

ORIGINAL PAPER

Hypertonic saline cough provocation test with salbutamol pre-treatment: evidence for sensorineural dysfunction in asthma

H. O. Koskela*, M. K. Purokivi*, K. M. Kontra†, A. H. Taivainen* and H. O. Tukiainen*

*Department of Respiratory Medicine and †Department of Pharmacy, Kuopio University Hospital, Kuopio, Finland

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Summary

Background Cough is one of the most common symptoms of asthma. However, studies using capsaicin, citric acid, or tartaric acid to document cough threshold have repeatedly failed to show statistically significant differences between asthmatic and healthy subjects. The studies using hypertonic aerosols as the cough stimulant have suggested an enhanced sensitivity in asthmatic subjects but the induced bronchoconstriction has made the interpretation of the results difficult.

Objective To determine the cough sensitivity to hypertonicity in healthy subjects, patients with chronic cough, and patients with asthma in a setting where the induction of bronchoconstriction is prevented.

Methods Nineteen healthy subjects, 21 non-asthmatic patients with chronic cough, and 26 asthmatic patients with chronic cough underwent an incremental hypertonic saline challenge including a pre-treatment with 0.4 mg of salbutamol. Spirometry was performed before the challenge, after salbutamol, and after the challenge. The patients with cough also underwent skin testing, histamine challenge, exhaled nitric oxide measurement, ambulatory peak flow monitoring, kept cough diary, and filled in the Leicester Cough Questionnaire. Eighteen patients repeated the saline challenge.

Results The challenge did not induce bronchoconstriction in any group. The osmolality to provoke 15 cumulative coughs was significantly smaller in the asthmatic patients than in the healthy subjects ($P < 0.001$) and in the cough patients without asthma ($P = 0.04$). According to multivariate analysis among all the 47 patients with cough, female sex ($P < 0.001$) and large spontaneous peak flow variation in the ambulatory recording ($P = 0.001$) were associated with high sensitivity to saline. The saline challenge response was well repeatable (intraclass correlation coefficient 0.90).

Conclusion The findings of the present study are not affected by induced bronchoconstriction. Asthma or, more specifically, spontaneous, reversible airway obstruction is associated with an enhanced sensitivity to the cough-provoking effect of hypertonic saline. This suggests a pathological function of the sensorineural apparatus in this disorder.

Keywords bronchial hyperreactivity, cough, receptors, sensory

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Correspondence:

Heikki O. Koskela, Department of Respiratory Medicine, Kuopio University Hospital, P. O. Box 1777, 70211 Kuopio, Finland.
E-mail: heikki.koskela@kuh.fi

Introduction

Asthma and chronic rhinitis may be considered as two manifestations of one syndrome, the chronic allergic respiratory syndrome [1]. In the nasal airways substantial evidence exists that the sensorineural apparatus is a central contributor to symptoms and hyperresponsiveness [2–6]. This would suggest that the sensorineural apparatus

might have a significant role also in the asthma pathophysiology but studies concerning this issue are scanty.

Cough involves a complex neural reflex that begins with a stimulation of cough receptors [7]. It is one of the most common and annoying symptoms of asthma [8] suggesting that this disorder might be associated with an enhanced excitability of the cough receptors. However, earlier studies using either capsaicin, citric acid, or tartaric

acid to document cough threshold have repeatedly failed to show statistically significant differences between asthmatic and healthy subjects [9–17]. The results of respective studies on patients with cough-variant asthma are conflicting [17–21]. Thus, an abnormal function of cough receptors has not been established in asthma and the prevalent coughing among asthmatics has been presumed to be due to bronchoconstriction as bronchodilating drugs have found to be helpful in asthmatic cough [22].

We have recently shown that asthmatic patients cough significantly more than healthy subjects during airway challenges with various hypertonic aerosols [23, 24]. The hypertonicity-provoked cough and bronchoconstriction seem to have different mechanisms [25] and the cough sensitivity decreases during the treatment with inhaled glucocorticoids [23]. The results suggest that there may be an abnormal cough reflex function in asthma which can be revealed by hypertonic challenges. However, these studies have one main limitation: The hypertonic challenges induce bronchoconstriction in asthmatic patients and it is difficult to separate the cough-provoking effect of induced bronchoconstriction from the effect of hypertonicity itself [24]. Another limitation is that the studies have only included healthy and asthmatic subjects but not patients with chronic cough of non-asthmatic origin [23–25]. The purpose of the present study was to determine the cough sensitivity to hypertonicity in healthy subjects, patients with chronic cough, and patients with asthma in a setting where the induction of bronchoconstriction is prevented.

Methods

Subjects

The group consisting of patients with chronic cough but without asthma was formed in the following way: There were 25 consecutive patients who had been referred to the authors' outpatient clinic due to chronic cough lasting at least 8 weeks [26]. In addition to cough, 11 of them also complained of dyspnoea and/or wheezing. In the great majority of the referred patients (21/25) spirometry and/or ambulatory peak expiratory flow (PEF) monitoring had been performed at the primary health care in order to rule out asthma. However, four out of those 25 showed features of variable airway obstruction during the study and were thus transferred to the asthmatic group. These features included at least one of the following: A more than 20% diurnal PEF variation in the ambulatory recording on at least 2 days, a more than 15% increase in PEF after salbutamol on at least 2 days, or a 12% or larger increase in forced expiratory volume in 1 s (FEV₁) spirometry after salbutamol. Chronic cough patients without these features formed the group 'cough, no asthma' (*N* = 21).

In addition, there were 22 well-described, previously diagnosed asthmatic patients from the authors' outpatient clinic. They met the American Thoracic Society criteria for asthma [27], had showed at least one of the above-mentioned features of variable airway obstruction at the time of diagnosis, and suffered of chronic cough as one of their symptoms. Together with the four referred cough patients with variable airway obstruction they formed the group 'cough and asthma' (*N* = 26). Besides cough, wheezing or dyspnoea was reported by 23/26 in this group.

Finally, there were 19 healthy subjects without any chronic respiratory diseases or symptoms. The characteristics of the subjects are presented in Table 1. The Finnish National Agency of Medicines and the Institutional Ethics Committee approved this study and all subjects provided their informed written consent for participation in the study.

Study design

In the patients with chronic cough the study consisted of two study days plus a 2-week observation period between them. The healthy subjects only underwent the second study day. On the first day airway responsiveness to histamine [28] was determined and skin prick tests were performed (Soluprick SQ, ALK-Abelló, Hørsholm, Denmark). During the observation period the subjects fulfilled a cough diary [29] and measured three PEF values at least twice daily. In case of respiratory symptoms additional PEF values were measured and repeated 15 min after 0.2 mg of inhaled salbutamol (Buventol Easyhaler, Orion Ltd, Espoo, Finland).

On the second study day the subjects filled in a validated cough-specific quality-of-life questionnaire (Leicester Cough Questionnaire [30]) and a questionnaire focusing on possible associated symptoms (rhinitis, symptoms of gastroesophageal reflux disease, wheezing, etc.). The exhaled nitric oxide concentration was measured (Sievers Model 280 NOA; Sievers Instruments Inc., Boulder, CO, USA). After that, an incremental saline challenge was performed to document the cough threshold to hypertonicity. Eighteen patients underwent a second saline challenge within 2–14 days to study the repeatability of the response.

The incremental saline challenge with salbutamol pre-treatment

The hypertonic solutions were made by adjusting the sodiumchlorid (Natrii chloridum Ph.Eur., Tamro Ltd, Helsinki, Finland) content of standard phosphate-buffered saline [31] [osmolality 300 mOsm/kg, measured by freezing-point depression (The AdvancedTM Micro Osmometer 3300-BR, Advanced Instruments Inc., Norwood, MA,

Table 1. Characteristics of the subjects

Characteristics	Healthy (N = 19)	Cough, no asthma (N = 21)	Cough and asthma (N = 26)	P-value
Age (years)*	48.6 ± 8.6	48.4 ± 12.3	53.1 ± 13.1	0.31
Female sex – number (%)	11 (57.9)	13 (61.9)	19 (73.1)	0.53
Body mass index (kg/m ²)	25.3 ± 2.9	26.9 ± 3.3	29.3 ± 5.4	0.008
Ever smoker – number (%)	10 (52.6)	7 (33.3)	11 (42.3)	0.47
Atopy – number (%)	3 (16.7)	7 (33.3)	7 (26.9)	0.50
Duration of cough (months)		45.7 ± 37.0	72.2 ± 69.4	0.12
Presence of chronic rhinitis – number (%)		13 (61.9)	15 (57.7)	0.77
Presence of symptoms of gastroesophageal reflux disease – number (%)		3 (14.3)	9 (34.6)	0.11
Global Initiative for Asthma severity classification – number (%)			Mild persistent 5 (19.2) Moderate persistent 21 (80.8)	
Treatment with inhaled corticosteroids – number (%)			18 (69.2)	
FEV ₁ (% predicted) [†]	101.9 ± 10.7	98.1 ± 14.4	94.5 ± 17.0	0.26
Increase in FEV ₁ after 0.4 mg of salbutamol (%)	3.1 ± 3.2	2.8 ± 3.5	6.1 ± 5.5	0.02
Exhaled air nitric oxide (p.p.m.)	16.1 ± 6.0	21.6 ± 11.4	23.8 ± 24.0	0.31
Histamine PD ₁₅ (mg)		1.43 ± 3.23	0.69 ± 5.10	0.09
Maximal PEF variation (%)		9.29 ± 4.74	14.9 ± 9.0	0.01
Leicester Cough Questionnaire total points [‡]		85.0 ± 17.9	99.2 ± 20.8	0.02
Cough diary mean score [§]		1.35 ± 0.61	1.42 ± 0.85	0.74

*The ± values are means ± SD.

[†]The predicted values are from Viljanen et al. [34].

[‡]A small number in Leicester Questionnaire indicates a great impact of cough on quality of life.

[§]A large cough diary score indicates frequent coughing.

FEV₁, forced expiratory volume in 1 s; PD₁₅, provocative dose of histamine to induce a 15% fall in FEV₁; PEF, peak expiratory flow.

USA)]. Phosphate buffer was used to avoid acidity, a known tussigenic stimulus [32, 33]. The pH values of the solutions were 7.00–7.63 (measured by potentiometric method, Mettler Toledo 320 pH metre, Mettler-Toledo Ltd, Leicester, UK). A high-output ultrasonic nebulizer (DeVilbiss Ultraneb 3000, Sunrise Medical Ltd, West Midlands, UK) with a one-way valve (Douglas Bag One-Way Air Valve, Harvard Apparatus, Holliston, MA, USA) was used. The sessions were video recorded to enable later cough counting.

First, spirometry was performed three times (Model M9449, Medikro Ltd, Kuopio, Finland) and the largest FEV₁ was recorded. Then the subject inhaled four puffs of 0.1 mg salbutamol (Ventoline Evohaler, GlaxoSmithKline Ltd, Stockley Park West, Uxbridge, Middlesex, UK) using a Volumatic chamber and the spirometry was repeated 15 min thereafter. The percentage increase in FEV₁ by salbutamol was recorded. After that the subject inhaled isotonic phosphate-buffered saline for 2 min using tidal breathing, wearing a nose clip. The subjects were free to interrupt the inhalation in case of cough. The coughs occurring during the inhalation and 2 min after it were counted up. Subsequently, they similarly inhaled solutions with osmolalities of 600, 900, 1200, 1500, 1800, and 2100 mOsm/kg. The challenge was stopped if a solution had provoked at least 20 coughs. Finally, the spirometry was repeated.

Non-permitted medications

All cough-relieving drugs, leukotriene antagonists, and antihistamines were stopped at least 3 days before the study. Short-acting β-2 adrenergic drugs were withdrawn for at least 8 h before the challenges. The asthmatic patients were allowed to use their inhaled corticosteroids and long-acting β-2 adrenergic drugs throughout the study but asked not to take them on the study days.

Statistical analysis

The occasional coughs provoked by the isotonic solution were counted up. This number was subtracted from the coughs provoked by each hypertonic solution. After that, osmolalities to provoke non-cumulative 5, 10, and 15 coughs (C5, C10, and C15) and cumulative 15, 20, and 25 coughs (CUM15, CUM20, and CUM25) were determined by linear interpolation. If the determined amount of coughs were not provoked even after the final solution, an arbitrary value of 2400 mOsm/kg was used for statistical analysis. A suitable outcome variable was then chosen on the basis of its ability to separate the three groups (Kruskal–Wallis and Mann–Whitney tests) and its repeatability (intraclass correlation coefficient).

To determine the independent variables associated with the chosen outcome variable several univariate analyses

of variance were performed considering the following variables: age, sex, body mass index, current and ever smoking, atopy, duration of cough, presence of asthma, presence of chronic rhinitis, presence of symptoms of gastroesophageal reflux disease, usage of inhaled corticosteroids, Global Initiative for Asthma severity classification, pre-challenge FEV₁ as percent of predicted [34], percentage increase in FEV₁ after salbutamol, saline challenge-induced change in FEV₁, exhaled nitric oxide concentration, histamine provocative dose to induce a 15% fall in FEV₁ as a log-transformed value, Leicester questionnaire total point, cough diary mean score, and maximal spontaneous PEF variation in the ambulatory recording. Only those independent variables that showed at least suggestive association with the outcome variable ($P < 0.2$) were included in the MANOVA with backward selection procedure. However, due to the problem of multicollinearity, the interrelationships of the possible independent variables were analysed utilizing Pearson's correlation coefficient. If it was larger than 0.5, only the variable showing closer association with the outcome variable was included in the final model. After the multivariate analysis, the difference of the distribution of the residuals from the normal distribution was analysed with one-sample Kolmogorov–Smirnov test.

In addition, ANOVA, paired and unpaired *t*-tests, and χ^2 tests were used. The Bonferroni correction was applied in the case of multiple comparisons. The data were analysed with SPSS software version 14.0.1.

Results

In all groups, the FEV₁ after the challenge was larger than that before the challenge ($P < 0.002$). The challenge did not induce statistically significant changes to the post-salbutamol FEV₁ in any group (Fig. 1). The subjects

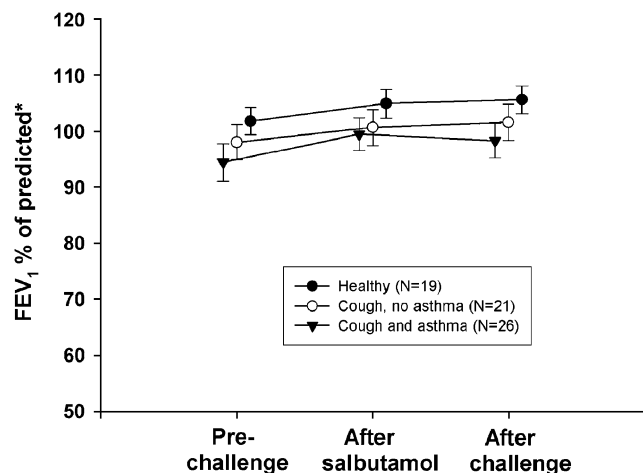


Fig. 1. The forced expiratory volume (FEV₁) values (means and standard errors) during the incremental saline challenge with salbutamol pre-treatment. *The predicted values are from Viljanen et al. [34].

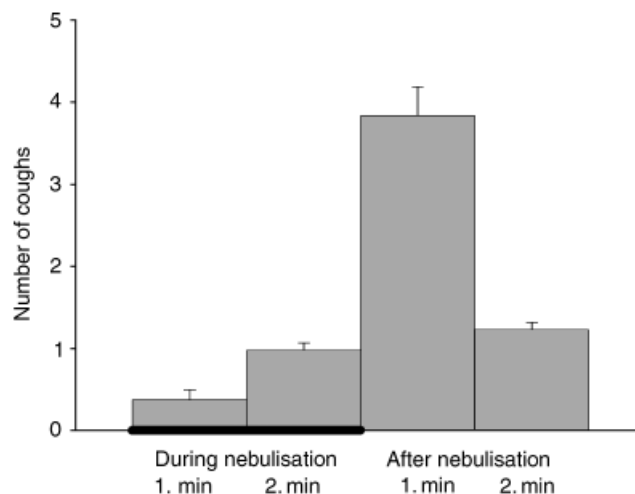


Fig. 2. The number of provoked coughs (means and standard errors) at each stage of all hypertonic saline nebulisations in the 47 patients with cough. The bold line on the time axis indicates the duration of nebulization.

tolerated the challenges well and rarely were forced to interrupt the inhalations due to cough. Saline-provoked cough usually appeared during the first minute after the inhalation, then ceasing (Fig. 2). However, some subjects continued to cough several minutes.

The asthmatic patients with cough started to cough at considerably lower osmolalities than the other subjects whereas the majority of the healthy subjects did not cough at all (Fig. 3). The ability of the various outcome variables to separate the three groups was generally the better the smaller was the required amount of coughs. In this respect, the best variable was the osmolality to provoke five non-cumulative coughs (C5, Fig. 4a). However, its repeatability was the worst (intraclass correlation coefficient of 0.51). By increasing the required amount of coughs the repeatability of the index increased. The osmolality to provoke 15 cumulative coughs (CUM15) was chosen as the final outcome variable due to its rather good ability to separate the groups (Fig. 4b) and its good repeatability (intraclass correlation coefficient of 0.90. The mean $\pm$ SE of CUM15 values of the first and the second challenge among the 18 patients who underwent two challenges were 1095 ± 146 and 1007 ± 122 mOsm/kg, $P = 0.30$).

The CUM15 values were significantly smaller in the asthmatic cough patients than in the healthy subjects (mean $\pm$ SE of CUM15 values 1380 ± 146 vs. 2270 ± 71 mOsm/kg, $P < 0.001$, Mann–Whitney test with Bonferroni's correction, Fig. 3) and in the non-asthmatic patients with cough (1380 ± 146 vs. 1864 ± 124 mOsm/kg, $P = 0.04$). The number of subjects demonstrating a measurable CUM15 value was 3/19 (16%) of the healthy subjects, 12/21 (57%) of the non-asthmatic cough patients, and 19/26 (73%) of the asthmatic cough patients ($P = 0.001$).

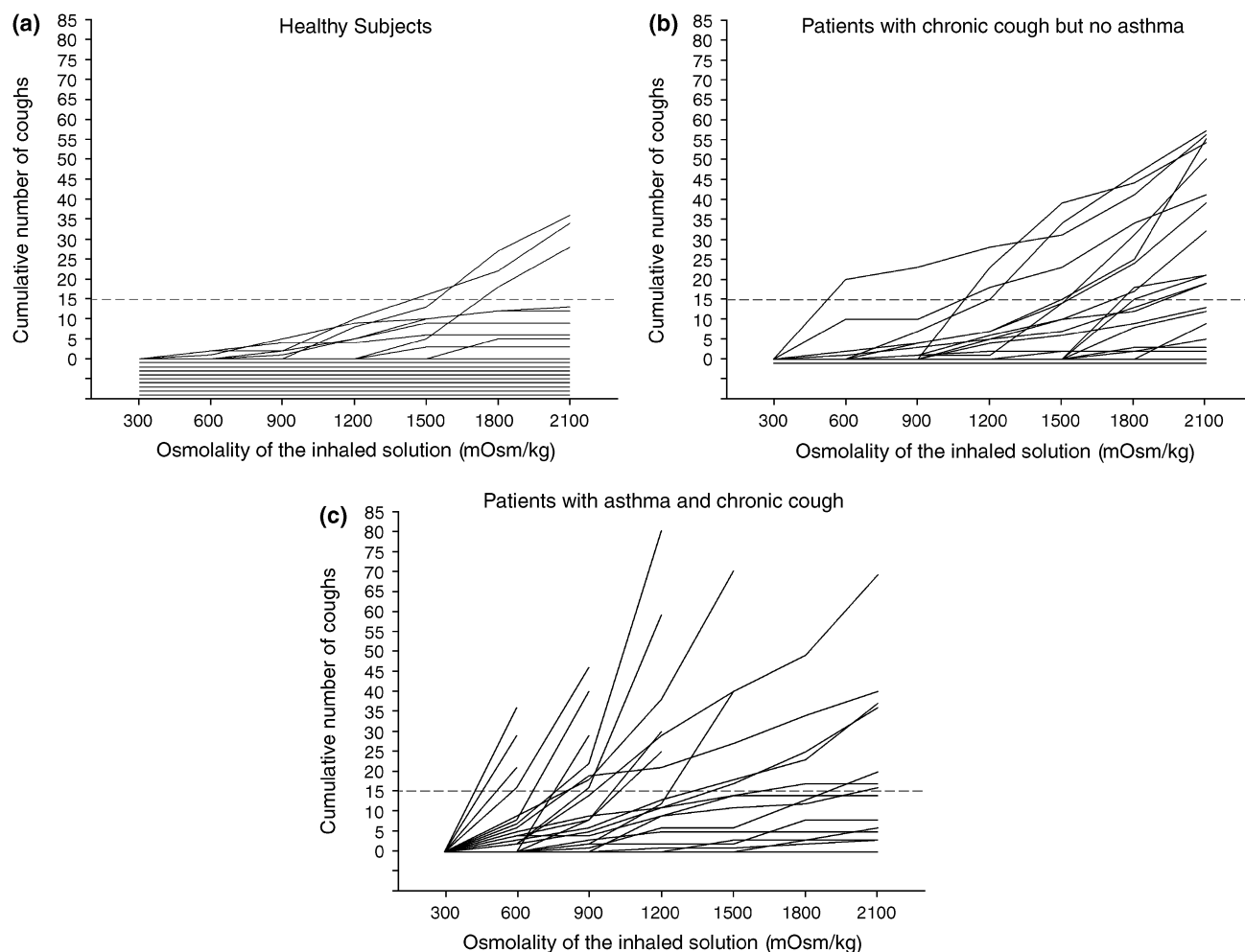


Fig. 3. The cumulative number of coughs evoked by hypertonic saline challenge in each healthy subject (a), patient with chronic cough but without asthma (b), and asthmatic patient with chronic cough (c). The dash line indicates 15 cumulative coughs. The horizontal lines at or below zero indicate subjects who did not cough at all.

In the multivariate analysis, female sex and large spontaneous PEF variation in the ambulatory recording were significantly associated with a small CUM15. This was true both if all the 47 cough patients were included ($P < 0.001$ and $P = 0.001$, respectively) or if only the 26 asthmatic patients with cough were included ($P = 0.002$ and $P = 0.01$, respectively). The residuals after both analyses showed close-to-normal distribution ($P = 0.50$ – 0.58 , Kolmogorov–Smirnov test).

Discussion

The present study demonstrated that asthmatic patients with chronic cough are more sensitive to the cough-provoking effect of hypertonic saline than healthy subjects and non-asthmatic patients with chronic cough. This was demonstrated in the absence of induced bronchoconstriction, as documented by FEV₁ measurements. The enhanced sensitivity to saline was associated

with female sex and with spontaneous, reversible airway obstruction, as documented by ambulatory PEF measurements.

Hypertonic saline induces bronchoconstriction in asthmatic, but not in healthy subjects [35] and bronchoconstriction is a well-known stimulus for cough [24, 36]. In the present study, a pre-treatment with 0.4 mg of salbutamol effectively inhibited bronchoconstriction in all groups and the demonstrated differences in saline cough sensitivity between the groups cannot be explained by differences in the level of induced bronchoconstriction. Thus, the enhanced cough sensitivity to saline in asthma seems to reflect a true dysfunction of the sensorineural apparatus. However, one potential source of error in the present study is a possible induction of small airways constriction as FEV₁ mainly measures large airway calibre [37]. The β_2 -agonist salbutamol was chosen as pre-medication due to its proven ability to inhibit hypertonic saline-induced bronchoconstriction [38] and because

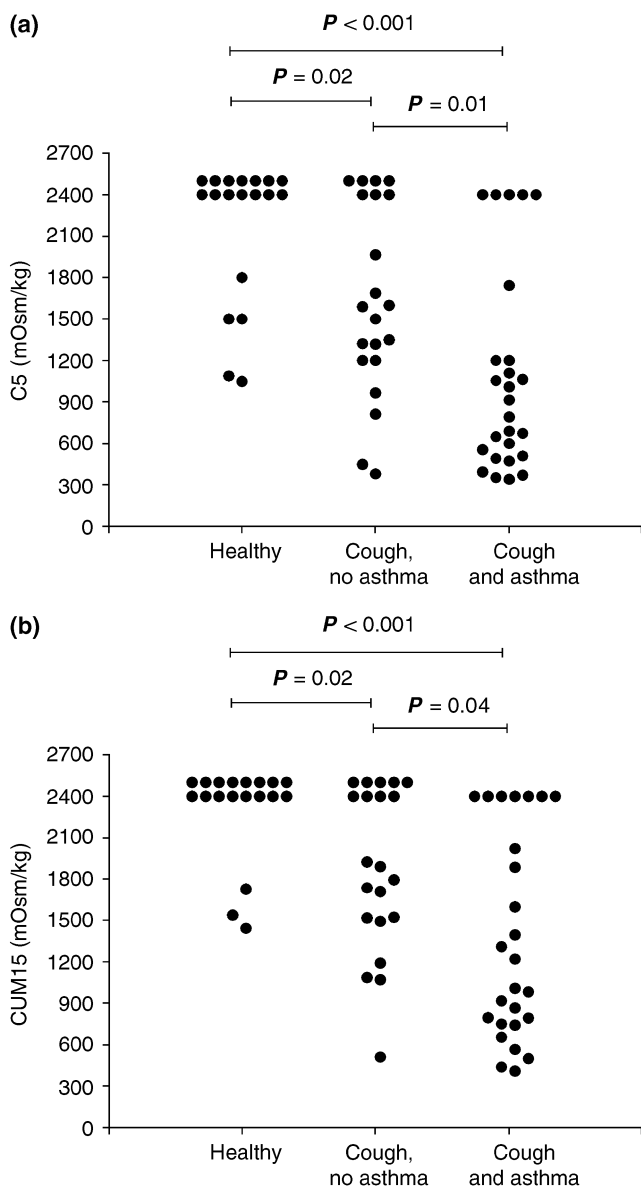


Fig. 4. The osmolality values to provoke five non-cumulative coughs (C5, a) and 15 cumulative coughs (CUM15, b).

inhaled β_2 -agonists do not have a significant effect on cough receptor function in man [39–41].

The statistically very significant difference in the saline sensitivity between healthy and asthmatic subjects is in sharp contrast to the studies using capsaicin and acid cough stimulants that have repeatedly failed to show respective differences [9–17]. Capsaicin and acids both activate directly a major sensory receptor for cough, the transient receptor potential vanilloid subfamily member 1 (TRPV1) [42]. These receptors are typical for nociceptors, i.e., vagal sensory nerves with cell bodies in the jugular ganglion [32]. TRPV1 can also be activated by a high concentration of extracellular Na^+ [43]. However, capsa-zepine, a TRPV1 antagonist, inhibits cough provoked by capsaicin and citric acid in guinea-pigs but does not affect

that provoked by hypertonic saline [44] suggesting different mechanisms of action. Neither do our results support direct TRPV1 activation by saline. The cough response to hypertonic saline typically started after the 2-min inhalations. This is in striking contrast to the cough response to capsaicin, which usually appears during or immediately after the first or second inhalation, and diminishes considerably after the first 30 s of inhalation [45]. The demonstrated delayed onset of cough after saline may indicate an indirect stimulation of TRPV1 or other cough receptors.

An indirect stimulation of TRPV1 by saline could explain the large difference in saline cough sensitivity between healthy and asthmatic subjects. Hypertonicity may induce a pathological series of events in the diseased mucosa that finally leads to a stimulation of TRPV1 or other cough receptors. The fact that asthmatic patients usually respond normally to direct TRPV1 stimulants [9–17] indicates that the receptors themselves function normally. We have previously shown that nasal hypertonic challenge with mannitol is associated with a release of 15-hydroxyeicosatetraenoic acid to nasal lavage fluid in subjects with allergic rhinitis, but not in healthy subjects [46]. Interestingly, 15-hydroxyeicosatetraenoic acid is one of the most potent endogenous activators of TRPV1 [42, 47].

From the clinical point of view the significant difference in saline sensitivity between the asthmatic and non-asthmatic cough patients was interesting. However, as there is no 'gold standard' for the diagnosis of asthma, all asthma definitions are more or less arbitrary. It is possible that some of our 'non-asthmatic cough patients' actually had mild asthma based on the common co-existence of dyspnoea and/or wheezing in addition to cough. On the contrary, the finding that the sensitivity to saline was strongly associated with spontaneous, reversible airway obstruction is free from definition bias. Of note, reversible airways obstruction is one of the most classical functional abnormalities in asthma.

The lack of association between the inflammation index nitric oxide and saline cough sensitivity was unexpected since in our previous study the cough sensitivity to inhaled mannitol decreased during an anti-inflammatory treatment with inhaled glucocorticoids [23]. Most asthmatic patients used inhaled corticosteroids in the present cross-sectional study, which may have blunted the possible association. The lack of association between histamine responsiveness and saline cough sensitivity corroborates that airway hyperresponsiveness and cough sensitivity to hypertonic aerosols are two separate phenomena [23, 24].

Women with chronic cough were significantly more sensitive than men to the cough-provoking effect of hypertonic saline. Accordingly, women are more sensitive than men to capsaicin and acid cough stimulants [48–51]. The reason for this sex difference in cough sensitivity to such a wide range of agents is unknown [50].

Can the present results be applied to asthmatic population in general as the asthmatic patients were selected on the basis of chronic cough? Cough is a very common symptom in asthma [8] and therefore the selection bias may not be large. Furthermore, almost all reported dyspnoea or wheezing besides cough, and all had showed features of variable airway obstruction at the time of diagnosis. Therefore, they do not represent patients with cough variant asthma [8] but classic asthma.

In conclusion, the present study has demonstrated that even when the induction of bronchoconstriction is prevented, asthmatic patients show an enhanced sensitivity to the cough-provoking effect of hypertonic saline. This sensitivity is associated with one of the most classical functional abnormalities of asthma, namely spontaneous, reversible airway obstruction. To the best of the authors' knowledge, this is the first direct proof that asthma is associated with a pathological function of the sensorineural apparatus, similarly to its upper airway counterpart, chronic rhinitis [2–6].

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References

- 1 Togias A. Rhinitis and asthma: evidence for respiratory system integration. *J Allergy Clin Immunol* 2003; 111:1171–83, quiz 1184.
- 2 Sanico AM, Philip G, Proud D, Naclerio RM, Togias A. Comparison of nasal mucosal responsiveness to neuronal stimulation in non-allergic and allergic rhinitis: effects of capsaicin nasal challenge. *Clin Exp Allergy* 1998; 28:92–100.
- 3 Sanico AM, Philip G, Lai GK, Togias A. Hyperosmolar saline induces reflex nasal secretions, evincing neural hyperresponsiveness in allergic rhinitis. *J Appl Physiol* 1999; 86:1202–10.
- 4 Sanico AM, Koliatsos VE, Stanis AM, Bienenstock J, Togias A. Neural hyperresponsiveness and nerve growth factor in allergic rhinitis. *Int Arch Allergy Immunol* 1999; 118:154–8.
- 5 Wu X, Myers AC, Goldstone AC, Togias A, Sanico AM. Localization of nerve growth factor and its receptors in the human nasal mucosa. *J Allergy Clin Immunol* 2006; 118:428–33.
- 6 Sarin S, Undem B, Sanico A, Togias A. The role of the nervous system in rhinitis. *J Allergy Clin Immunol* 2006; 118:999–1016.
- 7 Irwin RS, Boulet LP, Cloutier MM *et al.* Managing cough as a defense mechanism and as a symptom. A consensus panel report of the American college of chest physicians. *Chest* 1998; 114:33S–81S.
- 8 Dicpinigaitis PV. Chronic cough due to asthma: ACCP evidence-based clinical practice guidelines. *Chest* 2006; 129:75S–9S.
- 9 Bickerman HA, Barach AL. The experimental production of cough in human subjects induced by citric acid aerosols. Preliminary studies on the evaluation of antitussive agents. *Am J Med Sci* 1954; 228:156–63.
- 10 Pounsford JC, Birch MJ, Saunders KB. Effect of bronchodilators on the cough response to inhaled citric acid in normal and asthmatic subjects. *Thorax* 1985; 40:662–7.
- 11 Fujimura M, Sakamoto S, Kamio Y, Matsuda T. Cough receptor sensitivity and bronchial responsiveness in normal and asthmatic subjects. *Eur Respir J* 1992; 5:291–5.
- 12 Chang AB, Phelan PD, Sawyer SM, Del Brocco S, Robertson CF. Cough sensitivity in children with asthma, recurrent cough, and cystic fibrosis. *Arch Dis Child* 1997; 77:331–4.
- 13 Schmidt D, Jorres RA, Magnussen H. Citric acid-induced cough thresholds in normal subjects, patients with bronchial asthma, and smokers. *Eur J Med Res* 1997; 2:384–8.
- 14 Fujimura M, Kamio Y, Hashimoto T, Matsuda T. Airway cough sensitivity to inhaled capsaicin and bronchial responsiveness to methacholine in asthmatic and bronchitic subjects. *Respirology* 1998; 3:267–72.
- 15 Millqvist E, Bende M, Lowhagen O. Sensory hyperreactivity – a possible mechanism underlying cough and asthma-like symptoms. *Allergy* 1998; 53:1208–12.
- 16 Di Franco A, Dente FL, Giannini D *et al.* Effects of inhaled corticosteroids on cough threshold in patients with bronchial asthma. *Pulm Pharmacol Ther* 2001; 14:35–40.
- 17 Prudon B, Birring SS, Vara DD, Hall AP, Thompson JP, Pavord ID. Cough and glottic-stop reflex sensitivity in health and disease. *Chest* 2005; 127:550–7.
- 18 Fujimura M, Kamio Y, Hashimoto T, Matsuda T. Cough receptor sensitivity and bronchial responsiveness in patients with only chronic nonproductive cough: in view of effect of bronchodilator therapy. *J Asthma* 1994; 31:463–72.
- 19 Cho YS, Lee CK, Yoo B, Moon HB. Cough sensitivity and extrathoracic airway responsiveness to inhaled capsaicin in chronic cough patients. *J Korean Med Sci* 2002; 17:616–20.
- 20 Fujimura M, Ogawa H, Nishizawa Y, Nishi K. Comparison of atopic cough with cough variant asthma: is atopic cough a precursor of asthma? *Thorax* 2003; 58:14–8.
- 21 Fujimura M, Nishizawa Y, Nishitsuji M, Abo M, Kita T, Nomura S. Longitudinal decline in pulmonary function in atopic cough and cough variant asthma. *Clin Exp Allergy* 2003; 33:588–94.
- 22 Irwin RS, Corrao WM, Pratter MR. Chronic persistent cough in the adult: the spectrum and frequency of causes and successful outcome of specific therapy. *Am Rev Respir Dis* 1981; 123:413–7.
- 23 Koskela HO, Hyvarinen L, Brannan JD, Chan HK, Anderson SD. Coughing during mannitol challenge is associated with asthma. *Chest* 2004; 125:1985–92.
- 24 Koskela HO, Kontra KM, Purokivi MK, Randell JT. Interpretation of cough provoked by airway challenges. *Chest* 2005; 128:3329–35.
- 25 Koskela HO, Martens R, Brannan JD, Anderson SD, Leuppi J, Chan HK. Dissociation in the effect of nedocromil on mannitol-induced cough or bronchoconstriction in asthmatic subjects. *Respirology* 2005; 10:442–8.
- 26 Irwin RS, Baumann MH, Bolser DC *et al.* Diagnosis and management of cough executive summary: ACCP evidence-based clinical practice guidelines. *Chest* 2006; 129:1–23S.

- 27 American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive lung disease (COPD) and asthma. *Am Rev Respir Dis* 1987; **136**:225–44.
- 28 Sovijarvi AR, Malmberg LP, Reinikainen K, Ryttilä P, Poppius H. A rapid dosimetric method with controlled tidal breathing for histamine challenge. Repeatability and distribution of bronchial reactivity in a clinical material. *Chest* 1993; **104**:164–70.
- 29 Hsu JY, Stone RA, Logan-Sinclair RB, Worsdell M, Busst CM, Chung KF. Coughing frequency in patients with persistent cough: assessment using a 24 hour ambulatory recorder. *Eur Respir J* 1994; **7**:1246–53.
- 30 Birring SS, Prudon B, Carr AJ, Singh SJ, Morgan MD, Pavord ID. Development of a symptom specific health status measure for patients with chronic cough: Leicester Cough Questionnaire (LCQ). *Thorax* 2003; **58**:339–43.
- 31 Sterk PJ, Fabbri LM, Quanjer PH *et al*. Airway responsiveness. Standardized challenge testing with pharmacological, physical and sensitizing stimuli in adults. Official statement of the European Respiratory Society. *Eur Respir J* 1993; **6**:53–83.
- 32 Udem BJ, Carr MJ, Kollarik M. Physiology and plasticity of putative cough fibres in the guinea pig. *Pulm Pharmacol Ther* 2002; **15**:193–8.
- 33 Kollarik M, Udem BJ. Mechanisms of acid-induced activation of airway afferent nerve fibres in guinea-pig. *J Physiol* 2002; **543**:591–600.
- 34 Viljanen AA, Halttunen PK, Kreus K-E, Viljanen BC. Spirometric studies in non-smoking, healthy adults. *Scand J Clin Lab Invest* 1982; **42**:5–20.
- 35 Anderson SD, Smith CM. Osmotic challenges in the assessment of bronchial hyperresponsiveness. *Am Rev Respir Dis* 1991; **143**:S43–6.
- 36 Chausow AM, Banner AS. Comparison of the tussive effects of histamine and methacholine in humans. *J Appl Physiol* 1983; **55**:541–6.
- 37 Pellegrino R, Viegi G, Brusasco V *et al*. Interpretative strategies for lung function tests. *Eur Respir J* 2005; **26**:948–68.
- 38 Jones PD, Hankin R, Simpson J, Gibson PG, Henry RL. The tolerability, safety, and success of sputum induction and combined hypertonic saline challenge in children. *Am J Respir Crit Care Med* 2001; **164**:1146–9.
- 39 Eschenbacher WL, Boushey HA, Sheppard D. Alteration in osmolarity of inhaled aerosols cause bronchoconstriction and cough, but absence of a permeant anion causes cough alone. *Am Rev Respir Dis* 1984; **129**:211–5.
- 40 Nichol G, Nix A, Barnes PJ, Chung KF. Prostaglandin F₂ alpha enhancement of capsaicin induced cough in man: modulation by beta 2 adrenergic and anticholinergic drugs. *Thorax* 1990; **45**:694–8.
- 41 Fujimura M, Sakamoto S, Kamio Y, Bando T, Kurashima K, Matsuda T. Effect of inhaled procaterol on cough receptor sensitivity to capsaicin in patients with asthma or chronic bronchitis and in normal subjects. *Thorax* 1993; **48**:615–8.
- 42 Morice AH, Geppetti P. Cough. 5: the type 1 vanilloid receptor: a sensory receptor for cough. *Thorax* 2004; **59**:257–8.
- 43 Ahern GP, Brooks IM, Miyares RL, Wang XB. Extracellular cations sensitize and gate capsaicin receptor TRPV1 modulating pain signaling. *J Neurosci* 2005; **25**:5109–16.
- 44 Laloo UG, Fox AJ, Belvisi MG, Chung KF, Barnes PJ. Capsazepine inhibits cough induced by capsaicin and citric acid but not by hypertonic saline in guinea pigs. *J Appl Physiol* 1995; **79**:1082–7.
- 45 Midgren B, Hansson L, Karlsson JA, Simonsson BG, Persson CG. Capsaicin-induced cough in humans. *Am Rev Respir Dis* 1992; **146**:347–51.
- 46 Koskela H, Di Sciascio MB, Anderson SD *et al*. Nasal hyperosmolar challenge with a dry powder of mannitol in patients with allergic rhinitis. Evidence for epithelial cell involvement. *Clin Exp Allergy* 2000; **30**:1627–36.
- 47 Hwang SW, Cho H, Kwak J *et al*. Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. *Proc Natl Acad Sci USA* 2000; **97**:6155–60.
- 48 Fujimura M, Sakamoto S, Kamio Y, Matsuda T. Sex difference in the inhaled tartaric acid cough threshold in non-atopic healthy subjects. *Thorax* 1990; **45**:633–4.
- 49 Fujimura M, Kasahara K, Kamio Y, Naruse M, Hashimoto T, Matsuda T. Female gender as a determinant of cough threshold to inhaled capsaicin. *Eur Respir J* 1996; **9**:1624–6.
- 50 Kastelik JA, Thompson RH, Aziz I, Ojoo JC, Redington AE, Morice AH. Sex-related differences in cough reflex sensitivity in patients with chronic cough. *Am J Respir Crit Care Med* 2002; **166**:961–4.
- 51 Nieto L, de Diego A, Perpina M *et al*. Cough reflex testing with inhaled capsaicin in the study of chronic cough. *Respir Med* 2003; **97**:393–400.