Mucosal injury and eicosanoid kinetics during hyperventilation-induced bronchoconstriction

ARTHUR N. FREED, YONGQIANG WANG, SHARRON McCulloch, TERESA MYERS, AND RYOICHI SUZUKI
Department of Environmental Health Sciences, The Johns Hopkins University, Baltimore, Maryland 21205

Freed, Arthur N., Yongqiang Wang, Sharron McCulloch, Teresa Myers, and Ryoichi Suzuki. Mucosal injury and eicosanoid kinetics during hyperventilation-induced bronchoconstriction. J. Appl. Physiol. 87(5): 1724–1733, 1999.—Bronchoalveolar lavage (BAL) of canine peripheral airways was performed at various times after hyperventilation, and BAL fluid (BALF) cell and mediator data were used to evaluate two hypotheses: 1) hyperventilation-induced mucosal injury stimulates mediator production, and 2) mucosal damage is correlated with the magnitude of hyperventilation-induced bronchoconstriction. We found that epithelial cells increased in BALF immediately after a 2- and a 5-min dry air challenge (DAC). Prostaglandins D₂ and F₂α, and thromboxane B₂ were unchanged immediately after a 2-min DAC but were significantly increased after a 5-min DAC. Leukotrienes C₄, D₄, and E₄ did not increase immediately after a 2- and a 5-min DAC but did increase until 5 min after DAC. Hyperventilation with warm moist air did not alter BALF cells or mediators and caused less airway obstruction that occurred earlier than DAC. BALF epithelial cells were correlated with mediator release, and mediator release and epithelial cells were correlated with hyperventilation-induced bronchoconstriction. These observations are consistent with the hypothesis that hyperventilation-induced mucosal damage initiates peripheral airway constriction via the release of biochemical mediators.

airway resistance; exercise-induced asthma; leukotrienes; prostaglandins; thromboxane

HYPERVENTILATION WITH DRY air increases airway tone in guinea pigs, rabbits, cats, dogs, monkeys, and humans (7). The transient airway obstruction produced by these stimuli is usually greatest 2–10 min after hyperventilation stops and spontaneously recovers 30–60 min thereafter. Although the exact mechanism(s) responsible for this hyperventilation-induced bronchoconstriction (HIB) remains unknown, hyperventilation with dry air increases airway surface fluid osmolality (10), damages the bronchial mucosa (15, 28), and stimulates local mediator release (16, 29) that results in airway smooth muscle constriction in a canine model of exercise-induced asthma (9, 10).

Numerous animal and human studies using pharmacological interventions and analyses of bronchoalveolar lavage (BAL) fluid (BALF) suggest that prostanoids (5, 16, 17, 34) and leukotrienes (LTs) (21, 29, 32, 36) contribute to the development of HIB. The purpose of this study was to document changes in BALF cells and eicosanoid mediators that occur in canine peripheral airways during and after hyperventilation with warm moist or cool dry air to evaluate the following hypotheses: 1) hyperventilation-induced mucosal injury stimulates mediator production, and 2) the quantity of mediator production and release is related to the severity of mucosal injury, and both are correlated with the magnitude of HIB. If mucosal injury stimulates production of biochemical mediators, then mucosal damage would be evident during the challenge before mediators were released. Warm moist air significantly reduces hyperventilation-induced mucosal injury (15) and should have little effect on mediator metabolism. However, the use of warm moist air does not typically abolish HIB, raising the possibility that a vagal reflex, and not mediator release, may be responsible for the residual response (9, 16–18). Finally, if increased mucosal injury resulted in greater airway obstruction via increased mediator release, then mediator release would be positively correlated with mucosal injury and HIB, and mucosal injury would be similarly correlated with the magnitude of HIB. The data presented in this report are consistent with all these hypotheses and predictions.

METHODS

Experimental Techniques

Animals were handled and maintained in accordance with the Policy and Procedures Manual published by the Johns Hopkins University School of Hygiene and Public Health's Institutional Animal Care and Use Committee.

Anesthesia and instrumentation. Colony-bred male mongrel dogs (19.8 ± 0.65 kg, n = 27) were anesthetized with thiopental sodium (25 mg/kg iv). A continuous infusion of thiopental (4–6 mg·kg⁻¹·h⁻¹) was supplemented with fentanyl citrate (25 µg iv) every 15–20 min to maintain anesthesia. Depth of anesthesia was assessed by heart rate (HR), mean arterial pressure (MAP), canthal reflex, presence of spontaneous movements, and breathing. Dogs were intubated and ventilated on room air with a Harvard constant-volume ventilator (17 ml/kg); end-expiratory CO₂ was monitored with a CO₂ analyzer (model LB-2, Beckman, Anaheim, CA) and maintained at ~4.5% by adjustment of respirator frequency. HR and MAP were monitored with a noninvasive blood pressure and HR monitor (Accutorr 1A, Datascope, Paramus, NJ). Body temperature was monitored with a telemetry belt and rectal probe (Yellow Springs Instrument, Yellow Springs, OH) and maintained with a warming pad.

Measurement of peripheral airway resistance. A fiber-optic bronchoscope (5.5 mm OD; Olympus BF type P10, Olympus, New Hyde Park, NY) was inserted through an airtight portal of the endotracheal tube and gently wedged into a sublobar...
branched. Airway pressure (P_b) beyond the tip of the bronchoscope was measured using a PE-90 catheter (1.19 mm ID, 1.7 mm OD) that was threaded through the suction port of the bronchoscope and connected to a pressure transducer (Statham, Gould, Oxnard, CA). Compressed dry room temperature 5% CO2 in air was delivered around the catheter and into the wedged sublobar segment at 200 ml/min (3.33 ml/s). Peripheral airway resistance (R_p) under quasi-static conditions was calculated from measurements made at functional residual capacity. After the ventilator is stopped, P_b plateaus at a pressure above the alveolar pressure (P_A) in the surrounding unobstructed lung. At this time, R_p = (P_b - P_A)/3.33 ml/s.

Airflow challenge. DRY AIR CHALLENGE. Bronchoconstriction was induced by increasing the flow rate of 5% CO2 in dry air from 200 to 2,000 ml/min for 2 or 5 min. The flow rate was returned to 200 ml/min, and the airway was then immediately lavaged with Hanks’ buffered saline solution (HBSS) or monitored until a specific time after the dry air challenge (DAC) and then lavaged.

WARM HUMIDIFIED AIR CHALLENGE. Insufflation of dry 5% CO2 in air was switched to warm humidified 5% CO2 in air, and the flow rate was increased from 200 to 2,000 ml/min for 2 or 5 min. The warm humidified gas was produced by bubbling dry room-temperature (23°C) 5% CO2 in air through a Plexiglas canister containing a heat exchanger submerged in warm (42°C) distilled H2O and delivered to the suction port of the bronchoscope via a heated water-jacketed tube and an in-line water-jacketed water trap. During the 5-min 2,000 ml/min warm humidified air challenge (WAC), relative humidity and temperature of the gas leaving the tip of the bronchoscope were 96 ± 1.2% and 31.2 ± 0.2°C, respectively. The method used to estimate in vivo airway temperature and relative humidity during hyperventilation has been described previously (17).

BAL, differential cell counts, and mediator analysis. Three 20-ml aliquots of warm (37°C) HBSS were infused into a wedged sublobar bronchus through the port of the bronchoscope, and each aliquot was recovered by gentle aspiration. The recovered BALF samples were pooled and stored at 4°C until the conclusion of the experiment. The BALF was then centrifuged at 4°C for 10 min at 1,300 rpm. The cell pellet from a 5-ml sample was resuspended in 1 ml of supernatant, and total cell number was determined from a 10-µl sample placed on a hemocytometer. Differential cell counts of macrophages, lymphocytes, neutrophils, eosinophils, and epithelial cells were done on cytocentrifuged specimens stained with a modified Wright-Giemsa stain. Trypan blue exclusion was used to evaluate cell viability.

Lavage fluid samples were concentrated using a Sep-Pak C18 cartridge (Waters, Milford, MA), eluted in 4 ml of methanol, and stored at -70°C. Aliquots of the eluted sample were analyzed as previously described (29) with commercially available ELISA kits for prostaglandin (PG) D2, PGE2 (Cayman Chemical, Ann Arbor, MI), PGF2α, thromboxane B2 (TXB2), and leukotriene (LT) C4, LTD4, and LTE4 (LT(C=)4E4; Neogen, Lexington, KY).

Experimental Protocols

Mucosal injury and mediator release during and after hyperventilation with wet and dry air. Six series of experiments were done with 17 mongrel dogs (19.6 ± 0.61 kg) to obtain six “snapshots” of the acute transient effects of hyperventilation on bronchial mucosal integrity and the local release of biochemical mediators (Fig. 1). In series 1, mucosal injury and mediator release were examined after 2 min of hyperventilation in 10 dogs (20.6 ± 1.19 kg). Initially, a bronchoscope was wedged in a sublobar airway of the cardiac lobe, baseline R_p was recorded, and the segment was lavaged. The bronchoscope was removed, and a clean bronchoscope was wedged in a second sublobar airway in a middle lobe. Baseline R_p was recorded, a 2-min WAC was done, and the sublobar segment was lavaged immediately after the challenge. Finally, a bronchoscope was wedged in a third sublobar airway in the contralateral middle lobe, baseline R_p was recorded, and a 2-min DAC was done. Postchallenge R_p was not recorded in series 1, because the sublobar segment was lavaged immediately after each 2-min challenge (WAC and DAC). This was the only time a 2-min exposure was used. Data from series 1 were assumed to reflect the condition of an animal immediately after challenge (0 min). Postchallenge R_p was recorded after 2 or 5 min of DAC and WAC were done for 5 min, and BAL was performed immediately after challenge (0 min). Again, postchallenge R_p was not recorded. Series 3 (n = 11): DAC and WAC were done for 5 min, R_p was recorded at 2 and 4 min (●), and BAL was done at 5 min postchallenge. Series 4 (n = 10): DAC and WAC were done for 5 min, R_p was recorded at 2, 5, and 9 min (▲), and BAL was done at 10 min postchallenge. Series 5 (n = 10): DAC and WAC were done for 5 min, R_p was recorded at 2, 5, 10, and 14 min (●), and BAL was done at 15 min postchallenge. Series 6 (n = 10): DAC and WAC were done for 5 min, R_p was recorded at 2, 5, 10, 15, and 29 min (●), and BAL was done at 30 min postchallenge. All WAC are depicted in 1 plot (□).
identical WAC was done in the same wedged segment. The efficacy of the dose used for muscarinic blockade was tested in two dogs with aerosolized ACh (30 mg/ml for 30 s).

Statistical analyses. Rp data from BAL experiments were analyzed using a Kruskal-Wallis one-way ANOVA with Dunn’s method applied to ranks to compare individual treatment means (unpaired, uneven samples). Rp data from the WAC atropine study were analyzed using a Friedman two-way ANOVA in conjunction with a Student-Newman-Keuls test for the comparison of individual treatment means (paired samples). BALF cell and mediator data at each time point were compared using a Kruskal-Wallis one-way ANOVA in conjunction with a Bonferroni correction for the comparison of multiple paired samples. Spearman rank analysis (r) was used to determine whether the concentration of biochemical mediators in BALF was correlated with the percentage of epithelial cells recovered in the BALF or the magnitude of HIB. Statistical significance was judged at P < 0.05. Values are means ± SE.

RESULTS

Hyperventilation-Induced Changes in Rp

Figure 2 is a composite of Rp data collected during the four series of experiments in which measurements of Rp were recorded and BAL was done at 5, 10, 15, or 30 min after hyperventilation. Comparison of the change in Rp (ΔRp) at each time point after WAC and DAC revealed that the ΔRp at 5 and 10 min after DAC was significantly greater than the ΔRp at 5 and 10 min after WAC (Fig. 2A). Rp increased ~95% 5 min after a DAC. In contrast, Rp in airways hyperventilated with warm humidified air increased only ~38% and peaked immediately after the WAC (Fig. 2B).

Effect of Atropine on WAC

Rp increased 0.28 ± 0.04 cmH2O·ml−1·s−1 (n = 5) 5 min after WAC (Fig. 3). Atropine reduced (P < 0.05) this response to WAC by ~30% (ΔRp = 0.20 ± 0.06 cmH2O·ml−1·s−1). Treatment with atropine did not significantly affect MAP (P = 0.625), which averaged 103 ± 6 mmHg before and 105 ± 8 mmHg (n = 5) after administration of the drug. The atropine-induced increase in HR (from 80 ± 14 to 125 ± 12 beats/min) was not statistically significant (P = 0.125). Aerosolized ACh increased Rp 0.42 ± 0.17 cmH2O·ml−1·s−1, and atropine abolished this response (ΔRp = 0.03 ± 0.04 cmH2O·ml−1·s−1, n = 2).

Effects of WAC and DAC on BALF Cell Profiles

The average volume of BALF recovered from the six control experiments was 43 ± 2 ml (~72%, range 38 ± 2 to 45 ± 2 ml). The recovered volumes for the WAC and DAC experiments averaged 43 ± 2.5 ml (~72%, range 39 ± 3 to 46 ± 2 ml) and 36 ± 4 ml (~61%, range 32 ± 4 to 41 ± 3 ml), respectively. The time-dependent recovery of macrophages, lymphocytes, neutrophils, and eosinophils in BALF was not affected by the WAC or the DAC (Fig. 4). In contrast to these leukocytes, there was a marked increase in the percentage of epithelial cells recovered at each time point from DAC airways compared with WAC or unchallenged control airways (Fig. 4; all P < 0.003). There were no protocol-dependent differences in the total cells per milliliter of BALF recovered at any time point from DAC, WAC, or unchallenged airways (all P > 0.412), although the total...
number of cells recovered in BALF 30 min after DAC tended to be elevated (Fig. 4). The viability of cells recovered in BALF was similar for all protocols (98 ± 1%).

Effects of WAC and DAC on BALF Mediator Concentrations

Time courses for the release of bronchoconstricting PGs are summarized in Fig. 5. The concentration of PGD$_2$ recovered in BALF immediately after 2 min of DAC (Fig. 5, -3 min) was not significantly increased compared with WAC or control (P = 0.284). In contrast, PGD$_2$ was markedly increased immediately after 5 min of DAC compared with WAC or control BALF samples (P < 0.001). Although the concentration of PGD$_2$ 5 min after the DAC was significantly increased compared with WAC and control BALF samples (P < 0.001), no significant differences were detectable at 10, 15, or 30 min after the DAC. Although the concentration of PGD$_2$ appeared to fluctuate in response to DAC, these changes over time were not significant (P = 0.324).

The concentration of PGF$_{2\alpha}$ after 2 min of DAC was not significantly increased compared with WAC or control (P = 0.339). PGF$_{2\alpha}$ was significantly increased immediately after 5 min of DAC (P < 0.001) and at 5 (P < 0.001) and 30 min (P = 0.003) after DAC. Although not statistically significant, PGF$_{2\alpha}$ remained elevated at the 10- and 15-min time points. The concentration of PGF$_{2\alpha}$ immediately after the 5-min DAC (Fig. 3, 0 min) was significantly greater (P < 0.05) than concentrations measured after a 2-min DAC (-3 min) and at 5 min after the DAC (5 min).

The concentration of TxB$_2$ recovered in BALF immediately after 2 min of DAC was not increased compared with WAC or control (P = 0.351). TxB$_2$ was significantly increased immediately after a 5-min DAC (P < 0.001) and at 10 (P < 0.005) and 30 min (P < 0.002) after DAC. Although the concentration of TxB$_2$ fluctuated in response to DAC, these changes over time were not significant (P = 0.051).

The bronchodilating prostanoid PGE$_2$ was not significantly altered by WAC (P = 0.202) or DAC (P = 0.215). There were no detectable treatment differences (Fig. 6).

Finally, a 2-min DAC did not increase the concentration of LTC$_{4\alpha}$-E$_4$ in BALF compared with WAC or control samples (P = 0.219). LTC$_{4\alpha}$-E$_4$ was significantly increased at 5 (P < 0.001), 15 (P = 0.006), and 30 min (P = 0.001) after DAC. LTC$_{4\alpha}$-E$_4$ appears to rise slowly over the 30 min after DAC, and its concentration at 30 min is significantly greater than that recorded after only 2 min of DAC (Fig. 7, -3 min). Although LTC$_{4\alpha}$-E$_4$ is significantly increased immediately after a 5-min WAC compared with that recorded immediately after a 2-min WAC (P < 0.05), no significant effects were observed after that time point (Fig. 7).
Fig. 6. Concentrations of PGE2 in BALF recovered from control bronchi (○) and bronchi exposed to WAC (○) and DAC (●). For each time point, n = 9–11.

Correlations Between BALF Epithelial Cells, Mediators, and HIB

WAC, DAC, and pooled data (WAC + DAC) were examined to determine whether 1) mucosal injury (percentage of epithelial cells) correlated with BALF eicosanoid concentrations, 2) HIB (percent increase in R_p at the time of BAL) correlated with eicosanoid concentrations, and 3) mucosal injury correlated with HIB regardless of dog or recovery time (Table 1). With one exception (DAC PGE2), mucosal injury correlated with all mediators regardless of treatment (WAC and/or DAC; Fig. 8, Table 1). With exceptions for WAC LTC4-E4 and DAC PGF2α, significant correlations were detected between HIB and the bronchoconstricting mediators, primarily when pooled data were analyzed (Fig. 8, Table 1). Finally, mucosal injury correlated with HIB only when WAC and DAC data were combined for analysis (Fig. 9, Table 1).

Pooled data were examined with respect to the time when BALF was collected after hyperventilation challenge (Table 2). No correlations between mucosal injury and any mediator were seen immediately after a 2-min challenge (–3 min). Correlations between injury and PGD2α, PGF2α, TxB2, and PGE2 were first detected immediately after a 5-min challenge (0 min). Correlation with LTC4-E4 was not observed until 5 min later. Significant correlations between HIB and PGD2α, PGF2α, TxB2, and LTC4-E4 first appeared at 10 min. No R_p data were available for correlation with mediators at –3 or 0 min. Correlations between mucosal injury and HIB were first detected at 5 min (Table 2).

DISCUSSION

The experiments described here provide for the first time snapshots of the sequence of events that are initiated during a 5-min period of hyperventilation and a 30-min postchallenge period, during which bronchial obstruction typically develops and begins to subside (9). Our data suggest that mucosal injury occurs during the DAC and that eicosanoid mediator release occurs after hyperventilation stops. The implications of these observations are discussed below.

A composite of all R_p data collected during the time series study (Fig. 2) shows that significantly greater increases in R_p are produced by DAC than by WAC. It also reveals for the first time that the use of warm moist air not only attenuates HIB but causes it to develop earlier. We confirmed this observation in five dogs by directly comparing WAC and DAC in the same sublobar segment (Fig. 3, A and B). This difference in time course is not due to differences in the magnitude of HIB, because dry air-induced changes in R_p (14) of a magnitude similar to that produced by WAC also peak ~5 min after the DAC ends (Fig. 3, C and D).

DAC, but not WAC, resulted in a marked increase in the number of epithelial cells recovered after hyperventilation (Fig. 4). It is important to note that 1) a 2-min DAC produces mucosal injury (Fig. 4), 2) epithelial cell number does not increase 5–30 min after DAC (Fig. 4), and 3) few if any epithelial cells are recovered in BALF 5 h after DAC (8). Thus epithelial cells recovered 5–30 min after DAC probably indicate that exfoliation occurred during or immediately after the DAC and that mucociliary transport requires 30–300 min to clear these cells after DAC. This hyperventilation-induced epithelial exfoliation was reported previously in dogs (14, 16, 38, 39) and asthmatic humans (32), and morphometric analysis was used to confirm that these data directly reflect damage of the bronchial mucosa (15). Finally, although there were no other significant timerealted changes in BALF cell profiles, total cell counts tended to increase by 30 min after a DAC and foreshadow leukocyte infiltration that is significant 1 h later (28).

The initial hyperventilation-induced increase in bronchoactive prostanoids (Fig. 5) coincides with the development of HIB (Fig. 2) and is consistent with previous studies showing that these mediators can account for as much as 50% of the response in dogs (16, 17) and asthmatic children (34). The lack of an increase in PGD2α, PGF2α, and TxB2 immediately after a 2-min DAC is notable (Fig. 5) and suggests that mediator release is delayed and occurs at a later time. In a previous study, PGD2 and TxB2 increased ~5 min after a 2-min DAC (39), suggesting that mediator production is initiated during a 2-min DAC but its release is delayed until after the challenge ends (Fig. 5).
In contrast to a 2-min DAC, BALF mediator concentrations rise dramatically immediately after a 5-min DAC, suggesting that prostanoid production and release occur during the last 3 min of a 5-min DAC, during the rewarming that inevitably occurs between the end of the DAC and the beginning of the BAL (~30 s), or during the lavage itself.

We were unable to detect any significant differences in the concentrations of PGE$_2$ recovered in BALF samples from WAC, DAC, and control bronchi (Fig. 6). However, PGE$_2$ was significantly increased at 5 min when DAC was directly compared with the control sample (50.6 ± 6.3 vs. 28.3 ± 5.4 pg/ml, n = 10, P = 0.021). This is consistent with the finding of Omori et al. (29), who reported that PGE$_2$ was increased in canine BALF samples, 5 min after DAC. Thus it is possible that the small changes in PGE$_2$ seen in Fig. 6 are physiologically meaningful and that PGE$_2$ functions as an endogenous antagonist of HIB in human (31) and canine airways (29).

### Table 1. Spearman rank correlation analyses of BALF epithelial cells and eicosanoid mediators and R$_p$ for airways exposed to WAC and DAC

<table>
<thead>
<tr>
<th>Mucosal Injury vs. Mediators</th>
<th>HIB vs. Mediators</th>
<th>Injury vs. HIB</th>
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<tbody>
<tr>
<td>PGD$_2$</td>
<td>PGF$_{2\alpha}$</td>
<td>TxB$_2$</td>
</tr>
<tr>
<td>WAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r_s$</td>
<td>0.281</td>
<td>0.420</td>
</tr>
<tr>
<td>P</td>
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<td>0.001</td>
</tr>
<tr>
<td>n</td>
<td>60</td>
<td>58</td>
</tr>
<tr>
<td>DAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r_s$</td>
<td>0.460</td>
<td>0.530</td>
</tr>
<tr>
<td>P</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>n</td>
<td>58</td>
<td>58</td>
</tr>
<tr>
<td>WAC + DAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$r_s$</td>
<td>0.580</td>
<td>0.640</td>
</tr>
<tr>
<td>P</td>
<td>0.000</td>
<td>0.000</td>
</tr>
<tr>
<td>n</td>
<td>118</td>
<td>116</td>
</tr>
</tbody>
</table>

BALF, bronchoalveolar lavage fluid; WAC, wet air challenge; DAC, dry air challenge; WAC + DAC, pooled data; HIB, hyperventilation-induced bronchoconstriction [percent increase in peripheral airway resistance (R$_p$)]; PG, prostaglandin; TxB$_2$, thromboxane B$_2$; LT, leukotriene; $r_s$, Spearman rank correlation coefficient; n, number of BALF samples. Mucosal injury is defined as percentage of epithelial cells recovered in BALF. Significant correlations are indicated by boldface italic type.

In contrast to a 2-min DAC, BALF mediator concentrations rise dramatically immediately after a 5-min DAC, suggesting that prostanoid production and release occur during the last 3 min of a 5-min DAC, during the rewarming that inevitably occurs between the end of the DAC and the beginning of the BAL (~30 s), or during the lavage itself.

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**Fig. 8.** Correlations between magnitude of hyperventilation-induced mediator release and magnitudes of hyperventilation-induced mucosal injury (percentage of epithelial cells in BALF) and hyperventilation-induced bronchoconstriction (percent increase in R$_p$) measured at time of lavage. Open symbols, data obtained after hyperventilation with warm moist air; solid symbols, data collected after hyperventilation with cool dry air.

**Fig. 9.** Correlation between magnitude of hyperventilation-induced mucosal injury (percentage of epithelial cells in BALF) and hyperventilation-induced bronchoconstriction (percent increase in R$_p$) recorded at time of lavage. ◦, Data obtained after hyperventilation with warm moist air; ●, data collected after hyperventilation with cool dry air.
As with the bronchoactive prostanoids, the initial hyperventilation-induced increase in BALF leukotrienes (Fig. 7) coincides with the development of HIB (Fig. 2) and is consistent with previous reports showing that these mediators account for ~60% of the response in dogs (29) and asthmatic humans (1, 33, 36). The gradual increase in LTC4-E4 15–30 min after DAC (Fig. 7) appears to paradoxically coincide with the recovery phase of HIB (Fig. 2) and may contribute to the development of a late-phase response that typically can be detected in canine peripheral airways within 5 h after a DAC (8). However, because LTC4 is metabolized to LTD4 and then to LTE4 and its conversion is accompanied by a loss of bioactivity but not immunoreactivity to the antibody used to detect it, this increase in LTC4-E4 may in part reflect this ongoing metabolic process. In addition, we cannot rule out the possibility that these mediators are active at this time but are accompanied by the local release of some counteracting factor(s). Whether the local concentration of PGE2 within the airway wall would be sufficient to counterbalance the LTC4-E4 activity cannot be determined from our data.

The kinetics of hyperventilation-induced mediator release are eicosanoid specific (Figs. 5 and 7). Concentrations of all three prostanoids are highest immediately after DAC, plummet at the 5-min time point, and tend to increase again at 10 min after challenge. We initially believed that the drop in mediator concentrations at 5 min after the DAC resulted from a technical error. However, the fact that LTC4-E4 concentrations in the same BALF samples were elevated at that time suggests that the low prostanoid concentrations were not due to errors in sample retrieval or sample preparation. Repeating the 5-min time point experiments produced similar results. Thus we conclude that fluctuations in the concentrations of PGD2 and TxB2 and, to a lesser degree, PGF2a during the 30-min postchallenge period reflect pulsatile production and release or enhanced degradation and bioconversion. Although we know of no other reports of in vivo pulsatile mediator release in peripheral airways, nasal lavage samples recovered from patients with allergic rhinitis at 20-min intervals after antigen challenge are suggestive of intermittent release for histamine and PGD2 (27). Although the dip in LTC4-E4 concentration 10 min after DAC may be physiologically insignificant (Fig. 7), the possibility that it reflects pulsatile release or enhanced catabolism similar to that seen with the bronchoconstricting prostanoids cannot be ruled out. In vitro experiments do indicate that different mast cell mediators exhibit different release kinetics, suggesting that the mechanisms controlling release are mediator specific (40).

Although hyperventilation with warm moist air increased Rp by ~30% (Figs. 1 and 2), it was not accompanied by mediator release (Figs. 4 and 6). There are four possible explanations for this finding: 1) the small increase in LTC4-E4 seen immediately after challenge

<table>
<thead>
<tr>
<th>Time</th>
<th>PGD2</th>
<th>PGF2a</th>
<th>TxB2</th>
<th>PGE2</th>
<th>LTC4-E4</th>
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<tr>
<td>0 min</td>
<td>0.790</td>
<td>0.870</td>
<td>0.890</td>
<td>0.635</td>
<td>0.341</td>
</tr>
<tr>
<td>5 min</td>
<td>0.770</td>
<td>0.800</td>
<td>0.720</td>
<td>0.621</td>
<td>0.634</td>
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<td>15 min</td>
<td>0.740</td>
<td>0.800</td>
<td>0.636</td>
<td>0.494</td>
<td>0.667</td>
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<td>30 min</td>
<td>0.509</td>
<td>0.556</td>
<td>0.760</td>
<td>0.603</td>
<td>0.444</td>
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</table>

See Table 1 legend for definition of abbreviations and explanation.
accounts for the increase in \( R_p \) (Fig. 7), 2) subthreshold levels of PGD\(_2\), PGF\(_{2\alpha}\), TxB\(_2\), or LTC\(_4\)-E\(_4\) contribute to this response, 3) mediators not monitored in this study contribute to this response, or 4) a vagal reflex is responsible for this increase in \( R_p \). Although we cannot comment on the first three scenarios, pretreatment with atropine reduces the response to WAC by only 30% (Fig. 3). Thus, although muscarinic receptor activity partially accounts for the mild airway obstruction that develops after WAC, the relatively large residual post-atropine response suggests that mediator release is likely to be the primary mechanism contributing to the increase in \( R_p \) stimulated by WAC.

All mediators examined in this study were characterized by a 2- to 5-min delay separating the initiation of DAC from the time of mediator recovery in BALF and may simply reflect the de novo biosynthesis of these mediators (Figs. 4 and 6). However, this delay in mediator detection is consistent with the hypothesis that airway cooling inhibits the production or release of biochemical mediators (12). Airway cooling inhibits HIB in dogs (11, 12) and asthmatic humans (26), suggesting that the transient temperature change that occurs during hyperventilation may alter the rate of mediator production and release. Thus, if mediator release does contribute to the increase in \( R_p \) stimulated by WAC, the warmer airway temperatures generated during this challenge (17) may account for the leftward shift in the peak response to WAC in Figs. 2 and 3. Studies specifically designed to examine the effect of airway cooling on local mediator release are needed to conclusively address this question.

A role for eicosanoids in the development of HIB (20, 22, 29, 32) and exercise-induced bronchoconstriction (6, 25) has been implicated via the use of a variety of pharmacological interventions and analyses of BALF. Although the latter technique reveals that hyperventilation increases eicosanoid mediators in asthmatic patients (20, 29, 32), exercise does not (3, 23). Because bronchial blood flow increases during hyperventilation (2, 30) and exercise is likely to increase it further, it is possible that a postexercise increase in bronchial blood flow augments mediator clearance (24, 37) and accounts for the discrepancy between these hyperpnea- and exercise-based studies. Alternatively, on the basis of mediator production/release kinetics depicted in Figs. 5 and 7, it is possible that BALF was collected at the wrong time, i.e., at a time of low mediator production/release or enhanced catabolism. These variations in time course may also explain why we have been inconsistent in our ability to detect significant increases in PGD\(_2\), PGF\(_{2\alpha}\), and TxB\(_2\) in canine BALF samples recovered 5–7 min after DAC (14, 16, 29, 38, 39).

Hyperventilation-induced mediator release is correlated with the magnitudes of mucosal injury and HIB (Fig. 8). Because temperature per se does not contribute to the development of HIB in this canine model (11, 12), BALF samples from WAC and DAC experiments were pooled for analysis (Table 1). This allowed us to evaluate relationships across two extremes of one continuous variable, i.e., evaporative water loss. The correlations between epithelial cells and bronchoconstricting eicosanoids may reflect injury-induced mediator production and release from epithelial cells or mucosal mast cells and are consistent with the idea that mucosal cells are local sources for these eicosanoids. Significant correlations were also found between PGE\(_2\) and epithelial cells, the latter being a well-known source for this bronchodilating prostanoid (13). Correlations between mucosal injury and mediator release were time dependent (Table 2). The lack of any correlation immediately after 2 min of hyperventilation (−3 min) is consistent with the proposed scenario that mucosal injury occurs during and mediator release occurs after a challenge ends. Significant time-dependent correlations also exist between HIB and PGD\(_2\), PGF\(_{2\alpha}\), TxB\(_2\), and LTC\(_4\)-E\(_4\), but not PGE\(_2\) (Fig. 8, Tables 1 and 2), supporting the hypothesis that these mediators contribute to the development of HIB. Finally, the severity of mucosal injury was significantly correlated with the magnitude of HIB (Fig. 9, Tables 1 and 2). The relatively weak correlation seen for the pooled data may in part reflect the fact that hyperventilation-induced mucosal injury initiates airway narrowing via a plethora of potential pathways that include the direct and indirect release of eicosanoids. In addition, correlations between the BALF-derived data (epithelial cells and mediators) tended to be much stronger than correlations involving the magnitude of HIB, suggesting that the weak relationship between these variables may in part result from a “mismatching” of the events in time. Despite these potential problems, all the relationships summarized above are consistent with the scenario in which hyperventilation with dry air damages the bronchial mucosa and stimulates the release of biochemical mediators that produce bronchoconstriction. Although no data are available to implicate a role for neuropeptides in our canine model of HIB, one potential pathway may involve an injury-induced local release of tachykinins from afferent C fibers, which in guinea pigs indirectly stimulates airway narrowing via the release of cysteinyl LTs (19, 41).

Finally, the exact cause of hyperventilation-induced mucosal injury remains unknown. The fact that hyperventilation with warm humidified air prevents mucosal injury and inhibits HIB in this (Figs. 2–4) and other studies (9, 15) suggests that the shear stress associated with DAC does not cause bronchial epithelial exfoliation. However, hyperventilation-induced airway cooling and drying do increase airway surface fluid osmolality (10), and airway surface fluid hypertonicity may directly damage the bronchial mucosa. Furthermore, hyperosmolality can initiate mediator release in vitro and in vivo (4, 14, 35) and may contribute further to mucosal injury.

In conclusion, our data provide evidence that mucosal injury precedes mediator release, which occurs late in the challenge or immediately after the hyperventilation stops. This suggests that endogenous mediator release is not responsible for the acute mucosal damage that occurs during DAC but is the result of hyperventi-
lation-induced mucosal injury. However, this does not rule out the possibility that mediator activity contributes to mucosal damage. Although WAC does not cause significant mucosal injury or detectable mediator release, it does cause HIB. Two-thirds of the response to WAC is nonvaginal in origin, which suggests that other mediators or subthreshold eicosanoid activity accounts for its development. The fact that HIB develops more rapidly after WAC than after DAC and that mediator release is delayed during DAC indirectly supports the hypothesis that airway cooling slows mediator production and release during hyperventilation and accounts for the gradual increase in $R_p$ that typically occurs after hyperventilation stops. Finally, bronchoactive eicosanoids are correlated with mucosal injury and airway obstruction, and mucosal injury is correlated with the magnitude of HIB. All these observations are consistent with the hypothesis that hyperventilation-induced mucosal damage initiates peripheral airway constriction via the release of biochemical mediators.

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Address for reprint requests and other correspondence: A. N. Freed, Div. of Physiology, Rm. 7006, School of Hygiene and Public Health, The Johns Hopkins University, 615 North Wolfe St., Baltimore, MD 21205 (E-mail: afreed@hsph.edu).

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