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Increased release of histamine in patients with respiratory symptoms related to perfume

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Summary

Background Environmental perfume exposure may cause respiratory symptoms. Individuals with asthma and perfume contact allergy report such symptoms more frequently than others. However, immunologic mechanisms have not been demonstrated and the symptoms are not associated with IgE-mediated allergy. The study aimed to investigate whether basophils from patients with respiratory symptoms related to perfume released more histamine in the presence of perfume as compared with healthy volunteers.

Methods Histamine release was measured by the glass fibre method. Blood was obtained from healthy volunteers ($n = 20$) and patients with respiratory symptoms related to perfume ($n = 17$) attending a dermatological outpatient clinic for patch testing. The effect of an international brand perfume was investigated using the basophil histamine release test with perfume. Furthermore, basophils from a healthy non-atopic donor were incubated with participant's sera and histamine release induced by perfume was measured.

Results In both groups incremental perfume concentrations showed a positive and significant ($P < 0.001$) dose-response effect on the release of histamine. At the highest perfume concentration, the basophils released significantly ($P < 0.05$) more histamine in patients as compared with healthy volunteers. No difference was found between the groups when sera were incubated with basophils from a healthy non-atopic donor.

Conclusion Perfume induces a dose-dependent non-IgE-mediated release of histamine from human peripheral blood basophils. Increased basophil reactivity to perfume was found in patients with respiratory symptoms related to perfume.

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Introduction

Most people are exposed to perfume ingredients every day from cosmetics and household products. This exposure may be unintentional by evaporation from persons or objects or by personal choice. Respiratory symptoms related to environmental perfume exposure are common in the population [1], and no specific mechanisms have been demonstrated. In a single case, anaphylaxis has been reported after spraying perfume in the eyes [2], indicating histamine receptor activation induced by degranulation of basophils and/or mast cells. In a population-based study, respiratory symptoms related to perfume were not associated with skin prick test (SPT) reactivity, suggesting mechanisms other than IgE-mediated allergy [1]. However, the symptoms were significantly over-represented in individuals with asthma [1], and also in individuals with contact dermatitis and or positive patch tests to perfume [3, 4], suggesting a link between respiratory symptoms to perfume and increased cellular reactivity to irritants and chemicals.

Fragrance chemicals may cause adverse effects upon contact with the skin. These effects include allergic contact dermatitis, a delayed hypersensitivity reaction [5] and contact urticaria, an immediate dermal weal and flare reaction [6]. The mechanisms of fragrance-induced

contact urticaria is still not fully understood [6] and may involve liberation of prostaglandins, leukotrienes, substance P and histamine [7, 8].

The present study aimed to investigate whether the basophils from patients with respiratory symptoms related to perfume released more histamine in the presence of perfume as compared with basophils from healthy volunteers.

Methods

Patients with respiratory symptoms related to perfume attending a dermatological outpatient clinic for patch testing during a 6-month study period were included. The patients were stratified according to patch test results, hence about 50% had positive patch tests to perfume. A total of 21 patients and 21 healthy volunteers were enrolled in the study. Exclusion criteria for healthy volunteers were respiratory symptoms related to perfume, atopic airway disease and smoking. Blood samples were obtained from all individuals and the effect of an international brand perfume was investigated using the basophil histamine release test. Unfortunately, measurements of total histamine were not performed in five individuals (four patients and one healthy volunteer), leaving 17 patients and 20 healthy volunteers for the study. All participants completed a symptom questionnaire on respiratory symptoms before patch-test and SPTs with allergens from standard panels were performed. Ethical committee approval was obtained, and all subjects gave informed consent.

The perfume

The perfume used for challenging was Opium®, parfum (Yves Saint Laurent, Paris, France). The perfume has been on the market since 1977, and has been among the top 10 most sold perfumes in Europe. Chemical analysis has shown that the fragrance chemicals in the perfume are frequently used in other brands [9, 10].

Perfume-induced histamine release

Two types of perfume-induced histamine release tests were performed on each subject included in the study: (1) direct histamine release from patients' own basophils, which is an indication of either IgE sensitization or increased cellular reactivity to the perfume and (2) indirect histamine release induced by perfume after passive sensitization of stripped basophils. Here, a histamine release induced by the perfume is a strong indication of transferable perfume specific IgE to the non-sensitized cells. In both types of histamine release, cells were incubated with the perfume in the following concentrations: 5%; 1,5%; 0.5%; 0.15%; 0.05% and 0.015%.

Direct histamine release: 5 mL heparinized blood was drawn from each patient according to the method previously described [11]. In brief, 25 μL washed blood cells (plasma substituted with buffer) in the presence of 2.0 ng IL-3/mL blood was incubated at 37 °C for 60 min with 25 μL perfume in the above-mentioned concentrations or anti-IgE (DACO, Copenhagen, Denmark) or buffer. Incubation was performed in glass fibre-coated microtitre plates (HR-Test, RefLab, Copenhagen, Denmark) and released histamine and total cellular histamine contents were determined according to the manufacturers' standard protocol. Histamine release was expressed as a percentage of the total cellular histamine content.

Indirect histamine release: passive sensitization was investigated by incubating IgE-stripped donor basophils and participant's sera with perfume. In brief, buffy coat blood samples (from healthy blood donors) were screened and selected for the capability to elicit an anti-IgE response [histamine release (HR) > 30%] and with no HR-reactivity towards 10 common inhalant allergens and 10 food allergens. Peripheral blood monocytes (PBMCs) from the selected buffy coat fulfilling the above-mentioned criteria were isolated by Lymphoprep gradient centrifugation and contained 1–2% basophils. Cell-bound IgE was removed by washing the PBMCs in a phosphate buffer (pH: 3.55). Stripped basophils were then incubated with sera form patients and healthy volunteers [12] and subsequently with the Opium® perfume in the same six concentrations as described earlier. The HR was determined as stated above. Results were expressed in percentage of total cellular histamine content. An HR > 10% was considered a positive response.

Skin prick test

The Soluprick SQ® system (ALK-Abelló, Hoersholm, 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 weal diameter of 3 mm or more and atopy was defined as a positive SPT.

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 [13]. A positive reaction (+) was defined as a

minimum of homogeneous redness and palpable infiltration in the test area. Perfume contact allergy was defined as a positive patch test to the fragrance mix (450 μg/cm^2) composed of α-amyl cinnamic aldehyde, cinnamic aldehyde, cinnamic alcohol, eugenol, geraniol, hydroxycitronellal, isoeugenol and oak moss absolute.

Statistical analysis

Statistical analyses were performed with SPSS version 14.0 for Windows. Statistical significance was defined as $P < 0.05$. Friedman's test and Wilcoxon's signed-rank sum test were used to investigate dose dependency between the perfume concentration and release of histamine from basophils. The Mann–Whitney test was used to investigate differences in HR at particular perfume concentrations between healthy volunteers and patients. The χ^2-test and Fisher's exact test were used to investigate inter-group comparisons of more than 10% HR at various perfume concentrations.

Results

The characteristics of patients and healthy volunteers are shown in Table 1. There were overlaps between the defined subgroups with perfume allergy, atopy or asthma. Out of the nine patients with perfume contact allergy, four had atopy and two had asthma. Thus, most of the patients except three had contact allergy, atopy or asthma.

All patients with atopy ($n = 8$) had clinical symptoms of allergic rhinitis and four had asthma. One patient without atopy took asthma medication regularly because of non-allergic asthma.

Table 1. Basic characteristics of cases and healthy volunteers

	Patients ($n = 17$)	Healthy volunteers ($n = 20$)
Mean age, years (range)	50 (38–68)	48 (22–70)
Women	15	14
Nasal symptoms*	17	–
Lower respiratory symptoms†	9	–
Symptoms related to		
Other persons' wearing of perfume	17	–
Air fresheners	14	–
Flowers	13	–
Perfume contact allergy‡	9	–
Atopy§	8	2¶
Asthma‖	5	–

*Sneezing, irritation, runny or blocked nose related to perfume.
†Troubled breathing, coughing or chest tightness related to perfume.
‡A positive patch test to Fragrance Mix.
§A positive skin prick test to a standard panel of allergens.
¶No clinical symptoms.
‖Atopic asthma ($n = 4$) non-atopic asthma ($n = 1$).

Incremental perfume concentration had a positive and statistical significant effect (Friedman's test) on the release of histamine in patients ($P < 0.001$) and healthy control persons ($P < 0.001$). However, at the three lowest perfume concentrations, the release of histamine was inconsiderable in both groups (Fig. 1).

At the highest perfume concentration, the release of histamine was significantly higher in patients than in healthy volunteers (Tables 2 and 3 and Fig. 1).

Within the patient group no significant difference ($P < 0.05$) in HR was found when comparisons were made between subgroups dichotomized according to perfume contact allergy, SPT reactivity or asthma (Table 4).

No difference in the perfume-induced basophil HR was found between the patients and healthy volunteers when sera were incubated with basophils from a healthy non-atopic donor during the indirect HR test.

Discussion

The present study demonstrated that fragrance chemicals have the capacity to induce HR from peripheral blood basophils in a dose-dependent manner. Furthermore, an excess of histamine was released at higher perfume concentrations in patients with respiratory symptoms related to perfume as compared with healthy volunteers. This result cannot be explained by inter-group variation in histamine content because adjustments were made for total histamine release.

The dose-response curve was substantially steeper in the patient group but was not displaced toward lower perfume concentrations as compared with the healthy volunteers (Fig. 1). This pattern indicates that the basophils had increased reactivity to perfume in the patient group although the sensitivity i.e. the threshold to release histamine was the same in both groups.

Because no difference was found in HR between the groups when sera were incubated with donor basophils, it is most likely that the increased reactivity to perfume in patients is not attributed to serum factors, including IgE, but to certain features of the basophils that may distinguish them from basophils of the healthy volunteers. Because antihistamine treatment seems to be rarely effective, the basophil reactivity to perfume may involve release of other cytokines e.g. prostaglandins, leukotrienes or substance P with importance for the development of clinical symptoms.

Respiratory symptoms related to perfume were mainly experienced as immediate reactions by the patients in the present study and in general [1]. Similar to contact urticaria [14], the symptoms in the present study appear independent of prior perfume allergic sensitization [3]. Nevertheless, respiratory symptoms related to perfume are positively associated with inflammatory conditions in the skin and airways [1, 3], which could be due to priming of

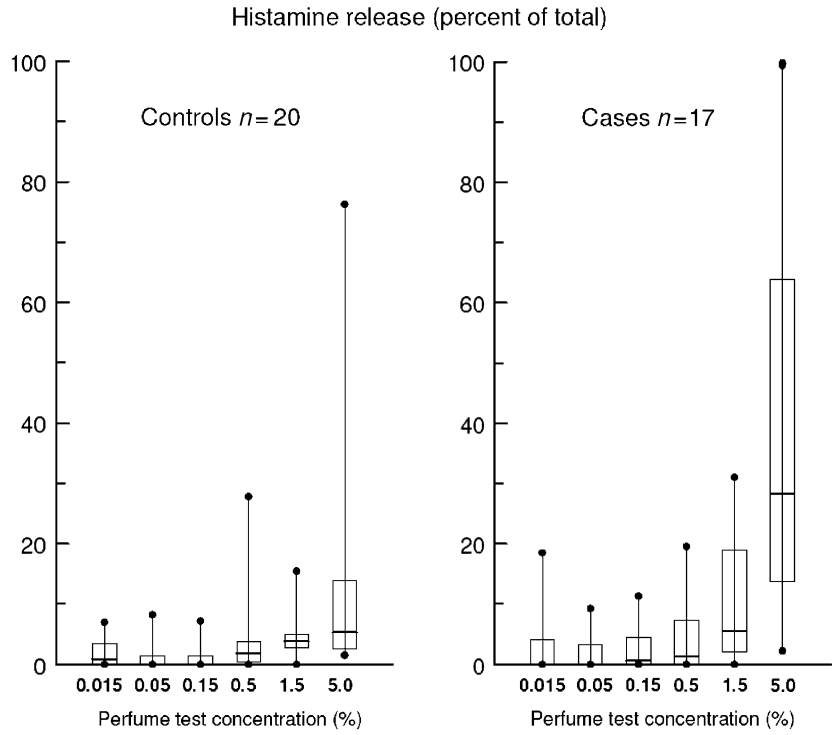

Fig. 1. Box plots showing histamine release (median, quartiles and range) at the different perfume concentrations in healthy volunteers (controls) and patients (cases).

Table 2. Comparisons of perfume-induced histamine response (percent of total) between patients and healthy volunteers

Perfume test concentration (%)	Median* (range)		Median differences and 95% CIs	Mann–Whitney's test, *P*-value
	Patients ($n = 17$)	Healthy volunteers ($n = 20$)		
5.0	28.4 (2.26–100)	5.33 (1.53–76.3)	20.8 (5.67–30.9)	0.0031**
1.5	5.49 (0.00–31.1)	3.78 (0.00–15.5)	2.28 (−0.57 to 9.61)	0.14
0.5	1.35 (0.00–19.6)	1.89 (0.0 0–27.8)	0.00 (−1.45 to 2.11)	0.99
0.15	0.57 (0.00–11.3)	0.00 (0.00–7.22)	0.56 (0.00–1.03)	0.19
0.05	0.00 (0.00–9.28)	0.00 (0.00–8.25)	0.00 (0.00–1.50)	0.36
0.015	0.00 (0.00–18.6)	0.84 (0.00–7.02)	0.00 (−1.35 to 0.68)	0.68

*Histamine response expressed as percentage of total cellular histamine content.
**The difference between patients and healthy volunteers was statistically significant ($P < 0.05$).
CI, confidence interval.

Table 3. Comparisons of perfume-induced histamine responses above 10% of total histamine content between patients and healthy volunteers

Perfume test concentration (%)	Healthy volunteers *n*/total*	Patients *n*/total*	*P*-value
5.0	7/20	14/17	0.007**
1.5	2/20	6/17	0.07
0.5	1/20	2/17	0.44
0.15	0/20	1/17	0.46
0.05	0/20	0/17	1.00
0.015	0/20	1/17	0.46

*Number of individuals in the group releasing more than 10% of the total histamine content.
**The difference between the groups was statistically significant ($P < 0.05$).

basophils by cytokines released during tissue inflammation resulting in enhanced perfume-induced HR. It is a well-known fact that IL-3, IL-5, granulocyte-macrophage colony-stimulating factor and nerve growth factor are able to prime basophils and enhance the IgE-mediated release of histamine [15] but little is known about factors priming non-IgE-mediated histamine release.

In summary, this is the first study suggesting that perfume induces a dose-dependent non-IgE-mediated histamine release from human peripheral blood basophils. Increased reactivity to perfume was found in basophils from patients with respiratory symptoms related to perfume, but the mechanism causing the increased reactivity is as yet unknown.

Table 4. Perfume-induced histamine responses (percent of total) in defined subgroups within the patient group ($n = 17$)

Perfume test concentration (%)	Median* (range) Perfume contact allergy ($n = 9$)	Atopy ($n = 8$)	Asthma ($n = 5$)	No allergy, atopy or asthma ($n = 3$)†
5.0	26.4 (2.26–100)	32.3 (5.00–100)	12.0 (5.00–80.4)	31.0 (26.7–85.4)
1.5	5.40 (0.00–31.1)	5.00 (0.00–23.7)	0.00 (0.00–6.19)	11.4 (5.49–20.4)
0.5	1.10 (0.00–19.6)	1.58 (0.00–19.6)	0.00 (0.00–2.06)	4.69 (1.10–7.77)
0.15	0.00 (0.00–11.3)	0.51 (0.00–11.3)	0.00 (0.00–1.03)	1.50 (0.00–8.74)
0.05	0.00 (0.00–9.28)	0.00 (0.00–9.28)	0.00 (0.00–4.12)	2.32 (0.00–3.88)
0.015	0.00 (0.00–18.6)	0.76 (0.00–18.6)	0.00 (0.00–9.28)	0.00 (0.00–4.80)

The same patient may appear in more than one of the defined subgroups.
*Histamine response expressed as percentage of total cellular histamine content.
†Patients without perfume contact allergy, atopy or asthma.

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