

The capsaicin cough reflex in eczema patients with respiratory symptoms elicited by perfume

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Respiratory symptoms elicited by perfume are common in the population but have unclear pathophysiology. Increased capsaicin cough responsiveness has been associated with the symptoms, but it is unknown whether the site of the symptoms in the airways influences this association. The aim of this study was to investigate the association between the site of airway symptoms elicited by perfume and cough responsiveness to bronchial challenge with capsaicin. 21 eczema patients with respiratory symptoms elicited by perfume were compared with 21 healthy volunteers in a sex- and age-matched case control study. The participants completed a symptom questionnaire and underwent a bronchial challenge with capsaicin. Lower, but not upper, respiratory symptoms elicited by perfume were associated with increased capsaicin cough responsiveness. Having severe symptoms to perfume ($n = 11$) did not relate to the site of the symptoms in the airways and was not associated with increased capsaicin cough responsiveness. In conclusion, respiratory symptoms elicited by perfume may reflect local hyperreactivity related to defensive reflexes in the airways, and measurements of the capsaicin cough reflex are relevant when patients with lower respiratory symptoms related to environmental perfume exposures are investigated.

Key words: capsaicin; cough; perfume; sensory hyperreactivity; sensory irritation. © Blackwell Munksgaard, 2006.

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Respiratory symptoms elicited by perfume and fragrance products are common in the general population. The mucosal sensations related to fragrances are various, mostly localized in the upper airways and less frequent in the lower airways. Although most people may experience such symptoms as mild and not bothersome, there are also many who report severe symptoms (1).

The pathophysiological mechanisms of respiratory symptoms elicited by perfume are unknown. In a population-based study positive associations were found between the symptoms and the bronchial obstruction reflex triggered by methacholine (1); the symptoms were also associated with hand eczema, and perfume contact allergy, but not with IgE mediated allergy to proteins. These associations were independent of sex, age, and psychological vulnerability, and suggest a link between skin and airways, e.g. tissue inflammation (1, 2). During tissue inflammation, sensory nerve activation is altered, which may lower the response threshold to some chemical stimuli that challenge airway defensive reflexes, e.g. chemicals triggering the cough reflex (3, 4). Cough is a normal indispensable reflex that is responsible for protecting

the airways from potentially harmful substances. Inhalation of capsaicin (the pungent principle in hot chilli pepper) stimulates the afferent C-fibres and A δ -fibres in the airways (4) and triggers the cough reflex in a dose dependant way in normal individuals (5). In nonasthmatic patients with lower respiratory symptoms elicited by perfume, increased capsaicin cough responsiveness has been observed, suggesting an underlying sensory hyperreactivity of the airways (6). However, it is unknown whether the site of the symptoms in the airways influences this association.

The aim of this study was to investigate the association between the site of perfume related symptoms in the airways and the bronchial cough responsiveness to capsaicin in eczema patients with respiratory symptoms elicited by perfume.

Materials and Methods

Study subjects and design

The study population consisted of 21 eczema patients attending a dermatological outpatient clinic for patch testing, and 21 control subjects.

The patients were recruited consecutively according to the inclusion criterion of having at least one respiratory symptom elicited by perfume. The patients were stratified according to patch-test results, 50% had contact allergy to perfume. A total of 22 patients was included, but one dropped out. The controls [healthy volunteers (HV)] were recruited by advertising and were selected to match the cases concerning sex and age. Exclusion criteria for the controls were atopic airway disease, eczema, smoking and respiratory symptoms elicited by perfume. All participants completed a symptom questionnaire and underwent lung function test, skin prick test (SPT) and bronchial challenge with capsaicin.

Questionnaire 1. The participant filled in a symptom questionnaire about localization and severity of the symptoms elicited by perfume before the capsaicin testing. The validation of the questionnaire has previously been described (1).

Questionnaire 2. Before the capsaicin test and during the 4-min intervals between inhalations, the participants rated 17 different symptoms according to their presence and severity.

Capsaicin challenge test

The capsaicin cough responsiveness was measured with a modification of the method described by Millqvist et al. (6). A stock solution of capsaicin (UNIKEM, Copenhagen, Denmark) 100 µmol/l was prepared. Aqueous solutions of 0.4, 2.0 and 10 µmol/l were made from the same stock solution of capsaicin by dilution with 0.9% saline containing 1% by volume of ethanol. Participants inhaled 1 ml of isotonic saline from a Paryboy® PARI LC PLUS nebulisor (Pari-Werk, Starnberg, Germany), breathing in tidal volume for 6 min, followed by 4 min of room-air breathing at ambient temperature and humidity. The particle median mass diameter was 3.5 µm, with at least 68% of the particles smaller than 5 µm. The nebulizers were operated by a Pari Turbobooy® N compressor (Pari-Werk). Subsequently, participants were provoked in the same way with incremental concentrations of capsaicin 0.4, 2.0 and 10 µM in 1-ml solutions. Lung function was measured before the saline provocation and after the end of inhalation of the last dose of capsaicin. The number of coughs during and 10 min after each dose of capsaicin was registered with a tape recorder. The capsaicin inhalation test was terminated if the number of coughs during and after one provocative dose exceeded fifty. Participants were instructed to withhold bronchodilator medication for 12 h

and antihistamines for 72 h and not to smoke for at least 4 h before the study visit. The capsaicin test was not carried out during or within 4 weeks after a respiratory tract infection. The cough responsiveness was expressed as the cumulative number of coughs during inhalation of three doses of capsaicin. Participants were instructed to focus on subjective symptoms during the capsaicin challenge test and were not informed that the number of coughs was the objective outcome-measurement of the test.

Lung function test

Forced expiratory volume in the first second (FEV₁) was measured with a dry bellow spirometer (Vitalograph®, Vitalograph Ltd, Buckingham, UK) (highest of 3 measurements within 100 ml). The spirometer was checked each study day with a 1-l calibration syringe (7).

SPT

The Soluprick SQ® system (ALK-Abelló, Hørsholm, Denmark) was used for the SPT. The panel of allergens comprised birch, grass (timothy), mugwort, horse, cat, dog and mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*). A negative control (diluent) and a positive control (10 mg/ml histamine) were included. A positive SPT was defined as giving a mean wheal diameter of 3 mm or more.

Patch test

Patch testing was performed with haptens from the European Standard Series by using Finn Chambers® (Epitest, Tuusula, Finland) on Scanpor® tape (Alpharma AS, Norgesplaster Facility, Vennesla, Norway). Test material was applied to the upper back and removed after 2 days, and reactions were recorded on days 2, 3 and 7. Reactions were classified according to international guidelines (8).

Definition of variables for analysis

The symptom localization was reported in *Questionnaire 1*. It was registered whether there had been at least one symptom, elicited by fragrance products, from the eyes, nose, mouth (including throat) and lungs, respectively, within the last 12 months.

Severe symptoms were defined as symptoms affecting daily life activities (i.e. influencing choice of shopping places, means of transportation or adjustments of working situations) and/or social

life activities (i.e. participation in social arrangement, celebrations or going to the theatre).

The median number of coughs during inhalation of 0.4, 2.0 and 10 μM capsaicin was used to express the cough reactivity to capsaicin.

A dichotomized outcome defined as a positive capsaicin challenge test was based on ROC curves for lower respiratory symptoms elicited by fragrance products, giving the highest possible sensitivity and specificity at 82 and 87%, respectively. Cut-values at 10, 20 and 45 coughs were selected, corresponding to inhalation of 0.4, 2.0 and 10 μM capsaicin, respectively. When the number of coughs was above one cut value, the capsaicin challenge test was regarded as positive.

SPT reactivity was defined as a positive SPT to at least one of the allergens in the panel. Participants who reacted to the negative control with a wheal greater than 3 mm were excluded from analysis where SPT was involved.

FEV₁ values were expressed as percentages of predicted value (9).

Smoking status was expressed as either present smoking, past or never smoking. Pack years of smoking were calculated in smokers and ex-smokers as: (years of smoking $\times$ daily cigarette consumption)/20.

Perfume contact allergy was defined as a positive patch test to one or more of the 3 test substances: fragrance mix, balsam of Peru and/or colophony (2).

Asthma and allergic rhinitis was defined as previously described (1).

Analysis

Statistical analyses were performed with SPSS version 12.0 for Windows. The χ^2 test and the Mann–Whitney *U*-test or Kruskal–Wallis *H*-test were used to compare the groups. The association of having a positive capsaicin challenge test and lower respiratory symptoms was further investigated in a

multivariate logistic regression model. Pack years of smoking and the cumulative number of respiratory symptoms were included in the model. Statistical significance was defined as $P < 0.05$.

Results

Table 1 shows the basic characteristics of the participants. All patients ($n = 21$) had at least one upper respiratory and/or eye symptom elicited by perfume within the last 12 months, and 11 patients (54%) also had lower respiratory symptoms (LRS) (Table 2). Thus, 10 patients had only upper respiratory and/or eye symptoms (URS). LRS comprised troublesome breathing ($n = 11$), chest tightness ($n = 6$), coughing ($n = 5$), wheezing ($n = 5$) and phlegm when coughing ($n = 4$). 9 of 11 individuals with LRS reported at least 2 of these symptoms.

In the capsaicin challenge test, the median number of coughs was significantly higher during each of the 3 inhalation series in the patient group compared with the group of HV. However, only slight and non-significant increases in the cough responsiveness were found between the patients reporting URS and the HV (Fig. 1). LRS were significantly associated with capsaicin cough responsiveness in each of the 3 inhalation series compared with both the group with URS and the HV. Reporting severe symptoms to perfume ($n = 11/21$) was not associated with either LRS or increased capsaicin cough responsiveness.

The mean number of pack years was significantly higher, and FEV₁ significantly decreased in individuals with LRS. No differences in mean age or sex were found between the group with URS and those reporting LRS.

A significant and positive association was found between a positive capsaicin challenge test and the number of pack years, as well as the cumulative number of respiratory symptoms related to perfume exposure. No association was

Table 1. Basic characteristics for cases and controls

	Patients with eczema ($n = 21$)	Healthy volunteers ($n = 21$)
Mean age, years (range) ^a	50.5 (38–68)	48.1 (22–70)
Women/men ^a	15/6 (75.6)	15/6 (75.6)
Contact allergy (percentage of total) ^b	10 (47.6)	–
FEV ₁ % predicted (range)	94.3 (69–122) ^c	103.2 (83–127) ^c
At least one positive skin prick test (percentage of total)	8 (38)	2 (9.5)
Allergic asthma (percentage of total) ^b	4 (19.1)	–
Non-allergic asthma (percentage of total) ^b	1 (5)	–
Allergic rhinitis (percentage of total) ^b	6 (28.7)	–
Present smokers (percentage of total) ^b	6 (28.6)	–
Past smokers (percentage of total)	11 (53.7)	8 (42.1)
Mean pack years (range)	13.8 (0–60) ^c	1 (0–5.2) ^c

^aMatching criteria for the healthy volunteers.

^bExclusion criteria for the healthy volunteers.

^cThe difference between the patients and healthy volunteers was statistically significant ($P < 0.05$).

Table 2. Characteristics of symptoms elicited by perfume, and the relation between localization of the various symptoms and a positive capsaicin challenge test

	Total (%) (<i>n</i> = 21)	URS ^b (%) (<i>n</i> = 10)	LRS ^c (%) (<i>n</i> = 11)
Localization			
Symptoms from the eyes elicited by perfume	20 (95) ^a	9 (90)	11 (100) ^a
Symptoms from the nose elicited by perfume	20 (95) ^a	9 (90)	11 (100) ^a
Symptoms from the mouth or throat elicited by perfume	13 (61) ^a	6 (60)	7 (64) ^a
Lower respiratory symptoms elicited by perfume	11 (52) ^a	–	11 (100) ^a
Time relations			
Age at début (range)	35 (10–58)	32 (12–58)	38 (10–57)
Symptoms occur within minutes after exposure	21 (100) ^a	10 (100)	11 (100) ^a
Symptoms occur at least once a week	14 (76) ^a	5 (50)	9 (82) ^a
Symptoms could persist for >24 h after elicitation	4 (19) ^a	2 (20)	2 (18) ^a
Elicitors			
Other persons' wearing of perfume	21 (100) ^a	10 (100)	11 (100) ^a
Air-fresheners	17 (81)	8 (80)	9 (82) ^a
Other persons' wearing of newly washed clothes	14 (67)	6 (60)	8 (73) ^a
Consequence			
Daily life activities were limited because of the symptoms ^d	11 (52) ^a	4 (40)	7 (64) ^a
Social life activities were limited because of the symptoms ^e	6 (29)	2 (20)	4 (36) ^a
Other symptoms elicited by perfume			
Symptoms with sensory character ^f	19 (90) ^a	8 (80)	11 (100) ^a
Perfume contact allergy ^g	10 (48) ^a	5 (50)	5 (55) ^a
Other eliciting agents			
Chemicals other than perfume ^h	19 (90) ^a	8 (80)	11 (100) ^a
Airborne proteins ⁱ	11 (52) ^a	5 (50)	6 (55) ^a

^a χ^2 test was significant ($P < 0.05$) comparing a positive capsaicin challenge test between the group in the row and the group of healthy volunteers ($n = 21$).

^bOnly upper respiratory symptoms (URS) elicited by perfume.

^cLower respiratory symptoms (LRS) elicited by perfume.

^di.e. choice of shopping places, means of transportation or adjustments of working situations were influenced by the symptoms.

^ei.e. participation in social arrangement, celebrations or going to the theatre were limited because of the symptoms.

^fHeadache, nausea, fatigue, trouble concentrating.

^gPositive patch test to fragrance mix, colophony or balsam of Peru.

^hEye or airway symptoms elicited by at least one of newsprint, drying paint, car exhaust or laser printers.

ⁱEye or airway symptoms elicited by at least one of furry animals, pollen, dust or mould.

found between a positive capsaicin challenge test and FEV₁% of predicted values. In logistic regression analysis, the association between capsaicin responsiveness and LRS was still significant when pack years and the cumulative number of respiratory symptoms related to perfume exposure were included.

The overall frequency of the symptoms provoked by capsaicin inhalation was significantly higher in individuals with LRS compared with the groups with no symptoms or URS elicited by perfume (Fig. 2). Similarly, the overall frequency of the symptoms released by capsaicin was significantly higher in the group with URS than in the group of HV.

In all individuals, the FEV₁ was unaffected by bronchial challenges with capsaicin ($n = 42$).

Discussion

Lower, but not upper, respiratory symptoms elicited by perfume were associated with increased capsaicin cough responsiveness. Despite normal

cough responsiveness to capsaicin, the patients with URS reported significantly more symptoms due to capsaicin challenge than the group of healthy control persons, suggesting that, nevertheless, they were more sensitive to capsaicin (Fig. 2).

Recent studies have dealt with selected groups of patients mainly with LRS. The distribution of symptoms, sex and age in the present study was not significantly different from that of a population-based sample (1). This representativeness of the patients might be explained by the fact that the patients in the present study were resigned to the dermatological outpatient clinic because of eczema and not because of respiratory symptoms elicited by perfume.

The model of capsaicin cough responsiveness used in the present study has shown good reproducibility after short-term retesting (6) and reliability when saline and capsaicin solutions are inhaled in randomized, double-blinded order (10). When measuring the cumulative number of coughs throughout 6 min of bronchial capsaicin

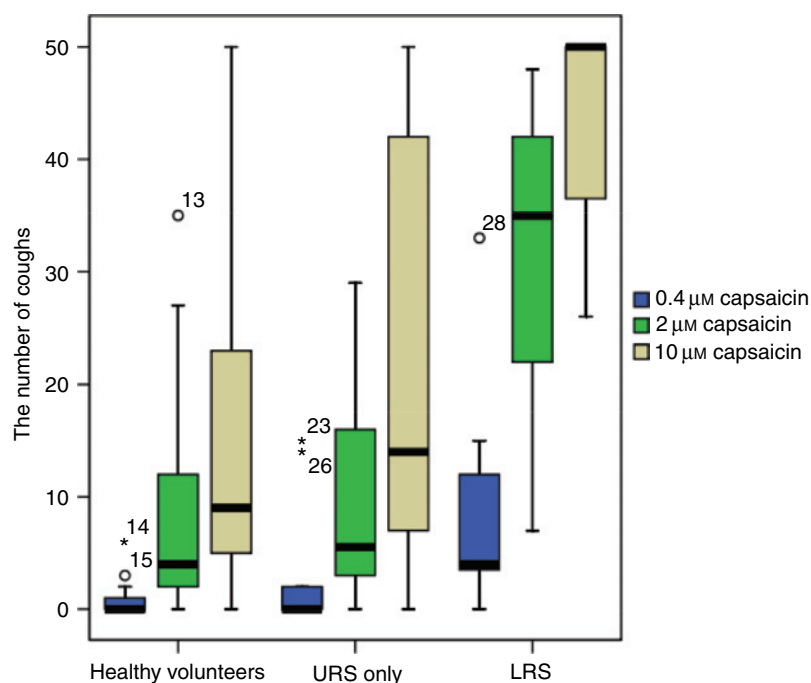


Fig. 1. Results from capsaicin bronchial challenge test. Number of coughs at the three concentrations of inhaled capsaicin (median, quartiles and range) for healthy volunteers, patients with only upper respiratory symptoms (URS) elicited by perfume and patients with lower respiratory symptoms (LRS) elicited by perfume.

challenge and 4 min of rest, the outcome of the test may reflect another aspect of the cough reflex than the exact threshold of elicitation. In normal individuals, tachyphylaxis occurs within the first minute of bronchial exposure to capsaicin (5), which could explain the low (expected) capsaicin cough responsiveness seen in our group of HV and individuals with URS. Exposure to inflammatory mediators and chemicals may lead to

sensitization of peripheral nerve endings (3), and neuronal sensitization of the cough receptor VR1 has been suggested as a possible physiological explanation for increased airway sensitivity to chemicals and strong scents (11). The method used in this study determined the tendency to continue coughing rather than a precise threshold for triggering the cough reflex. This indicates that damage or loss of normal inhibitory regulation of

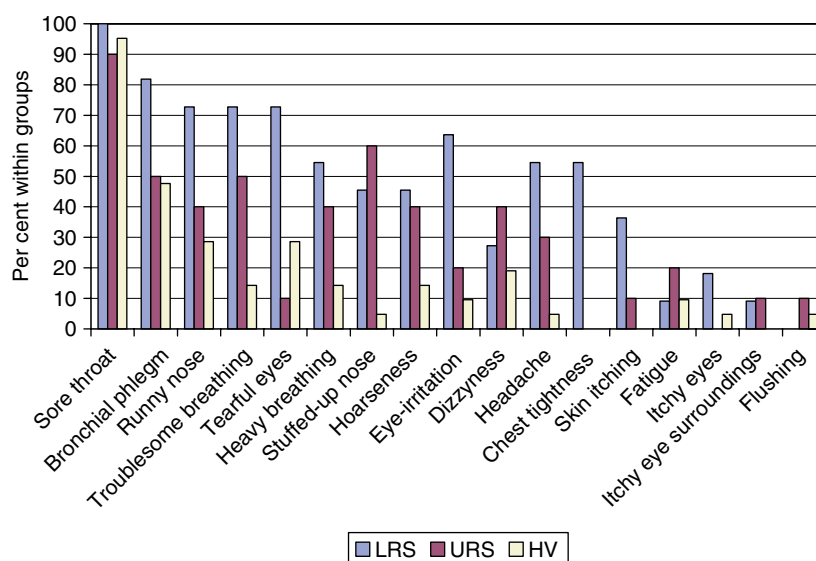


Fig. 2. Evaluation of 17 symptoms during the bronchial capsaicin provocation in healthy volunteers (HV), patients with only upper respiratory symptoms elicited by perfume (URS) and patients with lower respiratory symptoms elicited by perfume (LRS).

the cough reflex could also reflect a physiological explanation for the tendency to continue coughing in patients with LRS.

Sensory neurones play important roles in airway defensive reflexes as the physiological response to chemical stimulation in the upper (12) and lower airways (13). Hyperreactivity of airway sensory neurones may underlie cough and asthma-like symptoms related to perfume and airborne chemicals as suggested by Millqvist et al. (6). In addition, this sensory hyperreactivity may also include URS related to airborne chemicals in patients with clinical atopic airway diseases (11). Our findings support the observation stated by other researchers that respiratory symptoms related to perfume may be quantified by bronchial provocations with capsaicin. Further, our results suggest either that sensory hyperreactivity may be a local phenomenon only measurable in the relevant organ or that URS related to perfume may be caused by other mechanisms. Although 95% of the patients had perfume-related symptoms from the nose, only 28% had allergic rhinitis. Nasal application of capsaicin in normal individuals triggers protective reflexes of the upper airways such as sneezing, runny nose and lacrimation (14, 15), but it is unknown whether the responsiveness to nasal capsaicin provocation is altered in patients with nasal symptoms elicited by perfume. However, high doses of intranasal capsaicin reduce nasal hyperreactivity in idiopathic rhinitis (16), indicating the importance of capsaicin sensitive neurones of nonallergic nasal symptoms. Increased capsaicin cough responsiveness has been found in patients with multiple chemical sensitivity (MCS) (17, 18), a non-confined condition where symptoms from different organ systems are elicited by low-dose exposures to perfumes or other airborne chemicals (19). However, all patients in one study reported MCS symptoms from the lower airways (17), and whether adjustments of symptom localization of the lower airways (except for cough) were made in another study is unclear (18).

Capsaicin may be a relevant agent for use in diagnostic tools to investigate the reactivity of sensory neurones in different regions in the airways and skin.

The present study suggests that diagnostic interpretation of responsiveness to capsaicin should consider the localization of symptoms in the group investigated, together with the region capsaicin is distributed (e.g. skin, eyes, nose, mouth, throat and lower airways) and the physiological responses elicited.

In conclusion, measurements of the capsaicin cough reflex may be relevant when eczema

patients with annoyance from perfume and other airborne chemical exposures are investigated. Bronchial responsiveness to capsaicin was shown in this study to be of less importance when symptoms from regions other than lower airways are to be evaluated.

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