The effects of osmotic challenge on bronchial responsiveness to methacholine in non-asthmatic subjects

D. Antoniou, G. Pavlakou, P.J. Rees

ABSTRACT: We have measured the airway responses to methacholine on three days in sixteen non-asthmatic subjects. On the first day the methacholine challenge alone was performed. The other two days were randomized between pretreatment with distilled water or hypertonic saline administered by ultrasonic nebulizer. Distilled water and hypertonic saline did not affect baseline specific conductance (sGaw). Provocative dose producing a 35% fall in sGaw (PD_{35}sGaw) was slightly reduced by both distilled water and hypertonic saline (15.83 to 8.55 μmol with distilled water and to 11.80 μmol with hypertonic saline). Six out of 16 subjects reached a plateau of maximal response with methacholine. The level of this plateau was not affected by pretreatment with distilled water or hypertonic saline. These results show that distilled water and 3.6% saline produced small increases in non-specific reactivity in normal subjects and confirm that substantial osmotic challenge does not change airway calibre in non-asthmatic subjects.

Methods

Subjects

Asthmatic subjects develop bronchoconstriction in response to inhalation of ultrasonically nebulized distilled water and hypertonic saline [1]. The inhaled substance may produce its response through a change in osmolarity of the epithelial lining fluid, and it has been postulated that increases in osmolarity may be part of the mechanism producing exercise-induced asthma [2, 3]. These osmotic changes can result in the release of inflammatory mediators [4]. In asthmatic subjects responsiveness to methacholine has been shown to increase after distilled water nebulization [5, 6]. Smith et al. [7] confirmed this increase in methacholine response in asthmatic subjects after ultrasonically nebulized distilled water but did not find a significant change after hypertonic saline. This suggests that the mechanisms of response to hypo- and hyper-osmolar stimuli are not the same. Challenge with cold air inhalation has been shown to produce a small but significant increase in methacholine reactivity in normal [8] and asthmatic [9] subjects.

Non-asthmatic subjects are generally thought not to respond to osmotic challenges. We have compared the effects of ultrasonically nebulized distilled water and hypertonic saline on respiratory function and on subsequent methacholine response in non-asthmatic subjects. Where a plateau of response could be achieved we have looked at changes in maximal airway narrowing and in all subjects the dose of methacholine producing a standard reduction in specific airways conductance has been assessed before and after the osmotic challenge.

Results

Asthmatic subjects developed a significant increase in methacholine response after both distilled water and hypertonic saline. This increase was not observed in non-asthmatic subjects.

Conclusions

Osmotic challenges with distilled water and hypertonic saline did not affect baseline specific conductance (sGaw) in non-asthmatic subjects. Distilled water and 3.6% saline produced small increases in non-specific reactivity in normal subjects and confirm that substantial osmotic challenge does not change airway calibre in non-asthmatic subjects.
delivered through a Hudson nebulizer attached to a breath-actuated dosimeter. The dosimeter was driven by a compressed air source regulated at a pressure of 149 kPa (20 psi), and was actuated for 600 ms with each breath. Subjects inspired slowly from functional residual capacity to near total lung capacity with a 2–3 s breathhold. Five actuations of this system released 13 µl of solution with a mass median diameter of 1.6 µm.

Five inhalations were given, via the dosimeter, first of diluent then of increasing doses of methacholine. Baseline sGaw was taken as the mean of five measurements performed one minute after administration of diluent. Five measurements of sGaw were made one minute after the fifth breath at each methacholine dilution. Methacholine was given in doubling concentrations beginning with 0.2% w/v. Cumulative dose response curves were plotted on semi-log paper and the dose of methacholine producing a 35% drop in sGaw (PD<sub>sGaw</sub>) was calculated by linear interpolation between the two doses around this point. When at least two consecutive sGaw values after the 35% decrease differed by <10% this was regarded as a plateau. Reproducibility of the methacholine challenge in our laboratory gives 95% confidence intervals of less than 1.4 doubling dilutions.

The distilled water and 3.6% saline challenges were performed using a Devilbiss 65 ultrasonic nebulizer (Devilbiss UK, Feltham, Middlesex, UK). This produces a mist with a mass median particle size of 4.7 µm. The output of the nebulizer was inhaled through a 2.5 cm diameter tube, 1.4 m in length, by way of a low resistance two way valve (PK Morgan, Chatham, Kent, UK). The volume of expired air was measured by a Wrights respirometer (Medishield, Harlow, Essex, UK). After measurement of baseline lung function the osmotic challenge was given in six aliquots. Nebulizer output for these aliquots was 10, 20, 40, and then three 80 l volumes. Each 10 l delivers 1.6 g (mean, ± 0.06 g) of hypertonic saline or water to the subject at the mouthpiece. Measurements of sGaw were made 90 s after each aliquot of fog.

The sGaw measurements were made in a constant volume body plethysmograph using an automated system developed in our laboratory [10].

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After logarithmic transformation of the PD<sub>sGaw</sub> values the change after osmotic challenge was not related to the size of the measurement [11]. The mean difference in logPD<sub>sGaw</sub> was calculated and transformed back as an antilog giving a difference in geometric means with 95% confidence intervals.

### Results

There were no significant changes in baseline levels of sGaw after administration of ultrasonically nebulized distilled water or hypertonic saline (table 1). All subjects achieved a drop in sGaw of >35% with methacholine. Methacholine PD<sub>sGaw</sub> was significantly decreased after hypertonic saline and after distilled water. The geometric mean of the difference in PD<sub>sGaw</sub> after

<table>
<thead>
<tr>
<th>Subject</th>
<th>Methacholine only</th>
<th>After distilled water</th>
<th>After hypertonic saline</th>
</tr>
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<tbody>
<tr>
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<td>3.19</td>
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<td>2.30</td>
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<td>1.73</td>
<td>2.30</td>
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</tr>
<tr>
<td>15</td>
<td>2.38</td>
<td>2.25</td>
<td>2.14</td>
</tr>
</tbody>
</table>

Mean 2.47 2.22 2.19

sd 0.45 0.53 0.50

NS NS

Subjects 1–5 were atopic (positive skin tests to at least one common allergen). sGaw: specific airway conductance.

### Study protocol

All subjects gave informed consent and the study was approved by the Guy's Hospital Ethics Committee. Each subject visited the laboratory at the same time of day on three separate days. Each study was separated by at least 48 h and on study days 2 and 3 the double osmotic challenge was given in six aliquots. Subjects relaxed in the laboratory for 20 min before challenge. The maximum time between challenges was 30 days.

Day 1. Response to increasing doses of methacholine was measured. The challenge was terminated when the specific conductance (sGaw) response reached a plateau or when the subject felt unable to tolerate any further doses.

Days 2 and 3. On each day subjects were given either ultrasonically nebulized distilled water or 3.6% saline solution. Airway responses were measured and then a methacholine response was performed 20 min later in a similar fashion to day 1.

### Challenges

Serial dilutions of methacholine in normal saline were made for the methacholine challenge. This was delivered through a Hudson nebulizer attached to a breath-actuated dosimeter. The dosimeter was driven by...
Table 2. - Methacholine PD₃₅sGaw values µmol

<table>
<thead>
<tr>
<th>Subject</th>
<th>Methacholine only</th>
<th>After distilled water</th>
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<td>0.94</td>
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<td>1.45</td>
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<td>1.70</td>
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<tr>
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<tr>
<td>15</td>
<td>3.10</td>
<td>6.65</td>
<td>2.35</td>
</tr>
</tbody>
</table>

Mean 15.83  8.55  11.80

Table 3. - sGaw values at plateau level in six subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>Methacholine only</th>
<th>After distilled water</th>
<th>After hypertonic saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1.00</td>
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<td>0.79</td>
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<tr>
<td>6</td>
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<td>1.11</td>
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<tr>
<td>11</td>
<td>0.83</td>
<td>0.87</td>
<td>0.66</td>
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<tr>
<td>15</td>
<td>0.81</td>
<td>1.00</td>
<td>0.71</td>
</tr>
<tr>
<td>16</td>
<td>0.65</td>
<td>-</td>
<td>0.52</td>
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</tbody>
</table>

Mean (4-15) 0.88  0.83  0.81

Plateaus were only achieved in 6 of the 16 subjects (fig. 1). In five subjects plateaus were achieved with methacholine after each osmotic challenge, the sixth subject had a plateau with methacholine after hypertonic saline but did not perform the distilled water challenge. The level of the plateau did not change significantly after either distilled water or hypertonic saline (table 3). There was no significant change in thoracic gas volume at the plateau. In four subjects flow rate at 25% vital capacity was also measured from partial flow volume loops and gave similar plateaus.

Discussion

Airway responses to methacholine and histamine are less marked in non-asthmatic than in asthmatic subjects in terms of sensitivity and maximal response. This general hyperresponsiveness of asthmatics is reflected in lower levels of such indices of response as PD₃₅sGaw. Asthmatics also show airway narrowing in response to osmotic challenge with hypotonic or hypertonic solutions. This is thought to occur through direct osmotic changes in the mucosa or lining fluid changing wall thickness together with osmotically stimulated local mediator release. Non-asthmatic subjects in this study did not show airway narrowing in response to substantial osmotic challenges confirming the usefulness of such osmotic challenges in separating asthmatic from non-asthmatic subjects and showing the difference from direct challenge with histamine and methacholine.

Non-asthmatic subjects responded to histamine and methacholine at higher doses but some appear to reach a plateau of maximal airway narrowing which is not exceeded by increasing the dose of the challenging agent [12-14]. The plateau is similar for histamine and methacholine and the maximal response is not changed by the combination of the two stimuli in their maximum doses [15]. In asthmatics the plateau is lower or cannot be reached [12]. We achieved a plateau in only 6 out of 16 non-asthmatics perhaps because the subjects were given the opportunity to stop the challenge if they felt uncomfortable.
There is a disparity between airway muscle responses in vivo and in vitro, isolated airway smooth muscle from non-asthmatic subjects shows uninhibited contraction in vitro. The limitation of the action of non-asthmatic smooth muscle in vivo might be caused by mechanical coupling in the wall of the airways restraining the shortening of the muscle through its relation to cartilage or other structures in the wall. Structural changes in the airway wall would allow a greater response in asthmatic airways leading to release of the bronchial smooth muscle from its local mechanical inhibition.

Smith et al. [7] showed that responsiveness to methacholine in asthmatics was increased by distilled water and not by hypertonic saline but their results suggested a similar trend with both agents. In the present study of non-asthmatic subjects we found significant changes in methacholine PD₅₀sGaw after both osmotic challenges but the overall changes were not large. In those who achieved a plateau as defined in this study, this plateau was not significantly altered by prior osmotic challenge, although the number of subjects was small. Hypertonic and hypotonic aerosols would not be expected to release mediators from primed cells in the airway walls of non-asthmatics. The changes seen might be produced by fluid shifts in the airway walls although the lack of significant change in the plateau suggested that any mechanical linkage, which has been proposed in the wall of the airway [16], was not disturbed.

Specific conductance is not independent of volume but at the plateau of maximum response significant changes in thoracic gas volume changes did not occur. In four subjects flow at 25% basal lung volume was also measured and showed similar plateaus with hypotonic or hypertonic aerosols has no direct effect on airway size in non-asthmatics. This confirms the specificity of such tests in the diagnosis of asthma. Although maximal airway narrowing is not affected, the osmotic challenges do have a small secondary effect on some aspects of methacholine responses.

References


RESUME: Nous avons mesure les reponses des voies aerienne a la methacholine chez seize sujets non astmatiques. Le premier jour, l'on a pratiquer un test de provocacion isol a la methacholine. Pendant les deux autres jours, utilise un nebuliseur ultrasone et administre en pretreatment, de maniere randomisee, soit l'eau distillee et la solution saline hypertone. L'eau distillee et la solution saline hypertone n'ont pas modifie la conductance specifique de base (sGaw). PD₅₀sGaw et ses valeurs legemente diminu es, a la fois par l'eau distillee et la solution saline hypertone (de 15,83 a 8,55 j. mol avec l'eau distillee, et 11,80 j. mol avec la solution saline hypertone). Six de ces
16 sujets ont atteint un plateau de réponse maximale à la methacholine. Le niveau de ce plateau n'a pas été affecté par le traitement préalable à l'eau distillée ou à la solution saline hypertonique. Ces résultats démontrent que l'eau distillée et la solution saline à 3.6% provoquent de petites augmentations de la réactivité non spécifique chez les sujets normaux, et confirment qu'une provocation osmotique substantielle ne modifie pas le calibre des voies aériennes chez les sujets non asthmatiques.