Hyperpnea-induced bronchoconstriction is dependent on tachykinin-induced cysteinyl leukotriene synthesis

X. X. YANG, W. S. POWELL, M. HOJO, AND J. G. MARTIN
Mackins-Christie Laboratories, McGill University Montreal, Quebec, Canada H2X 2P2

The guinea pig is one of the most frequently studied models of hyperpnea-induced airway responses (37). In this species, the release of tachykinins, low-molecular-weight neuropeptides, from sensory C fiber nerve endings found in airways appears to contribute to the airway narrowing of HIB (36). Inhibition of neutral endopeptidase, an enzyme that degrades tachykinin in the airways and augments HIB and capsaicin, a neurotoxin that destroys tachykinin-containing nerve fibers, diminishes HIB (15). Furthermore, selective neurokinin (NK) antagonists abolish HIB (3). It is possible that tachykinins act through the production of other bronchoactive mediators such as cysteinyl leukotrienes (LTs). It has been shown both in vivo and in vitro that airway contractile responses to sensory nerve stimulation can be inhibited by cysteinyl LT antagonists and that different neural responses in vitro can be potentiated by the addition of exogenous LTD₄ (13). These findings suggest the possibility of LT involvement in HIB in guinea pigs. Various cyclooxygenase and lipoxygenase inhibitors and a LTD₄ antagonist have been shown to reduce the magnitude of HIB in the guinea pig (15). However, it is not known whether cysteinyl LTs act in parallel or in series with tachykinins to induce airway narrowing. It is possible that cysteinyl LTs and tachykinins may be involved in a cascade of reactions. Indeed, there is evidence that cysteinyl LTs can release tachykinins in airway tissue (5, 30).

We hypothesized that cysteinyl LTs mediate HIB and that their release is stimulated by the action of tachykinins on effector cells in the airways. To investigate the role of cysteinyl LTs in HIB, we took advantage of the fact that the bile is the major route for their excretion in the guinea pig (17, 26). Biliary cysteinyl LT levels were quantitated before and after hyperpnea challenge, and the effects of selective NK antagonists on cysteinyl LT levels were determined. We reasoned that cysteinyl LT levels after hyperpnea challenge would be unaffected by pretreatment of animals with NK antagonists if LT synthesis were independent of the endogenously released tachykinins. However, if our hypotheses were correct, the levels of cysteinyl LTs in bile should be reduced by pretreatment with NK antagonists. Furthermore, to evaluate the possible contribution of mast cells to the production of bronchoconstrictive mediators after hyperpnea, we measured histamine levels in bronchoalveolar lavage (BAL) fluid and tested the effects of an antihistamine on the degree of bronchoconstriction.

MATERIALS AND METHODS

Animal preparation. Forty-one male Hartley guinea pigs (400–550 g) were purchased from Charles River (St. Constant, Quebec, Canada). Guinea pigs were anesthetized with...
xylazine (7 mg/kg) and pentobarbital sodium (65 mg/kg) intraperitoneally. A high cervical tracheostomy was performed, and a short piece of polyethylene tubing (PE-240; 0.165 cm ID, 2.5 cm long) was inserted into the trachea below the second cartilaginous ring and connected to a small-animal respirator (model 360 rodent ventilator; Harvard, South Natick, MA). Silastic medical-grade tubing (Dow Corning Medical Products, Midland, MI) was used to cannulate the internal jugular vein for fluid replacement and administration of drugs. The inspiratory and expiratory tubes of the ventilator were attached to the tracheal cannula through a Y connector with a 3-cm common segment (total dead space 0.24 ml) to minimize conditioning of the inspired gas. The inspiratory port of the ventilator was connected to a warm (35–37°C) humidifier through which room air was passed. The tracheal pressure (P*tr) was measured at the tracheal cannula by a piezoresistive differential pressure transducer (model SCX010DN; SenSym, Sunnyvale, CA). The animals were mechanically ventilated with a tidal volume of 6 ml/kg and 60 breaths/min. PTR signals were amplified, passed through eight-pole Bessel filters (model 902 lpf, Frequency Devices, Haverhill, MA), and passed through an analog-to-digital converter (model D7-2801-a, Data Translation, Marlborough, MA) for final storage in an IBM compatible personal computer. A commercial software package (RHT Infodat, Montreal, Quebec, Canada) was used to analyze the changes in P*tr. Increases in P*tr, which reflect increases in respiratory system impedance, were interpreted to indicate airway narrowing and, for convenience, are referred to throughout the text as bronchoconstriction even though the possible contributions of microvascular leak and other mechanical changes were not quantitated.

Collection of bile. The bile duct was cannulated with polyethylene tubing (PE-60) through a small incision in the abdominal wall. After surgery, the animals were allowed to stabilize for a period of 90 min before challenge. For 1 h before and for 2 h after hyperpnea challenge, bile was collected under a stream of argon into Eppendorf tubes that were kept on ice. Bile was stored at −80°C before analysis by reverse-phase high-pressure liquid chromatography (RP-HPLC) and radioimmunoassay.

Experimental protocol. Dry gas hyperpnea (test; n = 7) was performed by introducing a dry mixture of 5% CO₂-95% O₂ from a balloon into the inspiratory port of the mechanical ventilator. The ventilatory rate was set at 150 breaths/min, and the tidal volume was increased to 4 ml; hyperpnea was continued for 5 min. Humidified gas hyperpnea challenge (control; n = 6) was performed in an identical fashion, except that the 5% CO₂-95% O₂ was bubbled through a water bath at 37°C before it passed through the ventilator. Baseline ventilator settings and gas mixtures were resumed after hyperpnea challenge for a period that lasted until baseline PTR was reestablished (usually 10–40 min). PTR measurements were obtained at 1-min intervals for the first 10 min into the recovery period, at 5-min intervals for the next 20 min, and at 15-min intervals thereafter for up to 2 h.

To evaluate the role of LTD₄ in HIB, a specific LTD₄ antagonist, MK-571 (2 mg/kg dissolved in 1 ml of 0.9% saline), was administered to a group of guinea pigs (MK-571; n = 5) through the jugular vein catheter 10 min before the dry gas hyperpnea challenge.

To deplete airway sensory nerves of neuropeptides, animals were pretreated with capsaicin (n = 4; total dose = 91 mg injected subcutaneously) in eight increasing doses over 5 days. Capsaicin was dissolved in 10% Tween 20 (Sigma Chemical, St. Louis, MO), 10% ethanol, and 80% saline. Each dosage was given as follows: day 1: 0.1 ml of 1.0 mg/ml capsaicin; day 2: 0.5 ml of 1.0 mg/ml capsaicin; day 3: 0.5 ml of 10 mg/ml capsaicin; day 4: 0.2 ml and subsequently 0.3 ml of 50 mg/ml capsaicin; and day 5: 0.5 ml and subsequently 0.7 ml of 50 mg/ml capsaicin. To counteract respiratory impairment caused by capsaicin, animals were treated with 2.5 mg/ml of aerosolized salbutamol for 5 min before each capsaicin treatment. All animals were anesthetized with 30 mg/kg ip pentobarbital sodium and were administered supplementary O₂. The dry gas hyperpnea challenge was performed 1 day after the final capsaicin pretreatment.

The role of tachykinins in HIB was also evaluated by using specific NK antagonists. The specific NK₁-receptor antagonist CP-99994 and the NK₂-receptor antagonist SR-48968 were dissolved in equal volumes of ethylene glycol and saline. All animals (NK antagonists; n = 6) were administered a combination of CP-99994 (1 mg/kg) and SR-48968 (1 mg/kg) into the jugular vein. Dry gas hyperpnea challenge was performed 10 min after the administration of the NK antagonists.

The role of histamine in HIB in guinea pigs (pyrilamine; n = 5) was assessed by using a specific histamine type 1-receptor antagonist. The histamine type 1-receptor antagonist pyrilamine was dissolved in saline (2 mg/ml in 1 ml) and administered intravenously through the jugular vein 10 min before dry gas hyperpnea challenge was performed. BAL fluid analysis from unchallenged control animals (n = 6).

Recovery of radioactive LT metabolites after tracheal instillation of [³H]LTC₄. Two guinea pigs were examined for the recovery of radioactive products in bile after instillation of 1 µCi [¹⁴,¹⁵-³H]LTC₄ in 100 µl of saline into the trachea. The bile was collected continuously for 4 h in 15-min fractions. The radioactivity in 30% of each bile sample was measured by liquid scintillation counting. The remaining volumes of each of the 15-min samples were pooled so as to obtain fractions corresponding to the following time periods: 0–1 h, 1–2 h, 2–3 h, and 3–4 h. Each fraction was analyzed by RP-HPLC as described below.

Separation of LTs by precolumn extraction/RP-HPLC. Methanol (1.6 ml) was added to an aliquot (0.4 ml) of each bile sample to yield a final concentration of 80% methanol. Samples were allowed to stand at room temperature for 30 min and were then centrifuged at 3,500 g for 10 min to remove protein. Distilled water (3.3 ml) was added to the supernatant to give a concentration of 30% methanol, and the pH was adjusted to 3.0 by using 1 M phosphoric acid. The sample was then filtered by using a 0.45-µm filter (Millex-HV, Millipore-Waters, Bedford, MA). With a Milton-Roy minipump, the sample (5.3 ml total volume) was loaded via a six-port switching valve onto a precolumn cartridge (µBondapak C₁₈ GuardPak, Millipore-Waters), which had been prequillibrated with 11 ml of 30% methanol adjusted to pH 3 by the addition of 1 M phosphoric acid. The precolumn was washed with 10 ml of 20% methanol containing 1 mM EDTA and 0.1% acetic acid and adjusted to a pH of 5.4 by the addition of ammonium hydroxide. The six-port valve was then switched to the "inject" position, placing the precolumn in line with an HPLC pump (model 510, Millipore-Waters) and an octadecylsilica HPLC column (Adsorbosphere HS C₁₈, 3-µm particle size, 4.6 × 150 mm, Alltech). The mobile phase consisted of methanol-water-acetic acid (64:36:0.1) containing 1 mM EDTA and adjusted to a pH of 5.4 by the addition of ammonium hydroxide. The flow rate was 0.7 ml/min. Ultraviolet absorbance was monitored by a variable wavelength ultraviolet detector (model 481, Millipore-Waters). Fractions collected every 1 min were monitored either for radioactivity by using a liquid scintillation counter (model LS 6000IC, Beckman).
Significant test was used. Differences were considered to be statistically significant at \( P < 0.05 \).

Radioimmunoassay. Column fractions were evaporated to dryness in a vacuum centrifuge, and the residues were dissolved in phosphate-buffered saline (0.1 ml at pH 8). Cysteinyl LTs were measured in each fraction by using a monoclonal antibody directed against LTC\(_4\), which cross-reacted with LTD\(_4\) (63.8 \pm 10.9\%), LTE\(_4\) (49.7 \pm 3.7\%), and N-acetyl LTE\(_4\) (59.5 \pm 7.4\%). [14,15-\(^3\)H]LTC\(_4\) was used as the radioactive ligand. All values were corrected for the degree of cross-reactivity of the LTs with the antibody, but they were not corrected for the recovery of cysteinyl LTs, which was \( \sim 60\% \) in the case of LTC\(_4\). The background values for materials displaying LTC\(_4\)-like immunoreactivity were quite low in the HPLC fractions and were not subtracted from the reported values for LTs. The total amounts of cysteinyl LTs in bile were calculated by adding the amounts (in pmol/time point) for LTC\(_4\), LTD\(_4\), LTE\(_4\), and N-acetyl LTE\(_4\).

Histamine assay. BAL was performed immediately after the peak increase in P\(_{\text{tr}}\) by instilling 5 ml of normal saline via the endotracheal tube. The saline was immediately reaspirated and kept at \(-40^\circ\)C until assayed. The histamine content in BAL fluid was measured by using a colorimetric assay based on the imidazole group of histamine. In brief, 0.5 ml of sample mixed with both 0.1 ml of 1% sulfanilic acid and 0.1 ml of 5% aqueous sodium nitrite solution was incubated for 10 min. Then 1.3 ml of aqueous 5% sodium carbonate solution were added. Two minutes later, 1 ml of 75% of ethanol was added. The absorbance of the orange-red complex in 200 µl of reaction mixture was measured at 530 nm within 20 min using a microplate reader (400 ATC, SLT Laboratories, Unterbergstrassel, A5082 Grödig/Salzburg, Austria). A standard curve was performed by using histamine dihydrochloride in concentrations ranging from 0 to 40 µg/ml.

Statistical analysis. All results are expressed as means ± SE. Comparison of two means was performed by using paired or unpaired t-tests or Wilcoxon’s signed rank test as appropriate. For comparison of several means, an analysis of variance (ANOVA) followed by the Fisher’s least significant differences test was used. Differences were considered to be statistically significant at \( P < 0.05 \).

Drugs and chemicals. LTC\(_4\), LTD\(_4\), LTE\(_4\), N-acetyl LTE\(_4\), monoclonal antibody to LTC\(_4\), and the specific LTD\(_2\)-receptor antagonist MK-571 were kindly provided by Dr. A. W. Ford-Hutchinson (Merck-Frosst, Pointe Claire, Quebec, Canada). Capsaicin, histamine hydrochloride, and sulfanilic acid were purchased from Sigma Chemical. The NK\(_1\)-receptor antagonist CP-99994 and NK\(_2\)-receptor antagonist SR-48968 were kindly provided by Dr. Ian Rodger (Merck-Frosst). Salbutamol was purchased from Glaxo Canada (Toronto, Ontario). Pentobarbital sodium was supplied by MTC Pharmaceuticals (Cambridge, Ontario, Canada). Pyrilamine was purchased from Research Biochemicals International (Natick, MA). Sodium nitrite and sodium carbonate were purchased from Fisher Scientific (Fair Lawn, N.J.).

RESULTS

Recovery of radioactive products in bile after tracheal instillation of [\(^3\)H]LTC\(_4\). The time course of the appearance of radioactivity in the bile of the two guinea pigs that received [14,15-\(^3\)H]LTC\(_4\) by tracheal instillation is shown in Fig. 1. The radioactivity in the bile samples increased almost linearly with time for the first 3 h and appeared to reach a plateau by 4 h. About 23% of the instilled radioactive material was recovered within 4 h (21 and 24% in each animal). The LT species contained in the radioactive material appearing in the bile samples were determined by RP-HPLC analysis. For this purpose, the bile samples were pooled to give four fractions, each corresponding to a collection period of 1 h, after instillation of [\(^3\)H]LTC\(_4\). The major species identified was LTD\(_4\) (38.4% of the total recovered radioactivity in the bile), whereas smaller amounts of LTC\(_4\) (10.7%), LTE\(_4\) (19.8%), and N-acetyl LTE\(_4\) (8.7%) were detected. The balance of the radioactivity was associated with unidentified polar species. There was no obvious change in the proportions of the different species over time.

Airway response to hyperpnea challenge. The baseline values of inspiratory P\(_{\text{tr}}\) were not significantly different among the various treatment groups. The time course of the change in the values of P\(_{\text{tr}}\) after isocapnic dry gas hyperpnea challenge was consistent with the development of bronchoconstriction (Fig. 2). The inspiratory P\(_{\text{tr}}\) showed an increase as early as 1 min after dry gas challenge compared with control animals challenged with humidified gas. The greatest change was observed by 10 min, so we chose this time point to test the statistical significance of the changes among groups (ANOVA and Fisher’s least significant differences test; \( P < 0.05 \)), and there was a gradual recovery over the subsequent 40–50 min. In contrast, no changes in P\(_{\text{tr}}\) were observed in control animals that received humidified gas. The constrictive response to dry gas was virtually abolished by pretreatment with either the LTD\(_2\)-receptor antagonist MK-571 or a combination of the NK\(_1\)-receptor antagonist CP-99994 and the NK\(_2\)-receptor antagonist SR-48968 (Fig. 2). The response was also abolished by depletion of NK\(_5\) from sensory nerves with capsaicin (Fig. 2). The peak value...
of Ptrafter challenge in the test group was significantly higher than the baseline value (paired t-test; \( P < 0.05 \)) and the peak values of Ptrafter challenge in the other groups (ANOVA and Fisher’s least significant differences test; \( P < 0.05 \)), with the exception of the pyrilamine-treated animals, which were not significantly different in their responses to the test group (Fig. 3).

Biliary cysteinyl LTs and hyperpnea challenge. To minimize the contribution of the surgical procedure to biliary LTs (12), we waited for 60 min before collecting bile for baseline measurements of cysteinyl LTs. The total cysteinyl LTs (expressed as the sum of LTC4, LTD4, LTE4, and N-acetyl LTE4) in bile before and after hyperpnea challenge are shown for the different treatment groups in Fig. 4. The biliary cysteinyl LTs were not analyzed for the MK-571-treated animals as it was not anticipated that the results in this group would be different from those for the dry gas-challenged test group. The total biliary levels of cysteinyl LTs before hyperpnea challenge were not significantly different among the groups (Fig. 4). There were few differences in the baseline levels of the various cysteinyl LTs among treatment groups (Table 1). However, LTD4 was significantly higher in the NK group compared with control and capsaicin-treated animals. After hyperpnea challenge, the total cysteinyl LT levels rose significantly (Wilcoxon’s signed ranks test; \( P < 0.05 \)) in the

| Table 1. Baseline prechallenge level of biliary cysteinyl leukotrienes |
|-------------------------|----------------|------------------|-------------------|------------------|
| Biliary Leukotrienes, pmol/h | Control (n = 10) | Test (n = 10) | Capsaicin (n = 10) | Neurokinin antagonist (n = 10) |
| LeukotrieneC4 | 0.6 ± 0.1 | 0.9 ± 0.5 | 0.2 ± 0.4 | 0.6 ± 0.3 |
| | (11.9) | (6.8) | (10.9) | (5.6) |
| LeukotrieneD4 | 1.4 ± 0.7 | 4.6 ± 1.6 | 0.5 ± 0.1 | 5.1 ± 0.9* |
| | (29.9) | (36.2) | (30.0) | (77.2) |
| LeukotrieneE4 | 2.0 ± 0.6 | 4.6 ± 2.5 | 0.7 ± 0.4 | 1.1 ± 0.6 |
| | (40.4) | (36.1) | (42.1) | (9.1) |
| N-acetyl leukotrieneE4 | 0.9 ± 0.3 | 2.6 ± 1.4 | 0.3 ± 0.16 | 1.0 ± 0.8 |
| | (17.7) | (20.8) | (18.0) | (8.1) |

Values are means ± SE; n, no. of animals. Percentage of total biliary cysteinyl leukotrienes shown in parentheses.
test group but not in the control group in which the challenge was performed with humidified air (Fig. 4). Nor did the levels of cysteinyl LTs increase after hyperpnea challenge in the groups of animals that were pretreated with either capsaicin or a combination of the NK₁- and NK₂-receptor antagonists. The bile was not analyzed from pyrilamine-treated animals. The change in cysteinyl LTs in the test group is attributable to LTD₄, which rose from 4.6 ± 1.6 to 12.5 ± 5.0 pmol/h (ANOVA and Fisher’s least significant differences test; P < 0.05). LTE₄ also tended to be higher in the test group, but the difference did not quite reach statistical significance (Fig. 5).

Histamine levels in BAL fluid. The concentration of histamine in the BAL fluid of unchallenged guinea pigs was 5.09 ± 0.6 µg/ml. There was no significant difference in the concentration of histamine in the lavage fluid of dry gas-challenged animals (6.43 ± 2.05 µg/ml; P = 0.24).

DISCUSSION

The principal aim of this study was to determine the relationship between the airway response to isocapnic dry gas hyperpnea challenge and the synthesis of cysteinyl LTs. The blockade of HIB by selective antagonists of both LTD₄ and NK receptors indicates that tachykinins and cysteinyl LTs are both important mediators of the bronchoconstrictive response to hyperpnea. The completeness of the blockade caused by the antagonists of either pathway suggests an important interaction between these two classes of mediators. The finding that a selective LTD₄ antagonist eliminated the airway response to hyperpnea suggests that tachykinins may evoke airway narrowing indirectly by the release of cysteinyl LTs from airway effector cells. The abolition of an increase in cysteinyl LT synthesis by pretreatment with NK antagonists confirms that airway tachykinins evoke cysteinyl LT release.

A limitation of our study is that we quantified the airway responses to hyperpnea challenge from changes in Pτ that reflect alterations in respiratory system impedance. The pulmonary response to hyperpnea challenge is clearly complex and involves changes in both airway and tissue resistance (31) as well as microvascular leak (15). However, we have assumed that most of the measured response is a consequence of airway narrowing resulting from airway smooth muscle contraction, which has been confirmed by morphometric studies (31).

Measurement of biliary cysteinyl LTs is a convenient method to evaluate the synthesis of these substances in vivo (17). The biliary recovery of radioactivity after intratracheal instillation of [³H]LTC₄ was ~23% in 4 h. Although this value is substantially less than the recovery of intravenously injected [³H]LTC₄, which has been reported to be 60% over 6 h, it is similar to the recovery of the intratracheally instilled label in rats (29). The conversion of LTC₄ to LTD₄ was almost complete, with only relatively small amounts of LTC₄ excreted unchanged. LTD₄ was the major cysteinyl LT identified in bile in most circumstances, in contrast to the rat, in which N-acetyl LTE₄ is the major identifiable species but in agreement with other studies on the metabolism of LTC₄ in the guinea pig. LTE₄ is the major cysteinyl LT metabolite in humans and other primates (19).

Our study and previous observations have demonstrated that HIB in guinea pigs can be entirely attributed to tachykinins (15). Sensory neuropeptides [substance P (SP); NKA, and calcitonin gene-related peptide (CGRP)] have been shown to be capable of producing constriction of airway smooth muscle, edema and plasma extravasation, and mucus hypersecretion both in animals and humans (2, 8, 27, 34). Tachykinins released by exogenous agonists or exogenously administered can cause bronchoconstriction (6, 16). Garland et al. (15) have shown that capsaicin pretreatment blunts the bronchoconstriction evoked by isocapnic dry gas hyperpnea. As expected, phosphoramidon, a neutral endopeptidase inhibitor, potentiates the effects of HIB, which is further, albeit indirect, evidence of participation of neuropeptides in the airway response (25). More recent data from studies that used the selective NK₁ (SP)-receptor antagonist CP-96345 and the NK₂ (NKA)-receptor antagonist SR-48968 have demonstrated attenuation of the airway response to HIB (39), providing more support for the role of tachykinins in this phenomenon. Our experiments have provided similar results: HIB is abolished after depletion of tachykinins by capsaicin and significantly blunted by pretreatment with a combination of the NK₁-receptor antagonist CP-99994 and the NK₂-receptor antagonist SR-48968.

The biliary LTD₄ level significantly increases after isocapnic dry gas hyperpnea challenge, indicating increased synthesis of cysteinyl LTs. The absence of such change after a similar challenge with humidified gas indicates the specificity of the response to the airway challenge. An important role for cysteinyl LTs in the
airway narrowing is confirmed by the demonstration that there is no significant change in $\text{Ptr}$ after hyperpnea challenge in guinea pigs after LTD$_4$ antagonist pretreatment. Cysteinyl LTs have been previously implicated in HIB in the guinea pig, since LT levels in BAL increase significantly after this treatment (21).

The completeness of the blockade of HIB by an LTD$_4$ antagonist and a 5-lipoxygenase inhibitor provide strong circumstantial evidence that cysteinyl LTs interact with tachykinins to cause airway narrowing (24, 28). The nature of this interaction has not yet been established. Bloomquist and Kream (5) showed that LTD$_4$ contracted guinea pig tracheal smooth muscle in part by releasing SP. Recently, Ellis and Undem (13) demonstrated that LTD$_4$ potentiated the capsaicin-sensitive peptidergic airway contraction and plasma extravasation that was induced both by vagal stimulation and electrical field stimulation of the trachea and the main bronchi of the guinea pig. Both effects were altered by the selective cysteinyl LT antagonists SKF-104353, ICI-198615 (LTD$_4$), and WY-48252. Exogenous substance P and NKA cause bronchoconstriction in the guinea pig that is not attenuated by an LTD$_4$ antagonist (13), which has led to the speculation that LTs release tachykinins in the airways and not the reverse (15). Because NK$_1$ and NK$_2$ antagonists block both the isocapnic dry gas HIB and the increase in biliary cysteinyl LTs, it appears that cysteinyl LTs are released by tachykinins and are the final mediators of HIB. The discrepancy between the effects of endogenous and exogenous tachykinins may reflect the fact that locally released neuropeptide may act principally on neighboring cells that are not smooth muscle cells.

Although we established the participation of cysteinyl LTs in HIB, our experiments did not permit us to identify the precise sources of cysteinyl LTs during HIB in guinea pigs. We considered the airway mast cell to be one of the most likely candidates. SP has been found to stimulate histamine release from the mast cell (23), and morphological studies have shown that there is a close association between SP-containing neurons and mast cells in rat lung (4). Nilsson et al. (32) have demonstrated the association of serotonin-positive cells and CGRP and NKA-immunoreactive nerves in the bronchi of the rat. Interestingly, the so-called mast cell stabilizers nedocromil sodium and sodium cromoglycate blunt tachykinin-induced bronchoconstriction in asthmatic subjects (33). The failure of pyrilamine to prevent the bronchoconstrictive response to dry gas hyperpnea challenge and the lack of an increase in lavage fluid histamine argues against significant mast cell degranulation. Unfortunately, so-called mast cell stabilizing agents have multiple potential sites of action so that their efficacy in preventing airway responses to hyperpnea challenge cannot be construed as implicating mast cells. Airway epithelial cells and macrophages are other potential sources of cysteinyl LTs in airways (14, 18).

The resemblance of the guinea pig reaction to dry air hyperpnea challenge and human asthmatic subjects to the same stimulus suggests that it is a potentially useful model of EIA. The results of our study demonstrate a further similarity to EIA in that cysteinyl LTs have been convincingly implicated in the airway narrowing triggered in this way. Pliss et al. (35) have showed that the concentrations of cysteinyl LTs in BAL fluid are significantly increased after isocapnic hyperpnea in asthmatic subjects. Israel et al. (24) have found that A-64077, a 5-lipoxygenase inhibitor, increased the tolerance of asthmatic subjects to hyperventilation with cold dry air and decreased the level of plasma LTB$_4$. Furthermore, the LTD$_4$-receptor antagonist MK-571 attenuates EIA in asthmatic subjects (28).

In conclusion, HIB in guinea pigs is mediated by cysteinyl LTs. The release of these mediators appears to be triggered by tachykinins that are presumably released from sensory nerve endings stimulated by altered osmolarity or thermal fluxes related to inhalation of dry air. Histamine was not involved in dry gas HIB in guinea pigs. The airway cells responsible for cysteinyl LT synthesis remain to be identified.

The authors thank Liz Milne for help in the preparation of the manuscript. This study was supported by the Medical Research Council of Canada Grant 7852 and the Respiratory Health Network of Centres of Excellence of Canada. Address for reprint requests: J. G. Martin, Meakins-Christie Laboratories, McGill Univ., 3626 St. Urbain St., Montreal, Quebec, Canada, H2X 2P2.

Received 7 November 1995; accepted in final form 16 October 1996.

REFERENCES


