Effect of furosemide on hyperpnea-induced airway obstruction, injury, and microvascular leakage

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Freed, Arthur N., Varsha Taskar, Brian Schofield, and Chiharu Omori. Effect of furosemide on hyperpnea-induced airway obstruction, injury, and microvascular leakage. J. Appl. Physiol. 81(6): 2461–2467, 1996.—Furosemide attenuates hyperpnea-induced airway obstruction (HIAO) in asthmatic subjects via unknown mechanism(s). We studied the effect of furosemide on dry air-induced bronchoconstriction, mucosal injury, and bronchovascular hyperpermeability in a canine model of exercise-induced asthma. Peripheral airway resistance (Rp) was recorded before and after a 2-min dry-air challenge (DAC) at 2,000 ml/min. After pretreatment with aerosolized saline containing 0.75% dimethyl sulfoxide, DAC increased Rp 72 ± 11% (SE, n = 7) above baseline; aerosolized furosemide (10–3 M) reduced this response by ∼50 ± 6% (P < 0.01). To assess bronchovascular permeability, colloidal carbon was injected (1 ml/kg iv) 1 min before DAC, and after 1 h, the vehicle- and furosemide-treated airways were prepared for morphometric analysis. Light microscopy confirmed previous studies showing that DAC damaged the airway epithelium and enhanced bronchovascular permeability. Furosemide did not inhibit dry air-induced mucosal injury or bronchovascular hyperpermeability and in fact tended to increase airway damage and vascular leakage. This positive trend toward enhanced bronchovascular permeability in DAC canine peripheral airways is consistent with the hypothesis that furosemide inhibits HIAO in part by enhancing microvascular leakage and thus counterbalancing the evaporative water loss that occurs during hyperpnea.

The attenuation of hyperpnea-induced airway obstruction (HIAO) in asthmatic subjects by furosemide is associated with concomitant reductions in airway cooling and rewarming (14). Inhaled furosemide could thus dilate the tracheobronchial vasculature, enhance heat delivery, and thereby reduce the thermal effects associated with hyperpnea. Although furosemide relaxes aorta and pulmonary artery rings in vitro (1), it fails to affect a sodium metabisulfite-induced increase in bronchial blood velocity in sheep (20). Thus furosemide does not appear to alter tracheobronchial blood flow. However, HIAO and injury are accompanied by an increase in bronchovascular permeability and extravasation of fluid into the airway wall (9, 10). As suggested by Rodwell et al. (26), furosemide may enhance dry air-induced bronchovascular leakage and attenuate HIAO by increasing paracellular water movement in response to an osmotic stimulus.

We designed the present study to address this basic hypothesis. In so doing, we first determined that furosemide inhibits HIAO in a canine model of exercise-induced asthma. We then documented the effect of furosemide on dry air-induced mucosal injury and bronchovascular hyperpermeability in peripheral airways.

METHODS

Experimental Techniques

Dogs were handled and maintained in accordance with the standards set forth in the Policy and Procedures Manual published by the Animal Care and Use Committee of the Johns Hopkins University School of Hygiene and Public Health. The effect of furosemide on HIAO was studied using an experimental protocol in which dogs were anesthetized with sodium thiopental and fentanyl citrate. An alternative protocol was devised to study dry air-induced changes in airway morphology in which the dogs were anesthetized with sodium pentobarbital (details below).

Measurement of peripheral airway resistance (Rp). Male mongrel dogs were intubated after assessment of the depth of anesthesia by heart rate, blood pressure, canthal reflex, presence of spontaneous movements and breathing. Mechanical ventilation was initiated on room air with a Harvard constant-volume ventilator (17 ml/kg). End-tidal CO2 was monitored with a CO2 analyzer (Beckman LB-2, Beckman, Anaheim, CA) and maintained at ∼4.5% by adjusting respiratory frequency. Heart rate and blood pressure were recorded by using a noninvasive monitor (Datascopc Accutorr 1A; Datascopc, Paramus, NJ). Rectal temperature was monitored with a telethermometer (Yellow Springs Instrument, Yellow Springs, OH) and maintained with a warming pad. A bronchoscope (Olympus BF Type P30, Olympus of America, New Hyde Park, NY) was inserted through an airgirt portal of the
endotracheal tube and wedged into a sublobar segmental bronchus. A dual-lumen catheter was threaded through the suction port of the bronchoscope, and one lumen was used to deliver compressed dry, 5% CO2 in air at room temperature at a rate of 200 ml/min into the wedged sublobar segment. The other lumen was connected to a pressure transducer (Statham, Gould, Oxnard, CA) and used to measure airway pressure at the tip of the bronchoscope (Pb). Pb was measured by an ultrasonic transducer at functional residual capacity of the lungs. Under these conditions, Pb plateaued at a pressure greater than the alveolar pressure (atmospheric) in the surrounding unobstructed lung so that Rp = Pb - 200 ml⁻¹·min⁻¹.

Dry-air challenge (DAC). Insufflation of dry 5% CO2 in air was increased from 200 to 1,500-2,000 ml/min for 2 min. At the end of 2 min, it was reduced to the baseline flow rate of 200 ml/min.

Administration of furosemide aerosol. Either furosemide [Sigma Chemical, St. Louis, MO, 10⁻³ M in 0.9% saline with 0.75% dimethyl sulfoxide (DMSO); 366 ± 3 mmol/kg, n = 3] or 0.75% DMSO-saline (381 ± 4 mmol/kg, n = 3) aerosols were generated with the use of an ultrasonic nebulizer (Ultra-Neb 100, DeVilbiss, Somerset, PA) that delivered ~ 15 µl/min. The catheter was temporarily removed, and the aerosol was generated in air with 5% CO2 and was delivered for 2 min into the wedged segment at 200 ml/min via the suction port of the bronchoscope. Thus ~ 0.01 mg of furosemide was delivered to each sublobar segment. Rp was recorded after the administration of colloidal carbon, was expressed as square micrometers per square millimeters of airway tissue (10, 24).

Experimental Protocols

Effects of furosemide on dry air-induced bronchoconstriction. Six dogs (7 lobes; mass = 22.6 ± 1 kg) were initially anesthetized with sodium thiopental (25 mg/kg), followed by a continuous thiopental infusion (4–6 mg·kg⁻¹·h⁻¹) and supplemented with intravenous fentanyl citrate (25–50 µg) given every 15–30 min. DAC was done after establishment of a stable baseline Rp was reestablished.

Tissue removal and preparation. Dogs were exsanguinated, a median sternotomy was done, the pulmonary artery and left atrium were cannulated, and the pulmonary vasculature was perfused with Hanks’ buffer solution. The lungs were removed within 30–60 min after DAC and were prepared for morphometric analysis as follows. After each lobe was cannulated, Streck tissue fixative (Streck Laboratories, Omaha, NE) was instilled into each lobe to an inflation pressure of 20 cmH2O. Lobes were then immersed in the fixative for 24–48 h before dissection. After fixation, the parenchyma was dissected free from the bronchi, and the location of the bronchus was determined via an airway map that was constructed at the beginning of the experiment. The airway tree was photocopied, diagrammed, and cut serially into ~ 3-mm- long rings and labeled for image analysis. A continuous ethanol series was used to dehydrate the bronchial rings, which were embedded in glycolmethacrylate with the use of a JB-4 embedding kit (Polysciences, Warrington, PA). One 2- to 3-mm cross section of each airway ring was stained with periodic acid-Schiff (PAS) and one with toluidine blue (TB) and naphthol yellow S.

Morphometric analysis. Airways with cross sections ranging from 0.5 to 4.4 mm in diameter were examined, using light microscopy and an image-analysis software (Sigma Scan, Jandel Scientific, Corte Madera, CA). Airways ≥ 4.5 mm in diameter were excluded due to their proximity to the bronchus (≤ 5 mm in diameter). Control airways were unwedged sublobar segments that were not exposed to DAC, but were located adjacent to a wedged segment. All cross sections were categorized as either bronchi (cartilaginous airways) or bronchioles (noncartilaginous airways). The relative condition of the airway mucosa was categorized as either C + G (presence of ciliated and goblet cells), C − G (containing cells with either very few or no goblet cells) or damaged mucosa (containing either squamous or basal epithelia or exposed basement membrane) (10). The mucosal categories C + G and C − G were considered normal. The perimeter of the base-}

Statistical Analyses

Rp data were analyzed with the use of a repeated-measures analysis of variance (ANOVA) and Duncan’s multiple-range test. All morphometric data were evaluated using either a Student’s t-test, Mann-Whitney U-test, or the Kruskal-Wallis one-way ANOVA for comparison of treatment means. Nonparametric multiple comparisons were done based on the Newman-Keuls test by using rank sums instead of means to compare any two treatments. All values were

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 hipically relaxed bronchial diameter (B) (D = B/PM). Three to eight airway cross sections were evaluated from each experimental sublobar segment, and two airway cross sections were examined from each control segment. The following measurements were obtained on each airway cross section at a magnification of ×400, and the overall average per sublobar segment was calculated for each treatment. Ciliated cells per millimeter and goblet cells per millimeter of basement membrane were counted in normal mucosa of PAS-stained tissues, and goblet-to-ciliated cell (G/C) ratios were calculated. Goblet cells were identified by their shape and affinity for PAS. The number of mast cells in the lamina propria and submucosa of the airway wall located directly below either normal or damaged mucosa was counted in cross sections stained with TB. Mast cells were identified as cells that had granules stained with TB. The number of mast cells was expressed per square millimeter of lamina propria or submucosa located below either normal or damaged mucosa. The permeability of vessels in the lamina propria and submucosa of normal or damaged epithelium was estimated by measuring the area of the vascular wall occupied by colloidal carbon in each airway cross section stained with PAS. Bronchovascular leakage, as indicated by extravasation of colloidal carbon, was expressed as square micrometers per square millimeters of airway tissue (10, 24).

Experimental Protocols

Effects of furosemide on dry air-induced bronchoconstriction. Six dogs (7 lobes; mass = 22.6 ± 1 kg) were initially anesthetized with sodium thiopental (25 mg/kg), followed by a continuous thiopental infusion (4–6 mg·kg⁻¹·h⁻¹) and supplemented with intravenous fentanyl citrate (25–50 µg) given every 15–30 min. DAC was done after establishment of a stable baseline Rp was reestablished. Four dogs [5 lobes; mass = 20.5 ± 2 kg (SE)] were pretreated with the vehicle aerosol, and DAC was done as described above. Two lobes were exposed to a 1,500 ml/min DAC, and three lobes were exposed to a 2,000 ml/min DAC for 2 min.

Dry air-induced changes in airway morphology. Five dogs (10 lobes; 17.6 ± 1 kg) were anesthetized with pentobarbital sodium (30 mg/kg) and supplemented with 30 mg of this drug every 30–60 min. In each dog, DAC was performed in one sublobar segment ~ 15 min after pretreatment with aerosolized vehicle and in another sublobar segment ~ 15 min after pretreatment with aerosolized furosemide. Baseline Rp was recorded after aerosol delivery, and colloidal carbon (1 ml/kg) was injected into the femoral vein ~2 min after DAC was done in each sublobar segment. Dogs were exsanguinated 15 min after DAC.

Morphometric analysis. Airways with cross sections ranging from 0.5 to 4.4 mm in diameter were examined, using light microscopy and an image-analysis system (Sigma Scan, Jandel Scientific, Corte Madera, CA). Airways ≥ 4.5 mm in diameter were excluded due to their proximity to the bronchoscope (~5 mm in diameter). Control airways were unwedged sublobar segments that were not exposed to DAC, but were located adjacent to a wedged segment. All cross sections were categorized as either bronchi (cartilaginous airways) or bronchioles (noncartilaginous airways). The relative condition of the airway mucosa was categorized as either C + G (presence of ciliated and goblet cells), C − G (containing cells with either very few or no goblet cells) or damaged mucosa (containing either squamous or basal epithelia or exposed basement membrane) (10). The mucosal categories C + G and C − G were considered normal. The perimeter of the base-
expressed as means ± SE. Statistical significance was judged at P < 0.05.

RESULTS

Effects of Vehicle on HIAO

Mean baseline resistances preceding the first and second DAC were 0.76 ± 0.3 and 0.78 ± 0.3 cmH₂O·ml⁻¹·s⁻¹ (n = 5), respectively, and were not significantly different (Fig. 1A). The first and second DAC increased Rp by 69 ± 7 and 65 ± 10% over baseline, respectively. The average Rp recorded at each time point after each challenge was similar.

Effects of Furosemide on HIAO

Mean baseline Rp preceding each of the two DAC was similar (0.95 ± 0.2 and 0.98 ± 0.2 cmH₂O·ml⁻¹·s⁻¹; Fig 1B). The first DAC increased Rp by 72 ± 1%. In contrast, after treatment with furosemide, DAC increased Rp by only 34 ± 5%. Dry air-induced changes in Rp were significantly attenuated at 2 (P < 0.01) and 5 min (P < 0.01) postchallenge when compared with similar points in time after the first DAC.

Morphometric Analysis of DAC Airways

A total of 88 airway cross sections from five dogs were examined. The average diameter of bronchi and bronchioles was 3.5 ± 0.13 mm (n = 35) and 1.77 ± 0.11 mm (n = 53), respectively.

Hyperpnea-induced mucosal injury. The perimeter of control bronchi and bronchioles was quantified in terms of the percentage of C + G, C − G, and damaged mucosa (Table 1). Control bronchi had 1 ± 1%, whereas control bronchioles had 2 ± 2% damaged mucosa. DAC significantly increased the proportion of damaged mucosa in vehicle-treated DAC bronchi (P < 0.01) but did not do so in the bronchioles of that group. Similarly, the furosemide-treated DAC bronchi contained 34 ± 18% damaged mucosa (P < 0.05) compared with control bronchi, whereas their bronchioles did not show significant damage (Fig. 2). There was no significant drug effect.

Dry air-induced changes in goblet cell number. The number of ciliated cells per millimeter of basement membrane in unchallenged control, DAC vehicle-treated, and DAC furosemide-treated bronchi were similar (Table 1). The number of goblet cells per millimeter of basement membrane was also similar for bronchi in the three groups. The bronchioles in the three groups showed the same trend with similar numbers of ciliated cells per millimeter of basement membrane and comparable numbers of goblet cells per millimeter of basement membrane (Table 1). As seen in Fig. 3, although not significant, DAC tended to decrease G/C ratios.

Dry air-induced changes in mast cell number. Regardless of mucosal condition (Fig. 4), the distribution of mast cells in the lamina propria of the control, vehicle-treated, and furosemide-treated bronchi and bronchioles was similar (Table 1). No differences were detected in the number of submucosal mast cells in either bronchi or bronchioles. Mast cell number per square millimeter of lamina propria located below C + G and C − G mucosa was similar and was combined for analysis under the rubric of normal mucosa. Mast cell number per square millimeter of lamina propria was then expressed as a function of mucosal condition (normal vs. damaged) regardless of airway size (Fig. 5). The number of mast cells located below normal ciliated mucosa in control airways was 255 ± 21/mm² and was not affected by DAC in either vehicle-treated (195 ± 25/mm², P = 0.19) or furosemide-treated (182 ± 24/mm², P = 0.11) airways. In contrast, the number of mast cells located below damaged mucosa in the furosemide-treated DAC exposed airways was significantly reduced (69 ± 21/mm², P = 0.016) compared with unexposed control airways. Mast cell numbers also tended to be lower in vehicle-treated airways compared with control (P = 0.114).

Dry air-induced bronchovascular hyperpermeability. Regardless of mucosal condition, negligible quantities of colloidal carbon were observed within the walls of vessels in the lamina propria and submucosa of control bronchi and bronchioles (Fig. 6). The density of colloidal carbon increased from 1–2 to 11 ± 4.9 and 22 ± 13.6 μm²/10⁻² mm² (P < 0.05) in DAC-exposed bronchi pretreated with either vehicle or furosemide, respectively. No significant difference existed between the vehicle- and furosemide-treated airways. DAC did not alter bronchovascular leakage in the submucosa of bronchi, nor did it significantly affect bronchovascular permeability in bronchioles.

Dry air-induced vascular leakage in the lamina propria was also examined as a function of mucosal
condition regardless of airways size (Fig. 7). The density of colloidal carbon located below normal ciliated mucosa was 1.1 ± 0.3 µm²/10⁻² mm² in control airways and was not different from that observed in C + G mucosa of either DAC vehicle- or furosemide-treated airways (P = 0.421). Colloidal carbon located below C − G mucosa in DAC-exposed vehicle-treated (4.2 ± 1.2 µm²/10⁻² mm²) and furosemide-treated (2.8 ± 0.4 µm²/10⁻² mm²) airways were significantly increased when compared with normal mucosa in control airways (P = 0.016). Extravasation of carbon beneath damaged mucosa was even greater: 8.9 ± 4.0 µm²/10⁻² mm² in vehicle-treated airways and 34.6 ± 13.5 µm²/10⁻² mm² in furosemide-treated airways (P = 0.016). However, no significant difference between treatments was detected (P > 0.11).

**DISCUSSION**

Pretreatment with aerosolized furosemide reduces HIAO in canine peripheral airways by ~50% compared with its vehicle control (Fig. 1). This level of inhibition compares favorably with other drugs previously used in our canine model that have modes of action that are better understood: methoxamine (an α₁-adrenergic agonist) inhibited HIAO by ~25% (22), atropine by ~30% (12), indomethacin by ~50% (11, 12), noradrenaline by ~60% (22), aminophylline (33) and MK-0591 (a leukotriene biosynthesis inhibitor) (21) by ~65%, and salbutamol by ~75–100% (23, 30). The efficacy of furosemide to inhibit HIAO in our canine model is similar to that seen in the inhibition of hyperpnea- and exercise-induced airway obstruction in asthmatic subjects, which varies from 30 to 60% (4, 14, 15, 25, 26, 29).

In this study, morphometric analysis revealed that unexposed control airways were composed of normal ciliated epithelium and goblet cells, with no more than 2% of the airway mucosa appearing damaged (Fig. 2). Although DAC tended to decrease G/C ratios (an indicator of goblet cell degranulation and mucosal perturbation) in normal bronchi, this trend was nonexistent in bronchioles (Fig. 3). DAC induced significant mucosal injury only in bronchi. Approximately 21% of the perimeter of vehicle-treated bronchi were injured, whereas ~34% of the furosemide-treated perimeter was damaged after DAC (Fig. 2). The extent of mucosal injury reported here is relatively small compared with our previously published results (9, 23, 24), but this is a consequence of using a weaker stimulus [2-min rather than 5-min exposures (10)] for this study.

Furosemide did not reduce the extent of dry air-induced mucosal injury in bronchi (Fig. 3), suggesting that injury per se was not directly responsible for HIAO. However, furosemide may interfere at some point in the cascade of events that are initiated “downstream” from the site of airway injury. In contrast to furosemide, pretreatment with salbutamol signifi-

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**Table 1. Effect of DAC on mucosal condition, ciliated and goblet cell number, and mast cell density in canine airways**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C + G Mucosa, %</th>
<th>C − G Mucosa, %</th>
<th>Damaged Mucosa, %</th>
<th>No. of Ciliated Cells, cells/mm⁻¹</th>
<th>No. of Goblet Cells, cells/mm⁻¹</th>
<th>Mast Cells, cells/mm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bronchi</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>52 ± 16</td>
<td>45 ± 14</td>
<td>1 ± 1</td>
<td>103 ± 8</td>
<td>23 ± 4</td>
<td>136 ± 33</td>
</tr>
<tr>
<td>Vehicle + DAC</td>
<td>27 ± 12</td>
<td>51 ± 12</td>
<td>22 ± 11*</td>
<td>97 ± 8</td>
<td>19 ± 5</td>
<td>149 ± 19</td>
</tr>
<tr>
<td>Furosemide + DAC</td>
<td>24 ± 15</td>
<td>42 ± 14</td>
<td>34 ± 18*</td>
<td>113 ± 15</td>
<td>18 ± 7</td>
<td>88 ± 19</td>
</tr>
</tbody>
</table>

Bronchioles

| Control     | 20 ± 14        | 78 ± 13        | 2 ± 2             | 108 ± 4                          | 12 ± 4                        | 261 ± 56             |
| Vehicle + DAC| 22 ± 8         | 77 ± 3         | 1 ± 1             | 109 ± 8                          | 13 ± 3                        | 204 ± 32             |
| Furosemide + DAC | 19 ± 7         | 76 ± 5         | 5 ± 3             | 105 ± 6                          | 13 ± 4                        | 223 ± 34             |

Values are means ± SE. Mucosal condition of airway perimeters includes %airway perimeter containing ciliated (C) and goblet (G) cells (C + G), C cells with few G cells (C − G), or damaged mucosa and average C and G cell no. and mast cell (lamina prophia) density in control canine airways and in vehicle- and furosemide-treated airways exposed to dry-air challenge (DAC). *P < 0.05 compared with control bronchi.
cantly reduced mucosal injury even in the presence of a stronger desiccating stimulus (5-min exposure) (23). The fact that airway epithelial cells can generate inhibitory signals that decrease bronchial smooth muscle responsiveness to contractile agonists (5) is consistent with the greater protection against HIAO afforded by salbutamol (30) in comparison with furosemide. Thus these observations suggest that the magnitude of epithelial damage may indeed play an indirect role in the development of HIAO.

Unlike exposure to a 5-min DAC (9, 23, 24), challenge for 2 min did not significantly alter mast cell number in either the lamina propria or submucosa of airways of any size examines in this study (Fig. 4). Although the number of mast cells located below normal ciliated mucosa in DAC bronchi did not decrease, mast cell number was reduced in dry air-exposed damaged tissue compared with undamaged airway (Fig. 5). Because completely degranulated mast cells would not be detected by our morphometric analysis, a reduction in the number of mast cells found below damaged mucosa is interpreted as an increase in mast cell degranulation. Whether mast cell degranulation either contributes to or is a consequence of mucosal injury in this canine model remains unknown. The fact that mechanical removal of airway epithelium has been reported to disrupt mast cells (7) suggests that dry air-induced mucosal injury causes mast cell degranulation. In either event, treatment with furosemide did not affect dry air-induced mast cell degranulation in damaged regions of an airway. This is similar to the effect of salbutamol on mast cell degranulation in DAC airways (23).

Furosemide has been reported to reduce the magnitude of airway cooling and rewarming that occurs during and immediately after DAC, and, in so doing, ameliorate a thermal stimulus purported to initiate HIAO in asthmatic subjects (14). However, HIAO does not develop in canine airways when cooling and rewarming occur in the absence of hyperpnea-induced airway drying (8). The fact that canine (8) and human (17) airways respond similarly to hyperpnea suggests that abrupt changes in airway temperature are not a prerequisite for the initiation of HIAO. Thus, for this study, we focused on the potential efficacy of furosemide for counterbalancing hyperpnea-induced airway dehydratons.
tion. We previously suggested that bronchovascular leakage occurs in concert with airway narrowing and protects the bronchial mucosa from excessive losses of heat and water (9). Hyperpnea with dry air does increase bronchial blood flow in dogs (3) and, as seen in this (Figs. 6 and 7) and other studies (9, 10), results in bronchovascular hyperpermeability. Theoretically, furosemide can affect blood flow (1, 14) and water flux across the mucosal epithelium (6) and may in part attenuate HIAO by stimulating a fluid-replacement mechanism. If furosemide diminishes HIAO by enhancing dry air-induced bronchovascular leakage and increasing water delivery to airway tissue, it would reduce the strength of the osmotic stimulus produced during a DAC. As seen in Fig. 6, a 2-min 1,500–2,000 ml/min DAC does increase bronchovascular fluid extravasation in canine bronchi. Although there is a tendency for greater bronchovascular leakage in furosemide-treated airways, this trend is not statistically significant. Thus furosemide's mechanism of action may not be related to vascular hyperpermeability.

Examination of mucosal-dependent vascular leakage reveals significantly greater fluid extravasation occurring below goblet cell depleted (C − G) and damaged mucosa when compared with ciliated mucosa replete with goblet cells (C + G). The variability and magnitude of bronchovascular leakage is noticeably greater, albeit not statistically significant (P = 0.11), in furosemide-treated compared with vehicle-treated canine peripheral airways (Fig. 7). These results stand in stark contrast to the report that inhaled furosemide decreased metabisulfite-induced microvascular leakage in the proximal airways of guinea pigs (28). However, the fact that furosemide inhibits bradykinin- but not adenosine-induced microvascular leakage specifically shows that the effect of furosemide on vascular permeability is stimulus dependent (13). Thus we are hesitant to ignore the obvious positive trend seen in Fig. 7 that clearly demonstrates that furosemide does not decrease and may actually enhance bronchovascular permeability in DAC canine peripheral airways.

In summary, we have shown that furosemide inhibits HIAO in canine peripheral airways with an efficacy similar to that reported for human asthmatic subjects. Although furosemide inhibits HIAO, it does not do so by dry air-induced mucosal injury or mast cell degranulation. Whether or not the tendency of furosemide to enhance dry air-induced bronchovascular fluid extravasation plays a significant physiological role in the inhibition of HIAO remains in question. However, even modest changes in microvascular permeability may be sufficient to compensate for the water loss that normally accompanies hyperpnea. Thus the trend toward increasing microvascular leakage is consistent with the hypothesis that furosemide enhances dry air-induced bronchovascular leakage and attenuates HIAO by increasing paracellular water movement in response to an osmotic stimulus. Experiments examining changes in airway lining fluid osmolarity in vehicle- and furosemide-treated bronchi immediately after hyperpnea with dry air may provide data that would unambiguously address this hypothesis and provide further insight into the mechanisms underlying the development and expression of hyperpnea-induced asthma.

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