

Plasma histamine concentrations and complement activation during house dust mite-provoked bronchial obstructive reactions

H. F. KAUFFMAN, S. VAN DER HEIDE, J. G. R. DE MONCHY and K. DE VRIES

Departments of Allergology and Pulmonology, Clinic for Internal Medicine, State University Hospital, Groningen, The Netherlands

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Summary

Lung function and histamine levels in peripheral venous plasma samples were followed after challenge with house dust mite allergen. In eight patients the mean maximal histamine concentration showed a significant rise during the early obstructive reaction when compared with the concentrations at similar time intervals after inhalation of a control solution. In nine other patients treatment with disodium cromoglycate (DSCG) caused a significant fall in plasma histamine concentrations indicating that histamine liberation plays a role during the early obstructive reaction and was released from the lung compartment. Histamine during early obstructive reactions compared with the pre-inhalation values did show a rise in mean maximal histamine concentration (1.5–4.1 ng/ml) but this was statistically not significant. During the late reaction neither significant increase in histamine nor inhibition by DSCG was found. Measurement of complement degradation products did not support the role of complement activation during either early or late bronchial obstructive reactions.

Introduction

Inhalation of house dust and house dust mite (*Dermatophagoides pteronyssinus*) may induce both early and late bronchial obstructive reactions. The pathogenesis of the early obstructive reaction (EBOR) is now well documented and in general is attributed to both the presence of elevated circulating IgE (Gökemeyer, 1976; Kerrebijn, Degenhart & Hammes, 1976; Pauli *et al.*, 1979; Spector & Farr, 1979), and the state of reactivity of the bronchial airways (Gökemeyer, 1976; Killian *et al.*, 1976; Neijens *et al.*, 1979). The mechanism underlying the late bronchial obstructive reaction is less clear. A type III reaction has been proposed for late reactions occurring after inhalation of *Aspergillus* allergen (Stevens, Hilvering & Orie, 1970). However, late reactions occurring after house dust and house dust mite inhalation do generally not

Correspondence: Dr H.F. Kauffman, Department of Allergology, Clinic for Internal Medicine, State University Hospital, Groningen, The Netherlands.

show either fever, leukocytosis and/or decrease in PaO_2 nor changes in radiological findings (Booij-Noord, Orie & Vries, 1972; Gökemeyer, 1976; Kerrebijn *et al.*, 1976; Warner, 1976). Whether an immune complex mechanism is involved in this late type reaction is still unresolved nor has evidence been found for the involvement of mast cell degranulation during the late reaction.

Information about mediator release both during early and late reactions may be obtained from the measurement of histamine in plasma as a marker of degranulation of mast cells after inhalation of an allergen. Elevations in plasma histamine were reported during early reactions after inhalation of different allergens (Allen *et al.*, 1979; Bath *et al.*, 1979; Martin *et al.*, 1980). Elevation of plasma histamine both during the early and late obstructive reaction was reported for one patient after inhalation of rat serum (Allen *et al.*, 1979).

In order to clarify the role of mast cell degranulation during both early and late obstructive reactions, histamine levels were followed in a larger group of patients after inhalation of house dust mite allergen. The effect of disodium cromoglycate (DSCG) pre-treatment was also studied on both lung function parameters and histamine concentrations. The involvement of complement in the pathophysiological process was studied by measuring the complement degradation products of C3 (C3b+c and C3d) in the same plasma samples used for histamine determination. As the occurrence of obstructive reactions is dependent on both the presence of homocytotropic antibodies and the state of reactivity of the bronchial airways, patients were characterized accordingly.

Methods

Twenty-two patients were studied who showed either early, late or combined bronchial obstructive reactions after inhalation of a house dust mite extract and further three patients with positive skin tests who did not react after inhalation of the antigen. None of the patients were treated with (oral) steroids and all medication was withheld 48 hr prior to inhalation. In eight patients the histamine concentrations during early obstructive reactions were compared with the corresponding values after inhalation of the control solution (Coca). In nine other patients the effect of DSCG was studied on both lung function and histamine concentrations. Additionally five patients were studied in order to extend the measurements during late obstructive reactions. Patients were selected and hospitalized in co-operation with the Department of Lung Disease.

Bronchial hyper-reactivity was measured by means of serial inhalations of histamine biphosphate in increasing concentrations using a 'Weisbadener Doppel inhaler' as described by Booij-Noord *et al.* (1972). The lowest concentration at which a fall in FEV_1 of 15% or more was found, was called the threshold value.

House dust mite (200 u/ml) challenge was performed as described for house dust by Booij-Noord *et al.* (1971), using challenges of 1, 4 and 5 min, successively at 5-min intervals. Challenge was terminated when the lung function decreased 15% or more and the FEV_1 was followed in time. Blood samples were taken from the antecubital vein in the presence of EDTA before challenge and 5 and 30 min, and 1, 3, 5, 7 and if possible 24 hr after the start of the provocation. The effect of disodium cromoglycate (DSCG) on house dust mite induced obstructions was studied as described by Booij-Noord *et al.* (1972) while blood samples were taken at similar time intervals as described above in order to study the effect on histamine liberation.

The blood samples were centrifuged directly and the plasma for histamine assay

stored at -20°C . Plasma for the determination of complement breakdown products was stored at -80°C . In a second sample leucocytes were counted.

Histamine was measured in five-fold by the single isotopic-enzyme assay as described by Beaven, Jacobsen and Horakova (1972), with a minor modification: for the isolation of ^3H -methylhistamine by extraction with chloroform, also solid NaCl was added (2.6 mg/ml), giving a better recovery of the ^3H -methylhistamine. Measurement of histamine in three healthy non-allergic individuals showed histamine concentrations below 1 ng/ml and did not rise above this value during the time periods mentioned above. Complement split products C3b + c and C3d were determined by the RIA and radial immunodiffusion according to Hannema *et al.* (1977) and Perrin, Lambert and Muscher (1975) respectively.

Specific IgE antibodies to house dust mite allergen were determined by RAST (Table 1). House dust mite extracts used in skin and inhalation tests were obtained from Diephuis Laboratories, The Netherlands.

Table 1. Characteristics of the patients with positive skin reactions against house dust mite who were challenged with the allergen

Patient No.	Age (years)	Histamine threshold (mg/ml)	Mite-specific IgE*	Obstructive reaction	
				Early (0-90 min)	Late (3-10 hr)
1	23	16	> 20	+	+
2	23	32	> 20	+	+
3	27	16	> 20	+	+
4	19	8	> 20	+	+
5	22	32	2-5	+	-
6	25	> 32	< 2	-	-
7	24	0.5	> 20	-	+
8	34	16	> 20	+	+
9	17	32	> 20	-	-
10	19	1	> 20	+	+
11	43	8	> 20	+	+
12	23	?	?	+	+
13	42	2	10-20	+	+
14	35	16	10-20	+	-
15	25	8	> 20	+	+
16	17	16	> 20	+	+
17†	39	?	?	+	+
18	26	16	> 20	+	-
19	17	8	> 20	+	+
20	22	8	nd	+	+
21	22	?	> 20	+	+
22	31	2	> 20	+	+
23	18	16	> 20	+	-
24	34	2	> 20	+	-
25	23	> 32	> 20	-	-

* Percentage binding of radio-active label. < 2 = Negative; 2-5, 5-10, 10-20, > 20 = increasing concentrations of IgE antibodies.

† Also obstructive after control solution.

Patients and reactions

It was possible to investigate plasma histamine after house dust mite inhalation in twenty-two reactors and three non-reactors. Histamine concentrations were measured after inhalation of the antigen at the time intervals as described above. After inhalation of the allergen five of the twenty-two reactors showed an isolated early reaction, one patient had an isolated late reaction and sixteen patients a combined early plus late reaction (see Table 1). All patients showed increased IgE antibodies against house dust mite except one (Patient no. 6), who did not react after inhalation of the allergen. All the patients who reacted with the antigen showed lowered threshold values for histamine. In the group of non-reactors the threshold value for histamine is negative twice and once slightly positive (32 mg/ml), reflecting a lower degree of non-specific bronchial hyperreactivity (Table 1). Calculations were done using Student's *t* statistics in general and additionally the sign test was used (Fig. 1) when the results did not show a normal distribution.

Results

In eight patients the maximal histamine concentrations during the early reaction after inhalation of house dust mite extract were compared with the maximal histamine concentrations measured at corresponding time intervals after inhalation of the control solution. Both the mean histamine concentration and the individual concentrations (seven out of eight) are increased after allergen inhalation when compared with the control values (Fig. 1). The differences are statistically significant when using the sign test, indicating histamine liberation after inhalation of the allergen.

The effect of DSCG on lung function and corresponding maximum histamine concentrations was also measured in nine patients after house dust mite inhalation with and without protection of the drug. In all patients DSCG treatment totally blocked the development of the obstructive reactions after allergen inhalation (not shown). In Fig. 2 it is shown that DSCG inhibited the elevations of plasma histamine during the time period of the early reactions till 60 min after inhalation, showing a statistically significant decrease of the mean plasma concentrations of 3.25–0.3 ng/ml. This indicates an inhibition of mast cell degranulation at the site of antigen application. By contrast, when studying the effect of DSCG during the recovery phase (60–180 min after inhalation) and the late obstructive reactions, the differences found in histamine concentration in the presence or absence of DSCG are statistically not significant (Table 2). The increase in histamine concentration found between 60 and 180 min after inhalation, indicates that histamine found during the period of recovery may not be derived from the lung compartment (Table 2). The mean maximal histamine concentrations in plasma of twenty-two reactors found before and after inhalation of the antigen are shown in Table 3. Increased histamine concentrations were found during early and late reactions when compared with values found during the pre-inhalation and recovery period.

However, the differences in histamine concentrations mentioned above are statistically not significant, due to the large inter-individual variations in histamine concentrations found both before and after inhalation of the allergen (Table 3). No consistent pattern was found between the maximum of the histamine concentration and the time of corresponding maximal fall in FEV₁ value. Maximal histamine values were found both before, during and after the minimum in FEV₁. Especially during the late reactions the increase in plasma histamine is often short in time falling back to zero

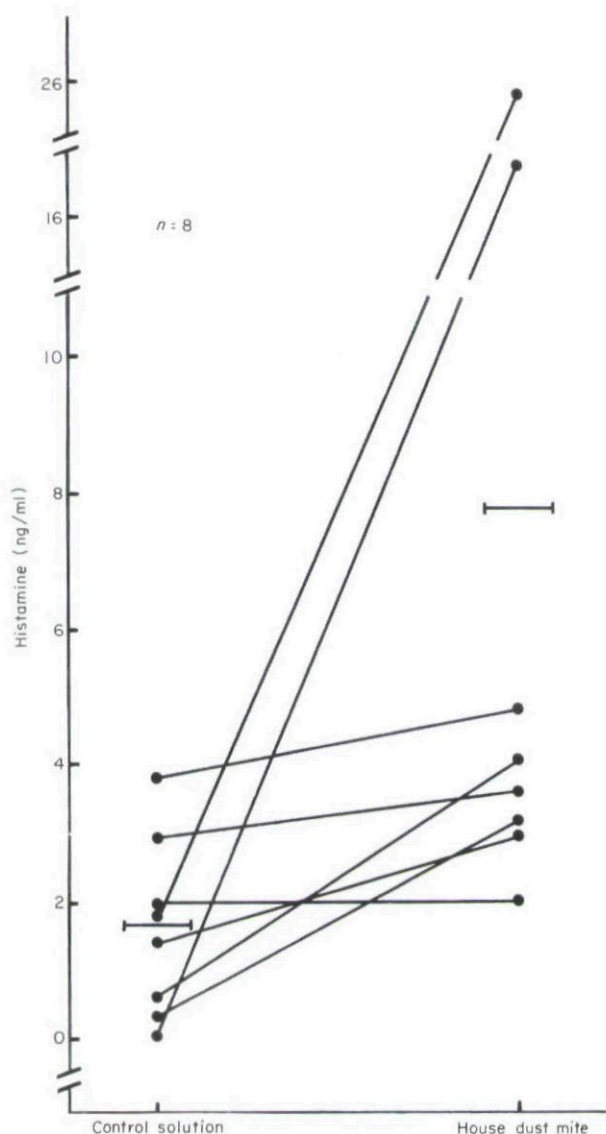


Fig. 1. Changes in individual ($n=8$) and mean maximum plasma histamine concentrations after house dust mite challenge during the early reaction compared with values found after challenge with control solution. — = mean values.

values when the obstruction is still maximal (FEV_1 minimal, not shown). Therefore, no correlation was found between the maximal fall in FEV_1 and the corresponding maximal plasma histamine concentrations found during the early and/or late reactions.

In nine patients the complement split products C3b+c and C3d were also determined at the same time intervals as the corresponding histamine measurements. From these nine patients, two showed an early reaction and seven a combined early plus late reaction. The concentrations of the complement split products did not show

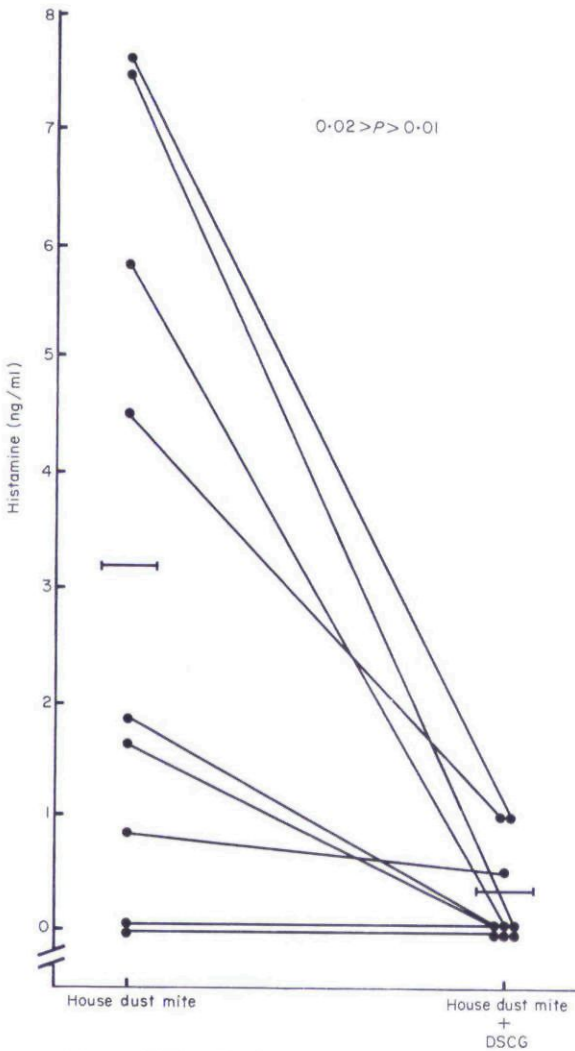


Fig. 2. Changes in individual ($n=9$) and mean maximum plasma histamine concentrations during the early bronchial obstructive reactions after house dust mite challenge with and without protection by DSCG. Histamine measurements were done at similar time intervals after allergen challenge. — = mean values.

Table 2. Concentrations of peripheral plasma histamine during early and late bronchial obstructive reactions and the intermediate recovery phase in nine patients after house dust mite challenge with and without DSCG treatment

	- DSCG			+ DSCG		
	Early	Recovery	Late	Early	Recovery	Late
Histamine (ng/ml)	3.25*	1.45	6.7	0.3*	4.05	1.35
s.d.	3.07	2.04	16.2	0.4	6.56	1.64

* $0.02 < P < 0.01$.

Table 3. Maximal histamine concentrations found in peripheral plasma during the early and late obstructive reaction and recovery period

Period	Time period		Histamine	
	(hr)	n	(ng/ml)	s.d.
Before inhalation	0	22	1.5	3.7
Early reaction	0-1½	21	4.1	6.2
Recovery period	1½-3	20	1.0	1.3
Late reaction	3-10	17	6.3	12.5

any relation with either early and/or late reactions and increased only incidentally slightly above normal values.

In accordance with previous observations, inhalation with house dust mite is generally not followed by fever and/or leukocytosis. However, one patient (Table 1, No. 3) with a dual reaction without a recovery phase between 1-3 hr after inhalation, also developed fever and leukocytosis and the corresponding histamine values were highly increased (Fig. 3). Also in this severely reacting patient no increase was found in the complement breakdown products during either early or late reaction.

Discussion

Plasma histamine concentrations were measured during bronchial obstructive reactions after house dust mite inhalation showing elevations during both early and late obstructive reactions. The mean plasma histamine concentration found before inhalation (1.5 ng/ml) is in accordance with similar values as reported by Simon *et al.* (1977) and Martin *et al.* (1980) in a sensitive group of patients using different allergens.

After allergen challenge a statistically significant increase was found in peripheral plasma histamine during the early reaction when compared with the values on a control inhalation day. The finding that treatment with DSCG significantly suppressed the elevation of histamine indicates that elevations in histamine concentrations found in the absence of this drug, are liberated from mast cells in the lung compartment. These observations are in accordance with those of Löwhagen (1980) measuring histamine metabolites in urine before and after treatment with DSCG. Although a rise in mean histamine concentration was also observed when compared with pre-inhalation values (Table 3), this increase in histamine was statistically not significant. This can be ascribed to: (1) the large inter-individual variation of the histamine values causing large standard deviations; and (2) in some individuals the pre-inhalation values were already relatively high and tended to remain constant or dropped to lower values after inhalation of the allergen (eight of twenty-one).

This latter observation suggests that a spontaneous liberation of histamine in the early morning has influenced the pre-inhalation histamine concentrations in accordance with the observations of Barnes *et al.* (1980).

The lack of relationship between the fall in FEV₁ and the corresponding increase in plasma histamine concentration could not be ascribed to differences in bronchial hyperreactivity as mentioned under Table 1. This indicates, that although hyper-reactivity to non-specific stimuli is an important factor for the development of obstructive reactions to allergens (Gökemeyer, 1976; Killian *et al.*, 1976; Neijens *et al.*, 1979), the

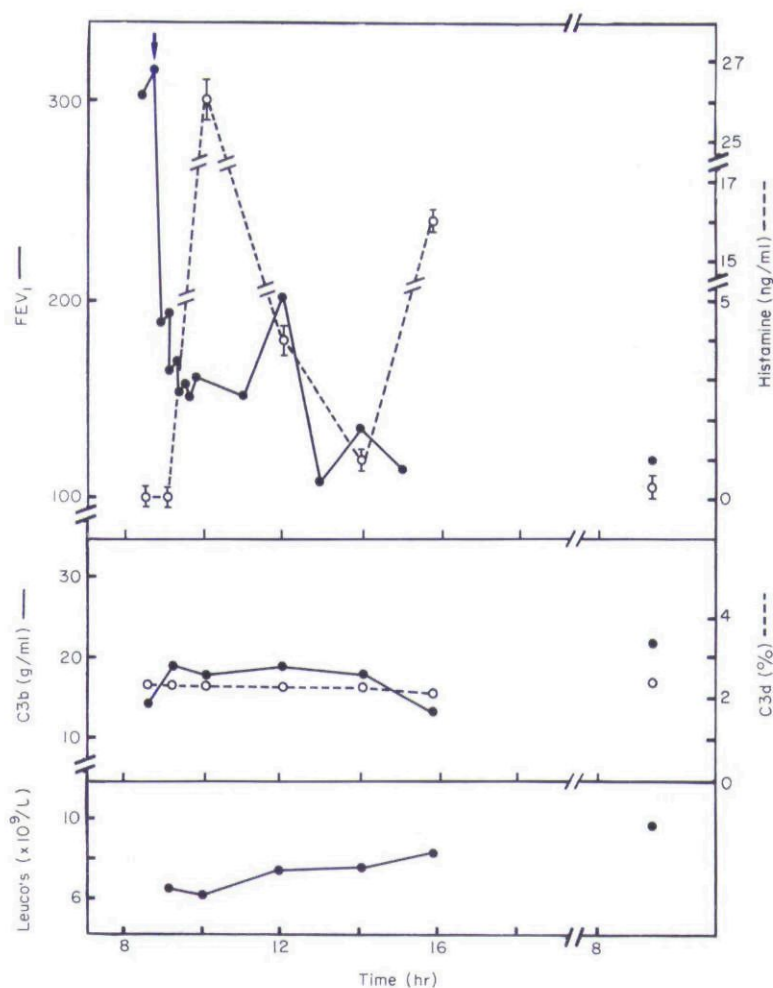


Fig. 3. Changes in lung function (FEV₁), plasma histamine, complement degradation products and white cell count during severe bronchial obstructive reactions following house dust mite challenge in Patient No. 3. → = challenge with house dust mite. Standard deviations of histamine measurements are indicated.

degree of hyper-reactivity alone does not determine the severity of the reaction. It is interesting to note that observations done by Martin *et al.* (1980) with pollen allergen-induced reactions did show more and longer lasting elevations in histamine when compared with the results obtained with house dust mite allergen (this study) or other allergens (Allen *et al.*, 1979; Bath *et al.*, 1976). These studies suggest that both dosage of allergen and nature of the allergen used, will play a role in the amount of histamine and other mediators (Martin *et al.*, 1980) to be released. Elevations of plasma histamine were also observed incidentally in our subjects during the recovery period between early and late reactions, which were not inhibited by DSCG treatment. This suggests a liberation of histamine from other sites than the lung compartment, possibly from circulating basophils (Allen *et al.*, 1979).

During the late reaction the increase of the mean maximal histamine concentration (Table 3) was statistically not significant and of short duration falling back to zero

values when the FEV₁ values were still decreasing (Fig. 3). Also the decrease of plasma histamine after DSCG treatment, was statistically not significant when compared with values in the absence of the drug (Table 2). These observations do not support the role of the mast cell degranulation during late obstructive reactions and suggest the involvement of other mediators. However, it remains possible that local binding and/or metabolism of histamine will prevent a rise in peripheral plasma histamine. Studies of both histamine and its metabolites (Keizer *et al.*, 1981) will possibly give more insight in the role of the mast cell degranulation during late obstructive reactions.

In agreement with similar observations of Hutchcroft and Guz (1978), the study of the complement breakdown products C3b + c and C3d does not support complement-mediated mechanisms of either early or late reactions with house dust mite allergen (Fig. 3). However, a limited local conversion of complement cannot be excluded.

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