Desiccation and hypertonicity of the airway surface fluid and thermally induced asthma

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Kotaru, Chakradhar, Rana B. Hejal, J. H. Finigan, Albert J. Coreno, Mary E. Skowronski, Lori Brianas, and E. R. McFadden, Jr. Desiccation and hypertonicity of the airway surface fluid and thermally induced asthma. J Appl Physiol 94: 227–233, 2003. First published September 13, 2002; 10.1152/japplphysiol.00551.2002.—To determine whether drying and hypertonicity of the airway surface fluid (ASF) are involved in thermally induced asthma, nine subjects performed isocapnic hyperventilation (HV) (minute ventilation 62.2 ± 8.3 l/min) of frigid air (−8.9 ± 3.3°C) while periciliary fluid was collected endoscopically from the trachea. Osmolality was measured by freezing-point depression. The baseline 1-s forced expiratory volume was 73 ± 4% of predicted and fell 26.4% 10 min postchallenge (P = 0.0001). The volume of ASF collected was 11.0 ± 2.2 µl at rest and remained constant during and after HV as the airways narrowed (HV 10.6 ± 1.9, recovery 6.5 ± 1.7 µl; P = 0.18). The osmolality also remained stable throughout (rest 336 ± 16, HV 339 ± 16, and recovery 352 ± 19 mosmol/kgH2O, P = 0.76). These data demonstrate that airway desiccation and hypertonicity of the ASF do not develop during hyperventilation-induced bronchial narrowing; therefore, other mechanisms must cause exercise-induced hyperventilation-induced airflow limitation.

METHODS

9 atopic asthmatic patients (6 men and 3 women), aged 28.4 ± 2.5 yr (mean ± SE), with documented exercise-induced asthma served as our subjects (Table 1). None used tobacco products or experienced an upper respiratory tract infection in the 6 wk preceding the investigation. All participants refrained from taking short-acting β2-agents for 12 h and antihistamines and antileukotriene compounds for 48 h and 4 days, respectively, before any challenge. The institutional review board for human investigation, at University Hospitals of Cleveland, approved the protocol, and all participants gave informed consent.

The study was performed in two parts. In the first, the individual minute ventilations (VE) associated with a reduction in the 1-s forced expiratory volume (FEV1) >20% (P VE20) was determined by having each subject generate stimulus-response curves to isocapnic hyperventilation of frigid air (HV) by use of standard techniques (10, 14–16, 28). The resultant P VE20 values were then employed in the subsequent experiments on airway surface fluid (ASF) volume and osmolality. During the challenges, VE was progressively increased in 4-min intervals while the participants inhaled air through a heat exchanger (10, 14–16, 28). The water content of the inspirate was <1 mgH2O/l, which for the purposes of this study was considered zero. The expired air was directed into a reservoir balloon that was being constantly evacuated at a known rate through a calibrated rotameter. The subjects were coached to keep the balloon filled, and, in so doing, their VE could be controlled at any desired value. End-tidal CO2

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concentrations were monitored with a Nellcor N-1000 analyzer (Malineckrodt, Kansas City, KS), and sufficient CO2 was added to the inspiratory port of the exchanger during hyperpnea to maintain end-tidal CO2 at eucapnic levels.

Table 1. Demographic and prechallenge clinical data

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age, yr</th>
<th>Sex</th>
<th>FEV1, liters</th>
<th>Medications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>29</td>
<td>F</td>
<td>2.02</td>
<td>Alb</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>M</td>
<td>4.05</td>
<td>Alb, IS, Anti L</td>
</tr>
<tr>
<td>3</td>
<td>44</td>
<td>M</td>
<td>3.69</td>
<td>Alb, Anti L</td>
</tr>
<tr>
<td>4</td>
<td>22</td>
<td>F</td>
<td>2.99</td>
<td>Alb</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>M</td>
<td>3.05</td>
<td>Alb</td>
</tr>
<tr>
<td>6</td>
<td>27</td>
<td>M</td>
<td>3.70</td>
<td>Alb, Anti L, AH</td>
</tr>
<tr>
<td>7</td>
<td>28</td>
<td>F</td>
<td>1.96</td>
<td>Alb, Anti L</td>
</tr>
<tr>
<td>8</td>
<td>19</td>
<td>M</td>
<td>2.72</td>
<td>Alb, Anti L</td>
</tr>
<tr>
<td>9</td>
<td>25</td>
<td>M</td>
<td>2.76</td>
<td>Sal, IS</td>
</tr>
</tbody>
</table>

Mean ± SE 28 ± 3 2.99 ± 0.26

F, female; M, male; FEV1, 1-s forced expiratory volume; Alb, inhaled albuterol; IS, inhaled steroids; Anti L, antileukotriene; AH, antihistamine; Sal, salmeterol.

After the tapered weights were recorded, 100 μl of double-distilled deionized water were added to the tubes, after which they were reweighed and centrifuged for 60 s to ensure thorough mixing (20–22, 32). The samples were left to stand overnight to allow the fluid on the paper to elute into the water. The osmolality of the mixture was measured by freezing-point depression (Advanced Micro-Osmometer, Advanced Instruments, Norwood, MA) (22).

After the prechallenge surface liquid was gathered, the subjects performed HV with the bronchoscope tip in the upper airway (22, 29, 30) at the individual P V˙E20 values previously determined to cause the index fall in FEV1. Perciliary fluid was collected as above during the last minute of HV and at the fifth minute of the recovery period.

Maximum forced exhalations were obtained in triplicate with a waterless spirometer, and the curve with the largest FEV1 was chosen for analysis. Spirometry was performed before anesthesia of the upper airway was undertaken, 15 min after its completion before endoscopy was begun, and again immediately on collection of the final fluid sample and removal of the bronchoscope. The last assessment occurred temporally at the 9–10th min of the recovery period and coincided with the height of the obstructive response.

The accuracy and reproducibility of the collection technique and osmolality measurements were assessed as in previous studies (22). The results from the filter paper technique were compared with direct measurements of an isosmolar standard (284 mosmol/kgH2O) and with two levels of hypertonicity (463 and 742 mosmol/kgH2O) known to be associated with mediator release from mast cells and basophiles (11, 36).

The influence of sample handling on evaporative water losses from the filter paper was determined by placing dry, weighed pledgets into 20 μl of the isosmolar standard for 30 s and serially reweighing them after exposure to air at room temperature (22.6 ± 0.6°C) and humidity (32 ± 3%) for 15, 30, 60, and 120 s (21, 22). This method was chosen to mimic as closely as possible the events transpiring when the collecting system was removed from the endoscope. An equation regressing water loss against time was constructed from 22 measurements at each experimental point. By knowing the handling time in each experiment and the slope of the regression line, the initial weight of the pledgets from each collection could be backextrapolated (21, 22).

The data were analyzed statistically by paired t-tests and one- and two-factor analyses of variance. The latter incorporated a feature for repeated measures. A two-tailed P value ≤ 0.05 was considered significant. The study was powered to detect an increase in osmolality of ≥30% (~100 mosmol/kgH2O) over baseline.

RESULTS

In vitro studies. A comparison of the accuracy and reproducibility of the osmolality measurements is presented in Fig. 1. There were no significant differences for the results between the direct and indirect techniques for any standard. The coefficients of variation of the measurements in the normal, moderate, and high standards with filter paper collection were 2.3, 2.4, and 3.1%, respectively.
Figure 2 displays the rate of evaporation of water from the filter paper pledgets. On average, pledget weight decreased 19.1 µg/s ($F = 2.47, P = 0.07$). At rest, the handling time was 18.9 ± 2.8 s and fell to 15.0 ± 1.2 and 12.6 ± 0.60 s during HV and the recovery period, respectively.

In vivo studies. The average prechallenge FEV1 was 2.99 ± 0.26 liters (73.2 ± 3.7% of predicted) (Table 1). All participants took inhaled $\beta_2$-agonists on an as-needed basis for control of their asthma. Two also used inhaled steroids, and five were treated with antileukotriene agents.

The mean $P \dot{V}E_{20}$ was 62.2 ± 8.3 l/min, and the temperature of the inspired air was −8.9 ± 3.3°C. The average temperature of the room during the ASF-collection experiments was 24.2 ± 0.4°C with a relative humidity of 29.4 ± 3.0%. Airway anesthesia caused a 3.9 ± 1.3% ($P = 0.02$) drop in the FEV1. After HV, there was a further fall of 23.4 ± 2.4% ($P = 0.0001$; total decrement 26.4 ± 2.5%, $P = 0.0001$; Fig. 3).

There were no significant differences between the quantities of ASF collected between the baseline, HV, and recovery phases by factorial analysis ($F = 1.79, P = 0.18$) for either the evaporation-corrected or uncorrected data (Fig. 4). By paired comparisons, the amounts of fluid gathered during rest and hyperpnea were identical (11.0 ± 2.2 and 10.6 ± 1.9 µl, $P = 0.88$); however, slightly less volume was collected (6.5 ± 1.7 µl) after obstruction developed ($P = 0.054$).
As with the collected ASF liquid, there were no significant differences between the corrected and uncorrected isosmolar values (Fig. 5). The prechallenge osmolality corrected for evaporation was 336 ± 16 mosmol/kgH2O and remained constant throughout the experiment (HV osmolality 339 ± 19, recovery 352 ± 19 mosmol/kgH2O; F = 0.27, P = 0.76).

There were no relationships between HV and the ASF collected at any time (r² = 0.05) or its osmolality (r² = 0.06), nor between ASF volume or tonicity and the fall in FEV₁ (r² = 0.03 and 0.04, respectively).

DISCUSSION

The results of the present study demonstrate that desiccation and hypertonicity of the ASF are not features of hyperpnea in asthma. Individuals with this disease do not have a defect in their ability to condition inspired air (15, 16) and, like normal people (22), can elevate V̇E without producing any measurable alterations in ASF physiology even when the evaporation of mucosal water is deliberately exaggerated with a dry inspirate as herein (10, 27, 29). Both the collectable volume and ionic activity of the ASF were within reported ranges during tidal breathing at the start of the study (18, 21, 22, 26, 33, 34) and remained constant despite ventilation increasing approximately sixfold. Nonetheless, symptomatic attacks of airflow limitation developed. Thus mucosal dehydration and hyperosmolality do not appear to be the mechanisms underlying thermally induced asthma.

These are the first measurements that directly relate ASF physiology to bronchial narrowing in humans, and they authenticate and extend earlier studies that implied a lack of association when using indirect assessments (16, 27). Gilbert and colleagues (16) mapped the temperatures in the lungs during the conditioning of inspired air in normal and asthmatic subjects and suggested that surface tonicity was unlikely to increase during hyperpnea. Their calculations indicate that the distributed nature of the thermal transfers would cause isosmolality to rise only 3–6% in the central airways even under the most severe ventilatory stress. In the present investigation, HV promoted an increase of <1% (Fig. 5). In addition, the quintessential tenet of the desiccation hypothesis cannot be met experimentally. If this theory were operational, the severity of obstruction should closely parallel the movement of water in the bronchi, yet it does not (27). When the consequences of progressive hyperpnea of dry inspirates at different temperatures were compared, identical degrees of evaporation of intrathoracic water did not yield similar degrees of bronchoconstriction (27). In fact, the greatest intrathoracic losses were actually associated with the smallest obstructive responses and not the largest. Consequently, other factors must be at work. These findings in combination with others in the literature demonstrate that even though evaporation is the major source of airway cooling and as such correlates with the severity of obstruction (4, 10, 16, 17), it serves only as a means of initiating the reaction. It seems that the dynamics of water movement is the critical factor and that the absolute losses are insufficient to materially alter periciliary liquid homeostasis.

None of the studies that profess a role for airway desiccation and thermally induced asthma has provided data directly linking the phenomena (1–3). To our knowledge, there are only two investigations that have actually examined the events at the airway surface during hyperpnea, and the applicability of the results to the pathogenesis of thermally induced asthma has been uncertain (13, 38). In one paper, mucosal tonicity rose in the noses of cold-sensitive subjects when they inhaled frigid air through their nostrils and exhaled out the mouth (38). In another, hypertonicity and a decrease in ASF volume developed in the segmental airways of dogs when dry air was forced over them through a bronchoscope (13). However, it has never been established whether the findings were representative of a normal physiological sequence or whether they were epiphenomena induced by the ventilatory paradigms employed. The present work resolves this issue. It is now clear that hyperpnea in asthmatic patients, as in normal individuals (22), has no measurable consequences on ASF homeostasis when inspiration and expiration proceed physiologically (Figs. 4 and 5). Rather, it was the unidirectional airflow and the associated ablation of water recovery that artifically caused drying to develop. When water-replenishment mechanisms are excessive, the lumens of the nares and lower airways narrow because the vessels respond to unregulated losses with hyperemia and edema formation to prevent thermal damage (7, 8, 31, 37). This appears to be a common defense mechanism and is seen in all vessels in the body that are exposed to the environment (35). Preliminary in vivo recordings of vascular surface events in the airways during HV suggest that a similar phenomenon occurs in asthmatic patients at far less thermal stress, perhaps because of pathology in the bronchial microcirculation (23, 24, 40).
Why is ASF desiccation not a feature of respiration in humans? The geometry of the respiratory tract in asthmatic and normal people and the countercurrent mechanism for water recovery effectively minimize losses. In fact, only 10–15% of the total amount of water evaporated from the entire tracheobronchial tree during the conditioning of inspired air derives from the central bronchi (16). Equally importantly, because the epithelium is permeable to water and cannot maintain an osmotic gradient (25), any transient increase in surface tonicity would pull water to the surface until the hyperosmolar state was eliminated. Moreover, fluid can also move easily through the paracellular pathways or be actively secreted from mucosal glands when needed (5). In aggregate, these passive and active recovery/replacement mechanisms are quite robust, and extraordinary circumstances are required to overcome them. For example, the entire upper airway must be bypassed for hours during tidal breathing through an endotracheal tube before the periciliary liquid in the trachea is reduced even when completely dry gases are inhaled (7, 8), and ≥45 min of hyperventilation are required to desiccate the nose under similar circumstances (31).

Could the airways themselves have become dehydrated and have missed it because it was not reflected in the ASF? We believe this to be improbable because of the intimate linkage of the vascular, epithelial, and secretory elements of the tracheobronchial tree in regulating water fluxes. Although it has been suggested that the mucosa and submucosal could dry out during hyperpnea (1), there are no recognized physiological mechanisms by which this can occur. However, even if there were, there is no readily apparent way for periciliary water and ion kinetics to remain unaffected. The bronchial circulation is the main source of water for the tracheobronchial tree, and blood flow is proportionally linked to \( V_e \), increasing and decreasing as thermal needs change (17, 21, 22, 37). Furthermore, asthmatic patients have a hypertrophic and hyperplastic microvasculature that augments the supply of heat and liquid to their airways (16, 40). Hence, there is always a large reservoir of fluid that can maintain epithelial and surface hydration (5, 25). Even if this were in deficit and the epithelium were desiccated, and even if it were possible to secrete an isosmotic or even hypoosmotic ASF in such conditions, the fluid at the air-mucosal interface could not remain in that physical state for long. The osmotic gradient that now existed across the epithelium would immediately cause water to flow into the cells from the lumen, thereby reducing the surface volume and increasing ionic concentration. These events were not observed.

Could there have been dehydration going on downstream from the trachea that we missed? This, too, seems unlikely. The region of the fifth tracheal ring is where the greatest thermal fluxes develop in the intrathoracic airways during hyperpnea, so it is where the maximum alterations in fluid volume and osmolality would be expected (14–16, 22, 29, 30). The magnitude of the exchanges distal to this point become progressively smaller because the temperature of the inspired air continuously rises, causing the gradients for water movement to continually decrease (15, 16, 29, 30). In an experiment with ventilations and inspired conditions such as ours, the air leaving the anterior segmental bronchi would have already received 85–90% of the water it was ultimately going to have (15, 16, 29, 30). This means that between this location and the alveoli, −7 \( \mu \)l of water per liter air or less would have been vaporized over a surface area of 1,000 \( \text{cm}^2 \) (39) containing potentially 100 ml or more of ASF (16). Consequently, even if the fluid evaporated in the lung periphery was not recovered or replaced, the loss per unit area would have been negligible. Hence, the fact that dehydration and hypertonicity did not develop upstream makes it virtually impossible for there to have been any greater changes distally that would have negatively impacted our conclusions.

The techniques we used to collect and analyze ASF have been developed by others (20, 33) and were verified in normal subjects in our laboratory (22). The potential pitfalls and sources of error were also identified and evaluated (22). We appreciate that our values for periciliary osmolality are slightly larger than those in serum, but they fit within the ranges found by previous investigators using a variety of techniques (18, 20, 21, 25, 32, 33). Knowles and associates (20) confirmed that the filter paper method accurately measures the ionic concentration of secretions on airway surfaces; yet, for reasons that are not clear, even in artificial airways, osmolalities are consistently higher than those simultaneously recorded by pipette (13). In our case, it is possible that the filter paper pledges drew macromolecules into the fluid from the subepithelium (12). An alternative thought is that inflammatory material was in the fluid from our subjects because of their underlying asthma. This too is uncertain, however, because the quantity and osmolality of the periciliary liquid did not differ from that seen in normal subjects or in patients with other forms of bronchial inflammation such as chronic bronchitis or cystic fibrosis (21, 22). Irrespective of the reason, the important point is that periciliary dynamics remained stable during hyperventilation whereas the \( \text{FEV}_1 \) fell.

It has been assumed that osmotic mast cell activation is a critical element in thermally induced asthma (2, 3, 11, 36), but our data imply that this too is unlikely to be the case. Although mediators can be released with osmolar stimuli, it requires levels greater than those found here to initiate the process in vitro (11, 36). For example, osmolalities approaching 600 mosmol/kgH\(_2\)O were required before histamine release from isolated basophiles, and mast cells significantly increased over baseline. Such values simply do not develop in vivo anywhere in the respiratory tract with normal ventilatory patterns. For example, even the total prevention of water recovery in the nose in the experiments above causes surface osmolality to rise only 25% (38).

Our subjects had typical and well-documented features of exercise-induced asthma, and their medica-
tion requirements were standard. Because there are no differences between the temperature and humidity profiles that develop in the lungs with exercise and voluntary hyperventilation when the appropriate variables are matched (15, 30), and because HV is less wearing on the subjects than physical exercise and voluntary hyperventilation when the appropriate variables are matched (15, 30), this was the stimulus employed (14–16, 22, 29, 30).

In summary, our data reveal that hyperpnea of sufficient intensity to produce bronchial obstruction is not associated with any abnormalities in the physiology of the ASF in asthmatic subjects. They also demonstrate that airway desiccation and hypertonicity do not seem to be part of the pathogenesis of thermally induced asthma.

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