Modeling the acute- and late-phase responses to peripheral airway cooling and desiccation

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HYPERPNEA INCREASES THE REQUIREMENT for the respiratory mucosa to warm and humidify the inspired air. Large increases in the minute ventilation, particularly when the inspired air is substantially below body temperature, can exceed the capacity of the upper airways to complete the conditioning process, resulting in peripheral airway cooling and desiccation. The acute-phase response to hyperpnea has been extensively characterized in both humans and numerous laboratory animals (20). However, whether this stimulus results in a second phase of airway obstruction is the subject of considerable debate (23). Some investigators argue that a late-phase response is a reproducible feature of hyperpnea (2, 4, 15, 18, 25), whereas others maintain that a late-phase response is not a feature per se of this stimulus but rather an epiphenomenon attributable to other factors (3, 5, 14, 17, 30, 31). The difficulty in clearly demonstrating a late-phase response in humans stands in contrast to data derived from a canine model of peripheral airway cooling and desiccation, in which a late-phase response can be reliably measured (9, 11). Given the consistency with which this animal model reproduces the comparable acute-phase response in humans (10), it seemed unlikely that the late-phase response could be unique to dogs. Rather, we hypothesized that the apparent differences between the canine model and the human data likely resulted from experimental design, specifically that the late-phase response is a feature of near-maximal challenge of the peripheral airways with unconditioned air and that submaximal challenges that produce an acute-phase response might not exceed the threshold necessary to produce a late-phase response. Thus we sought to model the dose-response effects of peripheral airway cooling and desiccation on the expression of the acute-phase and late-phase responses.

MATERIALS AND METHODS

Study Design

Male mongrel dogs (~20 kg, >6 mo old) were each used in two protocols. In each protocol, dogs were anesthetized as previously described (7), a bronchoscope was advanced into the lower airways until wedged in each of five separate sublobar airways, and baseline peripheral airway resistance (Rp) was measured. Each airway was then challenged once with one of five challenges: 200, 500, 1,000, 1,500, and 2,000 ml/min insufflation of room-temperature dry air with 5% CO2 added for 5 min. The acute-phase response after the challenge was then recorded for 10 min, and the bronchoscope was removed. Approximately 4.5 h after completion of the challenges, the dogs were reanesthetized and the challenged airways were relocated with the bronchoscope by using airway branching maps constructed at the beginning of the experiment. In the first protocol, only Rp was measured during the late-phase response. In the second protocol, bronchoalveolar lavage was performed in the challenged airways, and the recovered fluid (BALF) was analyzed for cellular and eicosanoid concentrations. All dogs were used in both protocols, and the same airways received the same challenges in both protocols.

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Methods

All animals were handled and maintained in accordance with the Policy and Procedures Manual published by the Oklahoma State University Institutional Animal Care and Use Committee. For the experiments, dogs were anesthetized with an intravenous bolus of thiopental (25 mg/kg) and fentanyl (1 μg/kg) and maintained under anesthesia with additional intravenous boluses of fentanyl (1 μg/kg) every 15 min. Depth of anesthesia was assessed by heart rate, blood pressure, canthal reflex, and the presence of spontaneous movements and breathing. Dogs were intubated and mechanically ventilated (17 ml/kg) with room air. End-tidal CO₂ was monitored with a CO₂ analyzer and maintained at ~4.5% by adjusting ventilator frequency.

Measurement of R̄p. A bronchoscope (5 mm OD) was inserted through an airtight portal of the endotracheal tube and gently wedged into a sublobar segmental bronchus. The suction port (1.2 mm) of the bronchoscope was connected to a pressure transducer used to measure airway pressure in the subtended lung segment (Pb). Compressed, dry, room-temperature 5% CO₂ in air was delivered at a rate of 200 ml/min through the suction port of the bronchoscope into the wedged sublobar segment. R̄p was measured by stopping the ventilator during exhalation such that the unobstructed areas of the lung equilibrated with atmospheric pressure at functional residual capacity. Under these conditions, Pb decays to a plateau at a pressure greater than the alveolar pressure (atmospheric) in the surrounding unobstructed lung so that R̄p = Pb/200 ml⁻¹·min⁻¹.

BALF recovery and analysis. Three 20-ml aliquots of warmed Hanks’ buffered saline solution were instilled through the bronchoscope into the wedged sublobar segment and then recovered by gentle aspiration. The recovered BALF was pooled and the volume determined. Nucleated cells were counted by using a hemacytometer. Differential counts of cytocentrifuged cells treated with a modified Wright-Giemsa stain were done. The balance of the sample was centrifuged, and the supernatant was filtered through a C₁₈ Sep-Pak exchange cartridge (Waters, Milford, MA) and eluted with 4 ml of methanol. Aliquots of the eluted sample were dried under vacuum, reconstituted with buffered saline, and analyzed with commercially available ELISA kits for thromboxane B₂, prostaglandin E₂ (Neogen, Lexington, KY), prostaglandin F₂α, prostaglandin D₂, leukotriene B₄, and sulfidopeptide leukotrienes C₄-E₄ (Cayman Chemical, Ann Arbor, MI).

Analysis

Analysis of variance procedures were utilized to assess the effect of challenge. Dog was included in the model to account for dog-to-dog variability. PROC MIXED in PC SAS Version 8.2 was used. Because challenge had meaningfully numeric levels, one degree of freedom contrasts were calculated to account for the linear and quadratic effects of challenge to response. Tests were performed using the type III sums of squares associated with these contrasts to test the significance of the quadratic effect given the linear effect in the model. If the quadratic effect was not deemed significant, it was removed and the linear-only effect model was fit. All contrasts and effects were judged significant if their respective P value was <0.05.

RESULTS

The acute-phase response data were best modeled as a binomial function expressing the response as a percentage of the prechallenge resistance and the challenge flow rate as the independent variable. The constant for the independent variable “challenge” was negative, causing the slope of the model to decrease with increasing challenge magnitude (Fig. 1). In addition, all challenges produced significant acute-phase airway obstruction compared with the control flow rate (200 ml/min). The late-phase response data were also modeled as a binomial function expressing the response as a percentage of the prechallenge resistance, but in this model, the constant for the independent variable challenge was positive, resulting in a model whose slope becomes steeper with increasing challenge magnitude. Furthermore, only the highest challenge (2,000 ml/min) produced late-phase obstruction that was significantly different from control.

All BALF cell and eicosanoid concentrations had significant linear relationships to challenge severity (Figs. 2 and 3). In the case of macrophages, lymphocytes, neutrophils, and sulfidopeptide leukotrienes, the slope of the relationship was sufficiently steep (relative to the variability of the values within each category) to result in significant differences between BALF from control lobes and that recovered from the two highest challenges. For eosinophils and the rest of the eicosanoids measured, only the BALF from the airways...
Fig. 2. Dose-response effects of peripheral airway hyperpnea on late-phase bronchoalveolar lavage fluid (BALF) nucleated cells. A: macrophage regression slope = 0.037, \( r^2 = 0.51, P = 0.0004 \). B: lymphocyte regression slope = 0.011, \( r^2 = 0.59, P = 0.0057 \). C: neutrophil regression slope = 0.011, \( r^2 = 0.46, P = 0.001 \). D: eosinophil regression slope = 0.0036, \( r^2 = 0.42, P = 0.0009 \). *Significantly different from control (200 ml/min), \( P < 0.05 \).

Fig. 3. Dose-response effects of peripheral airway hyperpnea on late-phase BALF eicosanoids. A: leukotriene (LT) B\(_4\) regression slope = \( 1.8 \times 10^{-3} \), \( r^2 = 0.45, P = 0.0239 \). B: LTC\(_4\) regression slope = \( 4.6 \times 10^{-3} \), \( r^2 = 0.46, P = 0.0010 \). C: thromboxane B\(_2\) (TxB\(_2\)) regression slope = \( 1.1 \times 10^{-2} \), \( r^2 = 0.36, P = 0.0031 \). D: PGD\(_2\) regression slope = \( 1.5 \times 10^{-2}, r^2 = 0.33, P = 0.0183 \). E: PGF\(_{2\alpha}\) regression slope = \( 2.7 \times 10^{-2}, r^2 = 0.44, P = 0.0010 \). F: PGE\(_2\) regression slope = \( 7.6 \times 10^{-6}, r^2 = 0.51, P = 0.0472 \). *Significantly different from control (200 ml/min), \( P < 0.05 \).
with the highest challenge had significantly greater eosinophil concentrations compared with control airways.

**DISCUSSION**

The widespread acceptance of the late-phase response to peripheral airway cooling and desiccation has been elusive because of the number of studies in humans that have failed to demonstrate that a second phase of airway obstruction occurred after exercise or isocapnic hyperpnea or that such a phenomenon occurring was directly associated with that challenge (3, 5, 14, 17, 30). The existence of a dose-response relationship between challenge severity and the acute-phase response, and the resulting need for standardization of exercise tests, has been previously described (1, 24). In this study, we have produced data to suggest that the late-phase response has a distinctly different dose-response relationship from the acute-phase response. These data help to reconcile the conflicting findings of those former studies and the studies that have demonstrated late-phase airway obstruction after exercise.

The mean airway wall temperature at any location in the respiratory tract represents a specific net heat loss over time. During normal ventilation, heat loss and recovery in the airway wall oscillate with the respiratory cycle: heat is lost when the relatively cooler air moves distally during inhalation, and some (but not all) of that heat is recovered when the relatively warmer air moves proximally during exhalation (21). The difference between the heat lost during inhalation and the heat recovered during exhalation is the net heat loss, and this net heat loss directly corresponds to the magnitude of airway cooling. The amount of heat lost during ventilation is correlated with the magnitude and duration of ventilation and is inversely correlated with the temperature of the inspired air (19). Thus equivalent respiratory heat loss can be generated by short periods of hyperpnea with cold air and prolonged periods of hyperpnea with warm air. Similarly, large increases in ventilation with room temperature air may approximate the same magnitude of respiratory heat loss as smaller increases in ventilation with extremely cold air. From a practical standpoint, it is not necessary to use cold air to produce peripheral airway cooling if the magnitude of either of the other two variables is sufficiently great. However, the magnitude of airway cooling is maximized with the use of extremely cold air, large increases in minute ventilation, and long duration of hyperpnea. The canine wedged bronchoscope model used in this study results in less heat loss per volume of air during “inhalation,” but by eliminating heat recovery during exhalation, it produces the same net heat loss, the same mean airway wall temperature, and therefore the same initial stimulus as a comparable oscillatory pattern of air movement (12). The validity of this comparison is further supported by the fact that the canine wedged-bronchoscope model consistently reproduces virtually every aspect of the acute-phase response to hyperpnea in humans (10).

Our study demonstrates that the relationship between hyperpnea and the acute-phase response is not linear (Fig. 1). Rather, the slope of the relationship tends to be steeper at lower values of challenge severity, thus producing an easily detectable response at relatively lower levels of challenge. The relationship between challenge severity and the late-phase response is also not linear (Fig. 1), but in contrast to the acute phase, the slope of this relationship tends to be low at the lower challenge levels and only becomes steep under severe challenge conditions. The implications of these relationships for human studies of hyperpnea and exercise-induced asthma are that challenges that produce acute-phase obstruction may not be sufficiently severe to produce an episode of late-phase airway obstruction. Many human studies of the hyperpnea-induced late-phase bronchoconstriction have titrated the initial challenge to a percentage of the maximal heart rate for the test subject (3, 5, 14, 17) or to a specific minimum decrease in forced expiratory volume in 1 s during the acute-phase response (31), whereas others have allowed the subjects to inspire room-temperature air (3, 14). Our data suggest that this approach may preclude the development of a detectable late phase of airway obstruction because of insufficient respiratory heat loss. To reliably demonstrate late-phase airway obstruction, investigators need to select a study population that is capable of achieving very high minute ventilations and/or use frigid inspired air to produce a challenge that results in a maximal acute-phase response. Thus the phenomenon of late-phase airway obstruction after exercise or isocapnic hyperpnea may be a feature of only the most severe challenges.

So how severe must a challenge be to elicit late-phase airway obstruction? It is widely accepted that a valid indicator of the magnitude of the challenge is the magnitude of airway cooling and that this is in turn related to the velocity of air and the temperature of the air. Freed et al. (13) measured the airway wall temperatures produced by varying the unidirectional flow of room-temperature air through a bronchoscope wedged in a canine sublobar bronchus and found that 500, 1,000, 1,500, and 2,000 ml/min produced decreases in airway wall temperatures of 0.7, 2.3, 4.9, and 7.0°C, respectively. Based on similar modeling of the effects of inspired air conditions and minute ventilation on airway temperature in humans by McFadden et al. (22), these same decreases in airway wall temperatures would be produced by increasing minute ventilation by ~10, 33, 70, and 100 l/min over resting minute ventilation when the inspired air temperature is ~18.6°C. Thus the highest challenge is quite close to the maximal minute ventilation, and only strenuous activity while breathing subfreezing air could be reasonably expected to produce sufficient thermal challenge to elicit both an acute-phase and late-phase bronchoconstriction response.
Despite the apparent requirement for a near-maximal challenge to produce late-phase airway obstruction, airway inflammation appears to be more readily elicited by submaximal challenges (Figs. 2 and 3). A change in macrophage concentration was most readily elicited by submaximal challenges (Figs. 2 and 3). A large number of epidemiologic studies have suggested that repeated strenuous exercise in cold weather can lead to airway inflammation and hyperreactivity similar to asthma, a syndrome that has been referred to as ski asthma (16, 26–29). This hypothesis is supported by recent studies demonstrating that repeated challenge of canine peripheral airways with the highest challenge used in this study (2,000 ml/min) can produce many of the features of “ski asthma,” including airway injury and inflammation (6–8). However, the results of the present study suggest that repeated maximal challenges might not be necessary to produce airway inflammation. Rather, winter athletes may suffer airway injury and inflammation even during less strenuous activity.

The results of this study help explain the apparent discrepancies between human and animal studies regarding the existence of a late-phase response to exercise or isocapnic hypopnea. Based on our data, it appears that although an acute-phase response requires a relatively mild stimulus, the development of late-phase airway obstruction requires a considerably stronger stimulus and thus may not be achievable in many human subjects. However, airway inflammation secondary to exercise or isocapnic hypopnea may be more readily induced, a fact that is supported by the relatively high prevalence of chronic airway inflammation in winter athletes.

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REFERENCES


