Allergy, 1980, 35, 521–529

Studies on Histamine Metabolism in Allergen-Induced Asthma

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The excretion of histamine (Hi) and its metabolite methylhistamine (MeHi) was determined in separated fractions of urine up to 12h after standardized allergen provocations in 18 adult patients with defined extrinsic bronchial asthma. The main histamine metabolite, methylimidazoleacetic acid (MeImAA), was measured in six of the patients.

After positive provocations (decrease in $FEV_1 > 20\%$) the excretion of Hi was significantly increased during 3h and that of MeHi during 4h after challenge. Negative provocations (decrease in $FEV_1 < 20\%$) were not followed by any changes in the excretion of Hi and MeHi. MeImAA excretion increased in five out of six patients after positive provocation. It was calculated that the increased excretion of Hi and its metabolites after a positive provocation corresponded to a release of about 1 mg histamine in the body or about 1 μg/g lung tissue if all histamine was liberated in the lung.

Pretreatment with two anti-allergic drugs, disodium cromoglycate and ICI 74.917, giving significant allergen protection, resulted in a smaller increase of the excretion of both Hi and MeHi, indicating an inhibition of histamine release *in vivo*.

Key words: bronchial asthma; bronchial provocation test; histamine metabolism; histamine; methylhistamine; methylimidazoleacetic acid.

Accepted for publication 25 January 1980

Evidence for the role of histamine in allergic asthma has accumulated during recent years. The release of histamine (and other mediators) from sensitized mast cells, leucocytes and lung tissue after exposure to specific allergens is well documented (1, 2, 21).

In vivo studies of histamine in whole blood and urine have given conflicting results in asthmatic patients (10, 18, 24). However, with improved methods for analysing histamine Chiesa et al. (8) and Gold et al. (11) were able to demonstrate a pronounced increase of histamine in arterial and venous plasma in sensitized dogs after inhalation of specific antigen. Recently an increase of plasma histamine has also been reported in asthmatic patients during allergen-induced and spontaneous attacks (3, 6, 7, 26).

In a previous study on intrinsic asthma (18) we found an increase of the urinary excretion of histamine metabolites during acute attacks. This was also found in a preliminary study of extrinsic asthma after provocation with specific allergens (16). In an experimental study of the metabolism of intrabronchially administered ^{14}C-histamine in which urine was collected in separated fractions we were able to demonstrate a rapid excretion of labelled histamine and its metabolites (14).

The aim of this study was to test a model of

0105–4538/80/060521-9 $02.50/0

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Table 1.
Clinical details of 18 asthmatic patients included in the study of the urinary excretion of Hi, MeHi and MeImAA after bronchial allergen provocation with and without pretreatment (DSCG and ICI 74.917). The response to allergen provocation was expressed as the maximum per cent decrease in FEV_1, from pre-challenge value. In six patients positive provocations (>20° decrease) were performed on two different occasions. Pretreatment was given with DSCG in eight patients, ICI 74.917 in eight patients and placebo in 10 patients (corresponding FEV_1, values on the same line as the drugs).

				Max. % decrease in FEV_1				
				Neg.	Pos.	DSCG	ICI 74.917	
Pat.	Age	Sex	Allergen	provoc.	provoc.	(20mg)	0.2mg	1.0mg
1	16	F	cat		51			
2	20	F	dog		48	24	—	—
3	24	F	cat		62*	2	2	19
			birch		57*			
			dust	5				
4	25	F	dog	2	36	—	±0	2
			cat		27			
5	26	F	rabbit		28			
			mould	2				
6	27	F	dust		32	3	31	2
			birch		33			
7	27	F	rabbit		50	46	—	—
8	29	F	dog		68*			
9	30	F	cow		49	7	±0	17
10	50	F	dog	14	33			
	51	F	dog		46	17	26	55
11	31	F	dust	±0				
12	19	M	cat		32			
			cat		37			
13	23	M	cat		21			
14	31	M	dog		43*			
			dog		36	19	8	16
15	53	M	horse		22			
			cat	6				
16	64	M	cat		42	11	2	17
17	21	M	dog		30	—	5	16
18	44	M	mould	1				
			n	7	16	8	8	8
			mean	4.3%	40.4%**	16.1%	9.3%	18.0%
			s.e.	1.8	3.3	5.1	4.3	5.8

* Terbutaline by aerosol was given 30–60 min after challenge.
** In patients provoked twice the individual mean is first calculated.

fractionated urine collection for determination of endogenous histamine and metabolites after standardized bronchial allergen provocation, and to study the influence of anti-allergic drugs (disodium cromoglycate and ICI 74.917) which have an inhibitory effect on the release of histamine *in vitro* (5, 9).

PATIENTS AND METHODS

Eighteen adult patients, 11 men and seven women, with extrinsic bronchial asthma were selected for the study. The diagnosis was confirmed by a positive history of allergy, positive skin test and positive bronchial

Table 2.
Urinary excretion of Hi and MeHi before and after negative allergen provocations (< 20% decrease in FEV_1) in seven patients with extrinsic bronchial asthma

	Pre-challenge	Post-challenge periods (μg/h) 1	2	3	4	5-8h.
Hi						
n	7	7	7	7	7	6
Mean	1.1	1.0	1.0	0.8	0.8	1.2
S.E.	0.2	0.1	0.1	0.2	0.2	0.2
MeHi						
n	7	7	7	7	6	6
Mean	9	8	8	8	12	10
S.E.	3	2	1	2	3	2

provocation with one or more allergens. Selected clinical details are given in Table 1. At the time of the study the patients were in a symptom-free phase with values of FEV_1 within normal range. None of the patients was taking regular anti-asthmatic drugs. Occasional medication (terbutaline, salbutamol and disodium cromoglycate by inhalation) was not allowed during a period of 12 h. During the day of study the patients were given a standardized histamine restricted diet (13).

The allergen challenges were performed in accordance with the standardized method earlier described by Löwhagen & Lindholm (19). The allergen response was expressed as the maximum per cent decrease in FEV_1 from pre-challenge value during 30–40 min after challenge.

The study was divided into three parts, most patients being included in more than one part (Table 1). The provocations were performed with intervals of at least 1 week.

Part 1. Seven patients were challenged with clinically irrelevant or low concentrations of relevant allergens. The decrease in FEV_1 after provocation was less than 20% and was considered to be a clinically negative provocation result.

Part 2. Sixteen patients were challenged with clinically relevant allergens giving a decrease in FEV_1 of more than 20%, which was considered to be a positive provocation result. Six of the patients were challenged twice with the same or another relevant allergen.

Part 3. Eight patients were pretreated with disodium cromoglycate (DSCG), ICI 74.917 and placebo before challenge with relevant allergens (Table 1). DSCG, 20 mg (inhaled by a Spinhaler), ICI 74.917, 0.2 mg and ICI 74.917, 1.0 mg (inhaled by a metered aerosol) and placebo (inhaled by a metered aerosol of the same type) were given in random order 15 min before challenge, double-blind except for DSCG.

Laboratory procedures

Fractionated urine collection for determination of histamine (Hi), methylhistamine (MeHi) and (in six patients) 1-methyl-4-imidazoleacetic acid (MeImAA) was started 1–4 h before and continued for up to 12 h after challenge. During the first 4 h after challenge urine was collected in hourly fractions. During the next 8 h urine was collected in two fraction (5–8 h and 9–12 h). All urine samples were stored in glass bottles containing 100 ml of 1 mol/l hydrochloric acid. Before challenge diuresis was enhanced by giving the patients water to drink.

Histamine was determined by bio-assay on isolated guinea pig ileum after purification of the histamine by absorption on to a Dowex-50 cation exchange resin (28). All values were expressed as the "free base" and corrected for recovery. Methylhistamine was determined by reaction with 2,4-dinitrofluorobenzene, followed by separation of the reaction products by thin-layer chromatography and spectrophotometric measurements of the eluates from the chromatogram (13, 29). The values were corrected for recovery and expressed as the "free base".

Methylimidazoleacetic acid was determined according to the method of Granerus & Magnusson (12) with slight modification (15). After evaporation of the urine, esterification with ethanol, extraction with ether and

separation on a cellulose column, the final separation and quantification was done by thin-layer chromatography. The values were corrected for recovery using an internal standard of ^{14}C-MeImAA (15).

Statistical procedures

In patients challenged on two different occasions (part 2) the mean values of FEV_1 and excretion of Hi, MeHi and MeImAA for the corresponding fractions were first calculated. The mean difference between the excretion of Hi and MeHi before and after challenge was analysed by means of Wilcoxon's test for paired differences. The same test was used for analysing the mean differences in FEV_1, and excretion of Hi and MeHi between drug and placebo experiments. Correlations between urine volumes and excretion of Hi and MeHi were analysed by means of Pittman's test, where correlations within each patient were weighed together (23). Differences were considered significant for $P<0.05$.

RESULTS

Part 1. Negative provocations (Tables 1 and 2).

The maximum decrease in FEV_1 was 0–14%, mean 4%. The mean excretion of Hi and MeHi before provocation was 1.1 and 9 μg/h respectively. No significant change after provocation was observed.

Part 2. Positive provocations (Tables 1, 3, 4).

The maximum decrease in FEV_1 was 21–68% mean 40%. The mean excretion of Hi and MeHi before provocation was 1.0 and 7 μg/h respectively. After provocation the mean excretion of Hi and MeHi was increased during the whole observation period. A significant increase of Hi was observed during the 1st, 2nd and 3rd h. MeHi was significantly increased during the 1st, 2nd, 3rd and 4th h.

MeImAA was measured in six of the patients. The mean excretion was increased during the whole observation period compared with before challenge. Individually there was an increased excretion in five of the six patients and unchanged excretion in one patient.

There was a significant correlation between Hi and MeHi (r=0.21), Hi and MeImAA (r=0.59) and MeHi and MeImAA (r=0.46).

Part 3a. DSCG and placebo (Table 1 and Fig. 1).

In the eight patients the maximum decrease in FEV_1 was 32–62%, mean 46 % after placebo pretreatment and 2–46%, mean 16% after

Table 3.
Urinary excretion of Hi and MeHi before and after positive bronchial allergen provocations (>20% decrease in FEV_1) in 16 asthmatic patients

		Post-challenge periods (μg/h)					
	Pre-challenge	1	2	3	4	5-8	8-12h.
Hi							
n	16	16	15	15	14	12	9
Mean	1.0	2.3	2.1	2.1	1.6	1.5	1.1
S.E.	0.3	0.5	0.5	0.4	0.4	0.3	0.2
MeHi							
n	16	16	15	15	14	13	9
Mean	7	12	13	15	17	10	13
S.E.	2	2	2	3	5	1	3

Table 4
Urinary excretion of 1-methyl-4-imidazoleacetic acid before and after positive allergen bronchial provocation in six patients (Nos. 3, 4, 6, 9, 14 and 17 in Table 1) with a mean maximum decrease in FEV_1 of 40%.

		Post-challenge periods (μg/h)			
	Pre-challenge	1–2	3–4	5–8	9–12h
n	6	6	5	6	5
Mean	101	183	225	210	146
S.E.	18	12	44	39	19

DSCG. The mean difference between placebo and DSCG was statistically significant.

There was no difference in the pre-challenge excretion of Hi and MeHi between days with DSCG and placebo pretreatment.

Hi: After placebo pretreatment the mean excretion of Hi was significantly increased during the 1st and 2ndh. After DSCG the mean excretion of Hi was significantly increased only during the 1sth. Compared with placebo, the mean excretion of Hi was lower during all 8h. A significant difference was observed during the 4thh.

MeHi: After placebo pretreatment the mean excretion of MeHi was significantly increased during the whole observation period. After DSCG no significant increase was observed. Compared with placebo, a significantly lower excretion of MeHi was observed during the 2nd and 3rdh and during the last period (5–8h).

Part 3b. ICI 74.917 and placebo (Table 1 and Fig. 2).

In the eight patients the maximum decrease in FEV_1 after pretreatment with placebo was 30–62%, mean 42%. The corresponding value after the 0.2 mg of ICI 74.917 was 0–31%, mean 9% and after the 1.0 mg dose it was 2–55%, mean 18%. The difference between placebo and drug (both doses) was statistically significant. There was no significant difference in mean pre-challenge excretion of Hi and MeHi between placebo and ICI 74.917 (both doses).

Hi: After placebo the mean excretion of Hi was significantly increased during the 1st and 2ndh. After 0.2 mg and 1.0 mg of ICI 74.917 only small and insignificant increases were observed. Compared with placebo, lower excretion of Hi was seen during the whole observation period except the 3rdh, the difference being significant for the 0.2 mg dose during the 2ndh.

MeHi: After placebo pretreatment the mean excretion of MeHi was significantly increased during all 8h. After 0.2 mg and 1.0 mg of ICI 74.917 the increase in excretion was less marked and significant only for the 1.0 mg dose during the 3rd and 4thh. Compared with placebo, there was a consistently lower excretion of MeHi during

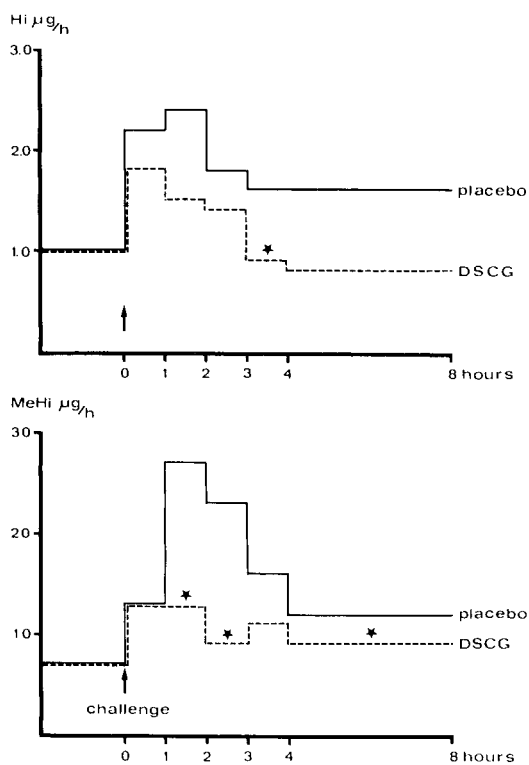

Figure 1. Urinary excretion (mean) of Hi and MeHi before and during 8h after bronchial allergen challenge in eight patients pretreated with placebo and disodium cromoglycate (DSCG) 15 min before challenge. Challenge (during 1–5 min) is indicated by arrow. Significant difference between drug and placebo in corresponding urine fractions is indicated by asterisk.

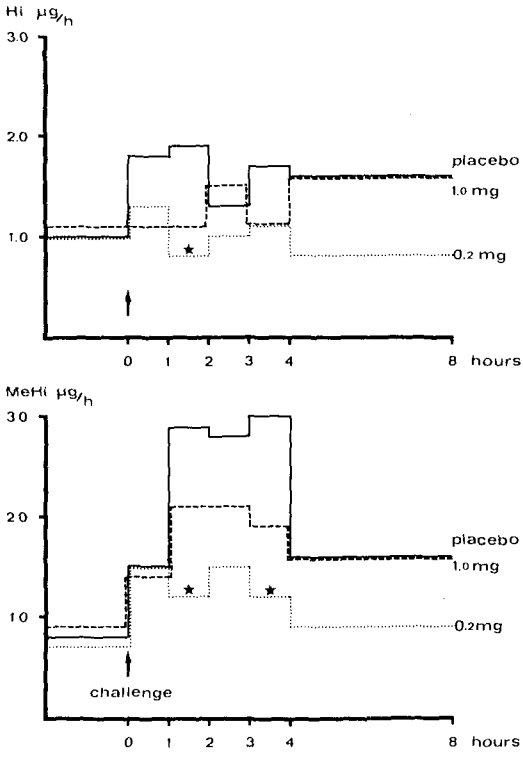

Figure 2. Urinary excretion (mean) of Hi and MeHi before and during 8h after bronchial allergen challenge in eight patients pretreated with placebo and ICI 74.917 (0.2 and 1.0mg). Challenge is indicated by arrow. Significant difference between drug and placebo in corresponding urine fraction is indicated by asterisk.

the whole observation period. A significant difference was registered for the 0.2 mg dose during the 2nd and 4th h.

Parts 1–3. Correlations to urine volumes and FEV_1

The correlations between urine volumes and excretion of Hi and MeHi were positive and significant in part 2 but not in part 1. There was no significant difference in urine volumes between part 1 and 2 in any corresponding urine fraction. After hydration the mean volumes were about 100 ml/h.

The correlations between maximum decrease in FEV_1 and excretion of Hi, 0–2h and MeHi, 0–4h after provocation, in parts 1 plus 2 were significant ($r=0.44$ and $r=0.49$ respectively). Corresponding correlations in parts 1,2 plus 3 were not significant.

DISCUSSION

In this study a model for fractionated urine collection for the determination of histamine and its metabolites after standardized allergen provocations has been tested in asthmatic patients. It has been shown that reaginic antigen-induced bronchospasm was associated with increased excretion of histamine and histamine metabolites in the urine, indicating a release of histamine in the lung. Negative provocation was not followed by any change in the excretion of histamine and MeHi. Pretreatment with two anti-allergic drugs, DSCG and ICI 74.917, giving significant allergen protection, resulted in a smaller increase in the excretion of both histamine and methylhistamine compared with placebo. The results obtained are in accordance with the plasma histamine changes found during allergen-induced bronchospasm in asthmatic patients by Bhat et al. (3) and give further support to the hypothesis that IgE-mediated histamine release is related in some way to allergen-induced bronchospasm in human bronchial asthma.

The collection of urine in separated fractions was guided by a previous study on the catabolism and excretion of intrabronchially given ^{14}C-histamine in asthmatic and non-asthmatic patients (14). According to this study, the urinary excretion of unmetabolized histamine corresponded to only 1–3% of the given dose. The main part was excreted within 1 h after administration. The intermediate metabolite MeHi accounted for 7% of the given dose and the main metabolite MeImAA for 40–50% within 6h after administration. Most of the remaining ^{14}C was excreted during the following 6h and more than 70% of the ^{14}C-activity could be expected to be MeImAA. Thus, by measuring the excretion of histamine and its methylated metabolites up to 12h after allergen provocation, it seems possible to estimate the release of histamine.

The urinary excretion of histamine and metabolites was about doubled after positive provocations compared with that before

challenge or compared with that found in the negative provocation group, in which the excretion remained at the pre-challenge level. The peak excretion of unmetabolized histamine was observed during the 1st h after challenge but the differences between the first 3 h were small. The peak excretion of MeHi was observed during the 4th h but the differences between the first 4 h were again small. The MeImAA excretion was increased during all 12 h after challenge, with a peak excretion 2–4 h after challenge. The excretion of histamine but not that of MeHi and MeImAA had returned to the pre-challenge level 9–12 h after challenge.

From these findings it can be calculated that during 12 h after challenge, histamine and its metabolites were excreted in amounts above the basal level corresponding to about 1 mg histamine base. Assuming that all histamine is liberated from the lungs, this will mean a release of about 1 μg/g lung tissue (2–3% of total histamine content). These figures can be compared with those found *in vitro* after allergen exposure in sensitized human lung tissue. Schild et al. (25) and Brocklehurst et al. (4) reported a release of 2.6–4.3 μg/g (8%) and 1.4 μg/g respectively in lung fragments obtained from allergic subjects. In passively sensitized human lung tissue a release of 16–20% of the total histamine content has been reported (20, 22, 27).

After allergen bronchial provocation in dogs sensitive to nematode antigen, Chiesa et al. (8) have reported much higher levels of plasma histamine in the aorta than in the pulmonary artery, indicating that the released histamine comes primarily, perhaps exclusively, from the lung. During spontaneous attacks of asthma in man, Charles et al. (7) could not detect significantly higher concentration of plasma histamine in arterial than in venous blood. However, their patients had been ill for several hours and higher arterial concentration at an earlier stage could not be excluded.

In the study of the metabolism of ^{14}C-histamine given intra-bronchially, it was observed that the excretion of ^{14}C-activity was dependent on the urine flow. A low diuresis caused a delayed excretion of total ^{14}C-activity. Especially unmetabolized histamine and the intermediate metabolite MeHi seemed to be more rapidly excreted if a certain level of diuresis was achieved. In the present study the patients were hydrated by giving them water to drink, making sure that a sufficiently large volume of urine would be voided during each hour after challenge. As in a previous study (18), intraindividual correlations between urine volume and excretion of histamine and MeHi were obtained in the positive provocation group. However, a high diuresis could not explain the increased excretion of histamine and metabolites as there was a similar diuresis in the positive and the negative provocation group.

After intrabronchial administration of ^{14}C-histamine during a symptom-free phase of asthma the urinary excretion of labelled histamine was found to occur mainly during the 1st h. In this study of endogenous histamine turnover in acute asthmatic attacks there was a similar onset of the histamine excretion, but this took place over a longer period, about 3 h. This may indicate a rapid induction of histamine release, which is in agreement with the findings of an increased plasma histamine level within a few minutes after provocation (3), but also points to a continued release of histamine for some hours. As the asthmatic symptoms after an allergen challenge last for some hours, there seems to be some relationship between symptoms and release of histamine but this was not studied in detail. However, there was a significant correlation between maximal decrease in FEV_1 within 30 min and the excretion of histamine and MeHi.

The experimental model was also used to study the influence on the excretion of histamine and MeHi of two anti-allergic drugs (DSCG and ICI 74.917), which have an inhibitory effect on the release of histamine *in vitro* (5, 9) and for which an allergen-protective effect has been demonstrated in clinical studies (5, 9, 17). In this limited number of patients

there was no significant correlation between allergen protection and reduction of urinary histamine and MeHi, but the increased excretion of both histamine and MeHi was generally reduced after pretreatment with the two drugs, indicating an inhibition of histamine release *in vivo* simultaneous with a significant clinical effect. Studies of this type might be useful in the evaluation of anti-allergic drugs in man.

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