

# Eicosanoid and muscarinic receptor blockade abolishes hyperventilation-induced bronchoconstriction

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**Freed, Arthur N., Sharron McCulloch, and Yongqiang Wang.** Eicosanoid and muscarinic receptor blockade abolishes hyperventilation-induced bronchoconstriction. *J Appl Physiol* 89: 1949–1955, 2000.—This study was designed to test the hypothesis that hyperventilation-induced bronchoconstriction (HIB) results from the combined effects of prostanoid and leukotriene metabolism. A bronchoscope was used in anesthetized dogs to record peripheral airway resistance and HIB before and after combined treatment with inhibitors of cyclooxygenase (indomethacin) and 5-lipoxygenase (MK-0591). Bronchoalveolar lavage fluid (BALF) cells and mediators from hyperventilated and control airways were also measured. Pretreatment with MK-0591 and indomethacin significantly attenuated, but did not abolish, HIB. However, addition of atropine nearly eliminated the residual response. Blockade of eicosanoid metabolism markedly reduced the concentrations of eicosanoids recovered in BALF after hyperventilation. Positive correlations between post-hyperventilation BALF prostanoid and epithelial cell concentrations are suggestive of mucosal injury-induced mediator production and release. We conclude that HIB is prevented in the presence of eicosanoid and muscarinic-receptor blockade and that both classes of eicosanoids contribute similarly to the development of HIB.

asthma; canine; leukotrienes; mucosal injury; prostanoids

PENETRATION OF UNCONDITIONED air into the lung periphery during hyperventilation or exercise causes bronchoconstriction in most individuals with asthma. Although the mechanism responsible for this transient airway obstruction is unknown, it has been hypothesized that evaporative water loss results in airway surface fluid (ASF) hyperosmolarity, which in turn stimulates the local release of biochemical mediators (2). Support for this hypothesis is provided by the fact that ASF osmolality increases during hyperventilation and that this change in osmolality correlates with the magnitude of obstruction that develops in canine peripheral airways (12). Further support comes from the numerous studies reporting a role for eicosanoids in the development of hyperventilation-induced bronchoconstriction (HIB) in animal models and in human subjects with asthma (10).

Of the two major groups of eicosanoids, i.e., prostanoids and leukotrienes, the latter are generally believed to exert the greatest influence on the development of HIB (34, 58). The mediation of HIB by leukotrienes has been demonstrated pharmacologically using leukotriene-receptor antagonists (8, 25, 27, 29, 30, 43, 44) and biosynthesis inhibitors (28, 31, 55). Similar results have been reported for several animal models, including rats (61), guinea pigs (22, 24, 60), and dogs (39). In contrast to leukotrienes, the mediation of HIB by prostanoids has received little attention. One recent study reported that inhaled indomethacin attenuated exercise-induced bronchoconstriction in asthmatic children (45), and another documented increased levels of urinary  $9\alpha,11\beta$ -PGF<sub>2</sub> (the primary metabolite of PGD<sub>2</sub>) after exercise challenge in adult asthmatic subjects (35), suggesting that prostanoids do play a significant modulatory role in the development of HIB. Earlier studies involving several animal models of HIB have reported similar results (17, 19, 22). Interestingly, the inhibition of 5-lipoxygenase-activating protein (FLAP) in dogs unmasked striking correlations between HIB and bronchoalveolar lavage fluid (BALF) prostanoids, further supporting a role for the local production and release of stimulatory prostanoids in the development of HIB (39).

HIB in the canine lung periphery mimics exercise-induced asthma (EIA) in humans. The time course over which HIB develops and subsides, the responses to variations in stimulus strength and duration (11, 19), the association with airway mucosal injury (16, 17, 57), and the release of biochemical mediators (39, 42) are all remarkably similar. So too is the protection provided by  $\alpha_1$ -adrenergic agonists (7, 36),  $\beta_2$ -adrenergic agonists (47, 51, 57), cyclooxygenase inhibitors (17, 19, 45), heparin (48), 5-lipoxygenase inhibitors (31, 39), furosemide (18), methylxanthines (23, 56), muscarinic-receptor antagonists (19, 47, 50), and warm-humidified air (2, 11, 19). Because inhibition of either cyclooxygenase (17, 19) or 5-lipoxygenase (39) was previously reported to reduce HIB by at least 50%, we hypothesized that blockade of both pathways would result in the abolition of HIB. However, data contained in this paper

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reveal that simultaneous inhibition of prostaglandin and leukotriene metabolism provides no more protection against HIB than does blocking either pathway alone. Because the magnitude of the remaining eicosanoid-independent bronchoconstriction was similar to that previously attributed to hyperventilation-induced stimulation of muscarinic receptors (19, 47, 50), we used atropine to determine whether the residual response was mediated by a vagal reflex. In doing so, we show for the first time that muscarinic blockade, in combination with the two eicosanoid antagonists, nearly abolishes HIB.

## MATERIALS AND METHODS

### Animals

Male mongrel dogs 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 Animal Care and Use Committee.

### Study Design

*Effects of cyclooxygenase and lipoxygenase inhibition on HIB, and bronchoalveolar lavage (BAL) cells and mediators.* Four lobes were wedged with a bronchoscope in each dog [ $19.6 \pm 1.1$  (SE) kg,  $n = 9$ ] for each experiment. In the first lobe (1), baseline peripheral airway resistance (Rp) was recorded and the sublobar region was then lavaged. Two contralateral lobes (2 and 3) were then wedged simultaneously, and Rp was recorded. Both sublobar airways were then exposed to dry air challenge (DAC), and lobe 2 was lavaged after Rp was recorded 5 min after the DAC. Lobe 3 was monitored until it recovered to within 10% of its original baseline value. During this time, a fourth sublobar airway (4) was wedged, and MK-0591 (2 mg/kg in 20 ml of 5% dextrose in 0.9% saline) was injected intravenously and then infused at a rate of  $8 \mu\text{g}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$  for the remainder of the experiment (5, 49). Forty minutes later, indomethacin (5 mg/kg) was injected in 10 ml of  $\text{Na}_2\text{CO}_3$  (1.67 mg/kg). Twenty minutes after the injection of indomethacin, sublobar airways 3 and 4 were simultaneously exposed to DAC. After Rp was recorded, sublobar airway 3 was lavaged 5 min after the DAC, and Rp in airway 4 was monitored for an additional 10 min.

*Effects of cyclooxygenase, lipoxygenase, and muscarinic-receptor blockade on HIB.* A second series of experiments was done at a later time using six different dogs ( $22 \pm 1.4$  kg). HIB was recorded before and after intravenous treatment with MK-0591, indomethacin, and atropine sulfate (0.1 mg/kg). Drug administration was similar to that described above, with atropine being injected ~15 min after indomethacin. This protocol was repeated in four dogs using the drug vehicles only.

### Methods

*Anesthesia and instrumentation.* Dogs were anesthetized with an intravenous bolus of sodium thiopental (25 mg/kg). Anesthesia was maintained with a continuous thiopental infusion ( $4\text{--}6 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$ ) and intravenous fentanyl citrate (25  $\mu\text{g}$ ) administered every 15–30 min. Depth of anesthesia was assessed by heart rate (HR), blood pressure, canthal reflex, and the presence of spontaneous movements and breathing. Dogs were intubated with a dual portal endotracheal tube and mechanically ventilated (17 ml/kg) with

room air. End-tidal  $\text{CO}_2$  was monitored with a  $\text{CO}_2$  analyzer (model LB-2, Beckman, Anaheim, CA) and maintained at ~4.5% by adjusting ventilator frequency. HR and mean arterial blood pressure (MAP) were recorded using a noninvasive monitor (Datascope Accutorr 1A; Datascope, Paramus, NJ), and body temperature ( $T_b$ ; measured as rectal temperature) was monitored using a telethermometer (Yellow Springs Instrument, Yellow Springs, OH).

*Measurement of Rp.* Two fiber-optic bronchoscopes (5.5 mm OD; Olympus BF type P10, Olympus of America, New Hyde Park, NY) were inserted through two airtight portals of an endotracheal tube and gently wedged into contralaterally located sublobar bronchi. Transducers (Statham, Gould, Oxnard, CA) were used to record airway pressure (Pb) via polyethylene 90 catheters (ID = 1.19 mm, OD = 1.7 mm) threaded through the suction ports of each bronchoscope. Compressed dry room temperature 5%  $\text{CO}_2$  in air was delivered around the catheter and into the wedged sublobar segment at 200 ml/min (3.33 ml/s). Rp was measured with the ventilator stopped at functional residual capacity. Collateral airflow in dogs allows the insufflation of 200 ml/min to proceed and at the same time allows us to assume that the pressure downstream from the bronchoscope is in equilibration with that of unobstructed lung. Under these conditions, Pb decays to a plateau pressure greater than atmospheric pressure (0 cmH<sub>2</sub>O), and  $\text{Rp} = (\text{Pb} - 0)/3.33 \text{ ml/s}$ .

*DAC.* Bronchoconstriction was induced by increasing the flow rate of 5%  $\text{CO}_2$  in dry air from 200 to 2,000 ml/min for 2 min. The flow rate was then returned to 200 ml/min, and Rp was monitored.

*BAL, differential cell counts, and mediator analysis.* BAL was performed 5 min after DAC by using three 20-ml aliquots of warm (37°C) Hanks'-buffered saline solution. The BALF was gently suctioned from the wedge segment by using a 20-ml syringe. BALF samples were temporarily stored on ice and centrifuged at 4°C for 10 min at 1,300 rpm. Total cell number in a 10- $\mu\text{l}$  sample of BALF was determined using a hemocytometer. Differential cell counts of macrophages, lymphocytes, neutrophils, eosinophils, and epithelial cells were done after staining with Diff-Quik. Trypan blue exclusion was used to evaluate cell viability.

BALF samples (20 ml) were concentrated by using a Sep-Pak C<sub>18</sub> 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 (39) using commercially available ELISA kits for  $\text{PGF}_{2\alpha}$ , thromboxane B<sub>2</sub> (TxB<sub>2</sub>), and leukotrienes C<sub>4</sub>, D<sub>4</sub>, and E<sub>4</sub> (LTC<sub>4</sub>-E<sub>4</sub>) (Neogen, Lexington, KY).

### Analysis

Rp, cell, and raw mediator data were analyzed using a Friedman two-way analysis of variance in conjunction with a Student-Newman-Keuls test for the comparison of individual treatment means (paired samples). Mediator data were analyzed with and without outliers ( $>\text{mean} \pm 2\text{SD}$ ) included, using a Kruskal-Wallis one way analysis of variance. HR and MAP data were analyzed using either a paired *t*-test or a Wilcoxon signed-rank test. Spearman rank correlation coefficient ( $r_s$ ) analysis was used to determine whether the concentration of biochemical mediators in BALF was correlated with either the magnitude of HIB or the percentage of epithelial cells recovered in BALF. Statistical significance was judged at  $P < 0.05$ . Data were expressed as means  $\pm$  SE.

## RESULTS

*Effects of Cyclooxygenase and Lipoxygenase Inhibition on HIB and BAL Cells and Mediators*

Mean baseline  $R_p$  preceding the two consecutive DACs were similar (Fig. 1). Before treatment, DAC increased  $R_p$   $\sim 140 \pm 21\%$  ( $n = 9$ ). After treatment with MK-0591 and indomethacin (MK-Indo), DAC increased  $R_p$  by only  $47 \pm 10\%$ . Dry air-induced changes in  $R_p$  were significantly attenuated at each time point recorded after the DAC (Fig. 1). There was a significant decrease in HR ( $12 \pm 4$  beats/min;  $P = 0.004$ ) and  $T_b$  ( $2.0 \pm 0.44^\circ\text{C}$ ;  $P = 0.002$ ) between the first and second DAC. MAP was not significantly affected ( $P = 0.400$ ).

Total cells per milliliter of BALF recovered from control, vehicle-treated, and MK-Indo-treated airways did not differ significantly ( $P = 0.685$ ; Fig. 2). Cell viability was also similar across treatments (98–99%). The numbers of macrophages, lymphocytes, neutrophils, and eosinophils recovered in BALF were not affected by either the DAC or the drug treatment. Although DAC markedly increased the number of epithelial cells recovered in BALF ( $P \leq 0.05$ ), no drug effect was detectable (Fig. 2).

Concentrations of  $\text{PGF}_{2\alpha}$ ,  $\text{TxB}_2$ , and  $\text{LTC}_4\text{-E}_4$  in BALF samples recovered from untreated DAC (DAC before iv administration of MK-Indo) and MK-Indo-treated DAC sublobar airways (DAC after iv administration of MK-Indo) were compared with unchallenged controls. It is important to note that our laboratory showed previously that HIB is repeatable over the time course of this study and that the magnitude of HIB is either unchanged or tends to increase in response to

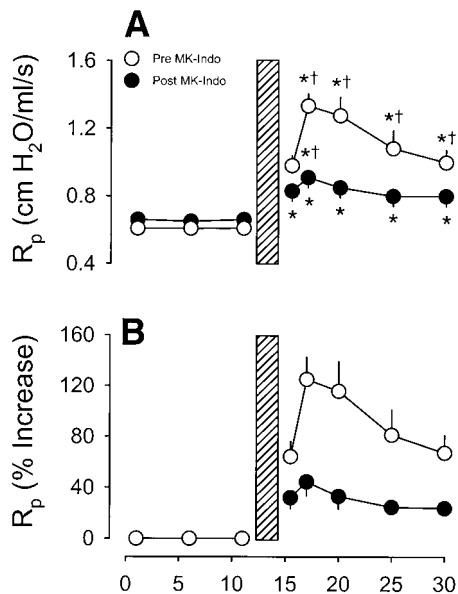


Fig. 1. A: peripheral airways resistance ( $R_p$ ) in sublobar segments hyperventilated with dry air [dry air challenge (DAC), 2,000 ml/min for 5 min; hatched bar] before (Pre;  $\circ$ ) and after (Post;  $\bullet$ ) treatment with MK-0591 + indomethacin (MK-Indo). B: data from A expressed as a percentage of baseline  $R_p$ . Values are means  $\pm$  SE;  $n = 9$  dogs.  $*P \leq 0.05$  compared with baseline.  $\dagger P \leq 0.05$ , before vs. after treatment.

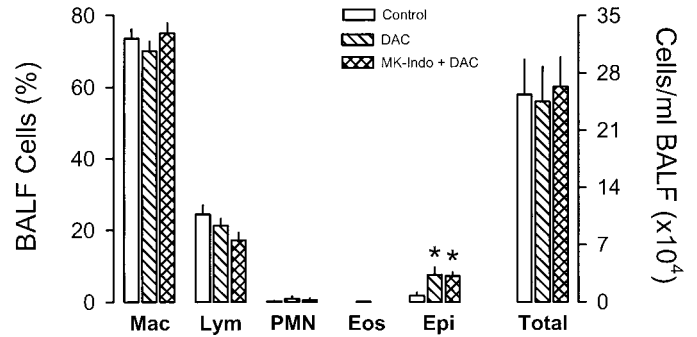


Fig. 2. Differential cell counts expressed as a percentage of total cells recovered in bronchoalveolar lavage fluid (BALF) from control, untreated DAC, and MK-Indo-treated sublobar segments 5 min after DAC (MK-Indo + DAC). Mac, macrophage; Lym, lymphocyte; PMN, neutrophil; Eos, eosinophil; Epi, epithelial cell. Total cell counts are expressed as cells/ml of BALF. Open bars, control; hatched bars, untreated DAC; crosshatched bars, DAC pretreated with MK-Indo. Values are means  $\pm$  SE  $*P \leq 0.05$  compared with control.

consecutive challenges (9, 11, 39). DAC significantly enhanced  $\text{PGF}_{2\alpha}$  and  $\text{LTC}_4\text{-E}_4$  compared with control, and treatment with MK-Indo significantly reduced all three eicosanoids compared with untreated airways exposed to DAC (Fig. 3).

*Correlations Between BALF Epithelial Cells, Mediators, and HIB*

The percentage of epithelial cells recovered in BALF was significantly and positively correlated with the concentration of  $\text{PGF}_{2\alpha}$  ( $r_s = 0.920$ ,  $n = 9$ ,  $P < 0.001$ ) and  $\text{TxB}_2$  ( $r_s = 0.767$ ,  $n = 9$ ,  $P < 0.012$ ) but was not correlated with  $\text{LTC}_4\text{-E}_4$  ( $r_s = 0.400$ ,  $n = 9$ ,  $P = 0.264$ ) (Fig. 4). No other cell type was positively correlated with any mediator (all  $P > 0.308$ ). The magnitude of HIB measured as the increase in  $R_p$  above baseline ( $\%R_p$ ) was significantly and positively correlated with the concentration of  $\text{PGF}_{2\alpha}$  ( $r_s = 0.691$ ,  $n = 8$ ,  $P < 0.047$ ). Neither  $\text{TxB}_2$  ( $r_s = 0.571$ ,  $n = 9$ ,  $P = 0.120$ ) nor  $\text{LTC}_4\text{-E}_4$  ( $r_s = 0.000$ ,  $n = 9$ ,  $P = 0.980$ ) was signifi-

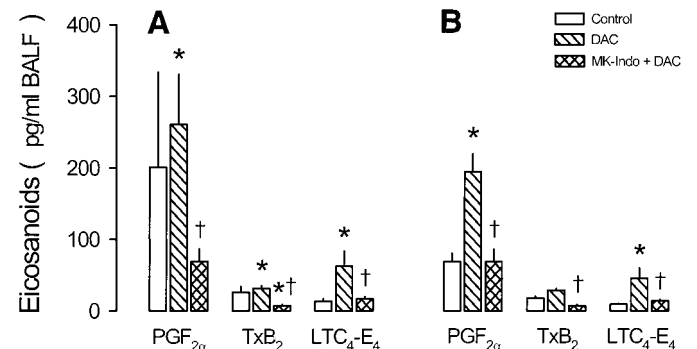


Fig. 3. Concentrations of  $\text{PGF}_{2\alpha}$ , thromboxane  $\text{B}_2$  ( $\text{TxB}_2$ ), and leukotrienes  $\text{C}_4$ ,  $\text{D}_4$ , and  $\text{E}_4$  ( $\text{LTC}_4\text{-E}_4$ ) in BALF recovered from control (open bars), untreated DAC (hatched bars), and MK-Indo-treated DAC (crosshatched bars) sublobar segments. A: raw data ( $n = 9$ ). B: data after removal of outliers ( $>$ mean  $\pm 2$  SD) for  $\text{PGF}_{2\alpha}$  (control,  $n = 8$ ; DAC,  $n = 8$ ; MK-Indo + DAC,  $n = 9$ ),  $\text{TxB}_2$  (control,  $n = 9$ ; DAC,  $n = 8$ ; MK-Indo + DAC,  $n = 9$ ), and  $\text{LTC}_4\text{-E}_4$  ( $n = 8$  for all groups).  $*P \leq 0.05$  compared with control.  $\dagger P \leq 0.05$  compared with untreated DAC.

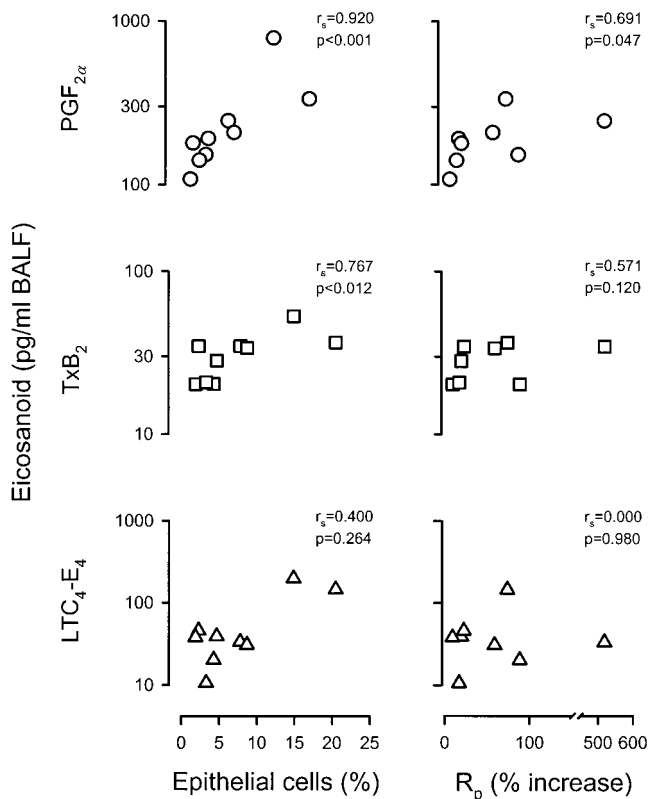


Fig. 4. Correlations between mucosal injury (*left*; %epithelial cells recovered in BALF) or dry air-induced bronchoconstriction (*right*; %increase in Rp) and the concentrations of PGF<sub>2α</sub>, TxB<sub>2</sub>, and LTC<sub>4</sub>-E<sub>4</sub> in BALF recovered from untreated DAC sublobar segments. *r<sub>s</sub>*, Spearman rank correlation coefficient.

cantly correlated with the increase in Rp (Fig. 4). Although not statistically significant, the percentage of epithelial cells recovered in BALF tended to correlate with %Rp ( $r_s = 0.600$ ,  $n = 9$ ,  $P = 0.077$ ; Fig. 5). Omission of the one outlier in Fig. 5 did not significantly alter this relationship ( $r_s = 0.643$ ,  $P = 0.072$ ,  $n = 8$ ). Treatment with MK-Indo abolished all of the significant correlations reported above (all  $P > 0.356$ ).

#### Effects of Cyclooxygenase, Lipoxygenase, and Muscarinic-Receptor Blockade on HIB

Before treatment, DAC increased Rp  $\sim 135 \pm 18\%$  ( $n = 6$ ). After treatment with MK-0591, indomethacin,

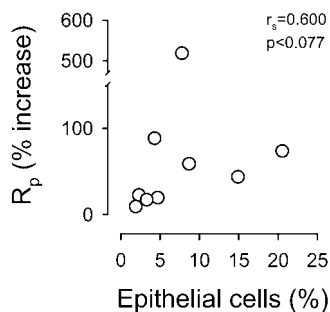


Fig. 5. Correlation between the magnitude of hyperventilation-induced mucosal injury (%epithelial cells recovered in BALF) and hyperventilation-induced bronchoconstriction (%increase in Rp).

and atropine, DAC increased Rp by only  $27 \pm 7\%$ . Dry air-induced changes in Rp were significantly attenuated at each time point recorded after the DAC (Fig. 6). Treatment with drug vehicles did not affect HIB ( $-14 \pm 3\%$ ,  $P > 0.05$ ,  $n = 4$ ).

Drug treatment significantly increased HR and MAP by  $80 \pm 14$  beats/min ( $P = 0.003$ ) and  $21 \pm 6$  mmHg ( $P = 0.018$ ), respectively. T<sub>b</sub> was not significantly affected ( $P = 0.063$ ).

#### DISCUSSION

Treatment with MK-Indo attenuates HIB in canine peripheral airways by  $\sim 67\%$  (Fig. 1). Surprisingly, the combined effects of cyclooxygenase and 5-lipoxygenase blockade on HIB do not appear to be additive. The level of inhibition is similar to that previously reported for dogs ( $-65\%$ ) treated with the FLAP antagonist alone (39). In asthmatic subjects, the use of either a 5-lipoxygenase or an LTD<sub>4</sub>-receptor antagonist reduces HIB by  $\sim 41 \pm 10$  (range 24–58%,  $n = 3$ ) (28, 31, 55) and  $49 \pm 4.6\%$ , (range: 31–73%,  $n = 9$ ) (1, 8, 25, 27, 29, 30, 43, 44), respectively. The use of indomethacin alone inhibits HIB in dogs (17, 19) and asthmatic children (45) by  $\sim 50\%$ . Previous work revealed that hyperventilation-induced prostaglandin release was not dependent on leukotriene production and that significant correlations between HIB and prostanoids emerged in the presence of FLAP antagonism (39). These correlations indicate that prostanoids contribute to the residual leukotriene-independent increase in Rp that occurs in response to DAC.

The increased number of epithelial cells recovered in BALF after DAC (Fig. 2) is similar to that previously reported for our canine model (14, 17, 39, 56, 57) and for asthmatic subjects (42) and suggests that DAC causes mucosal cell injury in canine and human airways. This conjecture has been confirmed in dogs by using morphometric analysis (16, 37). In addition to

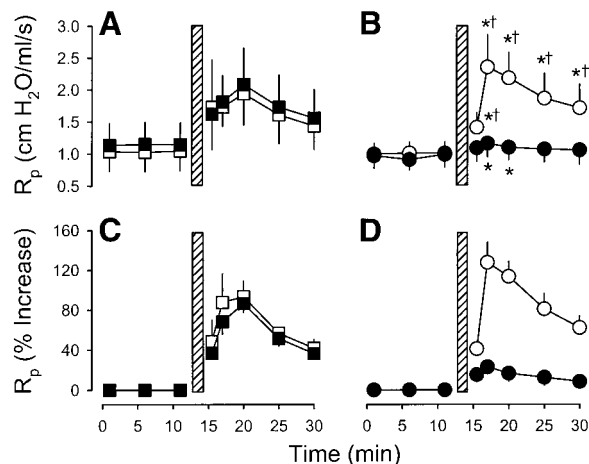


Fig. 6. A: Rp before (□) and after (■) treatment with drug vehicles ( $n = 4$ ). B: Rp before (○) and after (●) treatment with MK-0591 + indomethacin + atropine ( $n = 6$ ). Data from vehicle-treated (C) and MK-0591 + indomethacin + atropine-treated (D) airways expressed as a percentage of baseline Rp. Values are means  $\pm$  SE. \* $P \leq 0.05$  compared with baseline. † $P \leq 0.05$ , before vs. after treatment.

damaging bronchial epithelia, hyperventilation with cool dry air stimulates mast cell degranulation in dogs (15, 38) and human asthmatic subjects (6, 35). The fact that bronchial epithelial cells and mast cells can secrete a wide variety of proinflammatory eicosanoids (4, 35, 40, 59) is consistent with the hypothesis that the bronchial mucosa plays an important modulatory role in the development of HIB. However, inhibition of cyclooxygenase and 5-lipoxygenase activity does not alter BALF cell profiles, indicating that the inhibition of dry air-induced mucosal injury is an unlikely mode of action for these drugs.

Treatment with MK-Indo markedly inhibits eicosanoid metabolism (Fig. 3), suggesting that mediator inhibition is the primary mode of action responsible for the reduction in HIB. MK-0591 is a highly specific FLAP antagonist (5), inhibiting LTC<sub>4</sub>-E<sub>4</sub> but not prostanoid production stimulated during DAC of canine peripheral airways (39). Thus the reduction in prostanoid metabolism depicted in Fig. 3 is specifically attributable to the inhibition of cyclooxygenase by indomethacin (17). Our laboratory previously showed that the magnitude of HIB was significantly and positively correlated with the dry air-induced generation of LTC<sub>4</sub>-E<sub>4</sub> (39). However, in this study a similar relationship in untreated airways was found only between PGF<sub>2α</sub> and HIB (Fig. 4). This discrepancy probably reflects differences in the kinetics of mediator metabolism and the difficulties associated with matching functional and BALF data in terms of time and location (20). Positive correlations between PGF<sub>2α</sub>, TxB<sub>2</sub>, and BALF epithelial cells (Fig. 4) probably reflect injury-induced mediator production and release from either epithelial cells or mucosal mast cells (15, 38), and are consistent with the notion that mucosal cells are local sources for these prostanoids. Although other potential sources include macrophages, neutrophils, and eosinophils (13), no positive correlations were detected between any prostanoid and any leukocyte recovered in BALF (all  $P > 0.31$ ). In addition, mucosal injury tends to correlate with HIB (Fig. 5), supporting the hypothesis that injury-induced mediator production contributes to the development of airways obstruction. It is important to note that the exact cause of hyperventilation-induced mucosal injury remains a mystery. The fact that hyperventilation with warm-humidified air prevents mucosal injury and inhibits hyperventilation-induced bronchoconstriction (11, 16) suggests that the shear stress associated with DAC does not directly damage the bronchial mucosa. However, hyperventilation-induced evaporative water loss significantly increases ASF osmolality (12), and this may 1) directly damage airway epithelial cells and/or 2) initiate mediator release (14, 26, 46) that may contribute further to mucosal injury.

HIB in canine peripheral airways is nearly abolished when muscarinic-receptor blockade is combined with the inhibition of cyclooxygenase and FLAP activity (Fig. 6), indicating that a vagal reflex is primarily responsible for the residual peripheral airway obstruction that remains after treatment with MK-Indo. Our

laboratory previously showed that a parasympathetic component accounts for ~30% of the response to DAC in dogs (19, 50). Parasympathetic reflexes appear to contribute to the development of HIB in some but not all asthmatic subjects. Approximately 60% of asthmatic children exhibiting HIB benefit from treatment with a parasympatholytic agent (10, 23). Although the protection afforded by antimuscarinic therapy alone maybe relatively small, interference with both muscarinic-receptor activity and eicosanoid metabolism accounts for an 80% reduction in HIB, making this pharmacological intervention in canine peripheral airways as efficacious as treatment with  $\beta$ -agonists (~75–100%) (37, 51, 57).

At least three different groups of mediators appear to interact to modulate the development of HIB in dogs and humans: bronchoconstricting leukotrienes, bronchoconstricting prostaglandins, and bronchodilating prostanoids (10, 13, 58). The contribution of bronchoconstricting prostanoids to the development of HIB is generally viewed as secondary to the role of leukotrienes (34, 58). However, this view may directly reflect the number and specificity of antagonists available for evaluating these different eicosanoids. The fact that the pharmacological efficacy of indomethacin (17, 19, 45), montelukast (25, 27, 43), and zileuton (31) are very similar suggests that the excitatory leukotrienes and prostanoids exert similar modulatory effects in response to DAC.

Several other mediators in addition to the eicosanoids may contribute to the development of HIB. Although at this time there are no published data to implicate a role for excitatory neuropeptides in our canine model of EIA, one potential pathway may involve a local injury-induced release of tachykinins from afferent C fibers. This pathway has been suggested to indirectly stimulate airway narrowing via the release of cysteinyl leukotrienes (22, 60). However, just as neurokinins can stimulate leukotriene and prostanoid release in the lung (3, 32, 33), leukotrienes and prostanoids can stimulate neuropeptide release (21, 54). Thus it is possible that hyperventilation-induced mast cell degranulation (15, 38) results in eicosanoid-induced release of neuropeptides from sensory nerve fibers, resulting in bronchoconstriction. This scenario is consistent with our observation that treatment with MK-Indo (Fig. 1) provided no greater protection against HIB than either indomethacin (17, 19) or MK-0591 alone (39), suggesting that leukotrienes and prostanoids may be equipotent in stimulating neuropeptide release. The apparent lack of additivity of these drugs may reflect limitations in neuropeptide production. Experiments comparing eicosanoid concentrations in BALF recovered from canine airways treated with neurokinin-receptor antagonists would help to evaluate this potential excitatory pathway.

A variety of inhibitory mediators potentially counterbalance the action of these excitatory mediators. PGE<sub>2</sub> has been implicated as a primary endogenous antagonist of HIB in canine (39) and asthmatic subjects (41), and inhibition of its metabolism in this study

may account for any airway obstruction remaining after treatment with MK-0591, indomethacin, and atropine. The endogenous release of vasoactive intestinal peptide from dry air-stimulated parasympathetic afferents in dogs (50) and the endogenous production of nitric oxide in humans (52) may play similar roles.

In summary, cyclooxygenase and 5-lipoxygenase antagonists attenuate HIB in canine peripheral airways. However, on the basis of previous work by our laboratory (17, 19, 39), the combined effects of these two antagonists do not appear to be additive. This pharmacological intervention does not alter BALF cell profiles, but does inhibit hyperventilation-induced production and release of  $\text{PGF}_{2\alpha}$ ,  $\text{TxB}_2$ , and  $\text{LTC}_4\text{-E}_4$ . Addition of a muscarinic-receptor antagonist to the treatment regimen nearly abolishes HIB, indicating that a vagal reflex is primarily responsible for the residual peripheral airway obstruction that remains after treatment with MK-Indo. Finally, we speculate that hyperventilation with dry air directly stimulates leukotriene and prostanoid production and release from epithelial and mast cells and conclude that these mediators contribute equally to the development of HIB.

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