

Nasal histamine release following hyperosmolar and allergen challenge

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Allergic rhinitis is characterised by symptoms of sneezing, itching of the nose with watery secretions, and nasal obstruction. We have previously shown that patients can have the diagnosis of allergic rhinitis confirmed by nasal provocation tests and assessment of nasal inspiratory peak flow (NIPF) after specific allergen or hyperosmolar challenge. We now show that histamine is released into the nasal lavage fluid in response to such challenges. Saline lavage alone results in detectable histamine levels in the order of 5 ng/ml, but in the presence of allergen (HDM) there is a significant increase in histamine release in atopics but not in control subjects. With hyperosmolar challenge, atopics showed a biphasic response in that histamine release was increased with 1.8% and 3.6% saline but returned to baseline with 5.4% and 7.2% saline, then showing a further increase with 9.0% saline. This raises the possibility of two populations of responsive mast cells. Hyperosmolar challenge leads to symptoms of nasal itch and sneezing as well as histamine release in atopics but not in controls. This suggests that hyperosmolar challenge can be used as a simple diagnostic test for allergic rhinitis and may provide a model for nasal hyper-reactivity.

Key words: allergen; allergic rhinitis; histamine; hyperosmolar saline; mast cells; nasal provocation.

Accepted for publication 12 July 1988

Nasal challenge with allergen and a variety of other immunological and non-immune substances produces clinical symptoms (9) and the release of mast cell associated mediators (5) at a rate and concentration that support the idea that it is these mediators that are causing the observed symptoms.

We have previously shown that both allergen and hyperosmolar challenge produce clinical symptoms but their range and severity differ between the two challenge regimens (9). In this study we have now examined histamine release from the nasal mucosa of atopic and normal subjects; mediator release being a good indicator of mast cell activation (11). We have used allergen and hyperosmolar challenge to investigate the mucosal sensitivity of these two populations and find substantial differences in clinical

response and mediator production. This paper describes the pattern of histamine release which, following allergen challenge, leads to a dose-dependent response whereas with hyperosmolar solutions there is a biphasic pattern.

Both allergen and hyperosmolar challenge lead to mediator release in atopic but not in control subjects. This suggests that hyperosmolar challenge may prove useful as a simple test for the diagnosis of allergic rhinitis and provide a model for the study of hyperreactivity in the nose.

PATIENTS AND METHODS

Eleven patients (7 male), aged 18-40 years (mean 28.5 years) were selected from the Allergy Clinic at the Middlesex Hospital, Lond-

on, England, all having a clear history of perennial allergic rhinitis strongly suggestive of house dust mite (HDM) and with positive skin-prick test to HDM in nine.

The history of the HDM allergic rhinitis was confirmed by nasal provocation test (NPT) and specific IgE to *D. pteronyssinus* in the two patients who were skin test negative (see Table 1). In addition to rhinitis, three patients also had asthma.

All patients in this study were asymptomatic at the time of the study and none were taking any drugs.

Nine controls were studied (6 male) age 21–59 (mean 37.2 years) with no nasal symptoms and negative skin-prick tests to a panel of 16 common allergens. A summary of the clinical data is shown in Table 1.

The competitive enzyme immunoassay was performed as described by Chevrier et al. (4). Briefly, the histamine standards and samples were first treated with 1.4 benzoquinone to form histaminy-benzoquinone derivatives. The precoated monoclonal antihistamine/benzoquinone microelisa plate (Diagnostics Pasteur, Marnes la Coquette, France), was washed four times with Tris buffered saline (TBS) containing 0.1 % Tween. The benzoquinone-treated histamine samples and standards were added to the plate at a volume of 100 µl per well and incubated overnight at room temperature. The histamine/benzoquinone-HRP conjugate was diluted in TBS plus 0.1 % Tween and 1 %

BSA and then added to the plate at 100 µl per well and incubated for 2 h at room temperature. The plate was again washed four times with TBS/0.1 % Tween. 200 µl of the substrate solution containing citrate buffer, tetramethylbenzidine and 0.06 % hydrogen peroxidase was added to the plate. After 20 min the enzyme reaction was stopped with 50 µl 1M H₂SO₄. The plate was read at 450 nm.

In the nasal provocation test (NPT) all patients and controls were tested to three reagents, namely normal saline, hyperosmolar saline solution, and allergen (HDM).

NPT was performed at the same time of day with weekly intervals between each stimulus as suggested by Borum et al. (2). The patients were not told the nature of the challenge. When tested with saline, the patients and controls were challenged sequentially five times at 10-min intervals; otherwise, if tested to an allergen or a non-specific hyperosmolar stimulus, they were challenged up to the maximal dose as stated below, or to the dose provoking a 50 % fall from the starting NIPF whichever came first. The control group was tested on each occasion up to the maximal dose. For the purposes of this study, the maximum fall in NIPF with any of the saline challenges was taken as the control value, not the average value of all five, thus minimizing the difference between control and allergen challenges and thus making more rigorous the difference between patient groups.

Installation of solutions was done using a metered pump-spray delivering one puff/volume (100 µl) in each nostril, with the patients holding their breath in full inspiration to avoid bronchial provocation. After spraying, patients were allowed to wipe their noses but not blow. In week 1, the patients were tested with saline only, in week 2, with 0.9 hyperosmolar saline solutions in increasing concentrations of 1.8 %, 3.6 %, 5.4 %, 7.2 % and 9 %, and in week 3, with increasing concentrations of HDM extract: with 10, 100, 1000, 10,000 BU/ml. The control group was tested to HDM up to 10,000 BU/ml and salt solution up to 9 %. On each occasion, the patients were challenged with 0.9 % saline as control.

Table 1
Details of the atopic patients

No.	Age	Sex	Asthma	Skin test + ve (HDM)	RAST (HDM)
1	34	F	-	-	+
2	18	M	-	+	ND
3	40	M	-	+	ND
4	25	F	-	+	ND
5	30	M	-	+	ND
6	25	M	-	-	+
7	38	F	+	+	ND
8	22	F	-	+	ND
9	33	M	+	+	ND
10	20	M	-	+	ND
11	28	M	-	+	ND

Nasal blockage was measured using the Youlten nasal inspiratory peak flow meter (Air-med, Clement Clark Int. Ltd., 15 Wigmore Street, London W1. Patent No. 1463814). Sneezes, itching of nose and plate, and hypersecretion after challenge were also recorded. To become familiar with the peak flow meter, patients were instructed in its use and practised twice daily at home during the week prior to the first challenge. Before testing began and 10 min after each challenge, three NIPF measurements were recorded and the highest value obtained used to calculate the percentage fall NIPF from the formula: $\text{NIPF post-challenge} - \text{NIPF} \times 100$ (%), thus again minimizing the differences between challenges. Sneezes were counted during the 10 min following each challenge. Itch and rhinorrhoea following challenge were assessed by questioning the patient. Symptoms were only considered positive if moderate to severe. "Slightly" runny nose was not positive. Before testing, all solutions were warmed to room temperature.

Freeze dried purified HDM vials 100,000 BU/ml (Pharmacia Diagnostic AB, Uppsala, Sweden, lot No. KB31373) were diluted to different concentrations in saline immediately before testing. The saline solution for dilution, challenge and lavage was used at pH 7.0.

Nasal lavage was conducted with the patient's head tilted back, and 5 ml of 0.9 % saline was infused into the nose (2.5 mls/nostril) using a disposable plastic pipette. The fluid was collected immediately by putting the head in a forward position and blowing out. The collected fluid was spun at 2000 g for 5 min and the supernatant removed and stored at -20°C.

Statistics: Paired t-tests have been used throughout.

RESULTS

Allergen challenge

When lavage was performed 10 min after allergen challenge, histamine could be detected in some patients when challenged with HDM concentrations as low as 10 BU/ml and in all

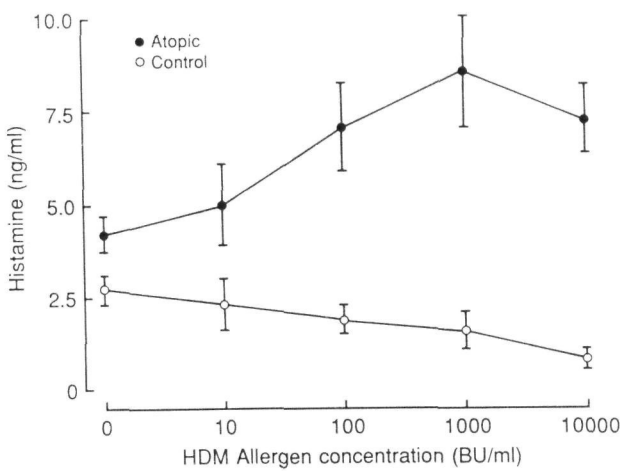


Fig. 1. Nasal lavage: Histamine release after allergen challenge.

patients at 100 BU/ml and over. No such release could be detected in the controls (Fig. 1). There was a dose-related release of histamine with a plateau at 1000 BU/ml of HDM. There was no significant difference in histamine release when saline was given ("0") but clear differences were seen following allergen provocation (Table 2).

Hyperosmolar challenge

With salt solutions all the patients released histamine in response to concentrations of 1.8 % NaCl. In the control group, an increase in histamine levels was detected only after challenge with 5.4 % NaCl (Fig. 2).

The patients gave a bi-phasic release of histamine with an early peak at 1.8-3.6 % followed by a trough at 5.4 % and a second peak at 9 %.

Table 2

Difference in histamine release following allergen challenge

Dose of HDM	P	No. controls	No. atopics
0 BU/ml	NS	8	9
10	< 0.01	8	9
100	< 0.001	8	8
1,000	< 0.001	8	8
10,000	< 0.001	8	5

After the first positive challenge some atopic patients declined further challenge with higher doses of HDM, therefore the number of patients at each point varies.

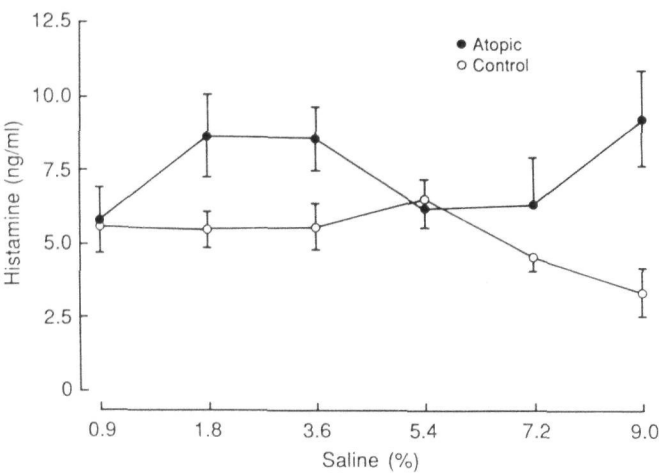


Fig. 2. Nasal lavage: Histamine release after hyperosmolar challenge.

The significance values are shown in Table 3 with the numbers of patients in each group.

DISCUSSION

We have previously shown that patients with allergic rhinitis respond reliably to nasal provocation with both allergen and hyperosmolar saline solutions (9). We now show that hyperosmolar challenge in the nose leads to histamine release in atopic subjects but not in controls (Fig. 2). The dose response curves of two challenge solutions are different. HDM gives a dose-dependent release of histamine with a plateau at 1000 BU/ml with no release in control subjects. Hyperosmolar saline leads to a marked biphasic release pattern in atopics with an early peak at 1.8–3.6% saline, followed by a trough at 5.4% with a second peak at 9.0% – again with no increase in histamine release in control subjects. These responses are clearly the hallmark of the atopic subjects and not of controls.

It has been reported that challenge with allergen leads to a fall in histamine due to a rise in the mean secretion volume (10). However, we found little change in fluid secretion after challenge. This difference is probably due to slight differences in methods used and indicated the difficulty in standardisation of such studies (1).

The nasal challenges were performed sequen-

tially at 10–15-min intervals. The early peak of histamine release suggests that one population of mast cells (and/or basophils) is more sensitive and perhaps more refractory to hyperosmolar saline than those that release following a 9% saline challenge.

A possible mechanism of such histamine release could be due to a possible differential cytotoxicity in mast cells lying at different levels within the nasal mucosa (7, 17).

The 1.8% and 3.6% hyperosmolar challenge could lead to a cytotoxic effect on superficial mast cells in the mucosa, with higher concentration needed to release histamine from those that are deeper. If this were the case we would have expected control subjects to release histamine at high concentrations of saline, which in fact they did not. Further evidence against a purely cytotoxic mechanism is the protective effect of sodium cromoglycate on histamine release following hyperosmolar challenge (8).

There was unlikely to be a significant redistribution of nasal mucosal mast cells in our atopic patients as they were asymptomatic at the time of study. Other groups using hyperosmolar nasal challenge have also demonstrated mediator release in atopic subjects (14). The histamine release obtained by these authors was similar to our findings. Hyperosmolar (14), allergen (5), dry air (15), and fog challenge (13) produce nasal symptoms by degranulating mast cells and releasing mediators including histamine, and these effects can also be blocked by sodium cromoglycate and nedocromil. Nasal symptoms following challenge can also be produced by methacholine (3), serotonin (16) and histamine (3) through mast cell-independent mechanisms. It is interesting that these agents

Table 3			
Difference in histamine release after hyperosmolar challenge			
% Saline	P	No. control	No. atopics
0.9	NS	9	11
1.8	< 0.005	9	11
3.6	< 0.01	9	10
5.4	NS	9	10
7.2	< 0.01	9	8
9.0	< 0.001	9	8

do not differentiate in an "all or nothing" manner between atopics and controls even though the atopics may show greater sensitivity to challenge. The mast cell-dependent agents produce symptoms only in atopics, and these responses are prevented by cromoglycate (8, 12, 13). This gives functional support to the central role of the mast cell in allergic responses of the nose and lung. Although we could not detect significant histamine release from controls with allergen or hyperosmolar challenge, our results and those of Lichtenstein's group (14) would indicate that the mechanism of mediator release with the two forms of challenge differs because of the different dose response curves which we have demonstrated and the different patterns of release of other mediators (14). This may indicate that different mast cell populations are involved in each case. Further work on this subject is now in progress.

ACKNOWLEDGEMENT

We are grateful to the American Academy of Otolaryngic Allergy for financial support.

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