$ produced by anaphylaxis of human lung was 102.5±32 ng/g lung tissue or 410±130 pg/mg protein (n = 14).

Preparation of human lung tissue for the determination of cyclic nucleotide levels. The cyclic AMP and cyclic GMP levels in human lung tissue were determined as described (12). To the replicates in 10% perchloric acid, 3,000 cpm of [^{14}C]cyclic AMP (0.1 pmol) and 3,000 cpm of [^{3}H]cyclic GMP (0.033 pmol) were added as a recovery label. The fragments were homogenized for 30 s at 4,000 rpm (Polytron homogenator, Brinkmann Instruments, Inc., Westbury, N. Y.) and centrifuged at 400 *g* for 15 min. The precipitates were retained and digested in 2 ml 0.1 N NaOH for 48–72 h for protein determination (14) and the supernates were neutralized with 5 N KOH with phenol red as pH indicator. After centrifugation (200 *g* for 15 min), the supernate was applied to 1.5 × 0.7-cm columns of Ag-1X8, equilibrated in 0.1 N formic acid. The columns were washed with 10 ml distilled water followed by 10 ml 0.1 N formic acid, the cyclic AMP was eluted with 10 ml 1 N formic acid, and the cyclic GMP was eluted with 15 ml 4 N formic acid. The eluates were lyophilized and then resuspended in acetate buffer (1.0 ml, 50 mM, pH 4.0-cyclic AMP or 0.5 ml, 50 mM, pH 6.2-cyclic GMP). The cyclic AMP level was determined with the protein binding assay (19) and the cyclic GMP by RIA (20). The values for cyclic nucleotides are reported as picomoles per milligram protein. Incubation of representative samples with cyclic 3′,5′-nucleotide phosphodiesterase before assay produced >95% hydrolysis of both cyclic AMP and cyclic GMP in all cases.

Statistical analysis. Statistical significance was determined by the Student's t test. Variations of experimental samples from control samples with a P value of <0.05 were considered statistically significant. Statistical significance is indicated in the figures by the use of asterisks: *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$. The n in each result indicates the number of individual experiments pooled to generate the data. Each result is presented as the mean±SEM.

RESULTS

Relationship between mediator release and cyclic nucleotide levels. The time course of the immunologic release of histamine, SRS-A, and $PGF_{2\alpha}$ from 16 individual experiments is shown in Fig. 1 (upper panel). Histamine was detected after 1 min (31±9% of maximal release, n = 11), reached 81±9% (n = 9) of maximal release by 5 min, and 98±2.5% of maximal release at 45 min (n = 6). Thus, the bulk of histamine release is rapidly completed (5 min), although some additional slower release is evident thereafter. IR-$PGF_{2\alpha}$ levels were significantly increased 2 min after challenge (43±14% of maximal release, n = 5, $P < 0.05$), peaked between 4 and 15 min, and declined to <50% of maximum by 60 min. SRS-A was not detectable until 2 min after challenge, the quantity released achieved statistical significance at 4 min (29±5% of maximum, n = 5, $P < 0.001$), peaked at 45 min (96±4% of maximum, n = 4), and declined thereafter.

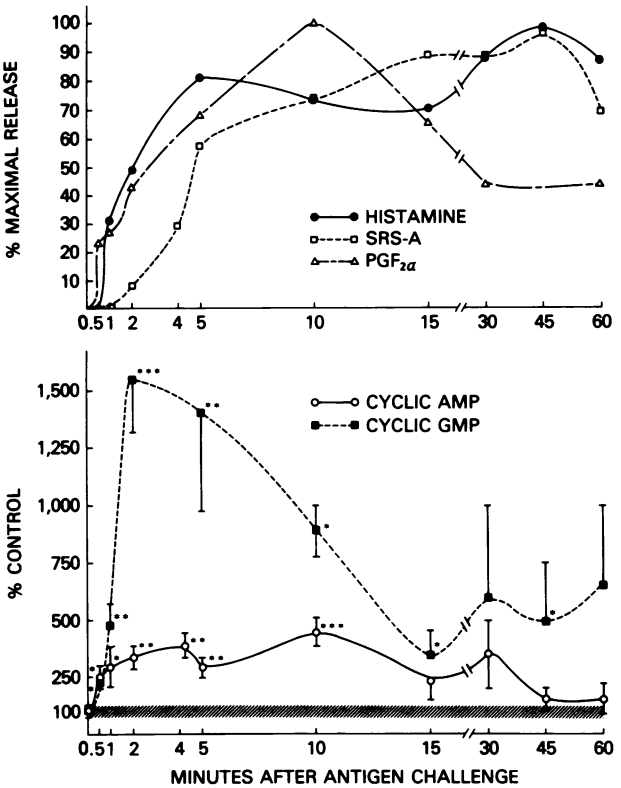

FIGURE 1 The time course of the immunologic release of mediators from human lung tissue and the concomitant changes in cyclic nucleotide levels. The mean release of histamine, $PGF_{2\alpha}$, and SRS-A after anaphylaxis is demonstrated along with the tissue levels of cyclic AMP and cyclic GMP (mean±SEM). The shaded area represents the control cyclic nucleotide levels: cyclic AMP = 5.1±1.4 pmol/mg protein and cyclic GMP = 0.89±0.2 pmol/mg protein. Maximal histamine release was 16.7±1.5% (n = 14), SRS-A release was 2,400±901 U/g (n = 16), and $PGF_{2\alpha}$ synthesis was 410±130 pg/mg protein (n = 14). The data represent the pooled observations from 16 separate experiments.

The cyclic nucleotide content of the lung fragments was simultaneously assessed (Fig. 1, lower panel). Cyclic GMP concentrations increased by 30 s, peaked at 2–5 min (15.2 times control levels), and returned toward base line thereafter. The base-line values of cyclic GMP were 0.89±0.20 pmol/mg protein (n = 10) which increased to an average maximum of 12.63±4.54 pmol/mg protein at 120 s after challenge (n = 7;

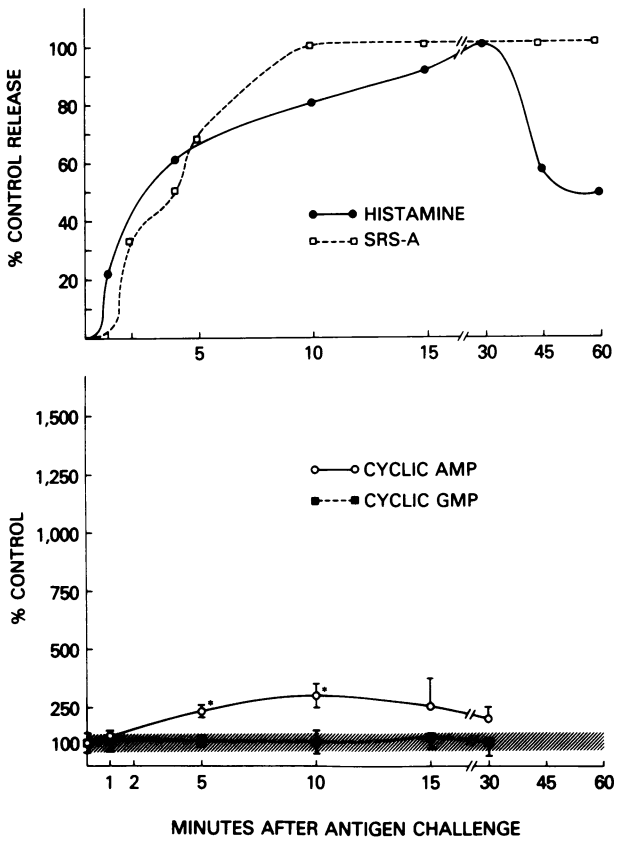

FIGURE 2 The effect of H-1 antagonists on the immunologic release of mediators from human lung tissue and the concomitant changes in cyclic nucleotide levels. The effects of incubation of IgE-sensitized lung fragments with 50 μM pyrilamine or diphenhydramine for 20 min before antigen challenge on the immunologic release of histamine and SRS-A and the changes in cyclic AMP and cyclic GMP are demonstrated. The shaded area represents the control cyclic nucleotide levels: cyclic AMP = 8.1±3.5 pmol/mg protein and cyclic GMP = 1.0±0.3 pmol/mg protein. Histamine release was 25.7±1.5% (n = 7) and SRS-A release was 1,333 ±640 U/g (n = 6). The data represent the pooled observations of eight separate experiments.

$P < 0.001$). The cyclic AMP concentrations also rose significantly within 30 s of anaphylaxis (2.5 times control levels, $n = 5, P < 0.05$) to a maximal increase of 3 to 4 times control levels between 2 and 10 min after anaphylaxis. The control levels for cyclic AMP were 5.1±1.4 pmol/mg protein (n = 12) and reached an average maximal increase of 22.44±3.06 pmol/mg protein ($n = 7, P < 0.001$) at 10 min after antigen challenge.

Histamine's actions can be separated into H-1 and H-2 responses (21) on the basis of selective receptor responses (22). H-1 effects include smooth muscle contraction and vascular permeability, whereas H-2 effects include gastric acid secretion and uterine contraction (22, 23). Pyrilamine and diphenhydramine are H-1 antagonists while cimetidine and metiamide are H-2 antagonists (23). The effects of the H-1 antagonists pyrilamine (50 μM; n = 6) and diphenhydramine (50 μM; n = 2) were examined (Fig. 2). Neither the pattern (Fig. 2, upper panel) nor the quantity of histamine or SRS-A released were significantly affected when compared to matched controls (Table I). Kinetic analysis (Fig. 2, lower panel) of the cyclic nucleotide responses revealed that the cyclic GMP elevation usually seen accompanying anaphylaxis was totally prevented by each of these H-1 antagonists, whereas the cyclic AMP response was muted but not prevented. Pyrilamine and diphenhydramine were equally effective.

The H-2 antagonists cimetidine and metiamide were next examined (Fig. 3). When an incubation of 20 min with 50-μM concentrations of these antagonists was employed, no significant alteration in either the pattern (Fig. 3, upper panel) or the amount (Table I) of mediator release was noted. The cyclic GMP response to anaphylaxis was also unchanged, whereas the cyclic AMP changes usually accompanying anaphylaxis were totally prevented (Fig. 3, lower panel and Table II). Both metiamide and cimetidine were equally effective.

The effects of ASA (10 μg/ml) (24, 25) upon the immunologic release of mediators and the concomitant cyclic nucleotide changes were examined (Fig. 4). The pattern (Fig. 4, upper panel) and the quantity (Table I) of histamine and SRS-A released were the same as in the absence of ASA, much as previously seen (12). In contrast, the increased synthesis of IR-$PGF_{2\alpha}$ usually

TABLE I
*The Effects of Antihistamines or ASA upon the Immunologic Release of Mediators**

Agent	Histamine		SRS-A		IR-$PGF_{2\alpha}$	
	% release	*n*	*U/g*	*n*	*% increase*‡	*n*
A None	22.1±0.9	7	1,075±500	6	550±117	8
H-1 antagonists	25.7±1.5		1,333±640		247±60 $P < 0.05$	
B None	18.5±2.0	7	1,177±451	9	640±110	9
H-2 antagonists	19.2±3.0		1,166±454		610±120	
C None	17.2±3.0	5	3,346±1,994	7	470±130	5
ASA	21.2±6.3		2,842±1,462		150±30 $P < 0.025$	

* The effects of the H-1 antagonists pyrilamine (50 μM, n = 6) or diphenydramine (n = 2), the H-2 antagonists cimetidine (50 μM, n = 6) or metiamide (n = 4), or the cyclooxygenase inhibitor ASA (10 μg/ml, n = 9) upon the immunologic release of mediators are compared with the untreated samples from the same individual experiments.

‡ The base-line values are: (A) none = 17.4±9.6 pg/mg protein, H-1 antagonists = 18.3±5.9; (B) none = 22.2±8.8, H-2 antagonists = 21.3 ±7.3; and (C) none = 22.1±8.2, ASA = 21.0±6.5. P values compare the differences between percent increases above base line of samples in the presence or absence of antihistamines or ASA.

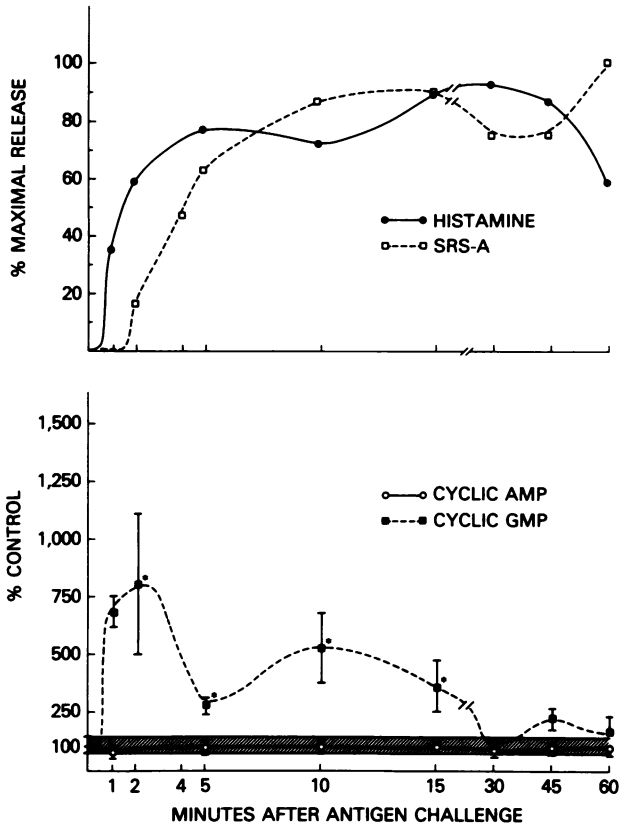

FIGURE 3 The effect of H-2 antagonists on the immunologic release of mediators from human lung tissue and the concomitant changes in cyclic nucleotide levels. The effects of 20-min incubations with 50 μM cimetidine or metiamide on the immunologic release of histamine and SRS-A and the changes in cyclic AMP and cyclic GMP are demonstrated. The shaded area represents the control cyclic nucleotide levels: cyclic AMP = 2.8±1.1 pmol/mg protein and cyclic GMP = 1.0±0.4 pmol/mg protein. Histamine release was 19.2±3.0% (n = 7) and SRS-A release was 1,166±454 U/g (n = 9). The data represent the pooled observations of nine separate experiments.

TABLE II

*The Effect of Antihistamines or ASA upon the Maximal Cyclic Nucleotides Increases Accompanying Anaphylaxis**

	Agent		Cyclic AMP	Cyclic GMP
		n	% increase‡	
A	None	6	440±108	796±182
	H-1 antagonists		350±92	152±41
				$P < 0.01$
B	None	5	380±60	1,230±382
	H-2 antagonists		110±10	870±280
			$P < 0.001$	
C	None	7	596±143	926±320
	ASA		880±70	1,292±480

* The effects of H-1 antagonists, H-2 antagonists, and ASA upon the maximum increases in cyclic AMP and cyclic GMP accompanying anaphylaxis are tabulated. These data represent the changes graphed kinetically on Figs. 2–4 (lower panels). The concentrations of antihistamines or ASA employed are the same as Table I. Each experimental manipulation is compared with the untreated samples from the same experiments.

‡ Base-line values for each set of figures are: (A) none (cyclic AMP) = 4.8±1.7 pmol/mg protein and (cyclic GMP) = 0.9 ±0.3; H-1 antagonists (cyclic AMP) = 8.1±3.5 and (cyclic GMP) = 1.0±0.3; (B) none (cyclic AMP) = 1.84±0.4 and (cyclic GMP) = 0.8±0.2; H-2 antagonists (cyclic AMP) = 2.8±1.1 and (cyclic GMP) = 1.0±0.4; (C) none (cyclic AMP) = 5.33±1.3 and (cyclic GMP) = 0.6±0.2; ASA (cyclic AMP) = 4.9±1.9 and (cyclic GMP) = 0.65±0.3.

accompanying anaphylaxis was significantly reduced by the incubation with ASA (Table I). In actual amounts, base-line IR-$PGF_{2\alpha}$ was 22.1±8.2 pg/mg protein and anaphylaxis induced an increase to 103.9±28.7 (n = 5; $P < 0.025$). After 30 min in 10 μg/ml ASA, base-line IR-$PGF_{2\alpha}$ levels were 21.0±6.5 pg/mg protein and anaphylaxis induced an increase to 31.5±6.3, (n = 5; $P > 0.30$) (Table I). The cyclic nucleotide pattern after anaphylaxis in the presence of ASA (Fig. 4, lower panel) was the same as matched control samples (Table II). The kinetic analysis of cyclic nucleotides was not carried out beyond 30 min after antigen challenge.

Effects of histamine upon lung cyclic nucleotides. The time course of the cyclic nucleotide response to 50 μM histamine was analyzed (Fig. 5). Within 30 s after the introduction of histamine, both cyclic GMP and cyclic AMP were elevated; peak responses were appreciated at 1–5 min and the levels returned to base line by 30 min. The peak increase in cyclic GMP was 5.8 times control and cyclic AMP was 4.4 times control. The peak effect of histamine appeared at 1–2.5 min. Therefore, a dose response of histamine (3–100 μM) was examined 1.0 min after stimulation. Histamine caused a significant ($P < 0.05$) increase in both cyclic AMP (240±15%, n = 5) and cyclic GMP (142±10%, n = 5) at 30 μM or more. The effects of antihistamines upon histamine-induced increases in cyclic nucleotides was studied (Fig. 6). The cyclic AMP increase produced by histamine was prevented by the H-2 antagonist cimetidine and the cyclic GMP increase was prevented by the H-1 antagonist pyrilamine.

Effects of histamine upon $PGF_{2\alpha}$ generation. In addition to preventing the cyclic GMP increase secondary to anaphylaxis, H-1 antagonists were also found to reduce the quantity of prostaglandins synthesized (Table I). Incubation of lung fragments with either pyrilamine (50 μM; n = 6) or diphenhydramine (50 μM, n = 2) for 20 min before immunologic challenge resulted in a 55% reduction in $PGF_{2\alpha}$ synthesis. This effect was consistent (seven out of seven experiments),

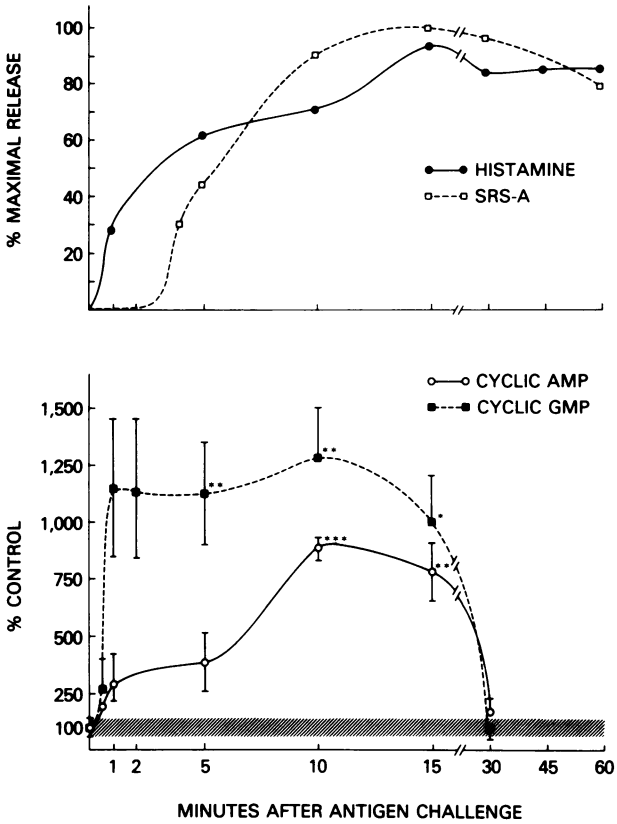

FIGURE 4 The effect of ASA on the immunologic release of mediators from human lung tissue and the concomitant changes in cyclic nucleotide levels. The effects of 30-min incubations with ASA (10 μg/ml) on the anaphylactic release of histamine and SRS-A and the changes in cyclic AMP and cyclic GMP are demonstrated. The shaded areas represent the control cyclic nucleotide levels: cyclic AMP = 4.9±1.9 pmol/mg protein and cyclic GMP = 0.65±0.3 pmol/mg protein. Histamine release was 21.2±6.3% (n = 5) and SRS-A release was 2,842±1,462 U/g. The data represent the pooled observations of seven separate experiments.

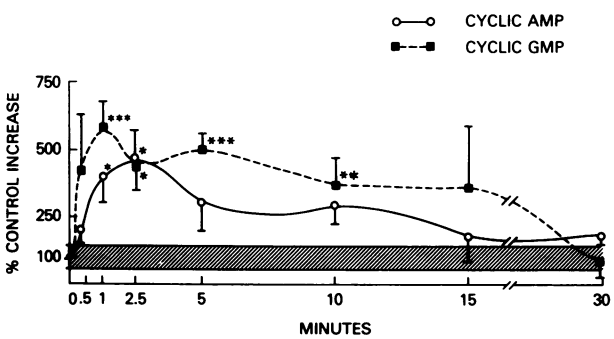

FIGURE 5 The time course of the effects of histamine on the cyclic nucleotide content of human lung tissue. The effect of 50 μM histamine on cyclic AMP and cyclic GMP is demonstrated. The shaded area represents control cyclic nucleotide levels: cyclic AMP = 11.1±5.0 pmol/mg protein and cyclic GMP = 0.9±0.4 pmol/mg protein. The data represent the pooled observations of eight separate experiments.

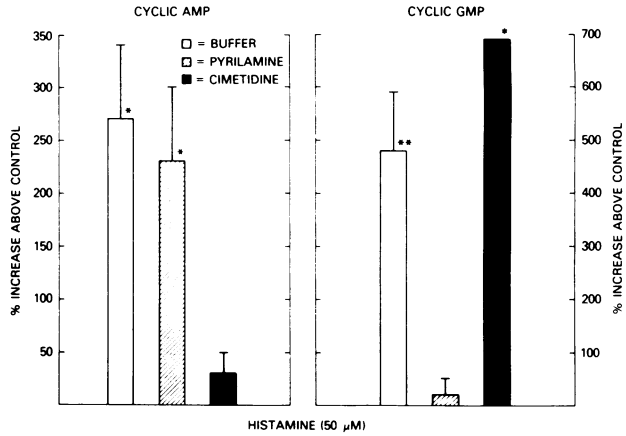

FIGURE 6 The maximal cyclic nucleotide responses of human lung tissue to histamine in the presence of antihistamines. The effect of histamine (50 μM) in the absence of antagonists (buffer) or in the presence of 50 μM pyrilamine or 50 μM cimetidine on cyclic AMP and cyclic GMP levels is demonstrated. The antagonists were added 20 min before histamine and the cyclic nucleotides measured 1 min after the introduction of histamine. Control value for cyclic AMP in the absence of antagonists was 5.8±1.8 pmol/mg protein, in the presence of pyrilamine was 5.2±3.0 pmol/mg protein, and in the presence of cimetidine was 4.6±1.0 pmol/mg protein. Control cyclic GMP value in the absence of antagonists was 0.9±0.3 pmol/mg protein, in the presence of pyrilamine was 0.5±0.2 pmol/mg protein, and in the presence of cimetidine was 0.7±0.1 pmol/mg protein. The data represent the average maximal increase above control pooled from observations obtained in 11 separate experiments.

ranged from 26 to 73% inhibition, was seen with two distinct H-1 antagonists, and was statistically significant ($P < 0.05$). The H-2 antagonists cimetidine and metiamide were without effect (Table I).

The capacity of exogenous histamine (1–100 μM) to induce $PGF_{2\alpha}$ synthesis was examined. Significant quantities of $PGF_{2\alpha}$ were generated by 10–100 μM histamine. The time course of $PGF_{2\alpha}$ synthesis in response to 50 μM histamine was determined (Fig. 7). Significant amounts of $PGF_{2\alpha}$ were induced 60 s after histamine stimulation, levels peaked at 15 min, and diminished thereafter. Incubation of the lung fragments with 50 μM cimetidine for 20 min failed to influence $PGF_{2\alpha}$ synthesis, whereas the H-1 antagonist pyrilamine (50 μM) completely prevented histamine-induced $PGF_{2\alpha}$ release.

Specific histamine agonists are available: 2-methylhistamine has 20% of the H-1 activity of histamine but only 2% of its H-2 activity (26). Dimaprit, on the other hand, has 20% of the H-2 activity and 0.001% of the H-1 activity (27). The capacity of these agonists (1–1,000 μM) to generate $PGF_{2\alpha}$ was, therefore, studied. Although 2-methylhistamine (10 μM or higher) generated significant quantities of $PGF_{2\alpha}$, dimaprit at no concentration caused increased $PGF_{2\alpha}$ synthesis.

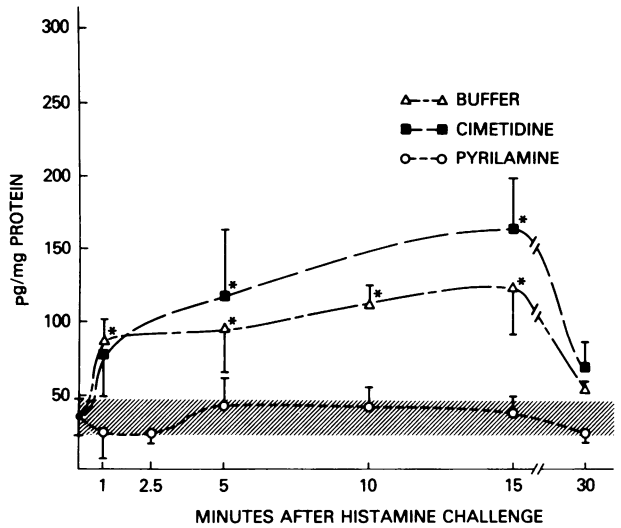

FIGURE 7 Kinetic analysis of the effect of antihistamines upon histamine-induced $PGF_{2\alpha}$ synthesis by human lung. The capacity of histamine (50 μM) to generate $PGF_{2\alpha}$ synthesis from human lung in buffer alone, in the presence of 50 μM cimetidine, or in the presence of 50 μM pyrilamine is presented from 1 to 30 min after histamine stimulation. Lung fragments were incubated with the antihistamines or in buffer for 20 min at 37°C before the addition of histamine. Control levels of 36.4±11.1 pg/mg protein (n = 7) are shown in the shaded area. The data represent the pooled observations of seven separate experiments.

The effects of pyrilamine on $PGF_{2\alpha}$ generation by added arachidonic acid (AA, 1 μg/ml) was studied to determine if the H-1 antagonist might have a nonspecific inhibitory capacity (Fig. 8). AA, the precursor

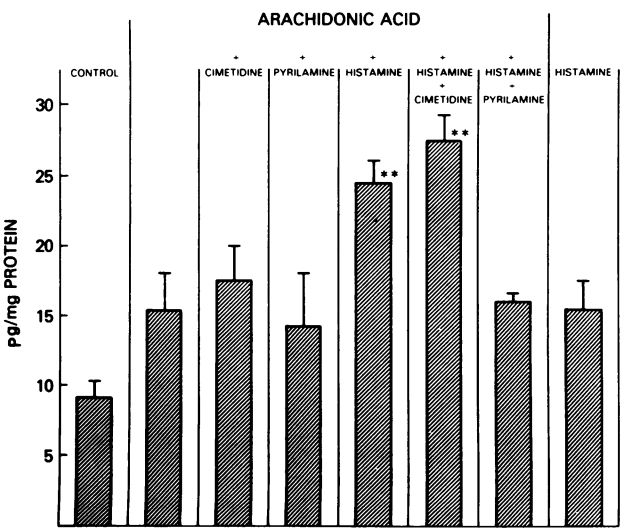

FIGURE 8 The effect of antihistamines upon histamine and (or) AA-induced $PGF_{2\alpha}$ synthesis by human lung. Lung fragments were incubated with 50 μM cimetidine or pyrilamine for 20 min before the introduction of either histamine (50 μM) or AA (1 μg/ml) or both agents. $PGF_{2\alpha}$ levels were determined 15 min later. The data represent the mean±SEM of an individual experiment.

molecule for prostaglandin formation, nonspecifically induces prostaglandin synthesis (28). $PGF_{2\alpha}$ was increased from 8.8 to 16 pg/mg protein by 1 μg/ml AA. The presence of neither pyrilamine nor cimetidine (both 50 μM) influenced the capacity of AA to generate increased $PGF_{2\alpha}$ synthesis. Histamine (50 μM), like AA, increased $PGF_{2\alpha}$ by 6 pg/mg protein and when combined with AA generated a greater than additive increase of 18.5 pg/mg protein ($P < 0.01$). Cimetidine failed to influence this increased production, whereas pyrilamine prevented a portion of the increased synthesis equivalent to that produced by histamine alone.

DISCUSSION

The IgE-dependent, antigen-induced secretion of the mediators of anaphylaxis from human lung is accompanied by significant increases in cyclic AMP and cyclic GMP. These changes occur simultaneously with the appearance of mediators and are related to histamine release. The evidence that suggests the causal role of histamine includes: (*a*) the capacity of histamine-receptor antagonists to prevent anaphylaxis-associated cyclic nucleotide changes, (*b*) the capacity of exogenously added histamine to reproduce these findings, and (*c*) the observations (7–10) that anaphylactically released histamine causes similar changes in guinea pig and dog lungs. While prostaglandins are capable of causing similar effects on the cyclic nucleotide levels of the human lung (11, 12), significant suppression of $PGF_{2\alpha}$ synthesis failed to prevent the phenomena.

Both endogenously released and exogenously added histamine stimulated increases in cyclic GMP, which were inhibited by two chemically distinct H-1 antagonists. Similarly, the histamine-induced increases in cyclic AMP were suppressed by two H-2 antagonists. The cyclic AMP increase accompanying anaphylaxis in the presence of H-1 antagonists appeared somewhat muted, although the maximum response was not significantly altered when compared to matched controls. A similar observation has been reported in guinea pig lung (7) and these findings suggest that a portion of the cell types in lung respond to H-1 stimulation with increases in cyclic AMP.

The prostaglandin synthesis accompanying lung anaphylaxis has been considered a secondary event (2, 29). A portion (≅50%) of this synthesis may be attributed to a secondary response to histamine interacting with H-1 receptor sites. This conclusion is based upon the following lines of evidence: (*a*) treatment of lung with H-1 antagonists significantly reduces the subsequent anaphylactically induced generation of $PGF_{2\alpha}$ while H-2 antagonists do not; (*b*) histamine, added exogenously, generates $PGF_{2\alpha}$ release from human lung and this effect is prevented by H-1 but not by H-2 receptor antagonists; and (*c*) H-1 agonists generate

$PGF_{2\alpha}$ whereas H-2 agonists fail to do so. Therefore, histamine stimulation of peripheral human lung tissue or guinea pig lung (30, 31), after either anaphylactic release from tissue mast cells or exogenous addition, causes $PGF_{2\alpha}$ synthesis through an interaction involving H-1 receptor stimulation. Some additional $PGF_{2\alpha}$ synthesis may occur directly from mast cells although isolated rat mast cells generate predominantly PGD_2 (32, 33) and little $PGF_{2\alpha}$ (33, 34).

The effect of nonsteroidal anti-inflammatory agents (25) on the immunologic generation of PG has been studied in several species including man (12, 35, 36). Agents such as ASA or indomethacin consistently suppress the synthesis of prostaglandins that accompany anaphylaxis. The effects of these agents on the release of the other mediators of anaphylaxis are less clear: the release of histamine has been reported as unaffected (12), inhibited (37), or enhanced (36), whereas SRS-A release may be unaffected (12), inhibited (28), or enhanced (35). Rat mononuclear cells stimulated with ionophore A-23187 synthesize a slow-reacting substance-like material through a cyclo-oxygenase pathway (38), whereas a rat basophilic tumor produces a similar material through a lipoxygenase pathway (39). It seems likely that antigen-induced SRS-A from human lungs is derived independently of ASA-sensitive cyclo-oxygenase enzymes but that the relationship to lipoxygenase enzymes needs a close evaluation.

One might have predicted that the selective increases in cyclic AMP or cyclic GMP seen after lung anaphylaxis in the presence of antihistamines would have resulted in modulation of mediator release. Histamine may have a cyclic AMP-dependent (13), H-2-mediated (40) autoinhibitory capacity in regard to basophil leukocyte histamine release. This observation may, in part, be species specific, because histamine is capable of inhibition of SRS-A release from bovine lung (41) but fails to influence mediator release from the rat peritonium (42). Histamine H-2 receptor antagonists augment reversed anaphylactic histamine release from monkey skin (41) but not from rat lung (43). The apparent enhancement of histamine release by H-2 antagonists may partly be a result of reduced histamine breakdown (44). In the present series of experiments, no alteration in either the pattern or the quantity of histamine or SRS-A release was appreciated after anaphylaxis in the presence of H-1 or H-2 antagonists. There are several possible explanations for this lack of effect: (*a*) the mast cell release reaction may be beyond the cyclic nucleotide-modulatable steps (3) before histamine-induced increases; (*b*) the mast cell may be experiencing simultaneous increases in cyclic AMP and cyclic GMP which negate each other; or (*c*) the lung mast cell may not be responsive to histamine.

Based upon these findings, mediator release in human lung might be expected to produce a profound generalized increase in the cyclic AMP and cyclic GMP concentrations of lung as well as inducing the bronchospasm, mucosal edema, and other events that contribute to asthma. It seems likely that individual variations exist in H-1 or H-2 responsiveness as has been observed in mice (45). Indeed, asthmatic subjects appear to have impaired H-2 responses (46). Therefore, antigen-induced mediator release in the lungs of an asthmatic subject might initiate a series of responses including a relatively selective increase in cyclic GMP levels. The pathologic consequences of such an alteration are not clear but conceivably might contribute to the asthmatic diathesis.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the skillful help of Russell Thompson in performing the radioenzyme assay of histamine, the advice of Michael Beaven, Ph.D., Peter Ramwell, M.D., and David Alling, M.D., Ph.D., and the excellent typing of the manuscript by Mrs. Patricia Silva and Ms. Susan Bolotsky.

REFERENCES

1. Kaliner, M., J. Blennerhassett, and K. F. Austen. 1976. Bronchial asthma. *In* Textbook of Immunopathology. H. J. Muller-Eberhard and P. A. Meischer, editors. Grune & Stratton, Inc., New York. 387–401.
2. Austen, K. F., and R. P. Orange. 1975. Bronchial asthma: The possible role of the chemical mediators of immediate hypersensitivity in the pathogenesis of subacute chronic disease. *Am. Rev. Respir. Dis.* **112:** 423–436.
3. Kaliner, M., and K. F. Austen. 1973. A sequence of biochemical events in the antigen-induced release of chemical mediators from sensitized human lung tissue. *J. Exp. Med.* **138:** 1077–1094.
4. Orange, R. P., M. A. Kaliner, P. J. LaRaia, and K. F. Austen. 1971. Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. II. Influence of cellular levels of cyclic AMP. *Fed. Proc.* **30:** 1725–1729.
5. Kaliner, M., R. P. Orange, and K. F. Austen. 1972. Immunological release of histamine and slow reacting substance of anaphylaxis from human lung. IV. Enhancement by cholinergic and alpha adrenergic stimulation. *J. Exp. Med.* **136:** 556–567.
6. Kaliner, M. 1977. Human lung tissue and anaphylaxis: Evidence that cyclic nucleotides modulate the immunologic release of mediators through effects on microtubular assembly. *J. Clin. Invest.* **60:** 951–959.
7. Mathé, A. A., L. Volicer, and S. K. Puri. 1974. Effects of anaphylaxis and histamine, pyrilamine and burimamide on levels of cyclic AMP and cyclic GMP in guinea pig lung. *Res. Commun. Chem. Pathol. Pharmacol.* **8:** 635–651.
8. Barrett-Bee, K. J., and L. R. Green. 1975. The relationship between prostaglandin release and lung c-AMP during anaphylaxis in the guinea pig. *Prostaglandins.* **10:** 589–598.
9. Mathé, A. A., S. S. Yen, R. Sohn, and P. Hedqvist. 1977. Release of prostaglandins and histamine from sensi-

tized and anaphylactic guinea pig lungs, changes in cyclic AMP levels. *Biochem. Pharmacol.* **26:** 181–188.
10. Gold, W. M. 1977. Neurohormonal interactions in asthma. *Am. Rev. Respir. Dis.* **115:** 127–137.
11. Tauber, A. I., M. Kaliner, D. J. Stechshulte, and K. F. Austen. 1973. Immunologic release of histamine and slow reacting substance of anaphylaxis from human lung. V. Effect of prostaglandins on release of histamine. *J. Immunol.* **111:** 27–32.
12. Kaliner, M. 1977. Human lung tissue and anaphylaxis. I. The role of cyclic GMP as a modulator of the immunologically-induced secretory process. *J. Allergy Clin. Immunol.* **60:** 204–211.
13. Bourne, H. R., K. L. Melmon, and L. M. Lichtenstein. 1973. Histamine augments leukocyte cyclic AMP and blocks antigenic histamine release. *Science (Wash. D. C.).* **173:** 743–744.
14. Lowry, O. H., N. J. Rosenbrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
15. Stechschulte, D. J., K. F. Austen, and K. J. Bloch. 1967. Antibodies involved in antigen-induced release of slow reacting substance of anaphylaxis (SRS-A) in the guinea pig and rat. *J. Exp. Med.* **125:** 127–147.
16. Beaven, M. A., S. Jacobsen, and Z. Horáková. 1972. Modification of the enzymatic isotopic assay of histamine and its application to measurement of histamine in tissues, serum and urine. *Clin. Chim. Acta.* **37:** 91–103.
17. Jaffe, B. M., J. W. Smith, W. T. Newton, and C. W. Parker. 1971. Radioimmunoassay for prostaglandins. *Science (Wash. D. C.).* **171:** 494–496.
18. Jaffe, B. M., H. R. Behrman, and C. W. Parker. 1973. Radioimmunoassay measurement of Prostaglandins E, A, and F in human plasma. *J. Clin. Invest.* **52:** 398–405.
19. Gilman, A. G. 1970. A protein binding assay for adenosine 3′,5′-cyclic monophosphate. *Proc. Natl. Acad. Sci. U. S. A.* **67:** 305–312.
20. Steiner, A. L., R. E. Wehmann, C. W. Parker, and D. M. Kipnis. 1972. Radioimmunoassay for the measurement of cyclic nucleotides. *Adv. Cyclic Nucleotide Res.* **2:** 51–63.
21. Ash, A. S. F., and H. O. Schild. 1966. Receptors mediating some actions of histamine. *Br. J. Pharmacol.* **27:** 427–439.
22. Chand, N., and P. Eyre. 1975. Classification and biological distribution of histamine receptor sub-types. *Agents Actions.* **5:** 277–295.
23. Beaven, M. A. 1976. Histamine. *N. Engl. J. Med.* **294:** 30–36, 320–325.
24. Piper, P. J., and J. R. Vane. 1969. Release of additional factors on anaphylaxis and its antagonism by anti-inflammatory drugs. *Nat. New Biol.* **223:** 29–35.
25. Vane, J. R. 1971. A mechanism of action for aspirin-like drugs: the inhibition of prostaglandin synthesis. *Nat. New Biol.* **231:** 232–235.
26. Black, J. W., W. A. M. Duncan, C. J. Durant, C. R. Ganellin, and E. M. Parsons. 1972. Definition and antagonism of histamine H_2-receptors. *Nature (Lond.).* **236:** 385–390.
27. Parsons, M. E., D. A. A. Owen, C. R. Ganellin, and C. J. Durant. 1977. Dimaprit-[S-[3-(N,N-dimethylamino)-propyl]isothiourea]-a highly specific histamine H_2-receptor agonist. Part I. Pharmacology. *Agents Actions.* **7:** 31–37.
28. Vargaftig, B. B., and N. Dao-Hai. 1971. Release of vasoactive substances from guinea pig lungs by slow reacting substance C and arachidonic acid. *Pharmacology (Basel).* **6:** 99–108.
29. Piper, P. J. and J. L. Walker. 1973. The release of spasmogenic substances from chopped human lung tissue and its inhibition. *Br. J. Pharmacol.* **47:** 291–304.
30. Liebig, R., W. Bernauer, and B. A. Peskar. 1974. Release of prostaglandins, a prostaglandin metabolite, slow reacting substance and histamine from anaphylactic lungs, and its modification by catecholamines. *Arch. Pharm. (Weinheim).* **284:** 279–293.
31. Yen, S. S., A. A. Mathé, and J. J. Dugan. 1976. Release of prostaglandins from healthy and sensitized guinea pig lung and trachea by histamine. *Prostaglandins.* **11:** 227–239.
32. Jakschik, B. A., C. W. Parker, and P. Needleman. 1978. Production of prostaglandin (PG)D_2 by rat basophilic leukemia (RBL-1) and purified rat mast cells. *Fed. Proc.* **37:** 384. (Abstr.)
33. Roberts, L. J., R. A. Lewis, R. Hansbrough, K. F. Austen, and J. A. Oates. 1978. Biosynthesis of prostaglandins, thromboxanes and 12-hydroxy-5,8,10,14-eicosatetraenoic acid by rat mast cells. *Fed. Proc.* **37:** 384. (Abstr.)
34. Strandberg, K., A. A. Mathé, and S. S. Yen. 1977. Release of histamine and formation of prostaglandins in human lung tissue and rat mast cells. *Int. Arch. Allergy Appl. Immunol.* **53:** 520–529.
35. Walker, J. L. 1973. The regulatory function of prostaglandins in the release of histamine and SRS-A from passively sensitized human lung tissue. *Adv. Biosci.* **9:** 235–240.
36. Dawson, W., and W. J. F. Sweatman. 1975. Probable role of prostaglandins in asthma. *Int. Arch. Allergy Appl. Immunol.* **49:** 213–216.
37. Gryglewski, R. J., B. Panczeko, R. Korbut, L. Grodzinska, and A. Ocetkiewicz. 1975. Corticosteroids inhibit prostaglandin release from perfused mesenteric blood vessels of rabbit and from perfused lungs of sensitized guinea pig. *Prostaglandins.* **10:** 343–351.
38. Bach, M. K., J. R. Brashler, and R. R. Gorman. 1977. On the structure of slow reacting substance of anaphylaxis: Evidence of biosynthesis from arachidonic acid. *Prostaglandins.* **14:** 21–38.
39. Jakschek, B. A., S. F. Alkenheim, and C. W. Parker. 1977. Precursor role of arachidonic acid in release of slow reacting substance from rat basophilic leukemia cells. *Proc. Natl. Acad. Sci. U. S. A.* **74:** 4577–4581.
40. Lichtenstein, L. M., and E. Gillespie. 1973. Inhibition of histamine release by histamine controlled by H-2 receptor. *Nature (Lond.).* **244:** 287–288.
41. Burka, J. F., and P. Eyre. 1976. Modulation of the release of SRS-A from bovine lung *in vitro* by several autonomic and autocoid agents. *Int. Arch. Allergy Appl. Immunol.* **50:** 664–673.
42. Smith, H., B. A. Spicer, and J. W. Ross. 1977. Further studies on passive peritoneal anaphylaxis in the rat. *Int. Arch. Allergy Appl. Immunol.* **54:** 414–421.
43. Chakrin, L. W., R. D. Krell, J. Mengel, D. Young, C. Zaker, and J. R. Wardell, Jr. 1974. Effect of a histamine H_2-receptor antagonist on immunologically induced mediator release *in vitro*. *Agents Actions.* **4:** 297–303.
44. Yamamoto, S., D. Francis, and M. W. Greaves. 1976. *In vitro* anaphylaxis on guinea pig skin: amplification by burimamide. *J. Invest. Dermatol.* **67:** 696–699.
45. Vaz, N. M., C. M. De Souze, M. M. Hornbrook, D. G. Hanson, and N. R. Lynch. 1977. Sensitivity to intravenous injections of histamine and serotonin in inbred mouse strains. *Int. Arch. Allergy Appl. Immunol.* **53:** 545–554.
46. Busse, W. W., and J. Sosman. 1977. Decreased H-2 histamine response of granulocytes of asthmatic patients. *J. Clin. Invest.* **59:** 1080–1087.