Hypertonic saline aerosol increases airway reactivity in the canine lung periphery

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Hypertonic saline aerosol increases airway reactivity in the canine lung periphery. J Appl Physiol 89: 2139–2146, 2000.—Hyperventilation with dry air increases airway surface fluid (ASF) osmolality and causes acute mucosal injury, leukocyte infiltration, and delayed airway obstruction and hyperreactivity in canine peripheral airways. The purpose of this study was to determine whether ASF hypertonicity per se can account for these hyperventilation-associated effects. We first measured ASF osmolality before and after normal (NSC) and hypertonic (HSC) saline aerosol challenges to document the magnitude of hypertonicity produced by these stimuli. We then measured canine peripheral airway resistance and reactivity to hypocapnia and aerosolized histamine before and after NSC and HSC. Cells and eicosanoid mediators recovered in bronchoalveolar lavage fluid at 5 and 24 h after NSC and HSC were examined. We found that HSC but not NSC caused acute ASF hyperosmolality, increased mediator release, and delayed airway hyperreactivity in the absence of mucosal injury and leukocyte infiltration. These observations suggest that ASF hyperosmolality contributes to the development of the late-phase response to hyperventilation and further suggest that hyperventilation-induced mucosal injury independently initiates leukocyte infiltration and late-phase airway obstruction.

Hypertonic saline aerosol increases airway reactivity in the canine lung periphery. J Appl Physiol 89: 2139–2146, 2000.—Hyperventilation with dry air increases airway surface fluid (ASF) osmolality and causes acute mucosal injury, leukocyte infiltration, and delayed airway obstruction and hyperreactivity in canine peripheral airways. The purpose of this study was to determine whether ASF hypertonicity per se can account for these hyperventilation-associated effects. We first measured ASF osmolality before and after normal (NSC) and hypertonic (HSC) saline aerosol challenges to document the magnitude of hypertonicity produced by these stimuli. We then measured canine peripheral airway resistance and reactivity to hypocapnia and aerosolized histamine before and after NSC and HSC. Cells and eicosanoid mediators recovered in bronchoalveolar lavage fluid at 5 and 24 h after NSC and HSC were examined. We found that HSC but not NSC caused acute ASF hyperosmolality, increased mediator release, and delayed airway hyperreactivity in the absence of mucosal injury and leukocyte infiltration. These observations suggest that ASF hyperosmolality contributes to the development of the late-phase response to hyperventilation and further suggest that hyperventilation-induced mucosal injury independently initiates leukocyte infiltration and late-phase airway obstruction.

Hyperventilation with cold, dry air and inhalation of aerosolized hypertonic saline induce bronchoconstriction in humans and dogs. The magnitude of obstruction created by one is significantly correlated with that of the other (14, 32). Although both are believed to initiate airway obstruction by increasing airway surface fluid (ASF) osmolality via the release of bronchial active mediators (1, 12, 14), hyperventilation-induced bronchoconstriction (HIB) differs from hypertonic saline-induced bronchoconstriction (HSIB) in terms of the time course over which airway obstruction develops. In contrast to HIB, which develops slowly after hyperventilation stops, HSIB peaks immediately after aerosol challenge in humans and dogs (12, 14, 32). This discrepancy in time course may be due in part to differences in airway cooling, which is present during hyperventilation but is absent during challenge with hypertonic aerosols. When canine bronchi are artificially cooled during hypertonic saline challenge (HSC), the onset of bronchoconstriction is delayed and resembles HIB (10). This observation is consistent with the hypothesis that hyperventilation with dry air and inhalation of hypertonic aerosol initiate acute airway obstruction by increasing ASF osmolality.

Late-phase changes in airway function occur 3–13 h after exercise in asthmatic subjects (2, 7, 23), although the mechanism responsible for this phenomenon is a subject of some debate (18). Hyperventilation-induced late-phase airway obstruction analogous to that reported in human subjects also occurs in canine peripheral airways (4, 8). In dogs, the late-phase response to hyperventilation is characterized by airway mucosal damage, vascular leakage, and inflammation that persist for at least 24 h after the dry air challenge (DAC) (29). These changes in airway morphology are associated with the development of bronchial hyperreactivity within 5 h after DAC (4). If hyperventilation-induced changes in ASF osmolality per se contribute to late-phase changes in airway structure and function, then aerosol challenge with hypertonic solutions should result in similar late-phase events. The fact that hyperosmolality induces interleukin-8 (IL-8) expression in human bronchial epithelial cells (17) is consistent with the hypothesis that HSC initiates neutrophil infiltration. Thus the purpose of this study was to test the hypothesis that HSC causes airway injury, inflammation, and late-phase hyperreactivity similar to that seen after hyperventilation with dry air. In doing so, we first had to confirm that the magnitude of the osmotic stimulus produced by HSC was comparable to that created during a typical hyperventilation challenge used in our canine model. We then documented the acute and delayed changes in canine peripheral airways exposed to that hypertonic stimulus and compared those results with previously published data characterizing hyperventilation-induced responses in our canine model (4, 8, 9, 12, 13, 29).

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Methods

Experimental Techniques

Dogs were maintained and treated 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.

Anesthesia and instrumentation. Male mongrel dogs (19.6 ± 1.1 kg; n = 9) were anesthetized with intravenous thiopental sodium (25 mg/kg). Intravenous fentanyl citrate (25 μg) was administered every 15 min and was supplemented with thiopental (50 mg) as needed to maintain anesthesia. Depth of anesthesia was assessed by heart rate, blood pressure, canthal reflex, and presence of spontaneous movements and breathing. Dogs were intubated and mechanically ventilated on room air. End-expiratory CO₂ was monitored with a CO₂ analyzer (model LB-2, Beckman, Anaheim, CA) and maintained at ~4.5% by adjusting respiratory frequency. Rectal temperature was monitored using a thermometer (Yellow Spring Instrument, Yellow Springs, OH), and a warming pad was used to maintain body temperature. Heart rate and mean arterial blood pressure were recorded every 2 min during each experiment with a noninvasive monitor (Accutorr 1A, Datascope, Paramus, NJ).

Measurement of peripheral airway resistance. A fiber-optic bronchoscope (5.5-mm OD; BF type P-10, Olympus of America, New Hyde Park, NY) was inserted through a port in the endotracheal tube and gently wedged into a sublobar bronchus. Airway pressure (Pb) was measured at the tip of a bronchoscope via a polyethylene catheter (PE-90) threaded through its suction port. Compressed, dry, room temperature 5% CO₂ in air was delivered around the catheter and into the wedged subsegment at 200 ml/min (3.33 ml/s). Pb was measured by stopping the ventilator during expiration and allowing the unobstructed areas of the lung to equilibrate with atmospheric pressure (Patm) at functional residual capacity and maintaining the volume of the airway at 200 ml by adjusting the ventilator. The unobstructed area of the lung was determined by stopping the ventilator during expiration and allowing the unobstructed areas of the lung to equilibrate with atmospheric pressure (Patm) at functional residual capacity. Under this condition, Pb decays to a plateau at a pressure greater than Plosm (0 cmH2O) in the surrounding unobstructed lung, and peripheral airway resistance (Rp) = (Pb – Patm)/0.33 ml/s.

Normal saline challenge and HSC. Solutions were prepared daily for each experiment, and osmolality was measured by using a Wescor vapor pressure osmometer 5500 (Logan, UT). Either 0.9% saline (308 mmol/kgH₂O, pH = 5.6, n = 9) or 14.4% NaCl (4,468 ± 35 mmol/kgH₂O, pH = 7.4, n = 9) was aerosolized (Ultra Neb 100, DeVilbiss Somerset, PA) and delivered into a wedged sublobar segment through the port of a bronchoscope. The PE-90 catheter was temporally removed from the bronchoscope, and the aerosol was delivered in 5% CO₂ and air at 200 ml/min for 60 s.

Airway reactivity to hypocapnia and histamine. After a stable baseline Rp was established, insufflation of 200 ml/min of 5% CO₂ in air was switched to 200 ml/min 0% CO₂ for 3 min. Rp at the end of the 3-min challenge was recorded. After a stable baseline Rp was reestablished, histamine (25 μg/ml) was nebulized in 5% CO₂ and dry air and delivered into the wedged segment for 30 s at 200 ml/min via the bronchoscope.

Bronchoalveolar lavage, differential cell counts, and mediator analyses. Bronchoalveolar lavage (BAL) was performed at 5 and 24 h postchallenge by using three 20-ml aliquots of warm (37°C) Hanks’ balanced salt solution. Hanks’ balanced salt solution was infused into a wedged sublobar bronchus through the port of the bronchoscope and was gently suctioned from the segment using a 20-ml syringe. The aliquots of BAL fluid (BALF) were pooled and stored at 4°C until the end of the experiment and then centrifuged at 4°C for 10 min at 1,300 g. Total cell number in a 10-μl sample of BALF was determined using a hemocytometer. After cytospin slide preparation and staining with a modified Wright-Giemsa stain, differential cell counts of macrophages, lymphocytes, neutrophils, eosinophils, and epithelial cells were done. Trypan blue exclusion was used to evaluate cell viability.

BALF was concentrated using a Sep-Pak C₁₅ cartridge (Waters, Milford, MA), eluted in 4 ml of methanol, and stored at −70°C. Aliquots of the eluate were analyzed as previously described (30) using commercially available ELISA kits for PGE₂ (Cayman Chemical, Ann Arbor, MI), PGF₂α, thromboxane B₂, and leukotrienes C₄, D₄, and E₄ (LT-C₄-E₄; Neogen, Lexington, KY).

Measurement of peripheral airway resistance. Bronchoalveolar lavage (BAL) was performed at 5 and 24 h postchallenge by using three 20-ml aliquots of warm (37°C) Hanks’ balanced salt solution. Hanks’ balanced salt solution was infused into a wedged sublobar bronchus in the right middle lobe (RML). The RML was then exposed to NSC, and Rp was recorded at 0.5, 2, 5, 10, and 15 min after baseline Rp was reestablished. After baseline Rp was reestablished, airway reactivity to aerosolized histamine was determined using a hemocytometer. After cytospin slide preparation and staining with a modified Wright-Giemsa stain, differential cell counts of macrophages, lymphocytes, neutrophils, eosinophils, and epithelial cells were done. Trypan blue exclusion was used to evaluate cell viability.

Effects of normal saline challenge and HSC on Rp, ASF volume, and ASF osmolality. After baseline Rp was recorded in eight dogs (21 ± 1.3 kg) using dry, room-temperature air, either normal saline challenge (NSC) or HSC was done, and Rp was recorded at 4, 8, 12, and 17 min postchallenge. ASF was sampled for 2 min before and at 1, 3, 5, 7, 9, 11, and 14–16 min after aerosol challenge. Placement of the pledget after aerosol challenge was delayed 1 min to dry the bronchoscope (using the biopsy forceps and a small strip of gauze sponge) before the pledget was passed through its port. A control pledget containing a volume of standard solution equal to that recovered on the sample pledget was placed in a preweighed airtight vial and weighed (model A200S, Sartorius, Bohemia, NY). After being unfolded, the pledget was placed in a vapor pressure osmometer (model 5500, Wescor) for analysis. The change in weight (Δ mg) was used to estimate the volume of ASF sample (μl) recovered on the pledget. The time to remove the pledget from the airway and place it in the osmometer was recorded as “handling time,” which averaged 39 ± 0.7 s (n = 24). A control pledget containing 10 μl of standard solution (290 mmol/kgH₂O) was analyzed and recorded just before the recovery of each ASF sample. Another control pledget containing a volume of standard solution equal to that recovered on the sample pledget was placed in a preweighed airtight vial for a time equal to the handling time and was analyzed within minutes after the ASF sample was recovered. ASF osmolality corrected for volume and handling time was calculated as follows: ASF osmolality = ASF sample × (standard sample/adjusted standard sample). In vitro validation of this methodology was previously reported (9).

Experimental Protocol

Effects of normal saline challenge and HSC on Rp, ASF volume, and ASF osmolality. After baseline Rp was recorded in eight dogs (21 ± 1.3 kg) using dry, room-temperature air, either normal saline challenge (NSC) or HSC was done, and Rp was recorded at 4, 8, 12, and 17 min postchallenge. ASF was sampled for 2 min before and at 1, 3, 5, 7, 9, 11, and 14–16 min after aerosol challenge. Placement of the pledget after aerosol challenge was delayed 1 min to dry the bronchoscope (using the biopsy forceps and a small strip of gauze sponge) before the pledget was passed through its port. Effects of NSC and HSC on airway injury, inflammation, and reactivity. A bronchoscope was wedged in the left lower lobe (LLL) of nine dogs (19.1 ± 1.1 kg). An airway map to that location was constructed for later use. After baseline Rp was recorded, airway reactivity to hypocapnia was measured at 0, 5, 10, and 15 min after hypocapnia. After baseline Rp was reestablished, airway reactivity to aerosolized histamine was recorded in the same sublobar bronchus at 0.5, 2, 5, 10, and 15 min after aerosol challenge. After baseline Rp was reestablished again, the LLL was challenged with normal saline (NSC), and Rp was recorded at 0.5, 2, 5, 10, and 15 min after challenge. The bronchoscope was then removed from the LLL and rewedged into a sublobar bronchus in the right middle lobe (RML). The RML was then exposed to NSC, and Rp was
recorded at 0.5, 2, 5, 10, and 15 min after challenge. The bronchoscope was then removed from the RML and rewedged into a sublobar bronchus in the left middle lobe (LML), where baseline Rp was recorded. The bronchoscope was then removed from the CL and exposed to NSC, and Rp was recorded at 0.5, 2, 5, 10, and 15 min after challenge. Finally, the bronchoscope was removed from the CL and rewedged into a sublobar bronchus in the cardiac lobe (CL). The CL was then exposed to NSC, and Rp was recorded at 0.5, 2, 5, 10, and 15 min after challenge. Finally, the bronchoscope was removed from the CL and rewedged into a sublobar bronchus in the left upper lobe, where baseline Rp was recorded, and the dog was allowed to recover. Dogs were reanesthetized 5 h later, and airway reactivity was measured again in the LLL, and BALs were done in the RML (NSC) and the LML (wedge control). The dog was allowed to recover again. Eighteen hours later (24 h after NSC), airway reactivity was measured a third time in the LLL, and the CL (NSC) and the left upper lobe (wedge control) were lavaged. A similar protocol using HSC in place of NSC was repeated in the same sublobar airways 2 wk later. The protocol sequence was reversed in one of the nine dogs studied to confirm that experimental sequence did not affect outcome.

Statistical Analyses

Baseline Rp, peripheral airway reactivity, ASF volume, and ASF osmolality data were analyzed by using a Friedman two-way ANOVA in conjunction with a Student-Newman-Keuls test for the comparison of individual treatment means. Mediators and cells from airways exposed to NSC and HSC were also compared using Friedman’s two-way ANOVA. Mediators and cells recovered at different times from the same wedge control airway (n = 18) were compared using a Wilcoxon matched-pairs signed-ranks test. These wedge control data were found not to be statistically different, and their averaged values (n = 9) were compared with either NSC or HSC data using the Mann-Whitney U-test. Spearman rank correlation coefficient ($r_s$) was used to examine relationships between Rp and changes in ASF volume and osmolality that occur immediately after aerosol challenge with either isotonic or hypertonic saline. All values were expressed as means ± SE. Statistical significance was judged at $P < 0.05$ in all cases.

RESULTS

Effects of NSC and HSC on Rp, ASF volume, and ASF Osmolality

HSC increased Rp by $207 \pm 61\%$ compared with only a $4 \pm 6\%$ increase after NSC (Fig. 1A). Rp after HSC was significantly greater ($P < 0.05$) than either baseline or Rp after NSC at 4, 8, 12, and 17 min after challenge. ASF volume increased ($P < 0.05$) above baseline at 1–3 min after NSC and HSC, and this increase did not differ between challenges (Fig. 1B). Only HSC increased ASF osmolality at 1–3, 5–7, 9–11, and 14–16 min after aerosol challenge compared with the baseline value (Fig. 1C). However, ASF osmolality after HSC was greater ($P < 0.05$) than that after NSC only at 1–3 min postchallenge. Change in osmolality was not correlated with peripheral airway reactivity after NSC ($r_s = 0.085, P = 0.568, n = 47$), but it was correlated with peripheral airway reactivity after HSC ($r_s = 0.405, P = 0.005, n = 47$). Because NSC and HSC reflect two extremes of a continuous variable (osmo-
lality), we combined all the data, which produced an $r_s$ value of 0.500 ($P < 0.001, n = 94$) (Fig. 2). Finally, change in volume was not correlated with peripheral airway reactivity after either NSC ($r_s = 0.114, P = 0.328, n = 48$) or HSC ($r_s = 0.056, P = 0.702, n = 48$).

**Effects of NSC and HSC on $R_p$**

HSC and NSC increased $R_p$ by $-95$ and $-30\%$ over baseline, respectively (Fig. 3). The peak response to HSC was significantly greater than the peak response to NSC. $R_p$ was significantly increased at 0.5, 2, and 5 min after HSC; $R_p$ was significantly increased only at 0.5 min after NSC. Although the acute responses to these two challenges were markedly different, $R_p$ recorded at 5 and 24 h after aerosol challenge was similar (Fig. 3).

**Effects of NSC and HSC on Airway Reactivity to Hypocapnia**

Reactivity to hypocapnia before NSC and HSC was similar. Neither NSC nor HSC altered airway reactivity to hypocapnia measured at either 5 or 24 h after aerosol challenge (Fig. 4). Responsiveness to hypocapnia tended to decrease at 5 and 24 h after NSC, but it remained relatively constant after HSC. The maximum response to hypocapnia before and at either 5 or 24 h after NSC (Fig. 5A) and HSC (Fig. 5B) confirms that neither treatment had a significant effect on airway reactivity to hypocapnia.

**Effects of NSC and HSC on Airways Reactivity to Histamine**

Airway reactivity to histamine before NSC and HSC was similar. Reactivity to histamine tended to increase 5 h after NSC (Fig. 6B) but returned to baseline levels by 24 h postchallenge (Fig. 6C). Reactivity to histamine also tended to increase 5 h after HSC, remained elevated at 24 h, and was significantly greater than that seen after NSC at that time (Fig. 6C). The maximum responses to histamine before and after NSC (Fig. 7A) and HSC (Fig. 7B) confirmed that histamine reactivity increased only in airways challenged with hypertonic saline. The maximum response to HSC occurred in four
dogs at 5 h and in five dogs at 24 h after the aerosol challenge. There was a statistically significant interaction between time and treatment ($P = 0.0174$).

**Effects of NSC and HSC on BALF Cell Profiles**

Five hours after bronchoscopy, the average volume of BALF recovered from control, NSC, and HSC airways was $43.1 \pm 0.85$, $43.6 \pm 1.03$, and $40.9 \pm 2.32$ ml, respectively ($P = 0.412, n = 9$). At 24 h, the recovered volumes for control, NSC, and HSC averaged $40.4 \pm 1.63$, $43.6 \pm 0.83$, and $43.1 \pm 1.45$ ml, respectively ($P = 0.216, n = 9$).

The recovery of macrophages, lymphocytes, neutrophils, and eosinophils in BALF was not affected by either NSC or HSC (Fig. 8). There was a small but significant increase ($P < 0.05$) in epithelial cells recovered at 24 h after HSC compared with the wedge control (Fig. 8B). There were no protocol-dependent differences in the total cells per milliliter of BALF recovered during any experiment (all $P > 0.5$).

**Effects of NSC and HSC on Eicosanoid Mediator Release**

The effect of either NSC or HSC on eicosanoid mediator release is summarized in Fig. 9. The concentrations of LTC$_4$-E$_4$ were increased ($P = 0.0062$) 5 h after HSC compared with the wedge control airway. LTC$_4$-E$_4$ increased 24 h after HSC compared with either the NSC airway ($P = 0.0195$) or the wedge control ($P < 0.0001$). PGD$_2$ ($P = 0.0171$) increased at 24 h after HSC compared with the wedge control airway (Fig. 9). Concentrations of PGF$_{2\alpha}$ at 5 and 24 h
HYPERTONICITY INCREASES AIRWAY REACTIVITY

**DISCUSSION**

This study shows that hyperosmolality per se causes a delayed increase in peripheral airway reactivity to histamine in dogs (Figs. 4 and 5). The development of airway hyperreactivity 5–24 h after inhalation of hypertonic saline is accompanied by an increase in the production of PGD$_2$ and cysteinyl leukotrienes (Fig. 9). Unlike hyperventilation with dry air (4, 8), it does not result in either leukocyte infiltration or late-phase airway obstruction (Figs. 3 and 8). The implications of these results are discussed below.

Changes in peripheral ASF osmolality have never been documented in vivo before and after either isotonic or hypertonic challenge of the lung periphery. We found that HSC increased ASF osmolality in dogs by 68 ± 22 mmol/kgH$_2$O ($n = 8$) at 1–3 min after the challenge ended (Fig. 1C). Although it is impossible to obtain an accurate measurement of ASF osmolality during an aerosol challenge because of technical problems associated with our method, it is likely that ASF osmolality would be considerably higher if measured at the end of the challenge period. Based on our value of 68 mmol/kgH$_2$O, the HSC used in this study generated an osmotic stimulus slightly greater than that produced in a similar-sized airway and at a similar time after hyperventilation with dry air (~40 mmol/kgH$_2$O) (9). Unlike HSC, it is possible to measure ASF osmolality during hyperventilation, and this increased to 140 mmol/kgH$_2$O at that time (9). The difference in these two measurements is likely to reflect the airways’ transepithelial compensatory mechanisms that have been reported to reduce the impact of hypertonic stimuli in canine airways (35) and is consistent with our assumption that ASF osmolality during HSC would be markedly greater than our postchallenge value. Thus it appears that the hypertonic saline aerosol and dry air hyperventilation challenges used in our laboratory result in similar osmotic stress for canine peripheral airways.

Figure 3 shows that Rp increases immediately after HSC, but, unlike the response to hyperventilation with dry air (4, 8), it does not increase 5–24 h after HSC. This suggests that transient ASF hypertonicity alone is insufficient to cause the development of late-phase airway obstruction. This observation is consistent with a recent study by Eder et al. (5) showing that HSC did not cause late-phase changes in airway function in children. However, that study examined changes in forced expired volume in 1 s only and did not evaluate other hallmarks of a late-phase response, such as inflammation or airway hyperresponsiveness. Our study suggests that changes in airway reactivity may occur in the absence of either diminished baseline airway function or inflammatory cell infiltration (Figs. 6–8).

We were surprised to find that HSC did not initiate leukocyte infiltration (Fig. 8). This stands in stark contrast to the late-phase inflammatory response reported for DAC (4, 8). Hashimoto and co-workers (17) reported that hyperosmolality induced IL-8 expression in human bronchial epithelial cells via activation of p38 mitogen-activated protein kinase and c-Jun NH$_2$ terminal kinase. Thus epithelial-derived IL-8 may account for the neutrophil infiltration that occurs within 5 h after DAC in dogs (4, 8). However, if HSC induces IL-8 production in vivo, it appears to be at levels insufficient to stimulate neutrophilia in canine peripheral airways. Although HSC does not produce significant acute mucosal injury in either asthmatic subjects or dogs (12, 27), DAC does (12, 31), and this difference may account for the disparity in these stimuli to initiate cell infiltration. On the basis of the recovery of epithelial cells in BALF, neither HSC (Fig. 8) nor DAC (4, 8) is associated with significant mucosal shedding 5–24 h after an exposure. However, the former appears to result from the lack of an acute airway injury (12), whereas the latter results from an active repair process (29).

In contrast to DAC (13), HSC does not significantly increase BALF mediator concentrations immediately after challenge in either humans or dogs (12, 27). However, the time at which BAL was done after the acute response to HSC may account for these negative results (13). In contrast to the acute response, leukotrienes are elevated at 5 and 24 h after challenge with a hypertonic aerosol. PGD$_2$, a relatively specific marker of mast cell degranulation (11), is also increased 24 h after HSC (Fig. 9). Although PGF$_{2\alpha}$ is increased at these times, a similar increase in BALF samples recovered after NSC suggests that this increase was not a direct result of HSC. It is possible that the rise in PGF$_{2\alpha}$ was the direct result of bronchoscopy, but our results with LTC$_4$ and PGD$_2$ suggest otherwise. Overall, our data are consistent with earlier reports that osmotic stimuli initiate mediator release from mast cells and basophils in vitro (6) and from...
canine (12) and human airways (15) challenged in vivo. These results are consistent with the hypothesis that eicosanoid mediators modulate the delayed increase in peripheral airway reactivity that occurs 5–24 h after HSC.

Hypocapnia-induced constriction in canine peripheral airways is reduced by pretreatment with either nifedipine or verapamil (24, 26), but not with atropine (24), indicating that hypocapnia-induced airway narrowing does not involve a vagal reflex. The fact that HSC increased airway eicosanoid concentrations (Fig. 9) without affecting airway responsiveness to hypocapnia (Figs. 2 and 3) confirms the work of Lindeman et al. (25), which showed that eicosanoids do not contribute to the development of hypocapnia-induced bronchoconstriction. Hypocapnia is believed to cause smooth muscle constriction by increasing intracellular pH (3), which causes conformational changes in voltage-sensitive calcium channels, and a concomitant influx of calcium (36). However, the lack of any changes in Rp peripheral airway reactivity after NSC indicates that low pH alone does not initiate late-phase alterations in airway function (Figs. 1–5). Numerous studies have reported that excess sodium increases calcium influx, which in turn results in greater smooth muscle constriction (16, 34). Jongejan et al. (21) reported that hypertonic-induced bronchoconstriction in vitro was in part dependent on intracellular calcium release and suggested that osmotic shrinkage in part stimulated constriction under hyperosmotic conditions. Hogman et al. reported that HSC caused subepithelial tissue edema, increased concentrations of Na$^+$, K$^+$, and Cl$^-$, decreased compliance (19), and increased airway responsiveness to histamine in rabbits (20).

Airway reactivity to histamine is enhanced 5–24 h after HSC (Figs. 6 and 7). The hypertonic saline solution used in this study was adjusted to a pH of 7.4, suggesting that the changes in airway reactivity that occurred may result from sodium-enhanced calcium influx unrelated to changes in intracellular pH. In canine central airways, histamine causes bronchoconstriction in part by directly stimulating either smooth muscle H$_2$ receptors, mediator release, or a vagal reflex (37). However, Kaplan et al. (22) reported that histamine-induced constriction in canine peripheral airways lacked a vagal component, suggesting that the development of hyperreactivity in our model primarily reflects HSC-associated mediator release. Thus the enhanced response to histamine 5–24 h after HSC (Figs. 6 and 7) may in part result from the increased synthesis and release of LTC$_4$, E$_4$ and PGD$_2$ at these times (Fig. 9). This scenario is supported by the observation that leukotrienes enhanced airway responsiveness to histamine in asthmatic subjects ~4 h after inhalation (28). Smith et al. (33) reported that airway sensitivity to methacholine in asthmatic subjects was not affected 1 h after challenge with hypertonic saline (4.5%), but they did not examine airway responsiveness at later times. We evaluated histamine reactivity at 5 and 24 h after aerosol challenge with 14.4% saline and observed peripheral airway hyperreactivity at either time point (Figs. 6 and 7). Although all dogs tested exhibited delayed hyperresponsiveness, they exhibited significant interanimal variation in the time required for bronchial hyperreactivity to develop. This individual variation is consistent with reported variability in the development of allergen- and exercise-induced late-phase responses in asthmatic subjects (2, 7, 23). Thus, despite the fact the HSC does not initiate late-phase changes in baseline lung function in either dogs (Fig. 3) or asthmatic humans (5), it does produce a delayed increase in canine peripheral airways reactivity and may have a similar effect in human subjects.

In summary, we found that HSC caused an acute increase in ASF osmolality and delayed increases in BALF eicosanoid concentrations and peripheral airway reactivity to histamine 5 to 24 h after exposure. HSC did not cause mucosal injury, cell infiltration, or airway obstruction. On the basis of our laboratory’s previously published studies (4, 8, 29), the response of peripheral airways to HSC differs from those seen after hyperventilation with dry air, in which airway injury, mediator release, and leukocyte infiltration accompany a delayed increase in airway resistance and reactivity. Thus we conclude that hypertonicity per se can stimulate the development of airway hyperreactivity in the absence of gross mucosal damage and inflammation. This suggests that the late-phase response to hyperventilation with dry air results from the simultaneous stimulation of two independent pathways. The first involves hyperventilation-induced mucosal injury, which initiates an inflammatory process and results in the development of late-phase airway obstruction (4, 8). The second involves hyperventilation-induced airways hypertonicity, which stimulates either a delayed or prolonged release of bronchoactive eicosanoids, and results in peripheral airways hyperreactivity.

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