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.

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, 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 9α,11β-PGF2 (the primary metabolite of PGD2) 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 α1-adrenergic agonists (7, 36), β2-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 lipoygenase inhibition on HIB, and bronchoalveolar lavage (BAL) cells and mediators. Four lobes were wedged with a bronchoscope in each dog [19.6 ± 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 ml/h. HIB was recorded before and after intravenous treatment (2 ml/kg) with an intravenous bolus of sodium thiopental (25 mg/kg). Drug administration was similar to that described with MK-0591, indomethacin, and atropine sulfate (0.1 mg/kg). HIB was recorded before and after intravenous treatment 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 ± SE.
RESULTS

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

Mean baseline Rp preceding the two consecutive DACs were similar (Fig. 1). Before treatment, DAC increased Rp ~140 ± 21% (n = 9). After treatment with MK-0591 and indomethacin (MK-Indo), DAC increased Rp by only 47 ± 10%. Dry air-induced changes in Rp were significantly attenuated at each time point recorded after the DAC (Fig. 1). There was a significant decrease in HR (12 ± 4 beats/min; P = 0.004) and Tb (2.0±0.44°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 ≤ 0.05), no drug effect was detectable (Fig. 2).

Concentrations of PGF$_{2\alpha}$, TxB$_2$, and LTC$_4$-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 consecutive challenges (9, 11, 39). DAC significantly enhanced PGF$_{2\alpha}$ and LTC$_4$-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 PGF$_{2\alpha}$ (r$_s$ = 0.920, n = 9, P < 0.001) and TxB$_2$ (r$_s$ = 0.767, n = 9, P < 0.012) but was not correlated with LTC$_4$-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 Rp above baseline (%Rp) was significantly and positively correlated with the concentration of PGF$_{2\alpha}$ (r$_s$ = 0.691, n = 8, P < 0.047). Neither TxB$_2$ (r$_s$ = 0.571, n = 9, P = 0.120) nor LTC$_4$-E$_4$ (r$_s$ = 0.000, n = 9, P = 0.980) was signifi-

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

![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 ± SE. *P ≤ 0.05 compared with control.](image)

![Fig. 3. Concentrations of PGF$_{2\alpha}$, thromboxane B$_2$ (TxB$_2$), and leukotrienes C$_4$, D$_4$, and E$_4$ (LTC$_4$-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 ± 2 SD) for PGF$_{2\alpha}$ (control, n = 8; DAC, n = 8; MK-Indo + DAC, n = 9), TxB$_2$ (control, n = 9; DAC, n = 8; MK-Indo + DAC, n = 9), and LTC$_4$-E$_4$ (n = 8 for all groups). *P ≤ 0.05 compared with control. †P ≤ 0.05 compared with untreated DAC.](image)
sightly correlated with the increase in $R_p$ (Fig. 4). Although not statistically significant, the percentage of epithelial cells recovered in BALF tended to correlate with $%R_p$ ($r_s = 0.600, n = 9, P = 0.077$; 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 $R_p$ by $135 \pm 18\%$ ($n = 6$). After treatment with MK-0591, indomethacin, and atropine, DAC increased $R_p$ by only $27 \pm 7\%$. Dry air-induced changes in $R_p$ 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. $T_b$ was not significantly affected ($P = 0.063$).

FIGURE 4. Correlations between mucosal injury (left; %epithelial cells recovered in BALF) or dry air-induced bronchoconstriction (right; %increase in $R_p$) and the concentrations of PGF$_{2\alpha}$, TxB$_2$, and LTC$_4$-E$_4$ in BALF recovered from untreated DAC sublobar segments. $r_s$, Spearman rank correlation coefficient.

FIGURE 5. Correlation between the magnitude of hyperventilation-induced mucosal injury (%epithelial cells recovered in BALF) and hyperventilation-induced bronchoconstriction (%increase in $R_p$). $r_s$, Spearman rank correlation coefficient.

DISCUSSION

Treatment with MK-Indo attenuates HIB in canine peripheral airways by $-67\%$ (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$_4$-receptor antagonist reduces HIB by $-41 \pm 10$ (range 24–58%, $n = 3$) (28, 31, 55) and 49 $\pm$ 4.6%, (range: 31–73%, $n = 9$) (1, 8, 25, 29, 30, 43, 44), respectively. The use of indomethacin alone inhibits HIB in dogs (17, 19) and asthmatic children (45) by $-50\%$. 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 $R_p$ 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
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 LTC₄-E₄ but not prostanooid production stimulated during DAC of canine peripheral airways (39). Thus the reduction in prostanooid 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 LTC₄-E₄ (39). However, in this study a similar relationship in untreated airways was found only between PGF₂α 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 PGF₂α, TxB₂, 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 prostanooids. Although other potential sources include macrophages, neutrophils, and eosinophils (13), no positive correlations were detected between any prostanooid 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 lead directly to mucosal damage (14, 26, 46). It 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 a 80% reduction in HIB, making this pharmacological intervention in canine peripheral airways as efficacious as treatment with β-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 prostanooids (10, 13, 58). The contribution of bronchoconstricting prostanooids 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 prostanooids 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 prostanooid release in the lung (3, 32, 33), leukotrienes and prosta-nooids 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 prosta-nooids 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. PGE₂ 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...
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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 antagonists do not appear to be additive. This pharmacological intervention does not alter BALF production and release of PGF₂α, Tx₂, and LTC₄. 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 prostaglandin production and release from epithelial and mast cells and conclude that these mediators contribute equally to the development of HIB.

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Eicosanoids Mediate Dry Air-Induced Bronchoconstriction