

Ragweed Antigen E and Anti-IgE in Human Central versus Peripheral Isolated Bronchi

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The ability of antigen to contract passively sensitized tissues was examined in human central (5 to 12 mm) and peripheral (0.5 to 2 mm) bronchi. Both central and peripheral bronchi contracted to ragweed antigen E (RW AgE), and these contractions were virtually abolished by a combination of indomethacin, cysteinyl-leukotriene, and histamine antagonists. There were, however, quantitative differences in contractile responses and in mediator release to RW AgE between central and peripheral bronchi. RW AgE was approximately 20-fold more potent in contracting peripheral bronchi compared with central bronchi. On a per weight of tissue basis, RW AgE released six-fold more histamine, 15- to 20-fold more immunoreactive leukotriene D₄ (i-LTD₄) and two- to 10-fold more prostanoids in the peripheral bronchi compared with central bronchi. Anti-IgE mimicked the effect of RW AgE with respect to inflammatory mediator release and with respect to the magnitude of the contractile response in peripheral and central bronchi. Anti-IgE, however, was more potent in contracting central than peripheral bronchi. Moreover, in peripheral bronchi, contractile responses to anti-IgE were only partially inhibited by a combination of indomethacin, cysteinyl-leukotriene, and histamine antagonists. These results indicate that the qualitative characteristics of antigen-induced mediator release and muscle contraction are similar in central versus peripheral bronchi. However, RW AgE is much more potent in causing smooth muscle constriction, and is capable of releasing a greater quantity of inflammatory mediators in peripheral bronchi/bronchioles than in the more central bronchi. **Ellis JL, Hubbard WC, Meeker S, Udem BJ. Ragweed antigen E and anti-IgE in human central versus peripheral isolated bronchi. Am J Respir Crit Care Med 1994;150:717-23.**

The immediate hypersensitivity response has been studied in human isolated bronchi. Typically, in these studies passively sensitized trachea, segmental, and large (> 3 mm diameter) subsegmental bronchi are challenged with antigen, and the smooth muscle contraction and/or inflammatory mediator release is quantified (1-6). The antigen-induced contractions of these bronchi can be blocked by a combination of histamine and cysteinyl-leukotriene receptor antagonists (4-6).

Relatively little is known about the immediate hypersensitivity response in more peripheral airways. To study peripheral airways *in vitro*, investigators have primarily relied upon lung parenchymal strips from various animal species including man. Using these preparations, differences in the immediate bronchoconstrictor response to immunologic stimuli have been noted between central bronchi and parenchyma. Thus in guinea pig parenchyma, unlike in larger bronchi, there is a contractile response resistant to histamine and cysteinyl-leukotriene receptor antagonists (7-9). Bronchi and parenchymal strips taken from humans also differ in their mediator release profiles to immunologic stimuli (10, 11).

The lung parenchymal strip may not be an appropriate model

of peripheral airway reactivity (12-14). The parenchymal strip is made up of several different tissues types including bronchi/bronchioles, blood vessels, alveoli, and pleura; therefore, any observations cannot be attributed to a particular tissue type. We therefore elected in this study to compare systematically the immediate hypersensitivity reaction in central bronchi with that in more peripheral bronchi/bronchioles isolated from human lung parenchyma.

METHODS

Macroscopically normal human lung tissue was obtained from 29 patients undergoing surgical resection and 22 organ donors (organ donor tissue was supplied by the International Institute for the Advancement of Medicine, Exton, PA). The surgical specimens were from patients with lung carcinoma. The organ donor specimens (mean age 35 ± 4 yr, 14 male, 8 female) were mainly from victims of head trauma (gunshot wounds, motor vehicle accidents, and intracranial bleeding). Surgical specimens were placed in RPMI 1640 solution (Gibco, Grand Island, NY) at 4° C within 90 min of resection for the 15-min transfer to the laboratory. The organ donor specimens were placed in ViaSpan tissue preservation solution (Du Pont Merck Pharmaceutical Co., Wilmington, DE) at 4° C and transferred overnight to the laboratory. On arrival at the laboratory, the specimens were placed in 4 L of modified Krebs' bicarbonate solution consisting of: NaCl (118 mM), KCl (5.4 mM), NaH₂PO₄ (1 mM), MgSO₄ (1.2 mM), CaCl₂ (1.9 mM), NaHCO₃ (25 mM), dextrose (11.1 mM), and gassed with 95% oxygen and 5% carbon dioxide at 4° C. Bronchi (0.5 to 12 mm outer diameter) were trimmed of surrounding parenchyma and blood vessels. The peripheral bronchi were trimmed using watchmakers' forceps and miniature vanna scissors, taking care to remove as much parenchyma as possible. Bronchi were either utilized immediately or placed in oxy-

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generated Krebs' solution at 4° C for use within the following 40 h. No differences were evident in responses to treatments between tissues that were used immediately and those used within the following 40 h. Tissues for sensitization were placed in a 1:10 dilution of serum from a ragweed-allergic individual. The serum was diluted with Krebs' solution, oxygenated, and kept at 21° C for the 16 h sensitization procedure. Tissues from both surgery and from organ donors were sensitized.

Organ Bath Studies

Central bronchi (5 to 12 mm outer diameter) were cut longitudinally and prepared as transverse strips 4 to 5 mm wide. Peripheral (0.5 to 2 mm outer diameter) bronchi were prepared as rings 4 to 5 mm in length and placed over two stirrups made of tungsten wire. Previous experiments have indicated that these peripheral airways contain a mixed population of bronchi/bronchioles. Bronchi were suspended in 10 ml organ baths containing Krebs' solution maintained at 37°C; tissues were connected to Grass FT03 force transducers for the measurement of isometric tension which was recorded on a Grass Model 7 polygraph (Grass Instruments, Quincy, MA). Preparations were suspended at an initial tension of 2 g (central) or 1 g (peripheral) and washed with fresh buffer every 15 min for a 60 min equilibration period. In preliminary studies, these resting tensions were found to be optimal for generating active tension.

Indomethacin (3 μ M) was added to the buffer solution at the beginning of the experiments to avoid the effects of prostanoids on responses to immunologic stimuli (15, 16). After the equilibration period, either anti-IgE (0.3 to 3,000 ng/ml) was added to naive tissues or RW AgE (0.1–300 ng/ml) was added to sensitized tissues in a cumulative fashion. The next concentration of agent was added when the response to the previous concentration had reached a plateau. At the end of the curve to either anti-IgE or RW AgE, tissues were maximally contracted with 30 mM BaCl₂. All results are expressed as a percentage of this maximal response to BaCl₂. The responses to these agents were examined in the absence or presence of a combination of pyrilamine (1 μ M) and SKF 104353 (10 μ M) added 30 min before the addition of either anti-IgE or RW AgE. In one set of experiments examining the response to anti-IgE in peripheral airways, zileuton (10 μ M) was added to the combination. The addition of pyrilamine and SKF 104353 was found to relax both central and peripheral airways. Experiments in our laboratory have indicated that both histamine and cysteinyl-leukotrienes mediate the intrinsic tone in human isolated airway (17). In experiments on the kinetics of the response, either 3,000 ng/ml anti-IgE or 300 ng/ml RW AgE was added to naive and sensitized tissues respectively, and the contractions followed for 30 min, at which time 30 mM BaCl₂ was added to maximally contract the tissues.

Release Studies

Peripheral and central bronchi including cartilage were cut into small pieces and divided into aliquots, each containing approximately 100 mg (wet weight). The bronchial tissue was incubated in 2 ml of Krebs' solution, which was gassed with 95% O₂/5% CO₂, and maintained at 37°C. The physiologic buffer was replaced at 15 min intervals for 90 min. Following this equilibration period, 2 ml of Krebs' solution, containing or lacking either 300 ng/ml RW AgE (sensitized tissues) or 3,000 ng/ml anti-IgE (naive tissues), was added for 15 min. After this, the supernatant was taken to assay histamine and eicosanoid release. In addition, to determine the total tissue content of histamine, 2 ml of 0.4 N perchloric acid was added to the tissue, which was then placed in a hot water bath for 15 min. The supernatant fluid was assayed to measure the total histamine content.

Histamine was assayed by the automated fluorometric technique described by Siraganian (18). Histamine release is expressed as a percentage of the total histamine content. Cysteinyl-leukotriene released from the bronchi was assayed using the radioimmunoassay previously described (19). Aliquots (100 μ l) were stored at 4°C and assayed without prior purification within 48 h of each experiment. The limit of sensitivity of this assay is approximately 0.03 pmole, as defined by that amount required to inhibit [³H]-LTC₄ binding by 10%. The anti-cysteinyl-leukotriene antibody is highly selective, with little affinity (cross-reactivity < 1%) for a variety of heterologous eicosanoids. The antibody does not, however, distinguish markedly among leukotriene C₄ (LTC₄), LTD₄, or LTE₄. Standard curves with authentic LTC₄, LTD₄, and LTE₄ were parallel; the amounts

of LTC₄, LTD₄, and LTE₄ required to inhibit [³H]-LTC₄ binding by 50% were found to be approximately 0.4, 0.5, and 0.6 pmole/0.1 ml, respectively.

Prostaglandin release into the supernatant fluid was assayed using combined gas chromatography (negative ion chemical ionization) mass spectrophotometry (GC/MS) as previously described (20). Briefly, a 50 μ l aliquot of the supernatant was added to 250 μ l of acetone in a silanized vial. A mixture containing a known quantity (about 1 ng) of 3,3,4,4-tetradeuterated PGE₂, PGD₂, PGF_{2 α} , TXB₂, and 6-keto PGF_{1 α} was added to provide internal standards for the identification and quantification of these prostanoids. In addition, the identification of 9 α , 11 β -PGF₂, was based on its retention times in relation to the tetradeuterated PGF_{2 α} . Samples were then dried under a stream of nitrogen, and the residue was treated with 2% methoxymine hydrochloride dissolved in pyridine. Excess pyridine was evaporated under nitrogen and the residue was subjected to sequential procedures for the synthesis of pentafluorobenzyl ester and trimethylsilyl ether derivatives as previously described (20). GC/MS analysis of the derivatized samples (1 μ l vol) was performed with a Finnigan Model 9611 gas chromatograph interfaced with a Finnigan MAT 4610B EI/CI mass spectrophotometer (Finnigan, San Jose, CA) supplied with a Superincos® data system. The sensitivity of this technique is < 0.1 fmole/injection for each of the six prostanoids assayed.

Statistical Analysis

The -log (μ g/ml) EC50 values were determined as the -log μ g/ml concentration of the agonist that produced 50% of maximum agonist contraction in each concentration response curve, converted to the negative logarithm prior to statistical analysis. Differences in the -log (μ g/ml) EC50 values for RW AgE and anti-IgE in central and peripheral tissues were evaluated using the Student's *t* test for unpaired observations. The differences in the time taken for RW AgE and anti-IgE to reach half the maximal response in peripheral and central airways were similarly evaluated. The release data were initially analyzed by a two-way ANOVA for repeated measures (StatView, Brainpower Inc., Calabasas, CA) followed by Student's *t* test for paired observations. Data that were found not normally distributed were subsequently analyzed by Wilcoxon signed-rank test. Probability values < 0.05 were considered significant.

Drugs

The following drugs were used: SKF 104353 (2(S)-hydroxy-3(R)-(2-carboxyethylthio)-3-[2-(8-phenyloctyl)phenyl]-propanoic acid and leukotriene B₄ were gifts from SmithKline Beecham Pharmaceuticals (King of Prussia, PA). Zileuton (N-(1-benzo[b]thien-2-ylethyl)-N-hydroxyurea) was a gift from Abbott Labs (Abbott Park, IL). WEB 2086 (3-[4-(2-chlorophenyl)-9-methyl-6H-thienol[3,2-f][1,2,4]triazolo-[4,3-a]-[1,4]diazepin-2-yl]-1-(4-morpholinyl)-1-propanone) was a gift from Boehringer Ingelheim (Ingelheim, Germany). Goat antihuman IgE was prepared as described previously (21); the antibody used for these studies represented the IgG fraction of goat serum prepared by DE-52 chromatography. Ragweed antigen E was obtained from Hollister-Stier (Spokane, WA). Barium chloride, indomethacin, ketanserin, and pyrilamine maleate were obtained from Sigma (St. Louis, MO). Indomethacin, WEB 2086, and leukotriene B₄ were dissolved in absolute ethanol. SKF 10433 and ragweed antigen E were dissolved in distilled water. Zileuton was dissolved in dimethylsulfoxide. The other drugs were dissolved in Krebs solution.

RESULTS

Tissue taken from surgical resection and from organ donors were used for all parts of this study. No differences were found in either the smooth muscle responses or in the release of mediators to antigen or anti-IgE between tissue obtained from the two sources. For example, the maximum response to 30 mM BaCl₂ in central airways for surgical specimens was 1.75 $\pm$ 0.20 g (*n* = 13) and from organ donors it was 1.95 $\pm$ 0.35 g (*n* = 8) (*p* > 0.5). The release of histamine evoked by anti-IgE in peripheral tissue was 2,984 $\pm$ 1,190 pmole/g (*n* = 11) and 3,164 $\pm$ 984 pmole/g (*n* = 4) (*p* > 0.9) for surgery- and organ donor-derived tissue, respectively, whereas for i-LT release it was 228 $\pm$ 60 pmole/g

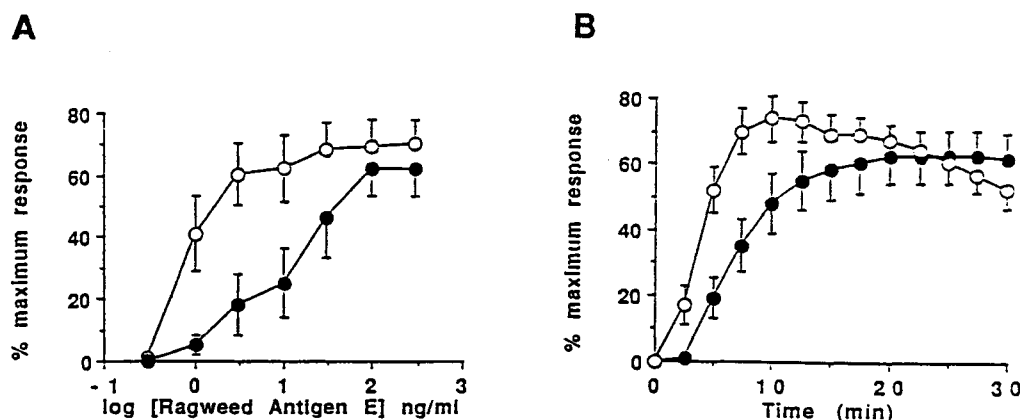


Figure 1. A) Concentration response curves to ragweed antigen E (0.1 to 300 ng/ml) in human central (closed circles) and peripheral (open circles) airways in the presence of indomethacin (3 μ M). Results are calculated as the percent of the maximum response to 30 mM BaCl₂, and they are given as mean $\pm$ SEM; n = 8 to 9 for central bronchi and n = 8 for peripheral bronchi. B) Kinetics of contractile response to ragweed antigen E (0.3 μ g/ml) in human central (closed circles) and peripheral (open circles) bronchi in the presence of indomethacin (3 μ M). Results are calculated as the percent of the maximum response to 30 mM BaCl₂, and they are given as mean $\pm$ SEM; n = 15 for central and n = 13 for peripheral bronchi.

(n = 11) and 364 ± 224 pmole/g (n = 4) (p > 0.4), respectively. The tissue data obtained from the two sources have therefore been pooled.

Responses to RW AgE

In passively sensitized tissues, RW AgE produced concentration-dependent contractions in both peripheral and central bronchi (Figure 1a). RW AgE was 14-fold more potent in peripheral compared with central airways. The $-\log$ (μ g/ml) EC₅₀ concentration of RW AgE averaged 3.01 ± 0.1 (n = 7) and 1.87 ± 0.19 (n = 7) (p < 0.01) in the peripheral and central airways, respectively. Responses in tissue obtained from surgery were not different from responses in tissue obtained from organ donors. For example, the $-\log$ (μ g/ml) EC₅₀ concentration of RW AgE in peripheral tissue was 3.12 ± 0.11 (n = 3) and 2.93 ± 0.15 (n = 4), respectively (p > 0.4). The time-course of the response to a maximally effective concentration of RW AgE (300 ng/ml) was also examined in peripheral and

central bronchi (Figure 1b). The response to RW AgE was more rapid in peripheral than in central airways; the time taken to half maximal response in peripheral airways was 249 ± 34 s (n = 13), whereas in central airways it was 541 ± 62 s (n = 15) (p < 0.001). The combination of pyrilamine (1 μ M) and SKF 104353 (10 μ M) greatly inhibited responses to RW AgE in both peripheral and central airways (Figure 2).

The profile of mediators released by a maximally effective concentration of RW AgE (300 ng/ml) was examined in central and peripheral airways (Table 1). As shown in Table 1, the release of inflammatory mediators, on a per wet weight basis, was much greater in peripheral than in central airways. There was about six-fold more histamine released from the peripheral as from the central airways. When expressed as a percentage of total histamine content, however, there was no significant difference in the amount of histamine released in the central versus peripheral airways. RW AgE released $11.2 \pm 1.8\%$ and $5.6 \pm 2.5\%$ of the total histamine

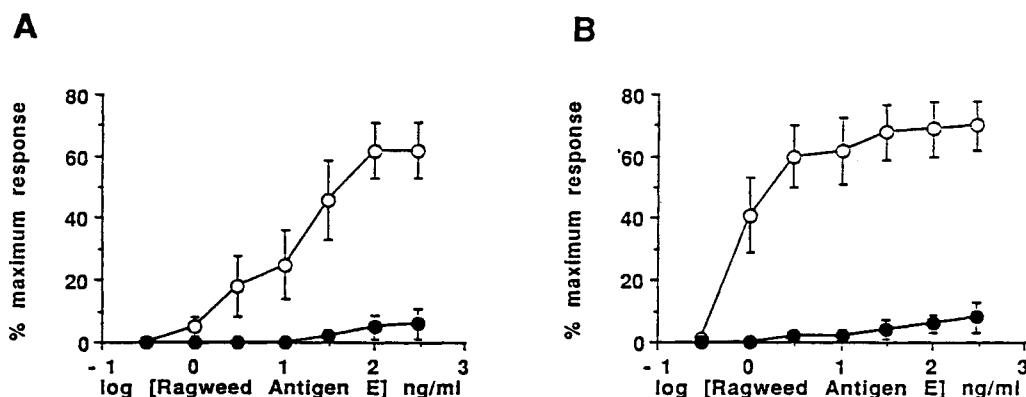


Figure 2. A) Contraction to ragweed antigen E (0.1 to 300 ng/ml) in human central bronchi in the presence of indomethacin (3 μ M). Control responses to ragweed antigen E (open circles) (n = 8 to 9) and inhibition of contraction by SKF 104353 (10 μ M) and pyrilamine (1 μ M) (closed circles) (n = 6). B) Contraction to ragweed antigen E (0.1 to 300 ng/ml) in human peripheral bronchi in the presence of indomethacin (3 μ M). Control responses to ragweed antigen E (open circles) (n = 8) and inhibition of contraction by SKF 104353 (10 μ M) and pyrilamine (1 μ M) (closed circles) (n = 6). Results are calculated as the percent of the maximum response to 30 mM BaCl₂, and they are given as mean $\pm$ SEM.

TABLE 1
ANTIGEN- AND ANTI-IGE-INDUCED MEDIATOR RELEASE FROM
HUMAN PERIPHERAL AND CENTRAL BRONCHI

	Release of Mediators (pmole/g tissue wet weight) ^a							n
	Histamine	i-LTs ^b	PGD ₂ ^c	PGE ₂	PGF _{2α}	TxB ₂	6-Keto PGF _{1α}	
Peripheral Bronchi								
Spontaneous release	2,028 ± 286	34 ± 7	1.1 ± 0.2	19 ± 3	2 ± 0.3	0.5 ± 0.1	14 ± 2	26
Net anti-IgE release	28,619 ± 7,275 [†]	227 ± 61 [†]	251 ± 51 [†]	14 ± 3 [†]	3 ± 0 [†]	6 ± 1 [†]	25 ± 5 [†]	19
Net RW AgE release	21,108 ± 4,298 [†]	276 ± 72 [†]	112 ± 38*	18 ± 5 [†]	3 ± 1 [†]	4 ± 1 [†]	36 ± 7 [†]	7
Central Bronchi								
Spontaneous release	450 ± 37	10 ± 2	0.4 ± 0.1	18 ± 3	2 ± 0.2	0.3 ± 0.05	6 ± 1	21
Net anti-IgE release	3,632 ± 1,297*	15 ± 5*	25 ± 6 [†]	8 ± 2 [†]	1 ± 0 [†]	1 ± 0 [†]	3 ± 1 [†]	14
Net RW AgE release	3,113 ± 1,619*	25 ± 17*	20 ± 12*	8 ± 3*	1 ± 0*	1 ± 0*	4 ± 2	7

^a Net release was calculated in each experiment by subtracting the spontaneous release of mediators from the release evoked by anti-IgE and RW AgE. The values represent mean ± SEM. The spontaneous release values reported reflect pooled values from experiments with RW AgE and anti-IgE.

^b Because 80% of the immunologically produced LTC₄ in human isolated bronchi is recovered as LTD₄ (19), the formula weight for LTD₄ was used to calculate the pmole/g tissue wet weight of the i-LTs.

^c A fraction of the PGD₂ was metabolized in the airways to 9α,11βPGF₂. The net release of 9α,11βPGF₂ from the peripheral bronchi evoked by RW AgE and anti-IgE was 9 ± 3 and 17 ± 4 pmole/g, respectively. The amount released from the central bronchi by RW AgE and anti-IgE was 3 ± 1 and 2 ± 2 pmole/g, respectively.

* p < 0.05.

† p < 0.01 response compared with spontaneous release. Initial analysis was by a two-way ANOVA for repeated measures (p < 0.05 for all comparisons) followed by Student's t test for paired observations except for the data for RW AgE in central airways for histamine, i-LTs, PGD₂, and TxB₂ which were analyzed by the Wilcoxon signed-rank test because release data did not fit a normal distribution.

stores in from the peripheral and central airways, respectively (p > 0.1). The total histamine content was 251 ± 37 nmole/g and 57 ± 8 nmole/g in the peripheral and central bronchi, respectively. RW AgE induced the production and release of about 11 times more i-LTD₄ and six times more PGD₂ from peripheral versus central airways. Approximately 7 and 12% of PGD₂ was recovered as 9α,11β PGF₂ in peripheral bronchi and central bronchi, respectively. We have previously noted that immunologically produced LTC₄ in the human isolated bronchi is recovered as LTD₄ (~80%) and LTE₄ (~20%) (19). There was a difference noted in the rank order of prostanoids released from central and peripheral bronchi. The rank order of release in peripheral airways was PGD₂ > 6-keto PGF_{1α} > PGE₂ > thromboxane B₂ ≥ PGF_{2α}, whereas in central airways it was PGD₂ > PGE₂ > 6-keto PGF_{1α} > PGF_{2α} ≥ thromboxane B₂.

Responses to Anti-IgE

Anti-IgE contracted both central and peripheral bronchi in a concentration-dependent manner. However, in contrast to RW AgE, it was five-fold more potent in central compared with peripheral airways (Figure 3a). The -log (μg/ml) EC₅₀ concentration of anti-IgE averaged 0.86 ± 0.16 (n = 12) and 1.61 ± 0.23 (n = 8) (p < 0.05) in the peripheral and central airways, respectively. Again, responses in tissue obtained from surgery were not different from responses in tissue obtained from organ donors; for example the -log (μg/ml) EC₅₀ concentration of anti-IgE in peripheral tissue was 0.90 ± 0.20 (n = 9) and 0.74 ± 0.30 (n = 3), respectively (p > 0.4). The kinetics of the response to 3,000 ng/ml anti-IgE response were similar in both sizes of airway (Figure 3b). The time taken to half the maximum response was 439 ± 95 s (n = 6)

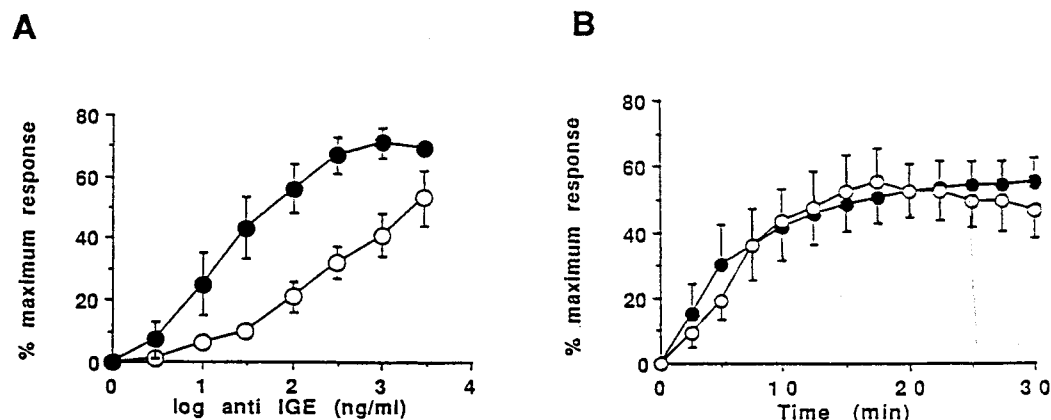


Figure 3. A) Concentration response curves to anti IgE (1 to 3,000 ng/ml) in human central (closed circles) and peripheral (open circles) airways in the presence of indomethacin (3 μM). Results are calculated as the percent of the maximum response to 30 mM BaCl₂, and they are given as mean ± SEM; n = 6 to 8 for central bronchi and n = 12 for peripheral bronchi. B) Kinetics of contractile response to anti IgE (3 μg/ml) in human central (closed circles) and peripheral (open circles) bronchi in the presence of indomethacin (3 μM). Results are calculated as percent of the maximum response to 30 mM BaCl₂, and they are given as mean ± SEM; n = 6 for central and peripheral bronchi.

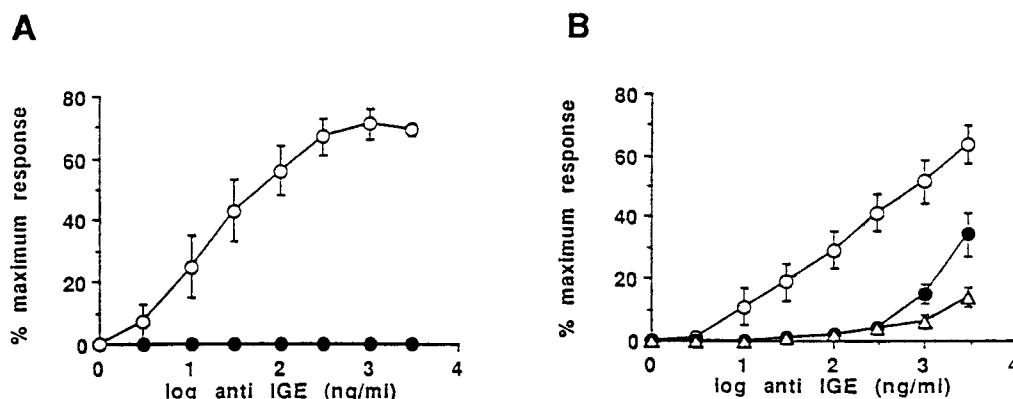


Figure 4. A) Contraction to anti-IgE (1 to 3,000 ng/ml) in human central bronchi in the presence of indomethacin (3 μ M). Control responses to anti-IgE (open circles) ($n = 6$ to 8) and inhibition of contraction by SKF 104353 (10 μ M) and pyrilamine (1 μ M) (closed circles) ($n = 8$). B) Contraction to anti-IgE (1 to 3,000 ng/ml) in human peripheral bronchi in the presence of indomethacin (3 μ M). Control responses to anti-IgE (open circles) ($n = 12$), inhibition of contraction by SKF 104353 (10 μ M) and pyrilamine (1 μ M) (closed circles) ($n = 6$ to 9) and inhibition by SKF 104353, pyrilamine, and zileuton (3 μ M) (triangles) ($n = 8$). Results are calculated as the percent of the maximum response to 30 mM BaCl₂, and they are given as mean $\pm$ SEM.

for peripheral airways and 398 ± 99 s ($n = 6$) ($p > 0.7$) for central airways. The contractile response in central airways was completely inhibited by the combination of indomethacin, pyrilamine (1 μ M), and SKF 104353 (10 μ M) (Figure 4a), whereas in peripheral airways this combination only partially inhibited responses (Figure 4b). Adding the 5' lipoxygenase inhibitor zileuton (10 μ M) to pyrilamine and SKF 104353 produced a further inhibition in responses to anti-IgE, but still did not abolish responses (Figure 4b). This response that remained after pyrilamine, SKF 104353, and zileuton treatment was not inhibited by either the platelet activating factor acether antagonist, WEB 2086 (10 μ M) or the 5-hydroxytryptamine antagonist, ketanserin (10 μ M). In the experiments with WEB 2086, the response to 3,000 ng/ml anti-IgE was $10 \pm 5\%$ ($n = 4$) in the presence of pyrilamine, SKF 104353, and zileuton and $13 \pm 5\%$ ($n = 4$) in the presence of these drugs and WEB 2086. In the experiments with ketanserin, the response to 3,000 ng/ml anti-IgE was $16 \pm 4\%$ ($n = 5$) in the presence of pyrilamine, SKF 104353, and zileuton and $15 \pm 3\%$ ($n = 5$) in the presence of these drugs and ketanserin.

The release of mediators to anti-IgE was also studied in central and peripheral airways. The profile of histamine and eicosanoid release in central and peripheral airways was similar to that seen with RW AgE (Table 1).

DISCUSSION

The immediate hypersensitivity response in human central bronchi was compared with that observed in more peripheral bronchi. In tissues treated with indomethacin, RW AgE was much more potent in contracting peripheral as compared with central airways, whereas the maximum effect elicited by antigen was similar in the two tissues. We considered three explanations for this observation: (1) the density of mast cells may be greater in peripheral than in central bronchi; (2) due to the potential heterogeneous population of mast cells, antigen may be evoking contractions by different mechanisms in the central versus peripheral airways; (3) the passive sensitization process may more effectively sensitize mast cells in the peripheral airways over the central airways.

The histamine content of a tissue is correlated to the number of mast cells in the tissue (22), and if we assume that the histamine content of individual mast cells found in the walls of the cen-

tral and peripheral airways is the same, then the data indicate that the density of mast cells is about 5 times greater in the peripheral airways than in the central airways. This explains the greater amounts of inflammatory mediator released in the peripheral airways, which may have resulted in a larger concentration of contractile mediators in the region of the smooth muscle for any given concentration of antigen. This would likely have the effect of increasing the potency of antigen for eliciting smooth muscle contraction.

We obtained little evidence for the hypothesis that differences in antigen potency are explained by differences in mast cell characteristics of the two tissues. In agreement with previous studies (4–6), we found that the contractile response to antigen in central bronchi is mediated by histamine and cysteinyl-leukotrienes. Responses to antigen in peripheral airways were also greatly inhibited by the combination of histamine and leukotriene-receptor antagonists. Mast cells in the lung have been divided into two types, those containing tryptase (T type) and those containing tryptase and chymase (TC type). Ninety percent of mast cells in dispersed lung cells are T type and 10% TC type (23). There is, however, variation in location for these different types. For example, in bronchial epithelium, 99% are T type and 1% are TC type, whereas in bronchial subepithelium 77% are T type and 23% are TC type (23). If these types are analogous to the rodent mucosal and connective tissue mast cells, respectively, as has been suggested (23), the mucosal mast cells, in comparison with connective tissue mast cells, would be expected to release low levels of histamine and PGD₂, but high levels of LTC₄ (24–27). In the present study there was little difference noted in the ratio of the release of histamine, PGD₂, and i-LTD₄ from the peripheral and central airways. The histamine: PGD₂:i-LTD₄ ratio was 125:1:1 and 250:2:1 in the peripheral and central airways, respectively. The amount of histamine released (as a percentage of total content) was also similar in the two airway groups.

Although the mast cells may be similar in the peripheral and central airways, the effect of mast cell mediators on secondary cells may differ in the two tissues. This may explain the difference in the prostanoid profile between central and peripheral airways. In the central airways, PGD₂ made up about 80% of the total prostanoid pool, whereas less than 50% of the prostanoid

pool from the peripheral bronchi was PGD₂. On a percentage basis, significantly more PGE₂ was released from the central airways (14% of the total pool) than peripheral airways (4% of the pool).

A larger percentage of unoccupied IgE receptors on the mast cells in the peripheral airways as compared with the central airways may also contribute to the increase in RW AgE potency in contracting the former tissues. This is based on the fact that RW AgE-induced responses were performed on passively sensitized tissues, and the amount of relevant antibody that binds to the mast cells is a function of the availability of unoccupied Fc receptors. To address this hypothesis, we evaluated the contractile potency of anti-IgE in unsensitized central and peripheral airways. In contrast to the effect with antigen, we found that anti-IgE was significantly less potent in contracting peripheral airways. This provides indirect evidence in favor of the hypothesis that there may be more nonspecific IgE bound per mast cell in the larger airways. Consequently, the passive sensitization procedure may have been less effective in sensitizing mast cells in these airways, accounting for the decrease in the potency of RW AgE.

It is possible that anti-IgE may stimulate cells that RW AgE does not. One scenario would be that high concentrations of anti-IgE cause the release of mediators from inflammatory cells that possess low levels of Fc receptors for IgE. Cells that fit this description and are found in the airway wall include macrophages (28, 29) and eosinophils (30). The observation that the mediator release profiles obtained with antigen and anti-IgE are virtually indistinguishable suggests, however, that these two stimuli act in a similar fashion in the airways. In the peripheral airways the contractile responses to anti-IgE are only partially blocked by a combination of cyclooxygenase inhibitor, and histamine and cysteinyl-leukotriene receptor antagonists. Responses to anti-IgE were further reduced by the addition of the 5' lipoxygenase inhibitor, zileuton, to the antagonist cocktail. One explanation for the further blockade of responses by zileuton is that the amount of cysteinyl-leukotrienes being produced by anti-IgE, in the absence of 5'-lipoxygenase blockade, was sufficient to overcome the competitive antagonism afforded by SKF 104353. This seems unlikely, however, because antigen challenge of the peripheral airways produced similar amounts of i-LTD₄, yet the contractions were virtually abolished by the histamine and leukotriene receptor antagonists. An alternative explanation is that 5' lipoxygenase products other than cysteinyl-leukotrienes are contributing to the contractile response. One such candidate is leukotriene B₄, which is known to elicit small contractions in human isolated bronchi (31). Even after treatment with SKF 104353, pyrilamine, and zileuton, a small contraction in response to anti-IgE remained in peripheral bronchi. It has been suggested that mediators other than histamine and leukotrienes are released upon anaphylaxis. These include platelet activating factor (PAF) (32) and serotonin (33). However, our results with the PAF receptor antagonist WEB 2086 and the 5-HT receptor antagonist, ketanserin, suggest that the residual anti-IgE-induced contraction is unlikely to be due to either PAF or serotonin.

In conclusion, the potency of RW AgE in eliciting contraction is greater in passively sensitized peripheral bronchi as compared with passively sensitized central bronchi. This is unlikely to be due to differences in the characteristics in the mast cells in the two groups of tissues. Rather, this may be secondary to the increase in mast cell density in peripheral airways. An increase in the ability of RW-specific IgE to occupy unbound IgE receptors on mast cells in the small airways may also contribute to this phenomenon. We also conclude that at high concentrations there

may be qualitative differences in the contractions elicited by antigen and anti-IgE, with mediators other than histamine and cysteinyl-leukotrienes contributing to the latter. Thus, some caution may be warranted when using anti-IgE as a model of antigen-induced responses in peripheral bronchi.

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