Effects of pH and osmolarity on aerosol-induced cough in normal volunteers

R. H. LOWRY, A. M. WOOD AND T. W. HIGENBOTTAM
Department of Respiratory Physiology, Addenbrooke's Hospital, Cambridge, U.K.

(Received 13 May/24 August 1987; accepted 14 September 1987)

SUMMARY
1. The chemosensitivity of cough receptors stimulated by inhalation of aqueous aerosols was evaluated in 21 normal volunteers in three experiments.
2. The pH of isotonic saline was altered using small amounts of phosphate or glycine buffers to produce solutions with a pH range of 2.6–10.0. These solutions were nebulized ultrasonically and breathed for 1 min periods by seven subjects in random order and on separate days. Cough frequency during each 1 min inhalation was recorded. Only the two solutions of extreme pH (2.6 and 10.0) caused cough.
3. The effect of altering the osmolarity of the inhaled aerosol on cough was assessed using D-glucose over a range of 77–1232 mosmol/l. Saline solutions over the same range of osmolarity were also tested. The pH of D-glucose was raised to match that of saline by adding small amounts of sodium hydroxide. All solutions were nebulized and inhaled by seven subjects as described above for 1 min periods during which cough frequency was recorded. Forced expired volume in 1 s was recorded after each inhalation and did not alter in any subject by more than 10%. Subjects coughed when inhaling all the D-glucose solutions over the whole range of osmolarity. Cough occurred with saline solutions only at low chloride concentration and at the highest concentration.
4. In order to clarify whether the response to hypertonic saline was due to the high ionic content of the solutions or to its hypertonicity, two other solutions were tested. These were an isotonic and a hypertonic mixture of D-glucose and saline, containing 'normal' (150 mmol/l) ionic content. Cough occurred with the hypertonic solution but not with the isotonic solution, suggesting that hypertonicity does stimulate cough.
5. The mechanism of cough induction by citric acid was studied. An aerosol of 0.68% citric acid in saline was compared with sodium citrate, both with and without chloride, with D-glucose and with water in seven subjects. Cough occurred in response to all aerosols except sodium citrate in saline solvent. Additive effects of low pH and lack of chloride, and not the citrate ion, are responsible for the irritant properties of citric acid.
6. The chemosensitivity of the cough reflex induced by inhaled aerosols mirrors that already described in vivo for laryngeal rapidly adapting receptors.

Key words: chemoreceptor, cough, osmolarity, pH.

Abbreviations: FEV₁, forced expiratory volume in 1 s; RAR, rapidly adapting receptor.

INTRODUCTION
The effective treatment of cough is hindered by insufficient knowledge of the underlying mechanisms involved in the reflex. Cough can be elicited by inhaling ultrasonically nebulized aqueous solutions of saline with a chloride concentration of less than 80 mmol/l [1]. This chloride dependency of certain respiratory reflexes has been observed by Boggs & Bartlett [2], who investigated apnoea in neonatal puppies. In addition to the chloride effect, they observed that saline solutions of pH < 4.5 and > 8.7 induced apnoea when installed into the larynx, while solutions of neutral pH lost their ability to induce apnoea when chloride was added. Osmolarity of the solutions appeared to be unimportant. In animal studies of isolated nerves, all solutions capable of inducing apnoea were found to stimulate laryngeal rapidly adapting receptors (RARs). The stimulating effect of water on RARs has been observed by other workers [3].

We have now studied the effects of altering the pH and osmolarity of inhaled aerosols on cough. Citric acid aerosols are frequently used to evoke cough. It has been proposed that the low pH of citric acid and the fact that it is often used in hypertonic solution may be responsible for initiating cough [4]. Citrate ions can also chelate...
calcium ions. This may be a mechanism of experimentally inducing citric acid bronchoconstriction [5], but in studies of isolated nerves, calcium chelation, and presumed reduction of concentration of calcium ions, does not appear to stimulate RARs of the larynx [2].

METHODS

Subjects

A total of 21 normal volunteers with no history of asthma or rhinitis were recruited from hospital staff (age range 25-39 years; seven were males and two were smokers). All had normal spirometric measurements. The volunteers were divided into three groups of seven to investigate the effects of altering osmolarity, pH and citrate concentration of inhaled aerosol on the cough response.

Cough challenge

The procedure has been described previously [1]. Briefly, aerosolized solutions were breathed tidally from an ultrasonic nebulizer run at maximal power setting (DeVilbiss 65; particulate aerosol liquid output 1.8 ml/min, mass median aerodynamic diameter 6-8 μm) for 1 min periods through a low resistance two-way valve. Expiratory flow and volume were recorded via a modified pneumotachograph connected to a two channel recorder (Brush 220, Gould, Coventry). Cough frequency was recorded from each 1 min inhalation.

Seven subjects inhaled the aerosols on separate days at the same time of day, for 1 min periods, in random order and single blind fashion.

Experiment 1

The effect of altered pH of inhaled aerosol on cough was studied using isotonic saline, the pH being manipulated by adding small amounts of phosphate buffers to achieve solutions with pH values of 4.8 and 8.0. To achieve a solution of pH 2.6, 2.1 ml of concentrated hydrochloric acid and 5.9 g of glycerine were made up to 1.5 litres with saline (154 mmol/l NaCl). A pH of 10.0 was achieved by adding 2.2 g of sodium hydroxide and 7.1 g of glycerine to 1.5 litres of saline.

Experiment 2a

In order to clarify the importance of osmolarity of inhaled aerosol on cough, the subjects inhaled D-glucose aerosols in a range from 77 to 1232 mosmol/l (all calculated values). For comparison, and to confirm the importance of chloride ion concentration, saline solutions of matched osmolarities were also inhaled. To exclude any effect the low pH of D-glucose may have on cough, small amounts of 0.1 mol/l sodium hydroxide were added to each solution in order to raise the pH to that of saline (pH 5-7). All aerosols were inhaled on separate days and forced expired volume in 1 s (FEV1) was recorded after each challenge.

Experiment 2b

To test the role of hyperosmolarity in inducing cough, two further solutions were tested as described above by seven subjects, three of whom had taken part in experiment 2a. The solutions were an isotonic and a hypertonic mixture of D-glucose and saline. The resulting solutions were 1.25% D-glucose in 0.68% saline (308 mosmol/l; 112 mmol/l Cl-) and 15% D-glucose in 0.9% saline (1232 mosmol/l; 150 mmol/l Cl-).

Experiment 3

The mechanism of the irritant properties of citric acid was examined by comparing the cough response to five aerosols. These were isotonic citric acid in saline, distilled water, isotonic D-glucose, isotonic sodium citrate and sodium citrate in saline.

Statistical analysis

Cough frequencies were first transformed by adding 1 and taking the square root, the usual transformation for data with a Poisson distribution. Two-way analysis of variance was then performed for experiments 1, 2a and 3, the factors being subjects and aerosol. The residual mean square was used to compute 95% confidence limits, which together with the resultant means were then back-transformed, i.e. squared and unity subtracted.

RESULTS

Experiment 1 (Table 1)

Cough occurred on inhalation of the two aerosols of extreme pH, 2.6 and 10.0 only. No significant difference in cough frequency at these two levels of pH was found (P>0.05). However, the cough response to both aerosols was significantly greater than the aerosols causing no cough (P<0.01).

Experiment 2a (Table 2)

No significant difference in cough response was found between any of the D-glucose aerosols (P>0.05). The dose-response relationship between decreasing chloride

<table>
<thead>
<tr>
<th>pH of saline...</th>
<th>2.6</th>
<th>4.8</th>
<th>8.0</th>
<th>10.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCF (coughs/min)</td>
<td>2.1 (0.6-4.2)</td>
<td>0.0</td>
<td>0.0</td>
<td>3.6 (1.7-6.0)</td>
</tr>
</tbody>
</table>

Table 1. Effect of altering the pH of the inhaled aerosol on cough frequency

The number of subjects studied was seven. The 95% confidence limits are shown in parentheses. Abbreviation: MCF, mean cough frequency.
concentration and increasing cough was confirmed ($P<0.01$). At the highest sodium chloride concentration occasional coughing was observed. FEV$_1$ values measured after each inhalation did not vary by more than 10% in any subject.

**Experiment 2b**

Cough occurred in response to inhalation of the hypertonic mixture of D-glucose and saline (mean 3.7 coughs/min, range 0–7 coughs/min) but not to the isotonic mixture.

**Experiment 3 (Table 3)**

Cough occurred in response to inhalation of the citric acid mixture, sodium citrate, D-glucose and water. No difference in cough frequency was found between these ($P>0.05$). Cough did not occur with sodium citrate in saline.

**DISCUSSION**

Aqueous solutions with low or no chloride ion concentration, when inhaled as aerosols, have been found to

<table>
<thead>
<tr>
<th>Osmolarity (mosmol/l)</th>
<th>d-Glucose</th>
<th>Saline</th>
</tr>
</thead>
<tbody>
<tr>
<td>77</td>
<td>7.6 (4.4–11.5)</td>
<td>8.1 (4.8–12.1)</td>
</tr>
<tr>
<td>154</td>
<td>9.5 (6.0–13.8)</td>
<td>2.1 (0.4–4.6)</td>
</tr>
<tr>
<td>308</td>
<td>12.0 (8.0–16.6)</td>
<td>0.0</td>
</tr>
<tr>
<td>616</td>
<td>11.5 (7.6–16.1)</td>
<td>0.0</td>
</tr>
<tr>
<td>1232</td>
<td>12.1 (8.1–16.8)</td>
<td>1.3 (0.0–3.6)</td>
</tr>
<tr>
<td>$P$</td>
<td>&gt;0.05</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Table 2. Effect of changes in osmolarity of inhaled saline and d-glucose aerosols on cough frequency**

Coughing in response to saline occurred only with hypotonic or markedly hypertonic aerosols. However, all d-glucose aerosols resulted in a similar cough response ($P>0.05$), showing the importance of chloride rather than osmolarity. The number of subjects studied was seven. The 95% confidence limits are shown in parentheses. Abbreviation: MCF, mean cough frequency.

<table>
<thead>
<tr>
<th>Aerosol</th>
<th>Osmolarity (mosmol/l)</th>
<th>pH</th>
<th>Chloride (mmol/l)</th>
<th>MCF (coughs/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.68% citric acid in 0.79% saline</td>
<td>308</td>
<td>2.0</td>
<td>130</td>
<td>11.4 (7.2–16.5)</td>
</tr>
<tr>
<td>Water</td>
<td>0</td>
<td>8.5</td>
<td>0</td>
<td>15.7 (10.8–21.6)</td>
</tr>
<tr>
<td>d-Glucose</td>
<td>308</td>
<td>3.6</td>
<td>0</td>
<td>18.1 (12.8–24.3)</td>
</tr>
<tr>
<td>Sodium citrate</td>
<td>308</td>
<td>8.6</td>
<td>0</td>
<td>12.5 (8.1–17.7)</td>
</tr>
<tr>
<td>Sodium citrate/saline</td>
<td>616</td>
<td>8.2</td>
<td>150</td>
<td>0.0</td>
</tr>
</tbody>
</table>

No cough occurred in response to the sodium citrate in saline aerosol. For the remaining aerosols, no significant difference in cough frequency was found ($P>0.05$). The number of subjects studied was seven. The 95% confidence limits are shown in parentheses. Abbreviation: MCF, mean cough frequency.

**Table 3. Cough frequency in response to aerosols of varying osmolarity, pH and chloride concentration**

Stimulate cough [1, 6, 7]. This response pattern is similar to that observed for laryngeal RARs in isolated nerve studies in animals [2, 3]. Our results in experiment 2 confirm that aerosolized solutions low in chloride induce cough.

Extremes of pH even in the presence of chloride were important stimuli for experimentally inducing cough. This pH sensitivity is similar to that observed by Boggs & Bartlett [2] and Boushey et al. [3] for laryngeal RARs.

While Eschenbacher et al. [7] suggest that alterations of osmolarity as well as absence of a permeant anion of inhaled aerosol induces cough, we found no change in the cough elicited by d-glucose aerosols over a range of osmolarity. This is in line with the observations of Boggs & Bartlett [2], who found that solutions of sucrose and urea stimulated laryngeal RARs regardless of their osmolarity. The 3.6% saline aerosol caused some coughing. This could be due to its high ion content or its hyperosmolarity. In experiment 2b, a solution of equal osmolarity but only a quarter of the ion content also resulted in occasional cough, suggesting that marked hyperosmolarity of inhaled aerosol is a further stimulus for cough.

It is possible that the performance of the nebulizer would be affected by hyperosmolar solutions and this was partly assessed by measuring the osmolality of isotonic and hypertonic solutions of saline and D-glucose before and after 1 min nebulizations. The values did not alter by more than 5%.

Our results suggest that there is an additive effect of low pH and lack of chloride on cough induced by inhaling aerosols of citric acid. Citric acid even in the presence of chloride stimulates cough while isotonic citric acid without saline is intolerable when inhaled from an ultrasonic nebulizer (T. W. Higenbottam, unpublished work). The irritant properties of acetic acid aerosol [8] can be attributed to a similar mechanism. Calcium chelation, thought to be responsible for the bronchoconstriction observed in Basenji-Greyhound dogs in response to citric acid [5], is unlikely to contribute to cough as sodium citrate in saline failed to elicit a response despite its being a calcium chelator. This is in line with the observations of Boggs & Bartlett [2].
In summary, we find cough to be stimulated by low chloride concentration, extremes of pH and very high osmolarity. These characteristics are similar to those described for laryngeal RARs in animal studies [2, 3]. In man, intra-epithelial RARs lying close to the airway lumen are located primarily in the central airways [9], the major site for cough provocation in man [10, 11]. It may be concluded that the reflexly induced cough from inhalation of aqueous aerosols results from stimulation of airway RARs which are located in the large conducting airways.

ACKNOWLEDGMENTS

This work was supported by a grant from the Asthma Research Council. We acknowledge with gratitude the advice and criticism given by Mr B. Milstein during the preparation of the manuscript.

REFERENCES