

Clinical aspects of allergic disease

Olfactory dysfunction in allergic rhinitis is related to nasal eosinophilic inflammation

Ludger Klimek, MD, and Georg Eggers, MD Mainz, Germany

Background: Olfactory dysfunction is a common finding in patients suffering from allergic rhinitis. However, little is known about the pathophysiology underlying this phenomenon and about the time course of hyposmia in seasonal allergy.

Methods: A prospective controlled study was performed on 17 patients with allergic rhinitis to grass pollen in order to evaluate olfactory function in correlation to the duration of allergen exposition, symptoms, eosinophil cationic protein (ECP) in nasal secretions, and nasal volume flow (NVF). Olfactory function was evaluated preseasonally and on days 3, 7, 14, and 21 of the season using a modified Connecticut Chemosensory Clinical Research Center testing procedure for threshold, identification, and discrimination. Twelve volunteers without allergy served as controls.

Results: Preseasonally, patients and controls performed equally in discrimination and identification testing, but not in threshold testing. No changes were found in the controls, but a significant decrease in threshold and identification from the 7th day of the season in patients with allergy was noted that was better correlated to ECP than to NVF. NVF was already maximally decreased from the 3rd intraseasonal day with no further changes. ECP increase became significant at day 14.

Conclusion: Patients with grass pollen allergy develop olfactory dysfunction during natural allergen exposure that might be related to allergic inflammatory mechanisms. (*J Allergy Clin Immunol* 1997;100:158-64.)

Key words: Seasonal allergic rhinitis, olfaction, eosinophil cationic protein, rhinomanometry

Hyposmia, a common condition associated with allergic rhinitis, is often neglected by patients and overlooked by physicians. This might be the reason why this condition has rarely been studied. Little is known in the literature regarding severity, pathophysiologic conditions, and time-course in seasonal allergies.

In this study, we examined olfactory function before and during the first 3 weeks of a grass-pollen season. The underlying mechanisms most often discussed for olfactory dysfunction in nasal allergy are a blockage of the olfactory cleft due to mucosal swelling and inflammatory mucosal changes. We therefore measured nasal volume

Abbreviations used

ECP: Eosinophil cationic protein
NVF: Nasal volume flow

flow (NVF) and nasal secretion levels of the inflammation marker eosinophil cationic protein (ECP) as well as scoring allergic symptoms. The time-course, severity, and quality of olfactory changes were studied using an established test for olfactory threshold, and identification and discrimination ability.

METHODS

The study was performed as a prospective controlled clinical trial. A total of 21 patients with allergy (10 females, 11 males; mean age 31.4 ± 6.0 years, range 20 to 42 years) were included. Four patients dropped out; two because of intake of nonallergenic concurrent medication (oral antihistamines), one because of the development of a viral rhinitis, and one because of insufficient compliance. Seventeen patients (eight females, nine males; mean age 32.7 ± 6.9 years, range 20 to 42 years) were included in the statistical analyses according to the study protocol. The control group consisted of 12 healthy volunteers (six females, six males; mean age 28.8 ± 4.3 years, range 22 to 37 years).

Inclusion criteria for the patients were the history of a seasonal allergic rhinitis to grass pollen requiring therapy for at least 2 years, positive skin prick test to a commercial grass pollen solution (ALK, Copenhagen, Denmark), specific serum IgE to grass pollen $\geq$ class II (CAP-system, Pharmacia, Uppsala, Sweden), and consent to avoid antiallergic therapy during the study period.

Exclusion criteria were the existence of any nasal disease other than allergic rhinitis, nasal allergy to perennial allergens or to seasonal allergens with natural allergen exposition that could be expected earlier than the grass pollen in our region (e.g., birch, hazel), chronic or acute paranasal sinus diseases, specific immunotherapy during the 3 years before study entry, age under 18 or over 45 years, pregnancy, any acute or chronic inflammatory disease, antiallergic therapy at study entry, presence of parasitic infections, hypereosinophilia syndrome, or refused consent.

Drop-out criteria were the intake of antiallergic medication during the study period, development of bacterial or viral rhinitis or of any exclusion criteria during the study period, or noncompliance with regard to the study schedule. Nasal or paranasal sinus diseases other than allergic rhinitis were excluded in every participant by history, nasal endoscopy,¹ and A-scan-sonography of the paranasal sinuses.² Informed consent was obtained from each volunteer before entering the study. The study was approved by the regional ethics committee.

From the Department of Otorhinolaryngology, Mainz University Hospital.

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Reprint requests: Ludger Klimek, MD, Department of Otorhinolaryngology, Mainz University Hospital, Langenbeckstr. 1, D-55101 Mainz, Germany.

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The controls had the same inclusion, exclusion, and drop-out criteria as the patients, except they were not allowed to have any kind of allergic diseases. These were excluded by history and skin prick test to common inhalation allergens.

Study design

Patients and controls were recruited in early 1994, and a preseasonal inclusion examination (D1) was performed. The D1 visit included history and diagnostic testing procedures to verify inclusion and exclusion criteria and olfactory function testing (see below). The patients received a diary to document their symptoms of allergic rhinitis (e.g., rhinorrhea, nasal obstruction, itching, sneezing) once daily by means of a scoring system on a 1 to 3 ordinal scale: 0 = free of symptoms, 1 = mild symptoms, 2 = moderate symptoms, 3 = severe symptoms. The total symptom score was composed by adding up all scores.

The patients were requested to contact the study coordinator at the beginning of their allergic complaints. They were then scheduled for a visit 2 days later, respectively the third intraseasonal day for the first intraseasonal visit (D2). The individual start point of the season was defined as total symptom score of 3 or more with increased grass pollen concentration at the same time. Alternatively, the beginning of the season was defined as an increase of the grass pollen concentration of 20 or more pollen grams per m³ air. If the patient had not contacted the study coordinator up to this date, the patient was scheduled by phone for the 3rd intraseasonal day (D2).

All further study visits followed in a previously determined scheme at the 7th (D3), 14th (D4), and 21st (D5) intraseasonal day. All patients were instructed not to take antiallergic therapy during the 3 intraseasonal study weeks.

Active anterior rhinomanometry was performed using NVF (cm³/s) with transnasal pressure difference of 150 Pascal (V_{150}) as a target parameter (Rhinomanometer 200, Atmos Inc., Lenzkirch, Germany).

Nasal secretions sampling

Nasal secretions were collected using standard size (14 × 14 × 4 mm) absorbent rubber-foam samplers. They were placed under rhinoscopic control between the septum and the head of the inferior turbinate on both sides. After 10 minutes they were placed into laboratory tubes on a platform and centrifuged at 2000g/0° C for 15 minutes. The nasal secretions were squeezed out. The clear sol phase was separated from mucus and cells and then pipetted into another tube.³ The amount of nasal secretions obtained was documented for all samples. As usual, albumin analyses were used to detect plasma leakage into the nasal secretions as a result of increased vascular permeability.⁴ Nasal secretions were immediately shock frozen in liquid nitrogen and stored at -80° C.

Determination of ECP

We assayed samples of 50 µl nasal fluid. ECP was determined by a competitive radioimmunoassay research kit (Pharmacia, Uppsala, Sweden) as described previously.^{5,6} All samples were subject to duplicated measurements. In short, samples were incubated with ¹²⁵I-labeled human ECP and rabbit anti-human ECP for 3 hours and then incubated with sheep anti-rabbit serum and decanted. Subsequently, radioactivity was measured in a gamma counter over 1 minute, and the sample values were calculated by spline interpolation. Samples containing over 200 ng/ml were diluted with zero standard.

Grass pollen concentration was measured using a Burkhard volumetric spore trap (courtesy of the German Pollen Informa-

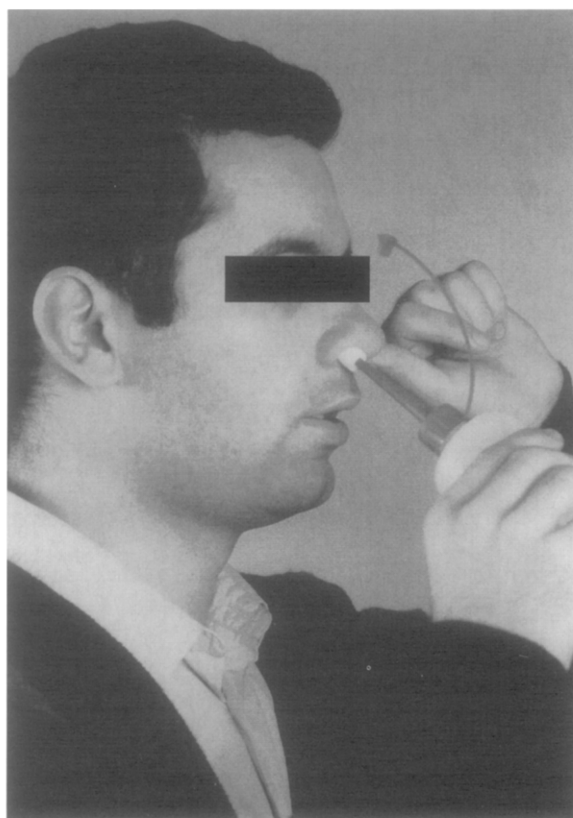


FIG. 1. Olfactory function testing with squeeze-bottle.

tion Service). Pollen counts were expressed as the mean daily number of pollen grains per cubic meter of filtered air.

Olfactory function testing

Olfactory function testing was performed by means of a modified Connecticut Chemosensory Clinical Research Center test⁷ (Fig. 1). In this test, 60 ml of the odorant was presented in a squeezable polyethylene bottle with a Teflon nasal adapter. Between tests, the bottle was closed by a pop-up spout. Testing was performed monorhinal using the results from the better nostril for statistical analysis. To sample a bottle, the person placed the adapter into the specified nostril, then squeezed and sniffed simultaneously. All tests were performed at the same time of day (between 8 and 10 AM) in the sequence of threshold testing, identification testing, and discrimination testing.

Threshold testing was performed with solutions of 1-butanol in deionized water, decreasing in 12 steps by a factor of 3 from a maximum aqueous concentration of 4% to a lowest concentration of 0.0000075%, from step 0 to step 12. Thus, a threshold value of 12 indicated the highest sensitivity. Starting with the lowest concentration, the patients received a bottle with odorant and a bottle filled with deionized water and had to decide which smelled stronger. The order of presentation was determined by randomization software. The patient received all bottles in a blinded manner. Once a verum bottle was correctly detected, the identical concentration was presented again. A concentration was defined to be the threshold concentration if it was correctly identified four times. If a false identification took place, the next higher concentrated butanol solution was applied. The test result was expressed as threshold score from 0 to 12.

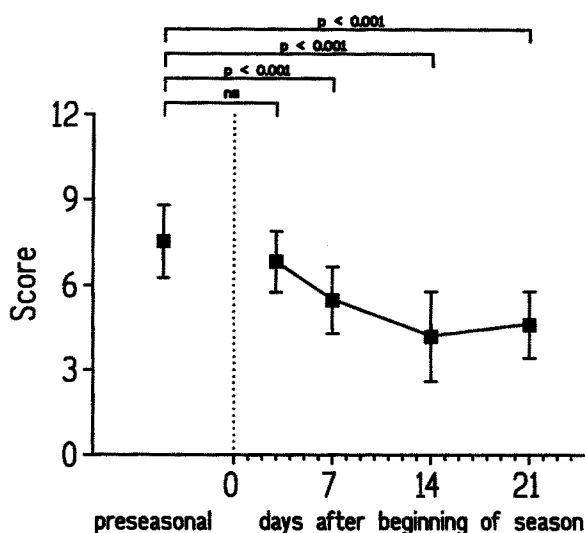


FIG. 2. Results of olfactory threshold testing (mean $\pm$ SD, patients; ns, not significant; p values after Bonferroni).

In the identification testing 12 well-known odorants (Table I) were presented to the test person in the same manner as described above. In a forced-choice-procedure, the person had to name the correct odor out of four answer possibilities. One point was awarded for each correct answer, with a maximum of 12 being achieved if all answers were correct.

The outcome of the threshold and identification testing was combined into a composite score by averaging the two into a single score as described by Cain et al.⁷ A composite score of 7.2 was defined to be the lower boundary of the zone of normosmia. Mild hyposmia was defined for values from 6.0 to less than 7.2, and moderate hyposmia for values of 4.5 to less than 6.0. Scores of at least 3.0 but less than 4.5 were designated severe hyposmia, and scores below 3.0 were designated anosmia.

In the discrimination testing the subject received three different odorants. Two were identical and the third was a different odor. The differing bottle had to be identified. A total of four triplets were tested in this manner. The person received one point for each correct answer, with a possible maximum of four points.

Study evaluation and statistics

A total of 98 patients were evaluated for the study. All but 21 were excluded, most because of coexisting allergy to mites, birch, and hazel, as determined by skin prick testing, or because of paranasal sinus diseases other than allergic rhinitis. Four additional patients were dropped from the study.

A total of 17 controls were evaluated for the study. Five had to be excluded, one because of paranasal sinus disease other than allergic rhinitis, one with positive skin prick test to *Dermatophagoides farinae*, and three refused consent during the study period. No healthy volunteer had to be dropped from the study. Twelve controls were included in the statistical analyses.

The statistical evaluations were performed using WinSTAT-Statistic Software (Kalmia Corp., MA). All data are expressed as means $\pm$ standard deviation. Statistical analyses for differences in olfactory threshold, identification, and discrimination for the entire study period and between the study visits were performed using an ANOVA procedure with analysis of variance after Bonferroni. Spearman's Rank correlation coefficient

TABLE I. Substances used for olfactory testing

Identification and discrimination testing			
Cinnamon	Lavender	Orange	Almond
Rose	Vanilla	Coffee	Peppermint
Cocoa	Anise	Banana	Eucalyptus
Threshold testing			
n-butanol			

TABLE II. Results of olfactory function tests

			D1	D2	D3	D4	D5
Patients							
Identification	Mean		9.8	8.9	7.3	6.8	7.2
	SD		1.3	1.4	1.3	1.4	1.4
Threshold	Mean		7.5	6.8	5.5	4.2	4.6
	SD		1.3	1.1	1.2	1.6	1.2
Discrimination	Mean		2.6	2.5	2.4	2.4	2.2
	SD		0.5	0.6	0.6	0.5	0.6
Healthy volunteers							
Identification	Mean		10.1	9.8	9.7	10.0	9.3
	SD		1.3	1.4	1.2	1.2	1.0
Threshold	Mean		9.1	9.4	8.6	8.8	9.2
	SD		2.2	1.8	3.5	1.8	2.0
Discrimination	Mean		2.7	2.8	2.8	2.7	2.8
	SD		0.4	0.4	0.4	0.3	0.2

(r_s) was determined to evaluate the correlation between olfactory measures versus symptom scores, NVF, and nasal secretion ECP levels. A range transformed, two-tailed, paired t -test according to Acritas (Acritas MG, Brunner E. Unified approach to rank tests for mixed models. J Stat Plann Interference 1996. Unpublished data) was used to analyze differences between the different correlation coefficients. A $p < 0.05$ was regarded as statistically significant.

RESULTS

From the 17 patients included in the statistical analyses, 15 contacted the study coordinator spontaneously to schedule the first intraseasonal visit (D2), because of having reached a daily total symptom score of 3 or more in their diary. The other two patients were contacted by the study coordinator, because a grass-pollen concentration of 20 or more pollen grams/m³ air had been measured at the pollen trap nearest their home. In both patients, on the day of the phone call a total symptom score of 3 or more in their diary was reached for the first time. The time interval for the D2 visit between the examination of the first until the last patient was 5 days. The volunteers were all tested during the same time period.

Olfactory function testing

The results of the threshold, identification, and discrimination testing are detailed in Table II. At the extraseasonal visit (D1) all but two patients and all but one control were normosmic. After 3 weeks of pollen exposure, all patients without exception had mild to

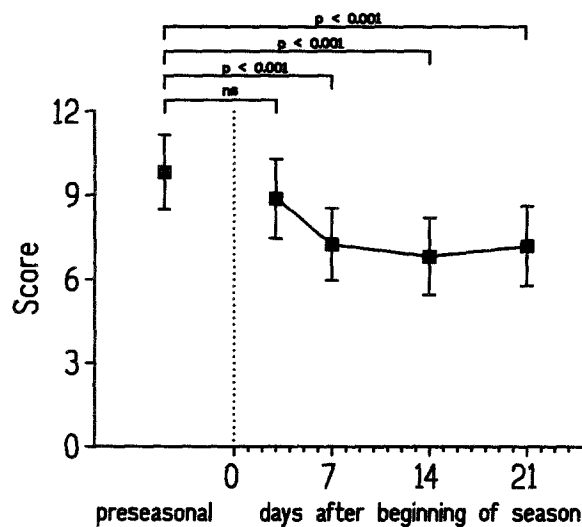


FIG. 3. Results of olfactory identification testing (mean $\pm$ SD, patients; ns, not significant; p values after Bonferroni).

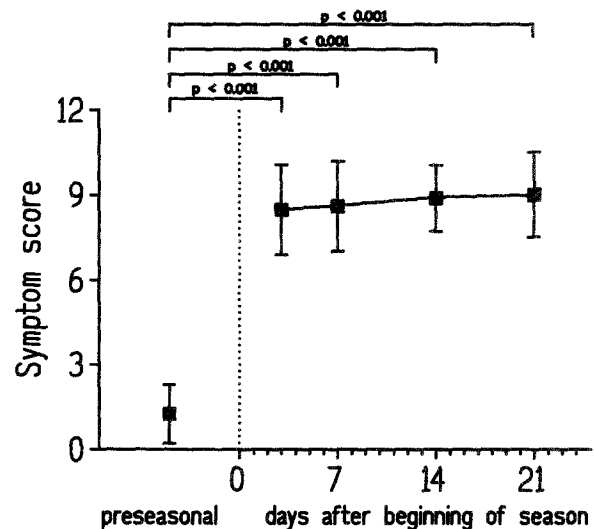


FIG. 4. Total symptom score from diary cards (mean $\pm$ SD, patients; ns, not significant; p values after Bonferroni).

TABLE III. Grading of olfactory function (according to reference 10 and our own unpublished data)

	Visit	No. of patients having:				
		Normosmia	Mild hyposmia	Moderate hyposmia	Severe hyposmia	Anosmia
Patients with allergy (n = 17)	D1	15	2	0	0	0
	D5	0	5	9	3	0
Controls (n = 12)	D1	11	1	0	0	0
	D5	10	2	0	0	0

severe hyposmia as measured by threshold and identification testing. No patient at this point in the study was anosmic. One additional control subject developed mild hyposmia, while all others remained normosmic (Table II).

When the patients were compared to the controls at the preseasonal study visit, a significant difference was found for threshold testing ($p = 0.02$, nonpaired t -test), but not for identification and discrimination ($p > 0.05$, nonpaired t -test).

In the controls there were no statistically significant changes in threshold, identification, and discrimination testing at any study period (all $p > 0.05$; Bonferroni), and over the total study time ($p > 0.05$; ANOVA).

In the patients there was a significant decrease in the threshold and identification testing over the total study time (D1 to D5) (both $p < 0.001$; ANOVA) (Figs. 2 and 3).

With the threshold testing, a significant decrease was first found at D3 (D1 vs. D2: not significant; D1 vs. D3: $p < 0.001$; D1 vs. D4: $p < 0.001$; D1 vs. D5: $p < 0.001$; Bonferroni). After D2, there was a further decrease in identification performance (D2 vs. D3: not significant; D2 vs. D4: $p < 0.001$; D2 vs. D5: $p < 0.001$; Bonferroni),

but not after D3 where any changes were considered insignificant.

With the identification testing, a significant decrease was initially found at D3 (D1 vs. D2: not significant; D1 vs. D3: $p < 0.001$; D1 vs. D4: $p < 0.001$; D1 vs. D5: $p < 0.001$; Bonferroni) if the preseasonal values were compared with those obtained after the onset of the season. After D2, there was a further decrease in identification performance (D2 vs. D3: $p < 0.05$; D2 vs. D4: $p < 0.001$; D2 vs. D5: $p < 0.01$; Bonferroni), but not after D3 where any changes were considered insignificant.

With the discrimination testing, there were no significant changes at any study period.

Symptom scores

In the patients, there was a statistically significant increase in the total daily symptom score over the entire study period (D1 to D5; $p < 0.001$; ANOVA; Fig. 4). This increase became significant between D1 and D2 (D1 vs. D2: $p < 0.001$; Bonferroni) and remained increased at all other study visits when compared with the preseasonal visit (D1 vs. D3, D1 vs. D4, D1 vs. D5: all $p < 0.001$; Bonferroni). The subjectively evaluated symptom score for nasal block-

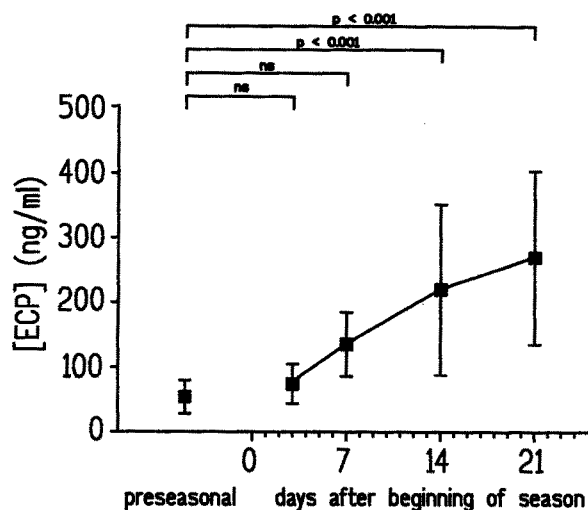


FIG. 5. Levels of ECP in nasal secretions (ng/ml, mean $\pm$ SD, patients; ns, not significant; p values after Bonferroni).

age increased in the same way (Bonferroni tests similar to total symptom score). There were no differences noted in the controls.

ECP measurements

ECP nasal secretion levels increased significantly over the total study time (D1 vs. D5: $p < 0.001$; ANOVA; Fig. 5). If compared with the preseasonal value, this increase became statistically significant at D4 (D1 vs. D2: not significant; D1 vs. D3: not significant; D1 vs. D4: $p < 0.001$; D1 vs. D5: $p < 0.001$, Bonferroni). However, there were also significant changes after D2 and after D3 (D2 vs. D3: not significant; D2 vs. D4: $p < 0.001$; D2 vs. D5: $p < 0.001$; D3 vs. D4: not significant; D3 vs. D5: $p < 0.01$, Bonferroni). In the controls there were no significant changes in any period (D1: 23.2 ± 20.6 ng/ml; D2: 26.9 ± 24.5 ng/ml; D3: 20.4 ± 28.1 ng/ml; D4: 23.7 ± 18.2 ng/ml; D5: 24.5 ± 21.3 ng/ml).

NVF

In the patients, NVF decreased significantly over the total study time ($p < 0.001$; ANOVA; Fig. 6). This decrease became statistically significant at D2 (D1 vs. D2: $p < 0.001$; Bonferroni) and remained changed at all other study visits if compared with the preseasonal visit (D1 vs. D3, D1 vs. D4, D1 vs. D5: all $p < 0.001$; Bonferroni). There were no significant differences in NVF after D2 ($p > 0.05$; Bonferroni).

In the controls there were no significant changes in NVF in any period (D1: 352 ± 229 ml/min; D2: 371 ± 174 ml/min; D3: 348 ± 261 ml/min; D4: 310 ± 274 ml/min; D5: 342 ± 208 ml/min).

Correlations

Olfactory threshold testing correlated well with ECP nasal secretion levels ($r_s = 0.83 \pm 0.24$, Spearman Rank), but not similarly well with NVF ($r_s = 0.65 \pm 0.23$,

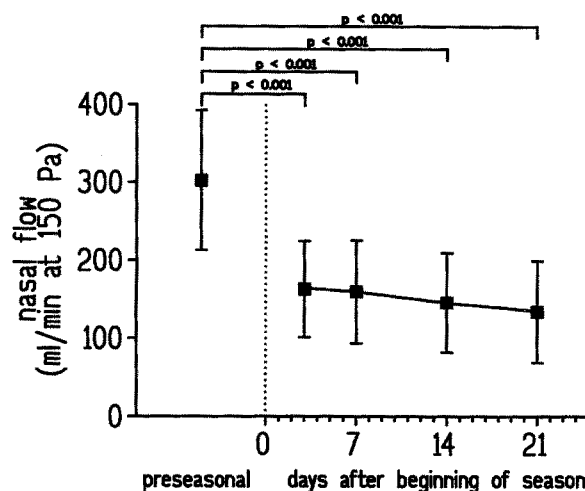


FIG. 6. NVF measured by active anterior rhinomanometry (ml/min, mean $\pm$ SD, patients; ns, not significant; p values after Bonferroni).

Spearman Rank) (Fig. 7). The correlation with ECP was significantly better than that with NVF ($p = 0.0002$; $T = 4.77$, 16 degrees of freedom; range transformed, two-tailed, paired t-test according to Acritas [see above]).

Olfactory identification testing correlated well with ECP nasal secretion levels ($r_s = 0.67 \pm 0.30$, Spearman Rank), but not similarly well with NVF ($r_s = 0.47 \pm 0.41$, Spearman Rank) (Fig. 8). The correlation with ECP was significantly better than that with NVF ($p = 0.0164$; $T = 2.68$, 16 degrees of freedom; range transformed, two-tailed, paired t-test according to Acritas [see above]).

DISCUSSION

Among all symptoms associated with nasal allergy, hyposmia is probably the least investigated, even though a complete communicative sense is influenced in this regard. To date there exist only few systematic studies regarding olfactory function in allergic rhinitis.^{7, 8-17}

The pathogenesis of this dysfunction is not fully understood. A blocked transport of the odor molecules to the olfactory epithelium because of nasal congestion is discussed, in addition to changes in composition and function of the olfactory mucus as a result of inflammatory hypersecretion and dyscrinia, along with inflammatory dysfunction of the olfactory receptor cells.^{10, 13, 18}

Seasonal allergic rhinitis might be ideal to study pathophysiologic factors of allergic hyposmia, because intraseasonal changes in the nose that are timely correlated to the development of hyposmia might be associated with this condition.¹⁹ In this study, we found no differences in olfactory identification and discrimination performance between patients with allergy and healthy volunteers at the extraseasonal visit. However, patients with allergy had higher olfactory thresholds in contrast to the results of previous studies that found no impairment in the olfactory function of patients with allergy out of season.¹⁸

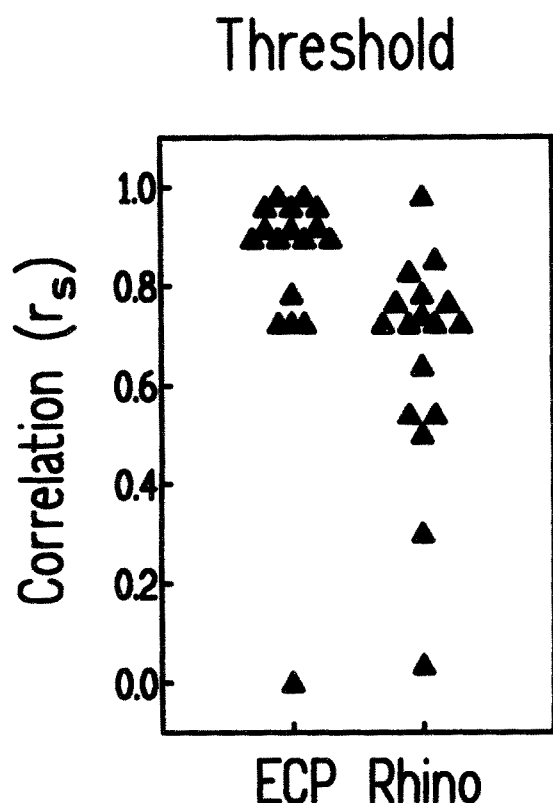


FIG. 7. Scattergram of correlations between olfactory threshold versus rhinomanometry (Rhino) and ECP nasal secretion levels.

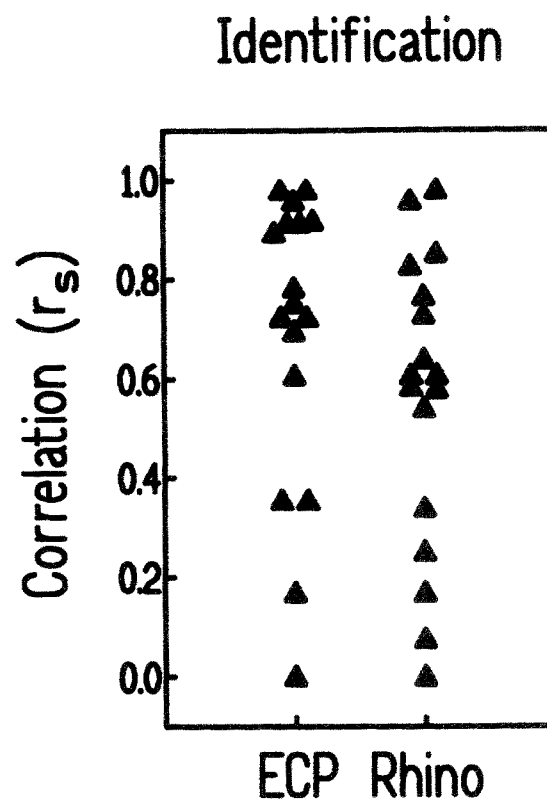


FIG. 8. Scattergram of correlations between olfactory identification versus rhinomanometry (Rhino) and ECP nasal secretion levels.

After 3 weeks of natural pollen exposure without antiallergic medication, the patients developed a moderate hyposmia in the mean, and no single patient remained normosmic. Therefore, we state that hyposmia is a common symptom in seasonal allergic rhinitis that can be detected if adequately looked for. The pathophysiologic conditions responsible for hyposmia in seasonal allergic rhinitis need a time period of less than 3 weeks to become clinically evident. The onset and time course of olfactory dysfunction might be of importance to explain this phenomenon.

Up to the third intraseasonal day we found only small, statistically insignificant changes in the tested olfactory qualities. The NVF was already significantly decreased at this visit. While there was no further change in NVF during the subsequent study period, we found a progressive olfactory dysfunction up to day 14 of the study. Cowart et al.¹² also reported that the degree of nasal obstruction is not directly related to olfactory dysfunction in allergic rhinitis. Additionally, the changes in nasal patency during the so-called nasal cycle do not influence olfactory function.²⁰ In another study, decongestion of the nasal mucosa with epinephrine nasal drops in allergic rhinitis did not normalize olfactory function. However, the drops may not have reached the olfactory region and thus would not be expected to improve olfactory airflow.¹⁰

The results of these previous investigations and our own study need to be assessed carefully because total NVF is not necessarily linear related to the volume that passes the olfactory cleft area. Therefore, it would be most interesting to determine exactly the part of the total NVF that passes the olfactory cleft. Currently, there exists no method to do this accurately under in vivo conditions. However, it is known from in vitro gas flow model studies on mock human nasal cavities that a relatively constant part of 15% of the total nasal airflow passes through the olfactory region provided that nasal anatomy is normal.²¹

The development of olfactory dysfunction was associated with a marked increase in nasal secretion ECP levels. The ECP is one of the four major granule proteins (major basic protein, eosinophil peroxidase, eosinophil-derived neurotoxin, and ECP) of the eosinophil granulocyte, a cell that is of major importance in allergic inflammatory conditions.^{8, 23} These proteins are all released in vitro to the extracellular environment upon stimulation of the eosinophil by various secretagogues.²² We chose this marker protein to monitor inflammatory changes of the nasal mucosa because ECP is a specific indicator of eosinophil activity; the content of this protein in other cells is negligible.²² The measurement of ECP in nasal secretions was demonstrated to be a sensitive marker of eosinophil activation either in

allergen challenge models²⁴⁻²⁶ or under natural pollen exposure.²⁷ In this study, ECP nasal secretion levels significantly increased up to day 14 of the season. This increase was closely correlated to the increase in olfactory dysfunction. After 3 weeks of pollen exposure, ECP levels were reached that in an in-vitro model were shown to reduce beat frequency of ciliated nasal epithelial cells, and that might even structurally damage or destroy these cells.^{22, 28}

While this is no proof for a direct influence of ECP on the olfactory epithelium, it gives evidence that the conditions leading to increased ECP release might also be associated with olfactory dysfunctions. The hypothesis that inflammatory changes are the reason for hyposmia in allergic rhinitis is also underlined by the fact that antiinflammatory treatment is efficient in this condition.^{16, 17, 29, 30}

As expected, we found no changes in olfactory discrimination during this study, because olfactory discrimination seems to be influenced by disorders of the central nervous olfactory system rather than by disorders of the nose and olfactory epithelium.³¹

Regarding the results of this study we state that patients with grass pollen allergy develop a moderate hyposmia during 3 weeks of natural grass pollen exposure. This is better correlated with inflammatory measures such as ECP nasal secretion level than with NVF measured by active anterior rhinomanometry.

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