

Variations in histamine concentration in nasal secretion in patients with allergic rhinitis

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The histamine concentration and content in nasal secretion and the volume of nasal secretion in nasal washing samples were measured under different conditions in 28 patients with allergic rhinitis sensitive to birch pollen. The mean histamine concentration was significantly lower after intranasal birch pollen challenge (2.08 µg/ml) than in prechallenge samples (6.96 µg/ml), and was also significantly lower in untreated patients during the birch pollen season (2.30 µg/ml) than off-season (7.18 µg/ml). The same relationship was found between the histamine content of the secretion samples obtained on these occasions. The mean secretion volume was greater after than before challenge, but not significantly higher during the season than off-season. A partial reversion of the changes in histamine concentration and content that occurred during the season was observed during intranasal corticosteroid therapy. The concentration and content of histamine in nasal secretion from symptomatic patients after intranasal histamine challenge did not differ significantly from those in asymptomatic subjects before challenge. It was concluded that although the histamine level in nasal secretion can be used as a marker of changes in the severity of allergic rhinitis, it is not ideal for this purpose.

Key words: corticosteroid; histamine; immediate hypersensitivity; nasal provocation test; nasal secretion.

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CLINICAL ASPECTS

There is a lack of objective measures that can be used to compare different phases of allergic rhinitis during clinical trials. Changes in the amounts of mediator substances in nasal secretion following challenge have been demonstrated in other studies. The recorded changes in the amount of histamine in the secretion indicate that biochemical investigations may also be applied to naturally occurring symptoms, although the findings do not make histamine a first choice as a biochemical marker.

Histamine is considered one of the principal mediators of allergic rhinitis. In accordance with this hypothesis, H₁-antihistaminic drugs are widely used in the clinic to minimize the symptoms of this disorder. Naclerio et al. (8) have furnished evidence that the nasal mucosa releases histamine together with arachidonic acid metabolites following intranasal allergen challenge. The release of leukotrienes from the human nasal mucosa *in vivo* after allergen challenge has also been demonstrated by Shaw et al.

(12). Both groups based their conclusions solely on the concentrations in diluted washing and did not calculate the actual concentration of mediators in nasal secretion.

Data from the previous study by Linder et al. (5) indicated that the histamine concentration in nasal secretion in allergic subjects is higher than has been reported hitherto, and suggested that the histamine concentration decreases and the secretion content of nasal spray washing samples increases after a positive allergen chal-

lenge and likewise during the pollen season. Drake-Lee & McLaughlan (2) found equally high histamine concentrations in nasal polyp fluid. The observation by Eggleston et al. (3) of a tendency towards lower histamine concentrations during infectious rhinitis is also interesting, since it exemplifies the dilution of the nasal secretion by the rhinitic response. The present study was undertaken to explore further the relationship between histamine levels in nasal secretion and the clinical manifestations of allergic rhinitis. The design was improved over the previous one in the following respects: 1) The absolute histamine content and the volume of secretion in the sample were recorded, 2) the effect of natural exposure to pollen was studied in untreated patients, 3) a number of patients also provided secretion samples while receiving standardized therapy, and 4) histamine challenges were performed to provoke symptoms resembling those caused by allergen.

MATERIAL AND METHODS

Study design

Two studies were carried out, one in 1983 and the other in 1984. Each study included seasonal exposure to birch pollen and an intranasal birch pollen challenge out of season. Fig. 1 shows the schematic plan for the visits, sampling and challenges. Each patient participated in one study only. The patients entered the study during the pollen season, before they started treatment. The challenge part of each study was scheduled to take place 4 to 5 months after the birch pollen season. Its main purpose was to investigate whether the results from the pollen season could be reproduced by pollen challenge.

In the 1983 study samples were also analyzed to determine whether the histamine level in nasal secretion was affected by intranasal treatment with beclomethasone dipropionate (BDP; Becotide Nasal®, Glaxo) 200 µg twice daily. This drug was used to treat the study group in standardized fashion during the season. A second nasal secretion sample was obtained after at least 1 week of treatment. At each visit, the

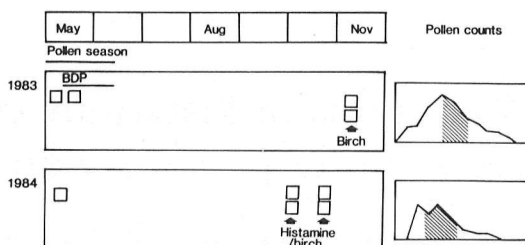


Fig. 1. The design of the study. Squares indicate the nasal secretion sampling and arrows the challenges. The birch pollen season and the period of treatment with intranasal beclomethasone dipropionate (BDP) are indicated. The timing of the sampling with respect to pollen occurrence is shown in the graphs to the right.

patient made an overall rating of the nasal symptoms as absent, slight, moderate, or severe. The ratings were converted to the scores 0, 1, 2, or 3 points, respectively. The difference in score between the two visits was used to indicate the change in the clinical condition. The challenge program in the 1983 study consisted of a single birch pollen challenge.

The patients in the 1984 study were seen only once during the birch pollen season, before starting treatment. Pharmacotherapy was prescribed for the rest of the season, as required in each individual patient. Four to 5 months after the birch pollen season, they underwent nasal challenges, once with birch pollen and once with histamine, the sequence being determined from random number tables. A minimum of 1 week was allowed for recovery after a positive challenge before the patient was rechallenged.

Patients

The numbers of patients screened for the investigations during the birch pollen season of 1983 and 1984, respectively, are given in Table 1. Patients who could be assumed on clinical grounds to be allergic to birch pollen, and in whom none of the exclusion criteria (see below) applied, were enrolled in the study after giving their informed consent. The final decision to include the patient was left until the diagnosis was confirmed by a positive birch pollen challenge.

Criteria for exclusion were the co-existence of a nonallergic nasal disease, chronic disease other than atopic allergy, pregnancy, immunotherapy for allergy during the last 3 years, age over 50 years, or refused content. Patients with asthma were excluded from the nasal challenges. Episodes of bronchospasm during the pollen season were regarded as asthma only if they were so severe or persistent that therapy was needed. Patients unsuitable for intranasal corticosteroid therapy were excluded from the 1983 study.

Sampling of nasal secretion

Nasal secretion samples were collected by means of nasal spray washing as described previously (4). Briefly, both nasal cavities were sprayed with repeated 100 μ l portions of a 0.9% sodium chloride solution containing lithium chloride as an exogenous marker. The subject then expelled the fluid onto a plastic dish and the sample was transferred to a pre-weighed plastic tube. The procedure was repeated until a sufficient amount was collected, which was usually achieved in less than 5 min. The samples were allowed to equilibrate at

approximately 20°C for 30–60 min. After weighing, the sample was filtered through a 3 μ m Millipore filter and frozen to -20°C pending assay.

The lithium concentration of the washing solution was increased from approximately 1 mM, as described in the original method, to approximately 2 mM, in order to improve the accuracy in analyses of samples containing small amounts of secretion. The calculated value for the secretion content was considered reliable if it was not below 3%. Lower values were rounded to 3%. This applied to five samples from unchallenged patients out of season, five samples from patients during seasonal corticosteroid therapy and one sample from an untreated patient during the season.

Histamine analysis

A sensitive fluorometric assay based on condensation with o-phthalaldehyde (OPT) at -20°C and modified to give optimal results in nasal secretion was used (5). The samples were precipitated with 5% trichloroacetic acid equivalent to half the sample volume and centrifuged, and the supernatant was neutralized. Duplicate 100 μ l aliquots from each supernatant were added to 1.9 ml of 0.05 M NaOH on an ice-salt cold mixture at -15°C. Thereafter 0.4 ml of 0.2% recrystallized OPT was added and the tubes were incubated at -20°C for more than 10 h. The tubes were then returned to the ice-salt mixture and the reaction was terminated with 200 μ l of 0.35 M H₂SO₄. After thawing, the aliquots were shaken and read in a spectrofluorometer at 360/450 nm. Technical mishaps occurred to three samples during the season, so that no values were obtained for the volume or for the histamine concentration in two of them and for the histamine content in one.

Nasal challenges

The criteria for positive allergen and histamine challenges were based on the subjective response. The patients had no symptoms of rhinitis before the start of the challenge. By means

Table 1

Numbers and ages of patients in the studies

	1983	1984
Screened	40	43
Entered study	22	30
Evaluated	14	14
Male/female	5/9	6/8
Mean age (range)	27 (17–48)	27 (16–40)
Birch pollen challenge	13	14
Birch pollen season	11	14
BDP in season	10	–
Histamine challenge	–	14

“Entered” patients provided at least one secretion sample. “Evaluated” patients completed at least the seasonal study or a birch pollen challenge. One patient in the 1983 study was excluded from the nasal challenge because of asthma, and three patients in the same study were excluded from the analysis of season values, because they had used disodium cromoglycate briefly before entering the study. One patient missed the visit during beclomethasone dipropionate (BPD) therapy.

of a syringe, 300 μ l of the allergen or histamine solution was instilled into each nasal cavity. After each dose the patient counted the number of sneezes and rated their rhinorrhea and nasal congestion on a four-level scale as absent, slight, moderate, or severe, as described by Pipkorn (10). A minimum positive response to the challenge was defined as one symptom rated as moderate or two symptoms rated as slight. The postchallenge nasal secretion sample was taken when the patient described a positive response, up to 10 min after the final dose. This protocol has been used in a previous study by Linder et al. (5).

The allergen challenges were carried out with birch pollen extract in albumin diluent (Spectralgen®, Pharmacia). The test was begun with a diluent blank and continued through 100, 1000, 10,000, and 100,000 biological units, or discontinued when a positive reaction was provoked. Intranasal histamine challenges were carried out with histamine hydrochloride in 0.9% sodium chloride solution. The challenge was begun with a dose equivalent to 12.5 μ g of histamine base in each nasal cavity. The dose was progressively doubled, and the test was carried on until a positive reaction was provoked or a dose of 400 μ g had been given. No diluent blank was included, since the challenge was preceded by washing with 0.9% sodium chloride for sampling purposes. The doses were identical to those used by Secher et al. (11), and differ marginally from those used by other investigators (1, 6, 9, 10).

Statistical procedure

Testing of the significance of changes in a variable between two measurements was carried out by means of Wilcoxon's matched-pair test.

RESULTS

Allergen exposure

The mean histamine concentration was significantly lower ($P < 0.002$) in samples obtained after intranasal birch pollen challenge (2.08 μ g/

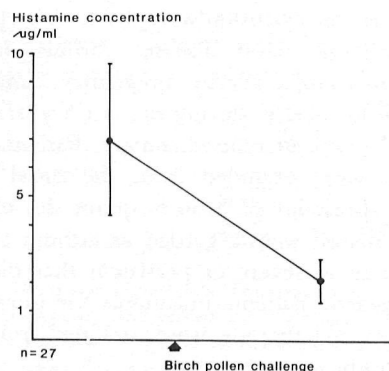


Fig. 2. The mean histamine concentration in nasal secretion before and after challenge. Combined results from the birch pollen challenges in the two studies (1983 and 1984). Error bars represent 95% confidence intervals.

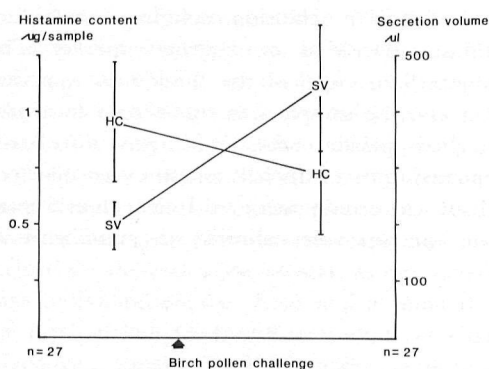


Fig. 3. The mean histamine content (HC) and the mean recovered secretion volume (SV) in nasal secretion samples before and after birch pollen challenge. Combined results from the birch pollen challenges in the two studies (1983 and 1984). Error bars represent 95% confidence intervals.

ml, SD 1.93) than in prechallenge samples (6.96 μ g/ml, SD 6.72), as seen in Fig. 2. The mean total histamine content, shown in Fig. 3, likewise decreased significantly ($P < 0.02$) following allergen challenge, from 0.96 (SD 0.68) to 0.73 μ g/sample (SD 0.69). The mean secretion volume which is also given in Fig. 3, was significantly higher ($P < 0.001$) after the challenge (440 μ l/sample, SD 280) than before (200 μ l/sample, SD 110).

The change in histamine levels between the

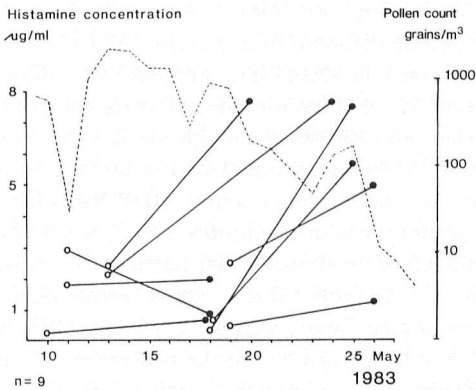


Fig. 4. Treatment with beclomethasone dipropionate during the birch pollen season in the 1983 study. The histamine concentration and the date of each sample are shown. Each line connects the sample before treatment (○) with that during treatment (●) for the same patient. Data from one patient are missing because of a technical fault. The broken lines shows the birch pollen values for the Stockholm region (Copyright for pollen values: Engström, Nilsson and Strandhede).

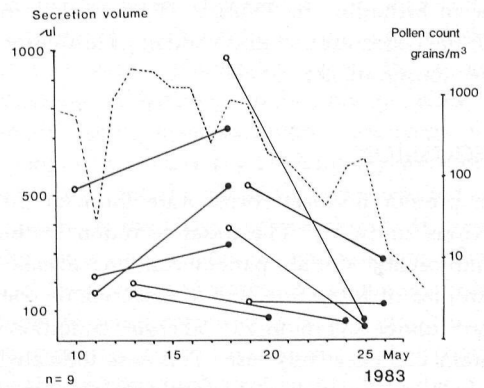


Fig. 5. Treatment with beclomethasone dipropionate during the birch pollen season in the 1983 study. The secretion volume and date of each sample is shown. Each line connects the sample before treatment (○) with that during treatment (●) for the same patient. Data from one patient are missing because of a technical fault. The broken line shows the birch pollen values for the Stockholm region (Copyright for pollen values: Engström, Nilsson and Strandhede).

visit before treatment during the seasonal part and the first postseasonal visit of the two studies (1983 and 1984) followed the pattern seen in the challenge model. The mean histamine concentration before the start of therapy during the season (2.30 µg/ml, SD 2.50) was significantly lower ($P < 0.01$) than that in the first postseason samples (7.18 µg/ml, SD 8.49). Similarly, a significantly lower ($P < 0.05$) histamine content was noted during the pollen season (0.46 µg/sample, SD 0.23) than after (0.89 µg/sample, SD 0.72). The mean secretion volume was higher during the season (320 µl, SD 220) than postseason (200 µl, SD 110), but this difference was not significant.

The mean symptom score was 1.4 of a maximum 3 at the first visit during the season, 0.6 at the second visit, and 1.1 after birch pollen challenge.

Treatment with beclomethasone dipropionate

A clinical improvement, observed as a mean decrease in the subjective overall rating of nasal symptoms by 0.8 points, occurred during the

period of corticosteroid therapy in the study of 1983. The mean histamine concentration in the nasal secretion (Fig. 4) was significantly higher ($P < 0.05$) during therapy (4.23 µg/ml, SD 1.15) than during the pollen season before therapy (1.55 µg/ml, SD 3.08) and at the same time significantly lower ($P < 0.02$) than postseason (10.59 µg/ml, SD 8.83). A significant increase ($P < 0.05$) in the mean absolute histamine content was noted during therapy in the season, compared with before therapy (450 µg/sample (SD 120) versus 350 (SD 116)). The mean histamine content during therapy was also significantly lower ($P < 0.01$) than postseason (820 µg/sample, SD 390). No significant difference was found either between the secretion volume during treatment and that before treatment during the season (Fig. 5), or those observed during and after the pollen season.

Histamine challenge

The histamine concentrations and contents in the secretion samples before and after histamine challenge are given together with the challenge

dose of histamine in Table 2. The changes in both the concentration and content of histamine were nonsignificant.

DISCUSSION

The present findings corroborate those of the previous study (5). The nasal secretion in the asymptomatic allergic patient contains sizeable quantities of histamine, but the secretions collected under symptomatic allergic conditions contain comparatively less. This was indicated by the finding of a decrease in histamine during deterioration of the rhinitis caused by allergen exposure and an increase during clinical improvement brought about by pharmacotherapy.

Two modes of allergen exposure were studied. Birch pollen challenge represents a standardized but artificial acute allergic rhinitis. During the natural pollen season, the conditions vary more between the samplings, and the natural disease probably involves a more pronounced element of inflammation. Both modes were followed by the same changes in the secretion samples: a fall in histamine concentration and content and a rise in secretion volume, although this rise was not significant during the pollen season.

One group of patients was studied during intranasal corticosteroid therapy. A period of 1 week was considered long enough to produce a reasonable therapeutic effect during the pollen season, and still short enough for all visits to be scheduled before the end of the birch pollination. In one challenge study, BDP had offered protection against symptoms after 1 week (13), although another study had failed to reproduce this (7). In the present study some clinical improvement was noted after 1 week of therapy, and at the same time the concentration and content of histamine in nasal secretion were intermediate between the pretreatment and postseason values. This leads to the same conclusion as the observations without therapy, that is, that the histamine concentration and content in nasal secretion are lower when the patients have clinical symptoms and increase as the condition improves.

There is an apparent conflict between the role that is generally assumed to be played by histamine in the allergic response and the present observations. However, if the secretion is regarded as a two-compartment system, where the newly secreted mucus makes up a more accessible compartment with a low histamine content, the observed decrease can be compa-

Table 2

Histamine challenges. Concentrations and contents of histamine in pre- and postchallenge samples of nasal secretion

Patient No.	Total dose μg (two sides)	Concentration $\mu\text{g/ml}$		Content μg	
		pre	post	pre	post
1	100	37.5	17.2	3.10	1.83
2	200	1.6	57.6	0.33	4.46
3	100	19.4	1.1	1.23	0.89
4	200	1.3	20.3	0.39	7.25
5	50	9.3	10.1	2.12	1.81
6	50	4.4	3.5	1.59	1.44
7	200	25.3	9.8	3.02	1.02
8	50	1.1	1.3	0.41	1.21
9	25	2.7	4.4	0.74	2.33
10	25	0.9	2.2	0.35	0.15
11	50	11.0	5.9	2.13	1.76
12	50	7.5	6.5	1.75	1.21
13	50	8.1	2.4	3.38	2.02
14	100	6.2	95.6	0.53	16.24
Mean		9.7	17.1	1.51	3.11

tible with an unaltered or even increased availability of histamine in a "deep" compartment. In other words, the secretion that is most easily collected is perhaps not in equilibrium with the biochemical environment of the effector organs in the mucosa. There is some support for such an assumption.

The report by Naclerio et al. (8) indicates that human nasal mucosa does in fact release histamine during allergic reactions *in vivo*, but the values obtained in their study refer to the diluted washings and give no information about the concentration of histamine in the secretion. Furthermore, in their method the prechallenge histamine concentration was reduced to low or undetectable levels by repeated washing of the nasal cavities. Thus, their method was designed to detect the release of mediators from the nasal mucosa in a challenge setting, whereas the purpose of the method of the present study was to measure the concentration of histamine in nasal secretion in any phase of the disease. The differences in approach and in the sample fluid may account for the divergent observations.

Exogenous histamine was used in the present study to provoke nasal symptoms. Reactions occurred in all patients thus challenged, but the postchallenge histamine concentrations in the collected secretion were found not to differ significantly from the prechallenge values, which were obtained under baseline conditions. When histamine triggers a reaction, the concentration at the receptor sites obviously must differ from the baseline level, and this latter observation strongly suggests that the histamine values are not representative of the activity at those sites. Another remarkable finding was that only an amount of histamine corresponding to a small fraction of the administered dose was recovered in the nasal washings. This again brings up the possibility of compartment, or pool, of histamine which is less accessible to the method of collection. It also raises the question whether there are homeostatic mechanisms which can protect the mucosa from prolonged exposure to an excess of the mediator.

To conclude, this study has demonstrated that the histamine level in nasal secretion can be used as a marker of changes in the severity

of allergic rhinitis, but that it is not ideal for the purpose. It should be required of such a marker that it varies predictably when the severity changes. Histamine can be said to fulfill this requirement, since variations concurrent with changes in clinical symptomatology were demonstrated, and the variations were detected adequately by the techniques employed. However, the association between symptomatic allergy and lower concentrations and contents of histamine in nasal secretion is purely empirical. A marker with a direct relationship to our concept of the pathophysiological events during allergic reactions would be much preferred, and the present results should give reason for a continued search for such markers.

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