STUDIES ON THE RELEASE OF LEUKOTRIENES AND HISTAMINE BY HUMAN LUNG PARENCHYMAL AND BRONCHIAL FRAGMENTS UPON IMMUNOLOGIC AND NONIMMUNOLOGIC STIMULATION

Effects of Nordihydroguaiaretic Acid, Aspirin, and Sodium Cromoglycate

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It now appears that slow-reacting substances of anaphylaxis (SRS-A)[1] and histamine are the most important mediators of bronchoconstriction in asthmatic symptoms (1–3). SRS-A have recently been shown to be LTC_4, LTD_4, and LTE_4 derived from arachidonic acid via the 5-lipoxygenase pathway (4, 5). Another important metabolite of this pathway is LTB_4 (6, 7). Studies on the biological activities of these metabolites suggested their involvement in several allergic and inflammatory diseases. The spasmogenic activity of LTC_4 and LTD_4 on guinea pig lung parenchymal strips is ~200–20,000-fold greater than that of histamine; on guinea pig trachea, they are 30 and 100 times, respectively, more potent than histamine (8). Furthermore, LTC_4 and LTD_4 have been shown to cause contraction of human bronchi in vitro and in vivo (9–13). In addition, LTC_4 and LTD_4 have been shown to increase mucous secretion in canine trachea (14) and human bronchia mucosa (15, 16). LTB_4 was shown to be a potent chemotactic and degranulating substance towards polymorphonuclear leukocytes studied in vivo and in vitro (17, 18). Synthesis of leukotrienes has been reported in several cells and tissues including human and guinea pig lung tissues (19). In human lung tissue, the major source of leukotrienes has been suggested to be the mast cells and the macrophages (20, 21). Nevertheless, a number of other cell types are located in either bronchioli or lung parenchyma, and their role in allergic symptoms is not known. Therefore, it is important to investigate the release of mediators of allergic reaction in both tissues to obtain a better understanding of the role of these mediators in allergic reactions in vivo. This investigation was

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[1] *Abbreviations used in this paper:* GC-MS, gas chromatography–mass spectrometry; Hete, hydroxyeicosatetraenoic acid; HPLC, high performance liquid chromatography; LT, leukotriene; NDGA, nordihydroguaiaretic acid; PBS, phosphate-buffered saline; PG, prostaglandin; RAST, radioallergosorbent test; SRS-A, slow-reacting substance of anaphylaxis.

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designed to provide a comprehensive study of the quantitative nature and the time course relationships between the release of 5-lipoxygenase metabolites of arachidonic acid and of histamine in both human lung parenchyma and bronchi after immunologic or nonimmunologic challenge. In addition, the action of a widely used antiallergic drug (sodium cromoglycate) on the release of histamine and leukotrienes was investigated and compared with the actions of a 5-lipoxygenase inhibitor (nordihydroguaiaretic acid [NDGA]) and a cyclooxygenase inhibitor (aspirin) in both allergen- and ionophore-stimulated human lung parenchyma.

Materials and Methods

Materials. High performance liquid chromatography (HPLC) grade organic solvents were purchased from Anachemia Chemical Co. (Montreal) and glass-distilled before use. [1-^{14}C]Arachidonic acid (50 mCi/mmol), [^{3}H]LTB_4 (100 Ci/mmol), [^{3}H]LTC_4 (20–60 Ci/mmol), and *S*-adenosyl-L-[methyl-^{3}H]methionine (75–85 Ci/mmol) were purchased from Amersham Corp. (Oakville, Ontario). Histamine was purchased from Sigma Chemical Company (St. Louis, MO), arachidonic acid from NuChek Prep Inc. (Elysian, MN), and ionophore A23187 from Calbiochem-Behring Corp. (San Diego, CA). FPL55712 was obtained from Fison Pharmaceutical Ltd. (Loughborough, England). Synthetic leukotrienes were kindly provided by Dr. Rokach (Merck Frosst Laboratory, Montreal). Histamine *N*-methyl-transferase was purified from rat kidneys as reported (22). Timothy allergen was obtained from Allergopharma Joachim (Ganzer KG D-2057; Reinbek, Federal Republic of Germany) and purified by dialysis before use.

Preparation of Human Lung Parenchymal and Bronchial Fragments. Lung specimens were obtained from patients undergoing surgery for lung carcinoma. Histologically normal tissue was used for this investigation. The parenchyma was separated from bronchi by surgical blades. Both the parenchyma and bronchioli (2–5 mm diam) were chopped finely into fragments of ~2 mm^2. The tissues were then washed five times with phosphate-buffered saline (PBS) and kept on ice until use.

Passive Sensitization of Parenchyma and Bronchi. Fragments of parenchyma or bronchi were incubated with serum from timothy-positive allergic patients (radioallergosorbent test [RAST], 30–40%) for 3 h at 37°C (1 ml of serum for 2 g of tissue wet weight and 5 ml of PBS) under an atmosphere of 95% O_2/5% CO_2 with constant shaking. Control tissues were incubated in the same manner with serum from timothy-negative subjects (demonstrating a negative skin test).

Incubation and Extraction Procedures. Passively sensitized or nonsensitized tissue fragments were washed five times to remove serum and incubated either with 0.5 μg/ml of timothy allergen (1 g tissue wet weight/10 ml of PBS) or ionophore A23187 (4 μM final concentration) for different periods of time. In some cases, the tissue fragments were incubated with both ionophore A23187 (4 μM final concentration) and arachidonic acid (30 μM final concentration). The incubations were ended by addition of 1 vol methanol containing prostaglandin B2 (PGB_2) (150 ng) as an internal standard. The incubation media were centrifuged (3000 *g*, 30 min) and the supernatant fluids were concentrated in vacuo. The residues were resuspended in 5 ml of 20% methanol and acidified to pH 3. The samples were then passed through a cartridge of octadecylsilyl silica (SEP-PAK, C18 cartridge; Waters Associates, Millipore Corp., Milford, MA). The extraction procedure was as reported (23) except that the metabolites of arachidonic acid were eluted with 10 ml of 90% methanol. Using this method of extraction, the recovery of [^{3}H]LTB_4 (1.7×10^5 dpm; mass, 34 ng) and of [^{3}H]LTC_4 (1.6×10^5 dpm; mass, 39 ng) were 84 ± 2.6% and 73 ± 3.1%, respectively (mean ± SEM; $n = 5$).

Reverse Phase HPLC. Chromatography was performed using a C_{18}–Radial Pak cartridge (100 × 8 mm inside diameter, 10 μm particle size; Waters Associates) as reported (24), with a modified gradient. The metabolites of arachidonic acid were detected by ultraviolet spectrophotometry at 280 and 229 nm and quantitated by comparing the areas

of their peaks with that of the internal standard (PGB_2) and correcting for differences in molar extinction coefficients and attenuation settings (25). For further confirmation of the identity of leukotrienes, their biological activities were tested on contractions of guinea pig parenchymal strips or ileum and by the incorporation of ^{14}C from [1-^{14}C]arachidonic acid. Further identification of hydroxy-eicosatetraenoic acid (Hete) and LTB_4 were carried out by gas chromatography–mass spectrometry (GC-MS) as reported (26).

Bioassay. The HPLC fractions corresponding to LTB_4, LTC_4, and LTD_4 were collected and evaporated to dryness in vacuo. The residues were collected in 250 μl of PBS containing 1% ethanol. Various fractions of these samples were added to 10 ml of oxygenated Krebs or Tyrode's buffer in an organ bath containing a suspended guinea pig lung parenchymal strip or ileum, as reported (27). The contraction of organs by leukotrienes was detected isometrically by a force displacement transducer and registered on a physiograph (desk model DMP-4A; Narco Bio-Systems, Inc., Houston, TX). For further confirmation of the contractions induced by the HPLC-eluted materials, the synthetic leukotrienes were used as references.

Histamine Assay. Evaluation of the amount of histamine in the incubations was carried out using a radioenzymatic assay as reported (22). Briefly, 25 μl of sample was added to glass tubes containing 25 μl of 0.5 M phosphate buffer, pH 7.8. A further 25 μl of 0.5 M phosphate buffer, pH 7.8, containing *S*-adenosyl-L-[methyl-^{3}H]methionine (1.25 μCi total) and 6 μg of histamine *N*-methyl-transferase was added to each tube. This mixture was incubated on ice for 1 h and the reaction was stopped by the addition of 1 vol of potassium borate (pH 11). After two organic solvent extractions, 125 μl of the extracted solution was counted in a β liquid scintillation spectrometer and the amount of histamine in each sample was determined by comparison with a standard curve obtained from known amounts of histamine.

Results are expressed as mean ± SEM. The statistical significance of differences between control and stimulated samples was determined using Student's *t* test.

Results

As shown in Fig. 1 *A*, unstimulated human lung parenchyma did not contain any significant amount of lipoxygenase metabolites. However, when fragments of human lung parenchyma were stimulated with ionophore A23187 and arachidonic acid, a number of lipoxygenase metabolites of arachidonic acid were detected (Fig. 1 *B*). Their identities were further confirmed by incorporation of ^{14}C from [1-^{14}C]arachidonic acid. Several unknown peaks are also seen on the chromatogram that did not correspond to any known metabolite of arachidonic acid. The peak absorbance at 280 and 229 nm with the retention time of ~51 min corresponded to the ionophore A23187. When fragments of human lung parenchyma were stimulated with ionophore A23187 alone, ω-COOH-LTB_4, ω-OH-LTB_4, LTB_4, 5-Hete, LTC_4, LTE_4, and LTD_4 were detected in incubation media. Small amounts of 12-Hete and 15-Hete were occasionally detected (Fig. 1 *C*). Similarly, when fragments of passively sensitized human lung parenchyma were challenged with timothy allergen, LTB_4, LTC_4, LTD_4, LTE_4, and 5-Hete were the major metabolites detected but in amounts less than the amounts detected after ionophore stimulation (Fig. 1 *D*). To further confirm the presence of LTB_4, LTC_4, and LTD_4, we tested the biological activity of the HPLC-eluted materials corresponding to each leukotriene on the contraction of guinea pig lung parenchymal strips and ileum. All three leukotrienes induced the contraction of guinea pig lung parenchymal strips comparable to that induced by standards (data not shown). LTD_4 induced contraction of both guinea pig lung parenchymal strips and of ileum, and its contractile activity was diminished when

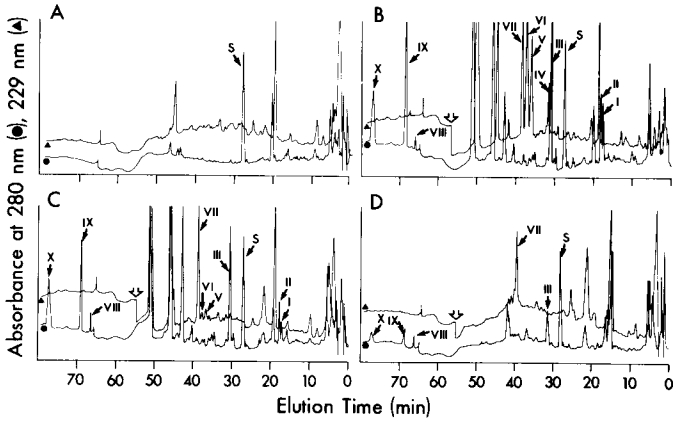

FIGURE 1. HPLC chromatograms obtained from analysis of incubation media of human lung parenchymal fragments (1 g, wet weight in 10 ml of PBS) without stimulation (*A*), stimulated with ionophone A23187 and in the presence of arachidonic acid (*B*), stimulated with ionophone A23187 alone (*C*), or challenged with timothy allergen (*D*) for 15 min at 37°C. The incubation was terminated with an equal volume of methanol containing 150 ng of PGB_2. The traces show the UV absorbance at 280 and 229 nm. The attenuation setting of UV spectrophotometers were 0.02 and 0.05 OD, respectively. HPLC analysis showed peaks corresponding to ω-COOH-LTB_4 (*I*), ω-OH-LTB_4 (*II*), PGB_2 (*S*), LTB_4 (*III*), HHT (*IV*), 15-HETE (*V*), 12-HETE (*VI*), 5-HETE (*VII*), LTC_4 (*VIII*), LTE_4 (*IX*), and LTD_4 (*X*). Open arrow at 56.5 min indicates the 229 nm baseline shift.

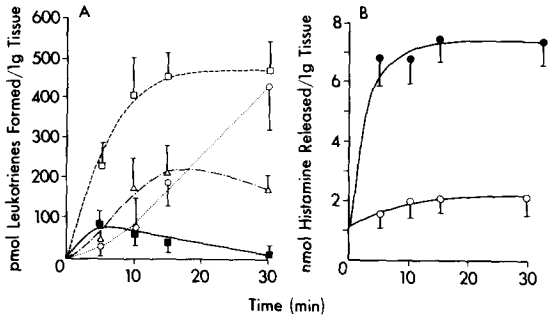

FIGURE 2. Time course of LTB_4 (□), LTC_4 (■), LTD_4 (△), and LTE_4 (○) formation by human lung parenchymal fragments, stimulated with ionophore A23187. Mean ± SEM, n = 12. (*B*) Time course of histamine release by human lung parenchymal fragments stimulated with ionophore A23187 (●) and control (○). n = 7.

the tissue was pretreated with 1 μM of FPL55712. LTC_4 also induced contraction of guinea pig lung parenchymal strips and a long-lasting contraction (>15 min) of ileum (data not shown). LTB_4 was less potent (>50 ng) in inducing contraction of guinea pig lung parenchymal strips, and failed to contract guinea pig ileum (data not shown).

Time Course of Leukotrienes and Histamine Release by Human Lung Parenchyma. Fig. 2*A* shows the release of LTB_4, LTC_4, LTD_4, and LTE_4 by 1 g (wet weight) of lung parenchyma stimulated with ionophore A23187. LTC_4 was released rapidly, reaching a maximum after 5 min of stimulation (83 ± 22.2 pmol/g tissue, wet weight), and then decreased. The release of LTB_4 and LTD_4 was, however, maximum after 15 min (438 ± 66.6 and 206 ± 68 pmol/g tissue,

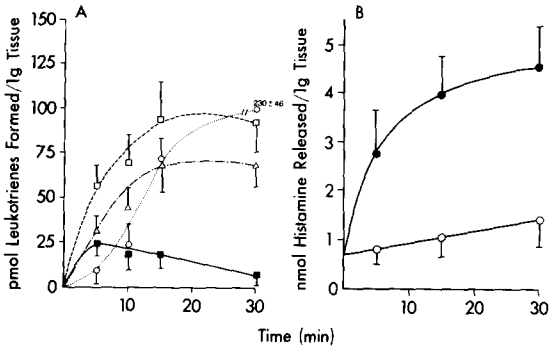

FIGURE 3. (*A*) Time course of LTB_4 (□), LTC_4 (■), LTD_4 (△), and LTE_4 (○) formation by passively sensitized human lung parenchymal fragments challenged by timothy allergen. n = 16. (*B*) Time course of histamine release by passively sensitized human lung parenchymal fragments incubated with timothy allergen (●), without allergen (○). n = 9.

respectively), followed also by a decrease. The formation of ω-OH-LTB_4, ω-COOH-LTB_4, and LTE_4 continued to increase through 30 min of incubation. The presence of these three metabolites was further confirmed by ultraviolet (UV) scanning and bioassay on guinea pig lung parenchymal strips (data not shown). In addition to releasing leukotrienes, human lung parenchyma also released histamine upon ionophore stimulation (Fig. 2*B*). The release of histamine reached a maximum after ~5 min (5.2 ± 0.95 nmol/g tissue wet weight).

A similar pattern of leukotriene and histamine release from allergen-challenged human lung parenchyma was observed. As Fig. 3*A* shows, LTC_4 was rapidly formed, reaching a maximum after 5 min (25 ± 7.1 pmol/g tissue), after which its concentration decreased. In contrast, maximum release of LTB_4 and LTD_4 occurred after 15 min of challenge (92.8 ± 21 and 67.8 ± 14 pmol/g tissue, respectively) while the amount of LTE_4 continued to increase through 30 min of incubation. The presence of ω-OH-LTB_4 and ω-COOH-LTB_4 could not be ascertained, since the amount of LTB_4 formed after allergen challenge was two- to fivefold less than the amount formed by ionophore stimulation. Human lung parenchyma also rapidly released histamine upon allergen challenge (Fig. 3*B*). However, in contrast to the action of ionophore, the release of histamine upon allergen challenge continued to increase up to 15 min (275 ± 70 nmol/g tissue).

Generation of Lipoxygenase Metabolites of Arachidonic Acid by Human Lung Bronchi. Fragments of human lung bronchi (1 g, wet weight) were stimulated with ionophore A23187 (4 μM) and arachidonic acid (30 μM). Analysis of the 15 min incubation media by reverse phase HPLC demonstrated the presence of LTB_4, LTD_4, and LTE_4 as well as 15-Hete, 12-Hete and 5-Hete (217 ± 65.6, 61 ± 22, 133 ± 53.7, 230 ± 76.5, 200 ± 48, and 350 ± 71 pmol/g tissue, respectively; n = 5). However, when fragments of human lung bronchi were stimulated with ionophore alone, LTB_4, LTD_4, LTE_4, 12-Hete, and 5-Hete, but not 15-Hete, were detected (data not shown). The amounts of leukotrienes and Hetes released by ionophore stimulation were constantly two- to fourfold less than the amounts formed upon stimulation with ionophore and arachidonic acid. When fragments of human lung bronchi were passively sensitized with serum

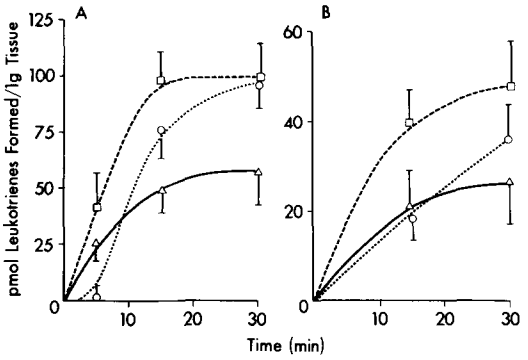

FIGURE 4. (*A*) Time course of LTB_4 (□), LTD_4 (△), and LTE_4 (○) formation by human lung bronchial fragments stimulated with ionophore A23187. $n = 7$. (*B*) Time course of LTB_4 (□), LTD_4 (△), and LTE_4 (○) formation in passively sensitized human lung bronchial fragments challenged with timothy allergen. $n = 9$.

from timothy-positive allergic patients and then challenged with timothy allergen, LTB_4, LTD_4, and LTE_4 were detected in amounts two- to fivefold less than the amounts formed by the ionophore-stimulated tissues (data not shown). Furthermore, no Hetes were detectable (below the limit of detection).

Time Course of Leukotriene and Histamine Release by Human Lung Bronchi. When human lung bronchi were stimulated with ionophore A23187, maximum release of LTB_4 and LTD_4 was observed after 15 min (100 ± 13 and 47 ± 10.6 pmol/g tissue, wet weight, respectively). However, the formation of LTE_4 continued to increase through 30 min (Fig. 4*A*). LTC_4, ω-OH-LTB_4, and ω-COOH-LTB_4 were not detected in any appreciable quantity. A similar profile of leukotriene release also was observed from passively sensitized human lung bronchi challenged with timothy allergen (Fig. 4*B*). However, the amounts of leukotrienes formed in bronchi upon allergen challenge were less than the amounts observed after ionophore stimulation. Furthermore, the maximum release of all the leukotrienes upon allergen challenge occurred after 30 min of incubation (LTB_4, 48 ± 10.3; LTD_4, 27 ± 9.7; and LTE_4, 36 ± 8.2 pmol/g tissue, wet weight). Human lung bronchi also rapidly released histamine upon both ionophore stimulation and allergen challenge (Fig. 5). The release of histamine upon ionophore stimulation was greater than the release induced by allergen challenge and, whereas release after ionophore stimulation reached completion after ~5 min (3.15 ± 0.9 nmol/g tissue), release induced by antigen challenge was not complete until 15 min (2.25 ± 0.65 nmol/g tissue, wet weight). In addition, a small amount of histamine was constantly released from the control incubations of both parenchymal and bronchial fragments, probably due to the manipulation of the tissues.

Effects of NDGA, Aspirin, and Sodium Cromoglycate on the Release of Leukotrienes and Histamine in Human Lung Parenchyma. The action of NDGA, aspirin, and sodium cromoglycate on the release of leukotrienes and histamine by human lung parenchyma upon challenge with ionophore or allergen were studied. NDGA inhibited (ID_{50}, 2×10^{-6} M) both the ionophore- and allergen-induced release of leukotrienes from lung parenchyma, whereas aspirin did not affect the

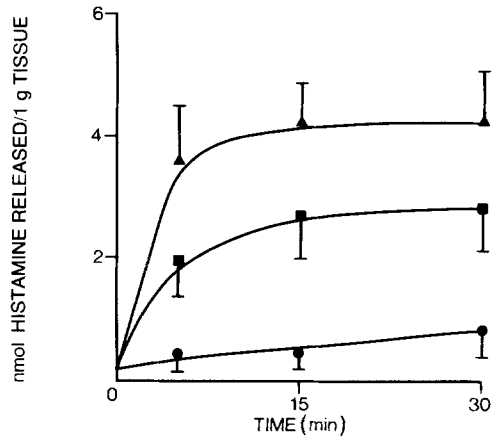

FIGURE 5. Time course of histamine release in human lung bronchial fragments control (●), challenged with timothy allergen (■) or stimulated with ionophore A23187 (▲). $n = 5$.

release of leukotrienes. Sodium cromoglycate (10^{-4} M) did not affect the release of leukotriene from tissue stimulated with ionophore, but it slightly decreased the allergen-mediated release of leukotrienes (LTB_4, 25 ± 5%; LTC_4, 50 ± 9%; LTD_4, 32 ± 8.3%; and LTE_4, 33 ± 7.5%; $n = 14$, $P < 0.05$; data not shown). The release of histamine by human lung parenchyma after challenge with allergen or ionophore was not affected by the action of either NDGA or aspirin. Allergen-mediated, but not ionophore-mediated, release of histamine by human lung parenchyma was also decreased (50 ± 6%, $n = 14$, $P < 0.05$), when the tissue was treated with 10^{-4} M of sodium cromoglycate (data not shown).

Discussion

The present data indicate that human lung parenchyma and bronchi release histamine and products of the 5-lipoxygenase pathway of arachidonic acid metabolism. Among the 5-lipoxygenase products, the SRS-A family (LTC_4, LTD_4, and LTE_4) are the predominant products. However, an appreciable amount of LTB_4 also is released. Previous investigations (28, 29) indicated that human lung parenchyma does not release LTB_4 in an IgE-dependent mechanism, whereas LTB_4 was shown to be released upon ionophore stimulation. In contrast, our results show that both human lung parenchyma and bronchi release LTB_4 through both IgE-dependent and ionophore-mediated mechanisms. The presence of LTB_4 was clearly defined not only by HPLC but also by bioassay and GC-MS. LTB_4 has several important biological activities: it is a potent chemotactic and chemokinetic for human neutrophils with an activity comparable to that of fmet-leu-phe, on a molar basis, and it is an aggregatory substance (18, 30, 31). In addition to these pro-inflammatory actions, LTB_4 causes contraction of lung parenchymal strips at concentrations lower than does histamine (32). Thus, the release of LTB_4 by lung parenchyma and bronchi may be an important causative factor in the inflammation of airway systems and may, in addition, cause bronchospasm. Another important aspect of this study is the finding that human lung parenchyma has the capacity to metabolize LTB_4 into ω-OH-LTB_4 and ω-COOH-

LTB_4. This demonstration was clearly defined, using HPLC, bioassay, and GC-MS. Although ω-OH-LTB_4 has been shown to be less chemotactic and aggregatory than LTB_4 (33–35), it is nevertheless as active as LTB_4 in contracting lung parenchymal strips (32, 35).

Other leukotrienes that were released by lung tissues were LTC_4, LTD_4, and LTE_4. LTC_4 and LTD_4 have been shown (36) to be more potent than histamine in causing constriction of the airway system, particularly the peripheral airways. In addition, they contract guinea pig trachea and human bronchi and, in both cases, are much more active than histamine (10, 37). These two leukotrienes were also found to induce mucous secretion in human bronchi (15).

We have observed that human lung parenchyma releases significant amounts of 15-Hete, 12-Hete and 5-Hete. Our results confirm previous findings on the formation of Hetes by human lung tissues (28). However, in contrast to a previous report (28), we noticed that 5-Hete and not 15-Hete was the major mono-hydroxy acid. These differences might be due to the amount of exogenous arachidonic acid added to the incubation medium or due to the time of incubation. Although the complete biological activities of these mono-hydroxy acids are unknown, 5-Hete and 12-Hete were shown to increase the anti-IgE–induced histamine release from rat mast cells (38) and from human basophils (39, 40). Furthermore 15-Hete was shown to inhibit 5-lipoxygenase activities in human PMNL (41).

An additional novel finding of this investigation is the demonstration that human bronchi also release appreciable amounts of biologically active 5-lipoxygenase metabolites of arachidonic acid. However, the amount of 5-lipoxygenase products (i.e., LTB_4, LTD_4, and LTE_4) formed by bronchi was less than that formed by parenchyma. In contrast to lung parenchyma, LTC_4 was not measurable in human lung bronchi, but this finding does not reflect an absence of LTC_4 synthesis by bronchi, since its metabolites (i.e., LTD_4 and LTE_4) were constantly detected. Neither ω-OH-LTB_4 nor ω-COOH-LTB_4 was detected.

The efficiency of sodium cromoglycate has been demonstrated with regard to the inhibition of immediate and late asthmatic responses, after antigenic challenges, and of exercise-induced asthma (42, 43). Our results show that sodium cromoglycate does not significantly inhibit either the leukotriene release or histamine release by lung tissues. Only at a high concentration (10^{-4} M) was sodium chromoglycate able to reduce the release of leukotrienes and histamine, but even so it did not completely block this release. This partial inhibition by sodium cromoglycate might be due to its action as a calcium antagonist (44, 45). Our studies with aspirin showed that this drug neither stimulates nor inhibits leukotriene formation or histamine release by lung tissues. These results do not support the hypothesis that allergy to aspirin stems from an increase of lipoxygenase metabolites caused by aspirin-induced inhibition of the cyclooxygenase pathway. However, we occasionally observed a slight, but not significant, decrease in the release of histamine from parenchyma treated with 10^{-4} M aspirin. The lipoxygenase inhibitor (NDGA) was shown to be the only inhibitor of leukotriene formation in lung tissues, indicating that a potent lipoxygenase inhibitor should be the most suitable drug in the treatment of leukotriene-mediated allergic reactions.

In conclusion, asthma is a complex disease resulting in airway narrowing because of combinations of airway muscle constriction, edema of the bronchial mucosa, mucosal infiltration with inflammatory cells, and excess mucous secretion (46). Leukotrienes and histamine are able to provoke these symptoms in vivo and in vitro. Our study shows that both lung parenchyma and bronchi release significant amounts of histamine and leukotrienes, suggesting that they are involved in human pulmonary allergic symptoms.

Summary

Fragments of human lung parenchyma or bronchi were studied by high performance liquid chromatography, gas chromatography–mass spectrometry, and bioassay for the biosynthesis of 5-lipoxygenase metabolites of arachidonic acid, and by radioenzymatic assay for the release of histamine, upon immunologic and nonimmunologic stimulation. Human lung parenchyma were passively sensitized with serum from timothy-positive allergic patients (radioallergosorbent test, 30–40%) and challenged with 0.5 μg/ml of timothy allergen. Analysis of the incubation media showed the presence of LTB_4, LTC_4, LTD_4, LTE_4, and histamine. Maximum release of LTB_4 and LTD_4 was observed after 15 min of challenge (92.8 ± 21, and 67.8 ± 14 pmol/g tissue wet weight, respectively; mean ± SEM) whereas maximum release of LTC_4 was observed after 5 min of challenge (25 ± 7.1 pmol). In parallel to leukotriene formation, histamine was released rapidly and reached a maximum after ~15 min of challenge (2.85 ± 0.76 nmol/g tissue).

When fragments of human lung parenchyma were stimulated with ionophore A23187 (4 μM), we observed a profile of leukotriene and histamine release similar to that seen in response to the allergen. Ionophore A23187 stimulated the release of two- to fivefold greater amounts of leukotrienes and histamine than did the allergen. Release of LTC_4 and histamine was maximal after 5 min of stimulation (83 ± 22.2 and 5.2 ± 0.95 nmol/g tissue, respectively), whereas LTB_4 and LTD_4 release reached a maximum after 15 min (438 ± 66.6 and 205 ± 68 nmol/g tissue, respectively). In addition, human lung parenchyma metabolized LTB_4 into ω-OH-LTB_4 and ω-COOH-LTB_4. This tissue also released 5-hydroxy-eicosatetraenoic acid (5-Hete), 12-Hete, and 15-Hete.

Fragments of human lung bronchi also released a similar profile of leukotrienes (except LTC_4) and histamine when challenged with the allergen or ionophore A23187. Maximum release of LTB_4 and LTD_4 by allergen or ionophore stimulation was observed after ~15 min (40 ± 7.5 and 21 ± 8 pmol/g tissue, respectively, upon allergen challenge; 100 ± 13 and 47 ± 10.6 pmol/g tissue, respectively, upon ionophore stimulation). The maximum release of histamine by bronchi was observed after ~15 min of allergen challenge and 5 min of ionophore stimulation (2.25 ± 0.65 and 3.15 ± 0.9 nmol/g tissue, respectively).

The release of leukotrienes but not of histamine by human lung parenchyma upon both allergen and ionophore challenge was inhibited by nordihydroguaiaretic acid (NDGA) (ID_{50}, 2×10^{-6} M). Aspirin neither significantly inhibited nor stimulated the release of leukotrienes or histamine by this tissue. Sodium cromoglycate did not inhibit the release of either leukotrienes or histamine by ionophore-stimulated parenchyma. However, at 10^{-4} M, it slightly decreased the

formation of leukotrienes (20–56%, $P < 0.05$) and release of histamine (50 ± 6%, $P < 0.05$) by allergen-challenged human lung parenchyma. These observations indicate that human lung parenchyma and bronchi release significant amounts of leukotrienes and histamine upon allergen or ionophore stimulation.

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References

1. Goetzel, E. J. 1980. Mediators of immediate hypersensitivity derived from arachidonic acid. *N. Engl. J. Med.* 303:822.
2. Ts'ao, C., W. J. Metzger, R. Patterson, and I. M. Suszko. 1976. Histamine-containing cells in bronchial lavage fluid. I. Ultrastructural characterization and comparison with mast cells in three types of tissues of rhesus monkeys. *Int. Arch. Allergy Appl. Immunol.* 52:315.
3. Schild, H. O., D. F. Hawkins, J. L. Mongar, and H. Herxheimer. 1951. Reactions of isolated human asthmatic lung and bronchial tissue to a specific antigen. Histamine release and muscular contractions. *Lancet* 2:376.
4. Murphy, R. C., S. Hammarstrom, and B. Samuelsson. 1979. Leukotriene C, a slow reacting substance (SRS) from murine mastocytoma cells. *Proc. Natl. Acad. Sci. USA.* 79:4275.
5. Morris, H. R., G. W. Taylor, P. J. Piper, M. N. Samhoun, and J. R. Tippens. 1980. Slow reacting substances (SRSs): the structure identification of SRSs from rat basophilic leukaemia cells. *Prostaglandins.* 19:185.
6. Borgeat, P., and B. Samuelsson. 1979. Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes. *J. Biol. Chem.* 254:2643.
7. Borgeat, P., and B. Samuelsson. 1979. Metabolism of arachidonic acid in polymorphonuclear leukocytes. *J. Biol. Chem.* 154:7865.
8. Drazen, J. M., K. F. Austen, R. A. Lewis, D. A. Clark, G. Goto, A. Marfat, and E. J. Corey. 1980. Comparative airway and vascular activities of leukotriene C-1 and D in vivo and in vitro. *Proc. Natl. Acad. Sci. USA.* 77:4354.
9. Hanna, C. J., M. K. Bach, P. D. Pare, and R. R. Schellenberg. 1981. Slow-reacting substances (leukotrienes) contract human airway and pulmonary vascular smooth muscle in vitro. *Nature (Lond.).* 290:343.
10. Dahlén, S. E., P. Hedqvist, S. Hammarstrom, and B. Samuelsson. 1980. Leukotrienes are potent constrictors of human bronchi. *Nature (Lond.)* 288:484.
11. Weiss, J. W., J. M. Drazen, N. Coles, E. R. McFadden, Jr., P. F. Weller, E. J. Corey, R. A. Lewis, and K. F. Austen. 1982. Bronchoconstrictor effects of leukotriene C in humans. *Science (Wash. DC).* 216:196.
12. Holroyde, M. C., R. E. C. Altounyan, M. Cole, M. Dixon, and E. V. Elliott. 1981. Bronchoconstriction produced in man by leukotrienes C and D. *Lancet.* 2:17.
13. Griffin, M., J. W. Weiss, A. G. Leitch, E. R. McFadden, Jr., E. J. Corey, K. F. Austen, and J. M. Drazen. 1983. Effects of leukotriene D on the airways in asthma. *N. Engl. J. Med.* 308:436.
14. Johnson, H. G., and M. L. McNee. 1983. Secretogogue responses of leukotriene C_4 and D_4: comparison of potency in canine trachea in vivo. *Prostaglandins.* 25:237.
15. Marom, Z., J. H. Shelhamer, M. K. Bach, D. R. Morton, and M. Kaliner. 1981. Slow reacting substances, leukotrienes C_4 and D_4, increase the release of mucus from human airways in vitro. *Am. Rev. Respir. Dis.* 126:449.

16. Coles, S. J., K. H. Neill, L. M. Reid, K. F. Austen, Y. Nil, E. J. Corey, and R. A. Lewis. 1983. Effects of leukotrienes C_4 and D_4 on glycoprotein and lysozyme secretion by human bronchial mucosa. *Prostaglandins.* 25:155.
17. O'Flaherty, J. T., M. J. Thomas, S. L. Cousart, W. L. Salzer, and C. E. McCall. 1982. Neutropenia induced by systemic infusion of 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid. *J. Clin. Invest.* 69:993.
18. Ford-Hutchinson, A. W., M. A. Bray, M. V. Doig, M. E. Shipley, and M. J. H. Smith. 1981. Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. *Nature (Lond.).* 286:264.
19. Samuelsson, B. 1982. The leukotrienes: an introduction. *Adv. Prostaglandin Thromboxane Leukotriene Res.* 9:1.
20. Peters, S. P., D. W. MacGlashan, Jr., E. S. Schulman, R. P. Schleimer, E. C. Hayes, J. Rokach, N. F. Adkinson, Jr., and L. M. Lichtenstein. 1984. Arachidonic acid metabolism in purified human lung mast cells. *J. Immunol.* 132:1972.
21. Fels, A. O. S., N. A. Pawlowski, E. B. Cramer, T. K. C. King, Z. A. Cohn, and W. A. Scott. 1982. Human alveolar macrophages produce leukotriene B_4. *Proc. Natl. Acad. Sci. USA.* 79:7866.
22. Verburg, K. M., R. R. Bowsher, and D. P. Henry. 1983. A new radioenzymatic assay for histamine using purified histamine *N*-methyl transferase. *Life Sci.* 32:2855.
23. Powell, W. S. 1980. Rapid extraction of oxygenated metabolites of arachidonic acid from biological samples using octadecylsilyl silica. *Prostaglandins.* 20:947.
24. Borgeat, P., B. Fruteau de Laclos, H. Rabinovitch, S. Picard, P. Braquet, J. Hébert, and M. Laviolette. 1984. Generation and structure of the lipoxygenase products: eosinophil-rich human polymorphonuclear leukocyte preparations characteristically release leukotriene C_4 on ionophore A23187 challenge. *J. Allergy Clin. Immunol.* 74:310.
25. Borgeat, P., and B. Samuelsson. 1979. Transformation of arachidonic acid by rabbit polymorphonuclear leukocytes. *J. Biol. Chem.* 254:2643.
26. Borgeat, P., S. Picard, P. Vallerand, and P. Sirois. 1981. Transformation of arachidonic acid in leukocytes. Isolation and structural analysis of a novel dihydroxy derivative. *Prostaglandins Med.* 6:557.
27. Sirois, P., S. Roy, J. P. Tétrault, P. Borgeat, S. Picard, and E. J. Corey. 1981. Pharmacological activity of leukotrienes A_4, B_4, C_4 and D_4 on selected guinea pig, rat, rabbit and human smooth muscles. *Prostaglandins Med.* 7:327.
28. Hansson, G., T. Bjorck, S. E. Dahlén, P. Hedqvist, E. Granstrom, and B. Dahlén. 1983. Specific allergen induces contraction of bronchi and formation of leukotrienes C_4, D_4 and E_4 in human asthmatic lung. *Adv. Prostaglandin Thromboxane Leukotriene Res.* 12:153.
29. Zijlstra, F., and J. E. Vincent. 1984. Determination of leukotrienes and prostaglandins in [^{14}C]arachidonic acid labelled human lung tissue by high-performance liquid chromatography and radioimmunoassay. *J. Chromatogr.* 311:39.
30. Smith, M. J. H., A. W. Ford-Hutchinson, and M. A. Bray. Leukotriene B: a potential mediator of inflammation. *J. Pharm. Pharmacol.* 32:517.
31. Dahlén, S. E., J. Bjork, P. Hedqvist, K. E. Arfors, S. Hammarstrom, J. A. Lindgren, and B. Samuelsson. 1981. Leukotrienes promote plasma leakage and leukocyte adhesion in post capillary venules: in vivo effects with relevance to the acute inflammatory response. *Proc. Natl. Acad. Sci. USA.* 78:3887.
32. Hansson, G., J. A. Lindgren, S. E. Dahlén, P. Hedqvist, and B. Samuelsson. 1981. Identification and biological activity of novel omega-oxidized metabolites of leukotriene B_4 from human leukocytes. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 130:107.
33. Jubiz, W., O. Rodmark, C. Malmsten, G. Hansson, J. A. Lindgren, J. Palmblad, A.

M. Udén, and B. Samuelsson. 1982. A novel leukotriene produced by stimulation of leukocytes with formyl methionylleucyl phenylalanine. *J. Biol. Chem.* 257:6106.

34. Camp, R. D. R., P. M. Woolard, A. I. Mallet, N. J. Fincham, A. W. Ford-Hutchinson, and M. A. Bray. 1982. Neutrophil aggregating and chemokinetic properties of a 5,12,20-trihydroxy-6,8,10,14-eicosatetraenoic acid isolated from human leukocytes. *Prostaglandin.* 23:631.
35. Ford-Hutchinson, A. W., Z. Rackham, R. Zamboni, J. Rokach, and S. Roy. 1983. Comparative biological activities of synthetic leukotriene B_4 and its omega-oxidation products. *Prostaglandins.* 25:29.
36. Lewis, R. A., J. M. Drazen, E. J. Corey, and K. F. Austen. 1981. Structural and functional characteristics of the leukotrienes. *In* SRS-A and Leukotrienes. P. J. Piper, editor. John Wiley & Sons, New York. 101–117.
37. Piper, P. J., M. N. Samhoun, J. R. Tippins, T. J. Williams, M. A. Palmer, and M. J. Peck. 1981. Pharmacological studies on pure SRS-A and synthetic leukotrienes C_4 and D_4. *In* SRS-A and Leukotrienes. P. J. Piper, editor. John Wiley & Sons, New York. 81–99.
38. Stenson, W. F., C. W. Parker, and T. J. Sullinan. 1980. Augmentation of IgE-mediated release of histamine by 5-hydroxyeicosatetraenoic acid and 12-hydroxyeicosatetraenoic acid. *Biochem. Biophys. Res. Commun.* 96:1045.
39. Peters, S. P., M. I. Siegel, A. Kagey-Sobotka, and L. M. Lichtenstein. 1981. Lipoxygenase products modulate histamine release in human basophils. *Nature (Lond.).* 292:455.
40. Peters, S. P., A. Kagey-Sobotka, D. W. MacGlashan, Jr., M. J. Siegel, and L. M. Lichtenstein. 1982. The modulation of human basophil histamine release by products of 5-lipoxygenase pathway. *J. Immunol.* 129:797.
41. Salari, H., P. Braquet, and P. Borgeat. 1984. Comparative effects of indomethacin, acetylenic acids, 15-HETE, nordihydroguaiaretic acid and BW755C on the metabolism of arachidonic acid in human leukocytes and platelets. *Prostaglandins Leukotrienes Med.* 13:53.
42. Pepys, J., R. J. Davies, A. B. X. Breslin, D. J. Hendrick, and B. J. Hutchcroft. 1974. The effects of inhaled beclomethasone dipropionate (Becotide) and sodium cromoglycate on asthmatic reactions to provocation tests. *Clin. Allergy.* 4:13.
43. Godfrey, S., and P. Konig. 1976. Inhibition of exercise-induced asthma by different pharmacological pathways. *Thorax.* 31:137.
44. Patel, K. R. 1981. Calcium antagonists in exercise-induced asthma. *Br. Med. J.* 282:932.
45. Craps, L. 1981. Ketotifen in the oral prophylaxis of bronchial asthma: a review. *Pharmatherapeutica.* 3:18.
46. Kaliner, M. A., J. Belnnerhasset, and K. F. Austen. 1976. Bronchial asthma. *In* Textbook of Immunopathology. P. A. Meischer and H. J. Muller-Eberhard, editors. Grune & Stratton, Inc., New York. 387–401.