A B S T R A C T  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)F₂α, 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 PGF₂α 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 antagonists prevented the cyclic AMP response to histamine, whereas H-2 antagonists prevented the cyclic AMP response.

H-1 antagonists prevented 50% of the PGF₂α synthesis accompanying anaphylaxis; H-2 antagonists had no effect. Exogenous histamine induced PGF₂α 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 PGF₂α was not influenced by antihistamines. Therefore, histamine interactions with human lung result in the synthesis of both PGF₂α and cyclic GMP in response to H-1 stimulation, and of cyclic AMP through H-2 stimulation.

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) 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-

**Abbreviations used in this paper:** AA, arachidonic acid; ASA, acetylsalicylic acid; GMP, guanosine 3',5'-monophosphate; IR-PGF₂α, immunoreactive PGF₂α; RIA, radioimmunoassay; SRS-A, slow-reacting substance of anaphylaxis.
ceptors resulted in elevations of cyclic GMP and prostaglandin (PG)F<sub>2α</sub> 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.); [3H]cyclic AMP (37.7 Ci/mmol), [8-14C]cyclic AMP (45 mCi/mmol), [2H]cyclic GMP (9.92 Ci/mmol), [3H]histamine (5–10 Ci/mmol), [3H]PGF<sub>2α</sub> (100–150 Ci/mmol), and Aquasol (New England Nuclear, Boston, Mass.); [methylyl-3H]adenosyl-L-methionine (40–50 mCi/mmol) (Amersham/Searle Corp., Arlington Heights, Ill.); monospecific rabbit anti-cyclic GMP antisera and 125I-2'-0-succinyl-cyclic GMP-tyrosine methyl ester (Collaborative Research Inc., Waltham, Mass.); iophosphor rabbit sera (ICN Pharmaceuticals Inc., Life Sciences Group, Cleveland, Ohio); goat anti-rabbit γ-globulin (N. L. Cappel Laboratories Inc., Cochrannie, Pa.); rabbit anti-PGF<sub>2α</sub> (Clinical Assays Inc., Cambridge, Mass.); silica gel 60 F-254 thin layer chromatography plates (EM Laboratories, Darmsstadt, West Germany); cyclic nucleotide phosphodiesterase-beef heart (Boehringer Mannheim Biochemicals, Indianapolis, Ind.); pyrilamine maleate (Merek 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-X, 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 is composed of (in grams per liter): NaCl, 8.0; KCl, 0.2; NaH<sub>2</sub>PO<sub>4</sub>, 0.05; glucose, 1.0; NaHCO<sub>3</sub>, 1.0; CaCl<sub>2</sub>, 0.2; MgCl<sub>2</sub>, 0.1; pH 7.8. All agents under study were prepared in Tyrode’s buffer just before use. The H-2 antagonists metiamide 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). Macrocopically normal areas of peripheral lung tissue were dissected free of pleura, large bronchi (>3–5 mm), and blood vessels, fragmented into 200–400 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 then assayed as described 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<sub>2</sub>O 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.

**Microenzymassay 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 [3H]methylhistamine by incubation with [methylyl-3H]adenosyl methionine. The [3H]methylhistamine is extracted into chloroform after alkalization and is quantitated by liquid scintillation counting. Recovery is calculated on the basis of 10 nCi [3H]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 microenzymassay 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. I 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 ± 90 I U (n = 16).

**Radioimmunoassay (RIA) of PGF<sub>2α</sub>.** The prostaglandins were determined by RIA (17). The assay involved incubating 100 μl of unknown with 6,000 cpm [3H]PGF<sub>2α</sub> and 50 μl of rabbit anti-PGF<sub>2α</sub> sera (diluted to permit binding of 35% of the [3H]PGF<sub>2α</sub> 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 [3H]PGF<sub>2α</sub> 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<sub>2α</sub>, 24 ± 10; PGE<sub>2</sub>, 3,700; PGE<sub>1</sub>, 140,000; 6-Keto-PGF<sub>1α</sub>, 200,000; and PGE<sub>2</sub> and PGF<sub>2α</sub> 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<sub>3</sub>-methanol: acetic acid:H<sub>2</sub>O (87:10:2:1) by employing a double-develop-
Cyclic nucleotides, Prostaglandins, and Anaphylaxis

RESULTS

Relationship between mediator release and cyclic nucleotide levels. The time course of the immunologic release of histamine, SRS-A, and PGF2α from 16 individual experiments is shown in Fig. 1 (upper panel). Histamine was detected after 1 min (31±5% 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-PGF2α 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.

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;
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 I). 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-PGF2α usually

### Table I

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

<table>
<thead>
<tr>
<th>Agent</th>
<th>Histamine</th>
<th>SRS-A</th>
<th>IR-PGFα</th>
<th>% release</th>
<th>U/g</th>
<th>% increase</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>A None</td>
<td>22.1±0.9</td>
<td>7</td>
<td>1,075±500</td>
<td>6</td>
<td>550±117</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>H-1 antagonists</td>
<td>25.7±1.5</td>
<td>1,333±640</td>
<td>247±60</td>
<td>P &lt; 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B None</td>
<td>18.5±2.0</td>
<td>7</td>
<td>1,177±451</td>
<td>9</td>
<td>640±110</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>H-2 antagonists</td>
<td>19.2±3.0</td>
<td>1,166±454</td>
<td>610±120</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C None</td>
<td>17.2±3.0</td>
<td>5</td>
<td>3,346±1,994</td>
<td>7</td>
<td>470±130</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>ASA</td>
<td>21.2±6.3</td>
<td>2,842±1,462</td>
<td>150±30</td>
<td>P &lt; 0.025</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The effects of the H-1 antagonists pyrilamine (50 μM, n = 6) or diphenhydramine (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±6.8, 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.

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 22.4±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).
accompanying anaphylaxis was significantly reduced by the incubation with ASA (Table I). In actual amounts, base-line IR-PGF2α 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, baseline IR-PGF2α 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 PGF2α 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 PGF2α synthesis. This effect was consistent (seven out of seven experiments),
The pooled observations of eight separate experiments. The shaded area demonstrated.

**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. The data represent the average maximal increase above control pooled from observations obtained in 11 separate experiments.

The capacity of exogenous histamine (1–100 μM) to induce PGF<sub>20</sub> synthesis was examined. Significant quantities of PGF<sub>20</sub> were generated by 10–100 μM histamine. The time course of PGF<sub>20</sub> synthesis in response to 50 μM histamine was determined (Fig. 7). Significant amounts of PGF<sub>20</sub> 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<sub>20</sub> synthesis, whereas the H-2 antagonist pyrilamine (50 μM) completely prevented histamine-induced PGF<sub>20</sub> 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<sub>20</sub> was, therefore, studied. Although 2-methylhistamine (10 μM or higher) generated significant quantities of PGF<sub>20</sub>, dimaprit at no concentration caused increased PGF<sub>20</sub> synthesis.
molecule for prostaglandin formation, nonspecifically induces prostaglandin synthesis (28). PGF₂α 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 PGF₂α synthesis. Histamine (50 μM), like AA, increased PGF₂α 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 PGF₂α 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 PGF₂α while H-2 antagonists do not; (b) histamine, added exogenously, generates PGF₂α 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
PGF₂α, 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 PGF₂α synthesis through an interaction involving H-1 receptor stimulation. Some additional PGF₂α synthesis may occur directly from mast cells although isolated rat mast cells generate predominantly PGD₂ (32, 33) and little PGF₂α (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 peritoneum (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.

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REFERENCES

alyzed and anaphylactic guinea pig lungs, changes in cyclic AMP levels. *Biochem. Pharmacol.* 26: 181–188.


