A comparison between the airway response to isocapnic hyperventilation and hypertonic saline in subjects with asthma

C.M. Smith, S.D. Anderson

ABSTRACT: We compared the response to isocapnic hyperventilation (ISH), where both cooling and drying of the mucosa occur, with the response to inhaling aerosols of hypertonic saline (HS), where airway osmolarity increases without airway cooling. We studied nine subjects on two days. For ISH, subjects ventilated at 70% of their estimated maximum voluntary ventilation (MVV). For HS, they inhaled aerosols of 2.7, 3.6, or 4.5% saline. The concentration that was used depended on the rate of ventilation during ISH. For both challenges the stimulus was given for one minute. Forced expiratory volume in one second (FEV1) was measured once between each minute, and the challenge ceased when the FEV1 did not change for two successive minutes. A plateau in FEV1 occurred after 8.1±2.4 (mean±1 sd) min of ISH, and 8.3±2.4 min of HS. The lowest FEV1 (% predicted) after ISH was 45±16% and after HS was 51±18% (r=0.93). However, the maximum responses occurred after the final challenge and were not the same as the plateau. For HS, the plateau represented 89±11% of the maximum response which developed within one minute of the final challenge. For ISH, the plateau was only 56±26% of the maximum response, which developed within 5.2±2.9 min after challenge. The similarities in the response to these challenges are consistent with the hypothesis that ISH induces asthma via hyperosmolarity. The delayed response to ISH suggests that cooling may delay the response to hyperosmolarity.


The severity of the asthmatic response which follows exercise and isocapnic hyperventilation is closely correlated with the amount of water that is lost from the respiratory tract during challenge [1, 2]. Evaporative water loss from the respiratory tract is associated with both cooling and drying of the respiratory mucosa. We have previously postulated that the respiratory water loss associated with hyperpnoea may result in the periciliary fluid becoming hyperosmolar, and that this may be the event that initiates an attack of asthma following exercise or isocapnic hyperventilation [3, 4]. The fact that inhaled hyperosmolar aerosols are potent agents for provoking an attack of asthma in vivo supports this hypothesis [4-8].

It has also been suggested that the rapid rewarming of airways that have been cooled during hyperpnoea may be the event that initiates the response to hyperpnoea [9]. The mechanisms postulated include vascular engorgement and oedema associated with reactive hyperaemia, or perhaps thermally-induced changes in smooth muscle contractility. This hypothesis is also attractive, because it is well known that the response to both exercise and isocapnic hyperventilation is delayed and does not fully develop until after the challenge has ended.

In a recent study with children [10] it was reported that cold air hyperventilation challenge, when performed in a series of one-minute periods resulted in a plateau in the airway response after ten one-minute periods had been completed. The advantage of using this technique for challenge is that it provides a sufficient number of points to examine the time-course of the response to isocapnic hyperventilation.

The aim of this study was to use this technique both with isocapnic hyperventilation and with hyperosmolar saline in a group of asthmatic adults. During hyperventilation both cooling and hyperosmolarity of the airways can be expected to occur. During challenge with hyperosmolar saline, subjects breathe at tidal volume, so that the increase in osmolarity should occur without airway cooling. An examination of the similarities and differences between the two stimulus-response curves may give some insight into the mechanisms by which the cooling and drying associated with evaporative water loss provoke an attack of asthma.
Subjects

Nine subjects (three females and six males) aged 18–34 yrs were recruited from the Respiratory Investigation Unit of the Royal Prince Alfred Hospital. All subjects had a clinical diagnosis of asthma and were known to have an abnormal response to hypertonic saline aerosols, isocapnic hyperventilation or exercise. With the exception of two subjects (nos 5 and 7) all aerosol medications were withheld for at least 6 h. Although the two remaining subjects took their aerosol medications 4–6 h before challenge, the time between medication and the two challenges was the same. No subjects required oral corticosteroids or theophylline for control of symptoms at the time of the study. Anthropometric details and current medication regimens are given in Table 1.

The protocol was approved by the Ethical Review Committee of the Royal Prince Alfred Hospital, and all subjects gave their consent to participate in the study after a full explanation of the protocol and the aims of the study had been given.

Methods

The subjects attended the laboratory on two occasions. On the first day they were challenged with isocapnic hyperventilation and on the second day with hyperosmolar saline aerosol. Eight of the subjects completed the tests within a period of two weeks. The remaining subject (no. 6) completed the tests within six weeks.

Challenge with isocapnic hyperventilation

The breathing circuit used was similar to that described by Phillips et al. [11]. Dry compressed gas was delivered by a demand valve to a target balloon. The demand valve could be set to deliver gas at 30–150 l/min. A calibrated rotameter was placed in the circuit between the target balloon and the subject to monitor the rate of flow. The subjects breathed through a low resistance two-way valve (Hans-Rudolph No. 2700, Kansas City Mo. USA), and were instructed to keep the target balloon filled at a constant volume. The rate of ventilation that was achieved was measured by passing the gas to a 350 l chain compensated gasometer (Warren E. Collins, Braintree Mass., USA) and recording the signals generated on a recorder (Devices M19, Herts, UK). The inspired gas contained a mixture of 5% CO₂, 21% O₂, and the balance N₂. This concentration of CO₂ produces near-normal end-tidal CO₂ at ventilation rates of 30–105 l/min¹ [11].

The protocol used was similar to that described by Zach and Polgar [10]. The subjects performed a series of one-minute challenges. During each challenge period the subjects were instructed to ventilate at 70% of their estimated maximum voluntary ventilation (MVV). The forced expiratory in one second (FEV₁) was measured two or three times before the challenge commenced and once only between each minute of challenge. The challenge ended when there was no further decrease in FEV₁ for at least two successive one-minute challenge periods, or until the FEV₁ had decreased by 60% of the initial value. In practice, a decrease in FEV₁ of less than 100 ml for at least two successive one-minute challenge periods after at least 5 min of challenge was accepted as the point at which to end the challenge. The MVV was estimated by multiplying the value for FEV₁ measured before challenge by 37.5.

Challenge with hypertonic saline

A Mistogen 143A nebulizer (Mistogen Equipment Co., Oakland, Ca, USA) was used to generate a dense aerosol of hyperosmolar saline, which was delivered to the subject via flexible tubing to a large two-way valve (Hans-Rudolph No. 2700). As for isocapnic hyperven-

<table>
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<th>Sex</th>
<th>Age (yrs)</th>
<th>Height (cm)</th>
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<th>Baseline FEV₁ (l)</th>
<th>ISH Concentration %w/v</th>
<th>Saline Concentration %w/v</th>
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<td>F</td>
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<td>S, B, SCG</td>
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S: salbutamol; B: beclomethasone dipropionate; SCG: sodium cromoglycate; ISH: isocapnic hyperventilation; FEV₁: forced expiratory volume in one second; *: as required.
tilation, the subjects were challenged in a series of one-minute periods.

This technique has not been described before, and it was not known whether there would also be a plateau in the response to hyperosmolar saline. It was decided that the challenges would end when there was no further decrease in FEV₁ for at least two one-minute challenge periods, or until the FEV₁ had decreased by 60%.

The concentration of saline used was determined by the rate of ventilation generated during isocapnic hyperventilation. If the airways become dehydrated during hyperventilation then the degree of hyperosmolality that develops will depend on the rate of ventilation. Thus, if the subject had a low predicted rate of ventilation (less than 70 l·min⁻¹) then they were challenged with a relatively low concentration of saline (2.7%). If the predicted rate of ventilation was 70–110 l·min⁻¹, 3.6% saline was used, and if it was greater than 110 l·min⁻¹ then 4.5% saline was used. The concentration of aerosol used for each subject is given in table 1.

Spirometry

The FEV₁ was measured (Minato AS-500, Osaka, Japan) before challenge at least twice, and where the values differed by more than 200 ml a third measurement was taken. The highest value recorded on each of these occasions was used in subsequent calculations of changes in FEV₁.

Between each one-minute challenge period the FEV₁ was measured only once. The time required to perform this manoeuvre was usually 10–20 s. After the last challenge period the FEV₁ was measured, as described, at 1, 3, 5 and 10 min, and then at two-minute intervals until the lowest value had been recorded.

Analysis of results

All values for FEV₁ were expressed in terms of percentage of predicted values taken from the tables of Goldman and Becklake [12]. The values are reported as the mean FEV₁ (%predicted) ±1 standard deviation. Paired t-tests were used to compare the baseline FEV₁, the minimum FEV₁ recorded after each challenge, the duration of each challenge, and the time taken to reach the maximum response after challenge. Pearson’s correlation coefficient was used to compare the relationship between the lowest value for FEV₁ provoked by each challenge.

To compare the time-course of the two challenges, the percentage of the maximum response after 25, 50, 75 and 100% of challenge had been completed was calculated as follows:

\[
\text{Percentage} = \left( \frac{\text{Initial FEV}_1 - \text{FEV}_1 \text{ recorded after } (x) \% \text{ of challenge time}}{\text{Initial FEV}_1 - \text{Lowest FEV}_1 \text{ recorded}} \right) \times 100
\]
Fig. 2. – Individual curves showing the forced expiratory volume in one second (FEV₁)(% predicted) after each minute during challenge with hypertonic saline (solid lines), and at various time intervals after challenge (dashed lines).

The challenge was divided this way because the duration of challenge was not the same for all subjects.

Results

The values for FEV₁ before challenge were similar on the two days. The mean FEV₁ (% predicted) before isocapnic hyperventilation was 85±22%, and before hypertonic saline was 84±19% (table 1).

Individual curves relating the FEV₁ (% predicted), to time both during and after challenge were drawn for isocapnic hyperventilation and hypertonic saline (figs 1 and 2).

For isocapnic hyperventilation eight of the nine subjects demonstrated a plateau in their response to repeated one-minute challenges (fig. 1). For subject no. 3 the curve prior to the last two points was linear, and without a third point to mark the plateau it is not clear in this subject whether a true plateau in the response had been reached. Only one subject (no. 1) showed no tendency to reach a plateau in the response to isocapnic hyperventilation. The curve remained linear and steep even after a 50% reduction in lung function.

Although there was a plateau in the response to isocapnic hyperventilation in most subjects, the mean (±1 sd) change in FEV₁ that occurred during challenge represented only 56±26% of the maximum reduction in FEV₁ that was ultimately recorded. After the completion of the challenge the FEV₁ continued to fall rapidly within the first 3 min and reached its lowest value 5–15 min following challenge. The mean (±1 sd) time taken to reach the lowest FEV₁ after challenge was 5.2±2.9 min.

For hypertonic saline seven of the subjects demonstrated a plateau in their response to repeated one-minute challenges (fig. 2). Again, for one subject (no. 1) the curve prior to the last two points was linear, and without a third point to mark the plateau it is not clear whether a true plateau had been reached. Two subjects (nos 7 and 9) showed no tendency to plateau in response to hyperosmolar saline before challenges were ceased due to poor lung function.

The maximum response to hyperosmolar saline had developed or was almost fully developed by the end of the challenge. The plateau for hypertonic saline was 89±11% of the maximum response. In two subjects (nos 3 and 7) the maximum response was observed within 20 s after the final challenge period. In the remaining seven subjects, the response had fully developed within the first minute after the final challenge period. The mean (±1 sd) time taken to reach the lowest FEV₁ after challenge was 0.85±0.3 min.

There was no significant difference between the two
challenges in the time taken to reach the plateau. The mean (±1sd) duration of the isocapnic hyperventilation challenge was 8.1 (±1.4) minutes and for hypertonic saline was 8.3 (±2.4) minutes. Although there was a highly significant difference in the time between end-challenge and the time the maximum response was recorded (p<0.001), the maximum response, once developed, was remarkably similar. The mean value for the lowest FEV1, following isocapnic hyperventilation was 45% (±16%) of predicted, and following hypertonic saline was 51% (±18% of predicted, and these values were highly correlated (r=0.93, p<0.001). Although these values were similar, the FEV1 was slightly lower following challenge with isocapnic hyperventilation compared with that measured following challenge with hyperosmolar saline in 8 of the 9 subjects studied, and the values were significantly different by paired t test (p<0.05).

The difference between the time-course of the responses relative to the maximum response to these two challenges was apparent from the first minutes of challenge. Figure 3 shows the percentage of the maximum response that had developed after 25, 50, 75 and 100% of the challenge had been completed. The response to hypertonic saline was rapid and apparent within the first minutes of challenge. By the time 50% of the challenge had been completed, 62±13% of the maximum response had developed. The response to isocapnic hyperventilation in the first half of the challenge was quite different to that for hypertonic saline. Of the nine subjects, six demonstrated bronchodilatation either in the first or second minute of challenge. Those subjects who were most obstructed at rest experienced the greatest degree of bronchodilatation during the first minutes of isocapnic hyperventilation (r=-0.79, p<0.01). The most obstructed subject (no. 7) actually maintained bronchodilatation throughout the challenge, and the entire response developed in the 5 min after the last challenge period. For the group, by the time 50% of the challenge had been completed only 29±23% of the maximum response had developed. This was less than half that of the response to hypertonic saline at the same point (fig. 3). This difference was maintained throughout the challenge, and it was not until 5–10 min after challenge with isocapnic hyperventilation that the response could be seen to be similar.

**Discussion**

Both isocapnic hyperventilation and hyperosmolar saline challenge, when performed in a series of one-minute periods resulted in a plateau to the response in the majority of subjects. Our findings confirm the findings of ZACH and POLGAR [10] that hyperventilation challenge with dry air, when performed in a series of one-minute periods, results in a plateau in the airway response after 8–10 min. ASSOURI et al. [13] have also shown, using an uninterrupted challenge, that the maximum response to isocapnic hyperventilation occurs when a challenge is performed for 5–7 min, and when the rate of ventilation is 60% of the predicted normal MVV.

The plateau in response to these challenges is quite different to that described for challenge with agents such as histamine and methacholine. In subjects with asthma, with the exception of subjects with mild asthma, the response to histamine and methacholine does not reach a plateau, even when the challenge has progressed to the point where there has been a 60% fall in FEV1. [14]. In the present study, only one subject had symptoms of mild asthma, and did not require regular
therapy with inhaled beta-sympathomimetic agents for control of asthma symptoms. The remaining subjects had symptoms of moderate and moderately severe asthma, and would almost certainly not have reached a plateau in their response to histamine and methacholine.

One explanation for the plateau that is observed with these challenges is that hyperpnoea and hyperosmolar saline may both provide an "osmotic" challenge to the airway. The observation that a maximum response can be recorded may relate to the decreasing potential for water loss or aerosol deposition to increase osmolarity in the more peripheral regions of the tracheobronchial tree. Figure 4 shows the estimated cumulative volume of water available at each airway generation [15]. These figures are based on the surface area of the airways, calculated from the data of WemEL [16], and on the assumption that the depth of the periciliary fluid is 10 μm, uniform throughout the tracheobronchial tree [17]. The total volume of water available increases exponentially as the surface area increases with each successive branching of the tracheobronchial tree. During ventilation, inspired air becomes progressively humidified as it passes through the airways to the alveoli. Thus, the requirement for water decreases towards the periphery, and the availability of water increases. The potential for evaporative water loss to increase the osmolarity of the periciliary fluid will, therefore, decrease exponentially in the more peripheral airways. Similarly, the potential for hyperosmolar aerosols to induce changes in the osmolarity of the periciliary fluid will decrease as they are deposited over a larger surface area. Thus, the response plateau observed in these forms of challenge may be due to the fact that the osmotic stimulus is limited to the more proximal of the intrathoracic airways.

**Fig. 4.** - The cumulative volume of water estimated to be available at each airway generation, calculated from the surface area [18], and using a depth of 10 μm for the periciliary fluid [19]. Reproduced from [17], with permission.

In this study the concentration of aerosol varied from 2.7–4.5%, and for each patient was determined by the rate of ventilation during isocapnic hyperventilation. It is likely that the potential for these aerosols to increase osmolarity of the periciliary fluid decreases exponentially in the peripheral airways. Thus, the choice of aerosol may have had little influence on the number of airways that will be affected by an increase in osmolarity, but may have had some influence on the duration of the challenge required to reach a maximum response. Of the seven subjects who reached a plateau, the duration of challenge for the subject inhaling 2.7% was 12 min; the mean duration of challenge for those subjects inhaling 3.6% was 9 min, and for those inhaling 4.5% was 8.3 min. Although the number of subjects studied is small, this trend suggests that the choice of aerosol within this concentration range may not have been important in terms of its effect on the maximum response observed, but may have had some influence on the duration of challenge.

It has recently been suggested that the response to hyperpnoea develops after challenge has ended, because of rapid rewarming of the airways that have been cooled due to evaporative water loss during hyperpnoea [9]. The postulated mechanisms include vascular engorgement and oedema in the submucosa, associated with reactive hyperaemia, or thermally-induced changes in smooth muscle contractility. Thus the event central to this hypothesis is airway cooling.

It is, however, interesting to compare the response to isocapnic hyperventilation and hypertonic saline. Both probably provide a hyperosmolar stimulus to the airway, but isocapnic hyperventilation provides a cooling stimulus as well. Both isocapnic hyperventilation and hypertonic saline induced a similar reduction in FEV₁. However, almost half of the response to isocapnic hyperventilation occurred after the final challenge period, whereas the response to hyperosmolar saline was almost complete at this time. Based on these findings an alternative hypothesis may be proposed: it is possible that airway cooling is actually a stimulus that causes relaxation, and acts to mask the bronchoconstriction provoked by hyperosmolarity. Immediately following a period of hyperpnoea there is rapid rewarming of the airways [18]. The rapid development of the response that typically occurs only after hyperpnoea has ceased may therefore be the "unmasked" response to hyperosmolarity.

That airway cooling can be an inhibitory stimulus has recently been reported by Freed et al. [19]. They described a dog model in which perfusion of the peripheral lung with dry air at high flow rates in anaesthetized dogs produced an increase in resistance to flow through collateral channels. The time-course of the development of the response was similar to that seen in humans after exercise and isocapnic hyperventilation. Using this model they compared the effect of airway cooling caused by high-flow ventilation with dry air in the left lower lobe (LLL), with the effect of airway cooling caused by perfusing the LLL with blood that had been cooled to about 30°C. Both protocols resulted in reduction of the temperature of the mucosa, which was identical in magnitude and time-course. Dry air ventilation, which will cause both cooling and
hyperosmolarity within the airways resulted in an increased resistance to flow through collateral channels. However, cooling of the airways by perfusing with cooled blood, which will cause airway cooling without hyperosmolarity, actually reduced the resistance to airflow.

There is also evidence from studies in human tissue in vitro that cooling of airway smooth muscle is associated with relaxation. BLACK et al. [20] reported that reducing the temperature of the organ bath from 37 to 20°C was associated with a decrease in baseline tension of approximately 47% in all specimens. Furthermore, the contractile response to both histamine and KCl was reduced at 20°C compared with that at 37°C. (It was, however, interesting to note that the contractile response to carbachol was potentiated at 20°C).

The bronchodilatation observed during the first one or two minutes of isocapnic hyperventilation may also be due to an inhibitory effect of airway cooling. Bronchodilatation was most pronounced in those subjects with the most obstruction at rest. This is a phenomenon that is also characteristic of exercise, and has often been attributed to an increase in levels of circulating catecholamines and/or a withdrawal of sympathetic tone [21]. However, these are events associated with exercise, and not with hyperpnoea. During isocapnic hyperventilation there is no significant increase in circulating levels of catecholamines [22], and no indication of withdrawal of sympathetic tone. It is possible that airway cooling results in initial bronchodilatation and that bronchoconstriction does not begin to develop until there has been sufficient respiratory water loss to induce hyperosmolarity within the airways.

In conclusion, challenge with both isocapnic hyperventilation and hyperosmolar saline results in a plateau to the response in the majority of subjects. This is probably explained by the fact that both of these challenges increase the osmolarity of the pericilliary fluid in the more proximal intrathoracic airways, where there is relatively little water available, but are limited in their potential to change the osmolarity of the pericilliary fluid in the more distal airways because of the relatively large amounts of water in this region of the tracheobronchial tree. Both challenges induced similar degree of bronchoconstriction, but the response to isocapnic hyperventilation was delayed until after the last challenge period had been completed. It is suggested that this inhibition may be related to airway cooling associated with evaporative water loss.

References
Comparaison entre la réponse des voies aériennes à l'hyperventilation isocapnique et à la solution saline hypertonique chez les sujets asthmatiques. C.M. Smith, S.D. Anderson.

RÉSUMÉ: Pendant l'hyperventilation, les pertes d'eau évaporée causent un refroidissement et un assèchement de la muqueuse respiratoire. Nous avons comparé la réponse à l'hyperventilation isocapnique (ISH), où se produisent à la fois un refroidissement et un assèchement de la muqueuse, et la réponse à l'inhalation d'aérosols de solution saline hypertonique (HS), où l'osmolarité des voies aériennes augmente sans refroidissement de l'air. Nous avons étudié 9 sujets pendant 2 jours. Pour ISH, les sujets ont ventilé à 70% de leur ventilation maxima/minute estimée. Pour HS, ils ont inhalé des aérosols de solution saline à 2.7, 3.6 ou 4.5%. La concentration utilisée dépendait du taux de ventilation pendant ISH. Pour les deux provocations, le stimulus a été administré pendant des périodes d'une minute. Le VEMS a été mesuré une fois entre chaque minute, et la provocation cessait lorsque le VEMS ne changeait pas lors de deux minutes successives. Les valeurs sont mentionnées comme moyennes avec ±1 ns. Un plateau de VEMS est survenu après 8,1±2,4 minutes d'ISH et 8,3±2,4 minutes de HS. Le VEMS le plus bas (en % des valeurs prédites) après ISH était de 45±16%, et après HS de 51±18% (r=0.93). Toutefois, les réponses maximales se produisent après la provocation terminale et ne sont pas les mêmes que le plateau. Pour HS, le plateau représente 89±11% de la réponse maximale qui se développe dans la minute après la provocation terminale. Pour ISH, le plateau ne représente que 56±26% de la réponse maximale, qui se développe dans les 5.2±2.9 minutes après la provocation. Nous concluons que les similitudes dans la réponse à ces deux provocations sont en accord avec l'hypothèse que ISH provoque l'asthme par hyperosmolarité. La réponse retardée à ISH suggère que le refroidissement pourrait retarder la réponse à l'hyperosmolarité.

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