Cough induced by low pH

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Citric acid has been used as a tussive agent in the investigation of the cough reflex. The mechanism of cough stimulation remains unclear. We conducted studies to compare the cough response of citric acid to an organic (acetic acid) and an inorganic acid (phosphoric acid). We also compared the cough response of citric acid to capsaicin, a non-acid tussive stimulus. In study 1, 26 subjects inhaled equimolar concentrations of citric and acetic acid. In study 2, 22 subjects inhaled citric acid and phosphoric acid in concentrations of similar pH. Eighteen subjects from study 2 completed a capsaicin cough challenge test. The cough response was recorded and the concentration causing two coughs, the cough threshold, calculated (D2). The correlation of cough thresholds between both citric and acetic acid (r=0.79, 95% CI 0.37-0.90, P<0.0001) and citric acid and phosphoric acid (r=0.68, 95% CI 0.37-0.86, P=0.0005) were significant. There was no correlation between the citric acid and capsaicin cough thresholds. These results show that an individual's cough response to acetic and phosphoric acid are similar to that caused by citric acid. Thus these acids may cause cough by a common mechanism such as disturbance of the pH of the airway surface liquid. Capsaicin does not appear to share this common pathway.

Introduction

Cough is one of the commonest respiratory symptoms and cough challenge testing has been used to study various aspects of the cough reflex. The earliest description of cough challenge testing was by Bickerman and Barach in 1954 (1). The tussive stimulus used was citric acid which remains one of the commonest tussive stimuli. Tussive stimuli used in cough challenge testing can be divided into three groups, the organic acids (citric and acetic acid), capsaicin, the pungent extract of chilli peppers and distilled water. There is still much debate regarding the exact mechanism of production of cough with these different stimuli (2). Citric acid is thought to stimulate cough via the rapidly adapting stretch (irritant) receptors. Capsaicin may cause cough through stimulation of the c fibres and distilled water through changes in the ionic milieu of the airway surface liquid (3,4).

Cough testing has been used to assess the cough reflex of patients with asthma and chronic obstructive airways disease (5–7) and chronic cough (8). It has been used in the pharmacological assessment of antitussive agents (9,10) and in understanding the physiology of the cough reflex (11). Recently in an epidemiological study of workers exposed to irritant fumes who complained of cough, a more sensitive cough response to citric acid and capsaicin was demonstrated (12). Thus cough challenges with tussive agents are being increasingly used as an investigational tool.

We hypothesize that citric acid causes cough due to the effect of pH and that this may be different to cough caused by capsaicin. To investigate further the mechanism of citric acid and capsaicin cough we undertook two experiments in normal individuals. We compared the cough response of citric acid to two different acids, one organic, acetic acid and the other inorganic, phosphoric acid. We also compared the cough response of citric acid to capsaicin.

Methods

SUBJECTS

Twenty-six subjects (11 men), mean age 34 years (range 18–60 years), were recruited for study 1 (citric acid vs. acetic acid cough response). Twenty-two different subjects (12 men), mean age 30 years (range 20–59 years) were recruited for study 2 (citric acid vs. phosphoric acid cough response). Eighteen subjects (10 men), mean age 30 years (range 20–59 years) from study 2 returned for the capsaicin cough challenge. Only a few of the subjects were naive to the cough challenge test.

All were not present smokers, had no history of perennial allergy or asthma and had not suffered a respiratory infections within the last 4 weeks. Spirometry (Vitalograph, U.K.) was performed in all subjects before and after the cough challenge and in all subjects the forced expiratory volume in 1 s (FEV1) was over 80% of predicted.
Table 1. pH of acid solutions

<table>
<thead>
<tr>
<th>Conc. CA/AA (mM)</th>
<th>CA pH</th>
<th>AA pH</th>
<th>Conc. PA (mM)</th>
<th>PA pH</th>
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</thead>
<tbody>
<tr>
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<td>3.1</td>
<td>0.5</td>
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</tr>
<tr>
<td>30</td>
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<td>2.76</td>
<td>1.5</td>
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<td>2.6</td>
<td>5</td>
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</tr>
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<td>2.35</td>
<td>15</td>
<td>1.26</td>
</tr>
<tr>
<td>1000</td>
<td>1.14</td>
<td>2</td>
<td>50</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Conc. = concentration; CA = citric acid; AA = acetic acid; PA = phosphoric acid.

Permission for the study was given by the South Sheffield Research Ethics Committee and all subjects gave written informed consent.

COUGH CHALLENGE METHODOLOGY

In study 1, stock solutions of equimolar concentrations of citric acid (1000 mM) and acetic acid (1000 mM) were diluted with 0.9% saline to produce log incremental doses (10, 30, 100, 300 and 1000 mM). In study 2, phosphoric acid at 50 mM was chosen as the stock inorganic acid. This had a similar buffering capacity to physiological pH as 1000 mM citric acid as determined by titration with sodium bicarbonate. Phosphoric acid was then further diluted in a similar fashion to citric acid (producing solutions of 0.5, 1.5, 5, 15 and 50 mM). The pH of these acid solutions are shown in Table 1. A stock solution of 1 mm capsaicin was diluted in a similar fashion (producing solutions of 1, 3, 10, 30 and 100 μM) for the capsaicin cough challenge test.

Cough challenge tests were performed as previously described (11). Briefly each stock solution was diluted with varying amounts of 0.9% saline to produce the five different concentrations as above. These solutions were arranged in ascending order of concentrations with two concentrations of saline inhalations randomly administered. The solutions were delivered by a compressed air driven nebuliser controlled by a breath activated dosimeter (Mefar, Brescia, Italy). The output of the dosimeter was 0.1 ml s⁻¹. The subjects were blinded as to which acid solution was being used. The subject received four 1-s inhalations of each solution with a 10-s interval between each. The cough response during this interval was recorded. Citric acid/acetic acid and citric acid/phosphoric acid cough challenge tests were completed on the same day with the type of acid which was inhaled first assigned in a random fashion. There was a 15-min interval between each separate cough test. Capsaicin cough challenge was performed on a separate day 1 week later. The coefficient of variation of the citric acid cough challenge at a 1-week interval in 90 subjects is 12% (unpublished observation).

STATISTICAL ANALYSIS

Log dose-response curves were plotted for all cough challenges. From this the cough threshold, D₂ (the concentration of tussive stimulus causing two coughs per inhalation) was determined by linear interpolation. Comparison of the D₂ values for citric acid and acetic acid, citric acid and phosphoric acid and citric acid and capsaicin were determined by correlation. Statistical significance was assumed at the 5% level.

Results

CITRIC ACID VS. ACETIC ACID

The geometric mean D₂s (SD) for citric acid and acetic acid were 437 mM (2.4 mm) and 391 mM (2.8 mm) respectively. There was a high degree of correlation between the two sets of cough D₂ values (r=0.79, 95% CI 0.56-0.90, P<0.0001). This is illustrated in Fig. 1(a).

CITRIC ACID VS. PHOSPHORIC ACID

The geometric mean D₂ (SD) for citric acid and phosphoric acid were 604 mM (2.0 mm) and 24 mm (2.0 mm), respectively. Again these cough D₂ data showed a high degree of correlation (r=0.68, 95% CI 0.37-0.86, P=0.0005, Fig. 1(b)).

CITRIC ACID VS. CAPSAICIN

Of the 22 subjects who took part in the phosphoric acid cough challenge, 18 returned for a capsaicin cough challenge.
challenge test. The geometric mean capsaicin D2 (SD) for these individuals was 8.2 ± 2 μM (2.0 ± 2 μM). No correlation was found when comparing these D2 values with their citric acid D2 values (r = 0.29, 95% CI = 0.21 - 0.67, P = 0.24).

Fig. 2 shows the mean cough response to the various tussive stimuli at the different concentrations.

Discussion

Chemical stimulation has been used to study different aspects of the cough reflex both in health and disease. Despite its widespread use, how citric acid stimulates cough remains unclear. Cough can be stimulated by the activation of rapidly adapting receptors (RARs or irritant receptors) located in the larynx and upper airways of the tracheobronchial (2). Citric acid has been considered to cause cough through this mechanism (11,13). That citric acid-induced cough is reduced in patients following laryngectomy (14) further supports this theory. The stimulus to cause firing of these receptors may be the opening of pH gated ion channels (13).

The citric acid cough response is subject to a degree of short and long term adaptation following repeated exposures (11,15). However the degree of adaptation is greatest after the first exposure (11). The majority of our subjects had previous experience of the cough challenge method and therefore adaptation was kept to a minimum. Also the acid that was inhaled first was randomly assigned to limit the degree of tachyphylaxis to any of the three acids. The cough response to citric acid is reproducible. The coefficient of variation of the cough response to a single dose of citric acid over a period of a few days is 12% (unpublished observation).

Acetic acid is an organic acid which has also been used as a tussive stimulus (16) and is thought to cause cough due to a low pH (5). In study 2 phosphoric acid was chosen as the inorganic acid as the stock solution had a similar buffering capacity to physiological pH as the stock solution of citric acid. Dilution of these stock solutions provided solutions of similar pH. Phosphoric acid is generally safe when inhaled and only irritation of the upper respiratory tract has been reported (17). Indeed this was a symptom reported by a few of our subjects but only at the higher concentrations.

Our study demonstrates a significant correlation between the citric acid cough response in normal individuals to both an organic acid, (acetic acid) and an inorganic acid (phosphoric acid). We believe that the tussive effects of acetic acid and phosphoric acid cough share a similar pathway to citric acid via the activation of pH sensitive rapidly adapting receptors. It has been shown that low extracellular pH (high proton concentration) stimulates spinal neurones of various animal species, possibly through proton-gated ion channels (18).

Capsaicin is another popular protussive agent. At a cellular level, in sensitive neurones, capsaicin opens a relatively nonselective cation channel (19), which has recently been cloned (20). This allows sodium and calcium ions to enter and potassium ions to leave the cell which results in depolarization and excitation of the neurone (19). There is some evidence that both capsaicin and protussae act at the same ion channel (21). capsain antagonists having been shown to inhibit both proton and capsaicin evoked responses (22,23). However the evidence is not consistent as some studies have shown a separation of the capsaicin and proton responses (21,24).

Other workers have previously shown a lack of correlation between citric acid and capsaicin cough challenges (25), although differences in inhalation techniques used in the study make interpretation of the results difficult. Our present study, using identical inhalation techniques, has shown no correlation between the citric acid and capsaicin cough thresholds in normal individuals. This would support the hypothesis that in humans acids act via a proton-sensitive mechanism and that this is different to capsaicin-induced cough.

References


