Different inhalation methods are used for cough reflex sensitivity (CRS) measurement. The single-breath method of tussigenic agent aerosol inhalation is widely used now. Comparison of two tussigenic agents - citric acid and capsaicin - in cough reflex sensitivity measurement was used in healthy volunteers. In 17 healthy volunteers (7 M, 10 F; mean age 21 years) without respiratory tract infection in last 3 weeks, without cardiovascular, allergic, respiratory metabolic diseases, and with normal spirometry, cough reflex sensitivity was examined by nebuliser ProvoJet (Ganshorn Medizin Elektronic, Germany) with doubled concentrations of capsaicin (SIGMA) from 0.49 to 1000 µmol/l and citric acid (LACHEMA) concentrations of 1, 3, 10, 30, 100, 300, 1000 µmol/l. Cough reflex sensitivity was defined as the lowest concentration of tussigenic agents which elicited 2 and 5 coughs (C2, C5). Geometric mean and 95% confidence interval (CI) of citric acid C2 was 454.5 (284.8-725.4) mmol/l in 88.2% of volunteers; citric acid C5 was 1000 µmol/l (0) in 47.1% of volunteers. Capsaicin C2 was 23.5 (8.2-67.5) µmol/l in 100% of volunteers and C5 was 263.7 (111.2-625.1) µmol/l in 76.5% of volunteers. We conclude that capsaicin single-breath test is more relevant for cough reflex sensitivity measurement in healthy volunteers than citric acid cough test.

Key words: capsaicin, citric acid, cough, cough challenges, cough reflex sensitivity

INTRODUCTION

Cough is a common symptom of many respiratory and non-respiratory disorders. It is useful from a diagnostic point of view to divide cough into acute (lasting less than three weeks) and chronic (lasting longer than eight weeks) types. The majority of cases of acute cough are due to upper respiratory viral
infections. The commonest causes of chronic cough are generally accepted to be gastro-oesophageal reflux disease, rhinosinusitis and asthma (1).

The anatomo-physiological approach to elucidating the cough reflex has led to the description in the guinea pig of a vagal afferent nerve subtype as being essential for defensive cough (2). The role of other airway sensory receptors might be to modulate, rather than initiate cough. Electrophysiological studies combined with immunohistochemistry are uncovering a heterogeneity of receptors and channels present on specialized receptors, such as the cough receptors. Centrally, the role of neurotransmitters such as tachykinins at the level of the ‘cough center’ may partially sensitise the cough reflex. This is an important observation, since patients with chronic cough have an increased cough reflex sensitivity, as measured by capsaicin or citric acid challenge (3).

A wide range of inhalation cough challenge methods has been applied in the studies of cough reflex. The more reproducible single breath method should be used (4). The choice of a tussigenic agent will frequently depend on local experience. However, when both citric acid and capsaicin cough challenges are performed within a short period of time, the cough response can be diminished by a quarter when citric acid is inhaled after capsaicin and by a third when capsaicin inhalation follows the administration of citric acid (5).

While testing and developing the inhalational cough challenges a large number of tussive agents have been tried including, sulphur dioxide, ammonia, and cigarette smoke (6). Although differing in their properties, only capsaicin and citric acid have stood the test of time, probably as a result of their greater reproducibility. In vitro studies have shown that capsaicin and citric acid act through the C fibres (7-10). In addition, citric acid has also been reported to stimulate RARs (rapidly adapting pulmonary stretch receptors) within the larynx and the upper airways. However, capsazepine a competitive inhibitor of capsaicin also reduces the effects of acids suggesting that both of these agents may be stimulating the same pathway (8).

Citric acid has been used to study pathology and the effects of pharmacological agents on the cough reflex for over 50 years (11). Capsaicin (trans-8-methyl-N-vanillyl-6-nonenamide) a pungent agent of hot pepper, has been the most commonly used non-acid tussigenic agent (12, 13). Capsaicin acts mainly on the afferent neurones of the non-myelinated C-fibres by opening a non-selective cation channel of vanilloid receptor resulting in a flow of calcium and sodium down their concentration gradient (13). This leads to depolarization and associated neurotransmitter release. The capsaicin induced calcium flow and desensitization is inhibited by a specific antagonist; an inorganic dye ruthenium red (14).

Symptom assessment in patients with lung disorders relies on subjective recording and therefore can be variable. Similarly, subjective measurements of cough are difficult to assess. In fact, one of the major difficulties in cough research has been inability to accurately quantify clinically relevant cough (4). The assessment of cough response during the inhalation cough challenge has been more
straight forward. The simplest approach involves manual recording carried out by one investigator or, as is preferred to minimize error, by two independent examiners. Usage of more elaborate system with high quality digitized cough recordings suitable for examination by pattern recognition algorithms is important (15, 16).

Cough sensitivity can be evaluated by inhalational challenge tests and it is significantly changed in some respiratory diseases (17-19). In addition, cough reflex sensitivity is also changed in diseases in which pathological processes are localized out of the larynx and tracheobronchial tree. Cough sensitivity is significantly increased in gastroesophageal reflux (20), allergic rhinitis (21), atopic dermatitis (22). But cough sensitivity is significantly decreased in patients with recurrent pneumonia (23) or in central nervous system disorders (24).

The aim of this study was to investigate the cough reflex sensitivity to capsaicin and citric acid in healthy volunteers and to estimate which tussigenic agent - capsaicin or citric acid - is more suitable for decreased and also increased cough reflex sensitivity measurement.

MATERIAL AND METHODS

Subjects

The study was carried out on 17 healthy volunteers (7 M, 10 F; mean age 21 years) - non-smokers - without respiratory tract infection in last 3 weeks, without cardiovascular, allergic, respiratory and metabolic diseases and with normal spirometry. Based on a structured, interviewer-led questionnaire, each subject was asked about respiratory symptoms and a past and family history of bronchial asthma, allergic rhinitis, gastroesophageal reflux, cardiovascular diseases, metabolic diseases, ACE inhibitor treatment.

The study was approved by Ethics Committee of Jessenius Medical School and informed consent was obtained from all subjects after the purpose of the test had been explained.

Subjects attended the laboratory to undergo a cough reflex sensitivity test and spirometry before and after the test. All patients were examined at the same time of day. Capsaicin or citric acid challenges were used in random order. Interval between capsaicin and citric acid cough reflex sensitivity testing was 48 hours in all volunteers. Subjects reported subjective feeling during cough challenge testing.

Assessment of cough reflex sensitivity

Measurement of cough threshold to inhaled tussigenic agent was carried out using modified single-breath method of Dicpinigaitis et al (25). Solutions of capsaicin (Sigma) were prepared to make a stock solution of 0.01 mol, and subsequently further diluted with physiologic saline solution to yield serial doubling concentrations ranging from 0.49 to 1000 µmol/l (25). Fresh dilutions were prepared on each day of testing. Citric acid (Lachema) was diluted in 0.9 % sodium chloride to obtain concentrations of 1, 3, 10, 30, 100, 300, 1000 mmol/l (4).

Subjects inhaled single breaths (from FRC to TLC) of capsaicin or citric acid aerosol administered via a nebulizer ProvoJet (Ganshorn Medizin Elektronic, Germany). The duration of aerosol delivery was programmed at 0.5 s. Single breaths of capsaicin or citric acid aerosol were administered in ascending order, with inhalations of saline solution randomly interspersed to
increase challenge blindness, until the concentrations inducing two or more coughs ($C_2$) and five or more coughs ($C_5$) were reached. Breaths were delivered at 1-min intervals. Subjects were unaware that the end point of the study was the number of coughs induced. The number of coughs in the first 15 seconds after each inhalation were counted by two experienced observers and also registered (cough recordings were transferred from the digital recorders to a personal computer and archived on a compact disc). Each concentration of tussigenic agent was inhaled once. The challenge was terminated at the concentration of tussigenic agent that resulted in 5 consecutive coughs ($C_5$). If subject did not reach $C_2$ and/or $C_5$ during the capsaicin or citric acid challenge, the subject was considered to be a non-responder (NR in Table 1).

Cough reflex sensitivity was defined as the lowest concentration of tussigenic agent that elicited 2 and 5 coughs ($C_2$, $C_5$ = concentration of capsaicin or citric acid inducing two or more coughs; $C_5$ = concentration of capsaicin or citric acid inducing five or more coughs).

Data analysis

Mean values for age, FVC (in liters and percent predicted), FEV1 (in liters and percent predicted) were calculated and compared with an unpaired Student $t$ test and a value of $P<0.05$ was considered to be significant. The results were expressed as the capsaicin or citric acid concentrations causing two or more coughs ($C_2$) and five or more coughs ($C_5$) in responded subjects. Geometric mean and 95% confidence intervals were calculated for each group. Data were analysed by chi-square test. The level of significance, power of the test and the proportions were evaluated. All cough challenge studies were performed by the author.

RESULTS

The induction of five or more coughs was not achieved in all subjects (Table 1). Cough reflex sensitivity was defined as the lowest concentration of tussigenic agent that elicited 2 and 5 coughs ($C_2$, $C_5$). $C_2$ was elicited by 3 concentrations of citric acid (15 subjects responded; 88.2%) and by 10 concentrations of capsaicin (all the subjects responded). $C_5$ was elicited by 1 concentration of citric acid in 8 responders (47.1%) and by 7 concentrations of capsaicin in 13 responders (76.5%). Capsaicin responsiveness was significantly greater than citric acid responsiveness (chi-square test).

Geometric mean and 95% confidence interval (CI) of citric acid $C_2$ was 454.5 (284.8-725.4) mmol/l in 88.2% of subjects; citric acid $C_5$ was 1000 mmol/l in 47.1% of subjects. Capsaicin $C_2$ was 23.5 (8.2-67.5) µmol/l in 100% of subjects and $C_5$ was 263.7 (111.2-625.1) µmol/l in 76.5% of subjects. Capsaicin and citric acid cough reflex sensitivity $C_2$ and $C_5$ concentrations in all subjects are demonstrated in Table 1.

There were no differences in baseline pulmonary function before and after cough reflex sensitivity testing in the subjects studied ($P>0.05$).

No serious adverse reactions to capsaicin inhalation challenge were reported. In 15 subjects (88.2%) after capsaicin aerosol inhalation and in 14 subjects (82.4%) after citric acid aerosol inhalation adverse reactions were limited to minor complaints - transient throat irritation. One subject (5.9%) reported some
degree of retrosternal discomfort after inhalation of both tussigenic agents. One another subject after capsaicin aerosol and 2 different subjects after citric acid aerosol inhalation reported no subjective feelings.

**DISCUSSION**

The aim of this study was to investigate the cough reflex sensitivity to capsaicin and citric acid in healthy volunteers and to estimate which tussigenic agent - capsaicin or citric acid - is more suitable for decreased and also increased cough reflex sensitivity measurement.

In vitro studies have shown that capsaicin and citric acid act through the C fibres (7-10). In addition, citric acid has also been reported to stimulate RARs within the larynx and the upper airways. However, capsazepine a competitive inhibitor of capsaicin also reduces the effects of acids suggesting that both of these agents may be stimulating the same pathway (8).

Capsaicin and citric acid cough challenges in our study were performed using single-breath method. A cough challenge test was conducted to assess the sensitivity of the cough reflex. Fewer subjects responded to citric acid cough challenge and a smaller number of concentrations of citric acid elicited cough response. Our data suggest that capsaicin responsiveness was significantly greater than citric acid responsiveness.

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*Table 1. Capsaicin and citric acid cough reflex sensitivity in healthy volunteers in this study (NR - non-responder).*
Cough test challenges can show functional change of airway afferent nerve-endings mediating cough. Cough reflex sensitivity can be evaluated by inhalational challenge tests and it is significantly changed in some respiratory diseases (17-19). Cough reflex sensitivity is also changed in diseases in which pathological processes are localised out of the larynx and tracheobronchial tree. Cough sensitivity is significantly increased in gastroesophageal reflux (20), allergic rhinitis (21), atopic dermatitis (22). But cough sensitivity is significantly decreased in patients with recurrent pneumonia (23) or in central nervous system disorders (24). There are many ways in which cough can be down-regulated, but in general they fall into two groups. The first involves cortical mechanisms, either the activation of inhibitory or the suppression of facilitatory pathways for cough; these conditions include voluntary control, sleep, placebo effect, general anaesthesia, and several clinical states such as coma, stroke and Parkinson's disease. Cough can also be down-regulated by afferent inputs to the brainstem, including from broncho-pulmonary and other visceral C-fibre receptors and from pathways activated in bronchopulmonary and other diseases. Presumably these reflex down-regulations of cough act at the brainstem level, but the involvement of cortical pathways cannot be excluded. Changes in blood gas tension levels that stimulate breathing also inhibit cough, confirming the view that brainstem regulation of breathing has features distinct from those that control cough. Much more research is needed on this potentially important subject (26, 27). In diabetes with autonomic neuropathy, the cough threshold to citric acid is increased five-fold and reflex cough may be even absent (28), indicating a down regulation.

The sensitivity of bronchopulmonary C fibers and RARs is enhanced by airway inflammation, which presumably heightens the airway defense function during the disease conditions. Although the mechanism underlying the enhanced sensitivities of these afferent endings is not yet fully understood, it probably involves local release of certain inflammatory mediators (e.g., prostaglandins, histamine) in the airways. By activating specific receptor proteins (e.g., subtypes of the prostanoid receptor) located on the membrane of the sensory terminals, these autocoids can alter the membrane properties and elevate the excitability of the afferent endings, which may lead to increased cough reflex sensitivity. The intracellular signal transduction pathways involving specific ion channels remain to be explored (29).

Our results suggest that capsaicin and citric acid could be used for increased cough reflex sensitivity measurement. Citric acid cough challenge test seems not well suitable for decreased cough sensitivity measurement in our modification. The subjects studied did not differ in baseline pulmonary function before and after cough reflex sensitivity testing. A review of the 20-year clinical experience has failed to uncover a single serious adverse event associated with capsaicin cough challenge testing in humans. Given the need for better antitussive therapies, capsaicin represents a vital component of future scientific inquiry in the field of cough. No serious adverse reactions to capsaicin inhalation challenge have been reported. In a small fraction of studies, minor adverse reactions to
capsaicin inhalation were limited to transient complaints due to throat irritation (30). No serious adverse reactions to capsaicin and citric acid were seen in the present study either.

Various cough challenges implemented by different centers have been discussed in a review article by Morice et al (4). Standardized guidelines for cough challenge testing should be introduced allowing for greater accuracy and comparability in pharmacological and physiological research.

Our data suggest that the capsaicin single-breath test is more relevant for cough reflex sensitivity measurement in healthy subjects than a citric acid cough test. Capsaicin also is suitable for the assessment of both increased or decreased cough reflex sensitivity.

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