

Tryptase and histamine release due to a sting challenge in bee venom allergic patients treated successfully or unsuccessfully with hyposensitization*

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Summary

Background Hyposensitization with bee venom leads to full protection in most, but not all patients with IgE-mediated systemic reactions to bee stings.

Objective To determine the relationship of clinical reactivity to the release of mediators and to changes of antibody concentrations in the peripheral circulation at a bee sting challenge test.

Methods Blood was sampled before (0 min) and at 15, 60 and 180 min after a sting challenge from 19 patients on hyposensitization. Of these, six still reacted and 13 were protected. Histamine, mast cell tryptase, bee venom-specific IgE and IgG in the serum, and histamine release from peripheral blood leucocytes (PBL) upon exposure to bee venom were determined.

Results Tryptase above the detection level was found only at 15 (60) min in 4/6 (1/6) patients who reacted. After the sting challenge there was a significant increase of the histamine levels in patients who reacted at 15 min ($P < 0.05$) and in patients who did react at 60 and 180 min ($P < 0.01$). The total histamine content of PBL was significantly decreased after 15 and 60 min in patients who reacted ($P < 0.01$) and in those that did not ($P < 0.05$). Bee venom-induced histamine release was significantly reduced in patients reacting and those that did not at 15 min ($P < 0.05$), and was significantly decreased in reactors also at 60 and 180 min ($P < 0.05/0.01$). Specific IgG antibodies showed a minor decrease ($P < 0.05$) after the sting challenge in both groups, whereas specific IgE did not change significantly.

Conclusion These results indicate that bee venom anaphylaxis is associated with the release of mediators from both mast cells as well as basophils. Successful hyposensitization does not induce a state of immunological non-reactivity, but rather alters the magnitude and the pattern of mediator release.

Keywords: anaphylaxis, hyposensitization, bee venom allergy, tryptase, histamine, basophils, specific IgG antibodies, specific IgE antibodies

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Introduction

In patients with IgE-mediated, systemic, anaphylactic

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reactions to bee or vespid stings prophylactic treatment with hyposensitization is highly effective. The outcome of venom hyposensitization has been assessed by sting challenge in treated patients, and complete protection, that is the absence of systemic clinical symptoms, has been found in, e.g. 77.6% [1], 80.4% [2], 97.5% [3] or 100% [4]. In most studies, a few patients did not respond adequately to hyposensitization.

The source of mediators released in anaphylactic reactions has not yet been definitely elucidated, basophils, mast cells or both types of cells being the most probable candidates [5,6]. Also, the renin angiotensin system may play a role in hymenoptera venom anaphylaxis, as allergic patients showed significantly reduced renin, angiotensin, angiotensin I and angiotensin II plasma levels as compared with controls [7]. Sting challenge provides a unique chance to determine mediator release in anaphylaxis. Also, it allows to compare the effects of allergen exposure in patients reacting no more with those in patients who continue to develop systemic symptoms. Thus, clues to the yet unknown mechanisms operative in hyposensitization may be obtained.

We have assessed the following parameters in bee venom-allergic, hyposensitized patients before and after a sting challenge in those who reacted and those that did not: the levels of mast cell tryptase and histamine in the circulation, the histamine content of peripheral blood leucocytes (PBL) and their release of histamine upon exposure to bee venom *in vitro*, and the serum levels of bee venom-specific IgE and IgG.

Materials and methods

Patients

Throughout 1991, 58 patients hyposensitized with bee venom (Reless, Scherax, Hamburg, Germany, 100 µg/4 weeks) were challenged with the sting of a living bee. Ten of these (17.2%) reacted systemically, 48 (82.8%) were found to be protected. Reactions were graded according to the system illustrated in Table 1. Of these 58 patients, six consecutive patients who reacted (four men, two women; aged 23–58 years) and 13 consecutive patients who did not react (seven men, six women; aged 17–58 years) were included in the study. The duration of hyposensitization had been 12–52 months in those showing reactions and 12–45 months in those that did not.

Table 1. Grading of anaphylactic/anaphylactoid reactions (after 8)

Severity grade 1:	only skin symptoms not restricted to the sting site (pruritus, flush, urticaria, angioedema)
Severity grade 2:	minor respiratory/cardiovascular/gastro-intestinal symptoms
Severity grade 3:	full shock
Severity grade 4:	cardiac/respiratory arrest

Skin testing

Threshold skin-prick testing with bee venom (*Apis mellifica*) (Reless, Scherax, Hamburg, Germany) was done on the ventral aspect of the forearm with incremental concentrations of 0.01, 0.1, 1.0, 10.0 and 100.0 µg/mL. A positive test result was defined as the presence of at least a weal with a diameter ≥ 2 mm. The lowest concentration causing a positive result was designated as the threshold concentration.

Sting challenge

For the sting challenge patients were hospitalized, the usual interval to the last bee venom injection being 3–4 weeks. After installation of an intravenous line and under anesthesiological supervision, a honey bee (provided by a bee keeper) was placed on the upper arm of the patient and stimulated to sting. The sting was left in the skin for at least 5 min. Symptoms occurring were treated and recorded. Patients were supervised on the ward for at least 18 h after the sting challenge.

Assays

Peripheral venous blood was drawn before the sting challenge (0 min) and at 15, 60 and 180 min thereafter. Serum samples were stored at -80°C . The sera were assayed with radioimmunoassays for mast cell tryptase (Pharmacia Tryptase RI-ACT, Pharmacia, Uppsala, Sweden) and for histamine (Histamin RIA, Immuno-tech S.A., Hermann Biermann, Bad Nauheim, Germany). Specific IgE was measured by a fluorescence enzyme immunoassay (Pharmacia CAP System, RAST FEIA; Pharmacia, Uppsala, Sweden). Specific IgG antibodies were determined by a radioimmunoassay (IgG RAST, Pharmacia, Uppsala, Sweden); values are given for IgE in kU/L and for IgG as percentage in relation to a reference sample as provided by the manufacturer.

The histamine content of PBL and the release of histamine from PBL upon stimulation with bee venom was determined as follows [9]: blood drawn into heparinized syringes was mixed with 6% dextran. After 90 min of sedimentation the leucocyte-rich supernatant was further isolated by centrifugation (4°C ; 300 g) for 10 min. The supernatant was discarded and the pellet was resuspended in 5 mL distilled water for 10 s; then 5 mL of 1.8% saline solution were added and centrifugation was repeated (4°C ; 300 g; 10 min). The supernatant was discarded and the pellet was resuspended in TCM buffer (NaCl 7.0 g; Tris 3.0 g; KCl 0.3 g; $\text{MgCl}_2 \times 6 \text{H}_2\text{O}$ 0.2 g; $\text{CaCl}_2 \times 2 \text{H}_2\text{O}$ 0.147 g; Aqua dest ad 1000 mL). Centrifugation and resuspension were repeated twice.

Table 2. Clinical data

No.	Patient	Sex	Age (years)	Severity grade of last reaction	Duration of HS (months)	Interval between last sting reaction and SC (years)	Threshold concentration at prick testing with BV (ug/mL)**	BV-specific IgE antibodies (RAST-class)**	Systemic symptoms at SC	Timepoint of intravenous drug* treatment (min after sting)
<i>Patients with systemic reactions at the SC</i>										
1	●	m	58	3	17	2	100	0	Flush, conjunctivitis	15
2	◇	f	26	2	15	2	100	3	Flush, stridor, conjunctivitis	9
3	■	m	35	2	12	1	100	2	Conjunctivitis, tachycardia	15
4	□	m	55	3	13	2	100	2	Conjunctivitis, nausea, drop in BP	10
5	★	f	23	1	52	1	10	3	Flush, angioedema, conjunctivitis	13
6	○	m	46	2	15	1	10	1	Flush, angioedema, drop in BP	19
<i>Patients without systemic reactions at the SC</i>										
1	●	f	30	3	15	3	100	2		
2	◆	f	51	2	41	5	⊖	0		
3	■	f	45	1	25	2	Not done	1		
4	□	m	35	3	14	2	100	1		
5	★	f	58	3	15	2	⊖	3		
6	○	m	48	2	38	4	100	2		
7	▲	m	37	2	18	2	100	1		
8	△	m	19	1	12	2	100	2		
9	▼	m	31	3	14	2	100	3		
10	▽	f	18	2	13	10	100	2		
11	★	f	17	2	17	1	100	3		
12	⊙	m	37	2	12	1	100	3		
13	◇	m	23	1	45	5	100	2		

* 8 mg dimetindene maleate, 400 mg cimetidine in all patients; additionally 750 mg prednisolone in patient 2 and 4.

** Before the SC.

f. Female. m. Male. BP. Blood pressure. HS. Hyposensitization. SC. Sting challenge. BV. Bee venom. ⊖. Negative up to 100 µg/mL.

Afterwards, the cell density of the suspension was adjusted to 10^6 cells/mL by the addition of buffer and 0.5 mL were incubated for 30 min with bee venom (Reless, Scherax, Hamburg, Germany) at a concentration of $0.08 \mu\text{g/mL}$ at 37°C . Supernatants were recovered by centrifugation on (4°C ; $300g$) for 10 min. The histamine content was measured spectrofluorometrically according to the method described by Siraganian [10] with an auto-analyser (Autoanalyzer für Histamin, Braun und Luebbe, Norderstedt, Germany). Blank values were obtained by handling bee venom-free samples as described. Total histamine content was measured after complete cell lysis induced by addition of 70% perchloric acid to the cell suspension. The histamine release induced by bee venom was calculated as percentage of the total histamine content according to the formula

$$\text{Histamine release} = \frac{\text{Histamine content of bee venom-containing sample} - \text{histamine content of blank}}{\text{Total histamine content} - \text{Histamine content of blank}} \times 100.$$

Statistical analysis and data presentation

If not indicated otherwise, the median and single values (characterized by different symbols for each individual) are given. The Wilcoxon test was used for comparisons within the groups of reactors or non-reactors, the unpaired Mann-Whitney *U*-test for comparisons between the groups (except for sex distribution). The chi square test was used for statistical analysis of the sex distribution. *P* values ≤ 0.05 were regarded as significant. Calculations were done using a computer program (GFA-Statistik für Atari von W. Buscher, G.P. Engels & J. Krauth).

Results

Clinical data

At the sting challenge, three of the reactors developed an

Table 3. Mast cell tryptase (U/L) in the serum before (0 min) and 15, 60 or 180 min after the bee sting. No tryptase could be detected in patients who did not react to bee sting challenge

Patient*	0 min	15 min	60 min	180 min
●	<2	3.6	<2	<2
◇	<2	<2	<2	<2
■	<2	<2	<2	<2
□	<2	27.4	<2	<2
★	<2	2.1	<2	<2
○	<2	4.1	3.0	<2

* Identification as in Table 2.

anaphylactic reaction of the severity grade 1, another three a reaction of the severity grade 2. Reactors and non-reactors did not differ significantly with regard to age, sex, severity grade of the last clinical systemic reaction, threshold concentration for bee venom in the

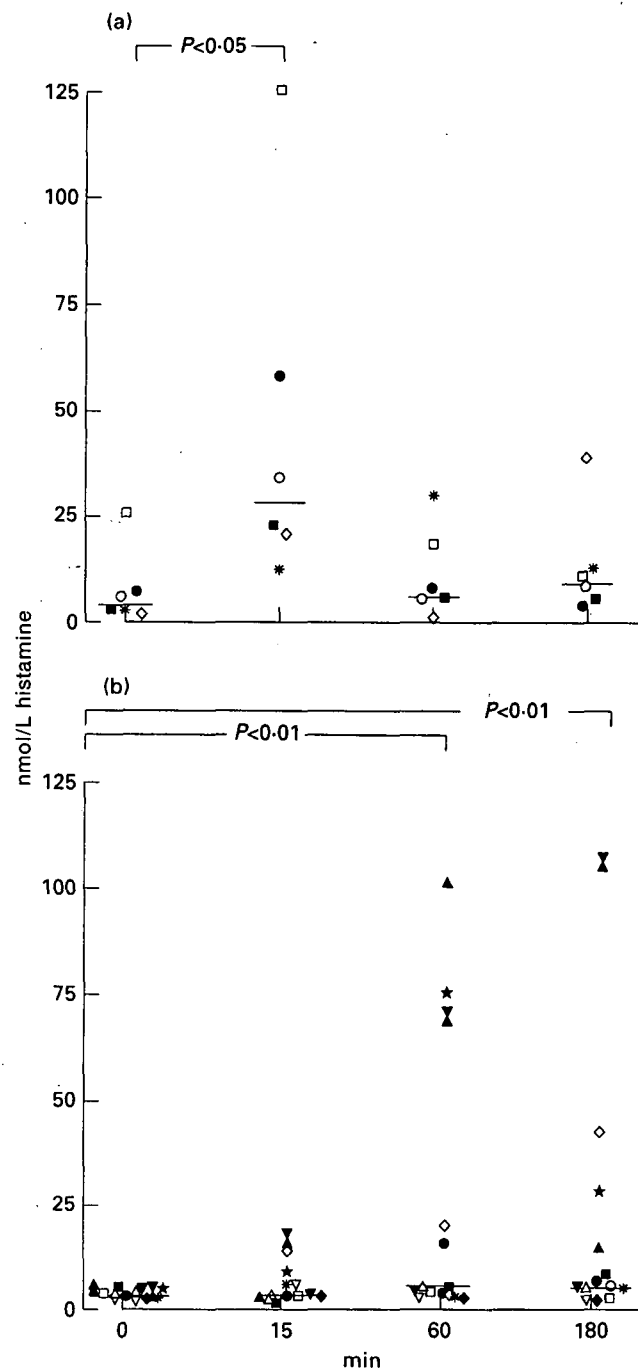


Fig. 1. Histamine in the serum of patients before and after the bee sting challenge. Patients identification is according to Table 2. —. Median. (a) Reactors. (b) Non-reactors.

skin-prick test or duration of hyposensitization. Details are given in Table 2.

Mast cell tryptase

Before the sting challenge, mast cell tryptase was below the detection level (2.0 U/L) in all patients. Four of six patients displaying a reaction exhibited an increase of mast cell tryptase at 15 min after the sting (2.1 U/L; 3.6 U/L; 4.1 U/L; 27.4 U/L), one of these also at 60 min (3.0 U/L) (Table 3). None of the non-reactors had demonstrable mast cell tryptase levels after the sting.

Histamine levels

Patients reacting had higher histamine levels than those that did not before the sting challenge (median: 4.7 nmol/L vs 3.0 nmol/L; $P < 0.05$). In those reacting, there was a significant increase of the histamine levels to 28.5 nmol/L (median) at 15 min; at 60 or 180 min levels did not differ significantly from the baseline values (median 7.4 nmol/L or 10.2 nmol/L). In non-reactors the histamine levels were not significantly different from the baseline values at 15 min and were increased at 60 min or 180 min (median 4.9 nmol/L or 5.8 nmol/L; each $P < 0.01$). At 15 min both groups of patients differed significantly with regard to their histamine levels ($P < 0.01$). For details see Fig. 1.

Histamine content of PBL

The total histamine content of PBL was not significantly different in reactors and non-reactors at 0 min (median 0.19 nmol/ 10^6 leucocytes vs 0.23 nmol/ 10^6 leucocytes). At 15 or 60 min it was decreased in those showing reactions (median 0.08 nmol/ 10^6 cells or 0.06 nmol/ 10^6 ; each $P < 0.01$) and in those that did not (median 0.19 nmol/ 10^6 cells or 0.21 nmol/ 10^6 ; each $P < 0.05$). At 180 min there was no significant difference from the baseline values in either group. At 15 or 60 min, the values of patients with reactions were significantly ($P < 0.01$) lower than the values of those who did not. Details are shown in Fig. 2.

Bee venom-induced histamine release from PBL

Before the sting challenge, the median bee venom-induced histamine release was 18.5% in patients reacting and 46.5% in those who did not (no significant difference). Compared with the baseline values, allergen-induced histamine release was significantly

decreased in reactors at 15 min (median 12.2%; $P < 0.05$), 60 min (median 11.0%; $P < 0.05$) and 180 min (median 8.0%; $P < 0.01$). Correspondingly, PBL from non-responsive patients released significantly less histamine at 15 min (median 19.8%; $P < 0.05$), but not at 60 min or 180 min. At 15 and 60 min, PBL from patients with reactions released significantly ($P < 0.01$ / $P < 0.05$) less histamine than PBL from patients who had none. Details are shown in Fig. 3.

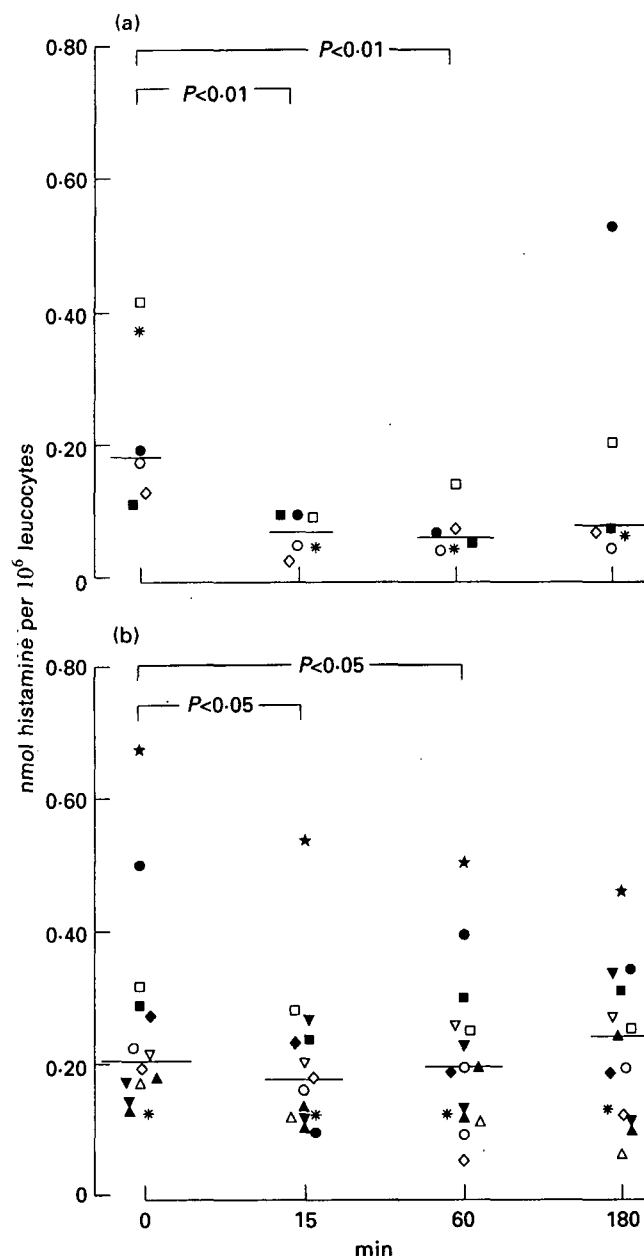


Fig. 2. Total histamine content of peripheral blood leucocytes before and after the bee sting challenge. Patients identification is according to Table 2. —. Median. (a) Reactors. (b) Non-reactors.

Bee venom-specific IgE

Median bee venom-specific IgE levels were 1.98 kU/L in patients reactions and 2.50 kU/L in those who did not at 0 min (no significant difference). They were not changed significantly at 15 min (median 1.18 kU/L/1.41 kU/L), 60 min (median 1.18 kU/L/1.01 kU/L), or 180 min (median 1.28 kU/L/1.23 kU/L). There was no significant difference of these values between both groups at any of the time points of determination.

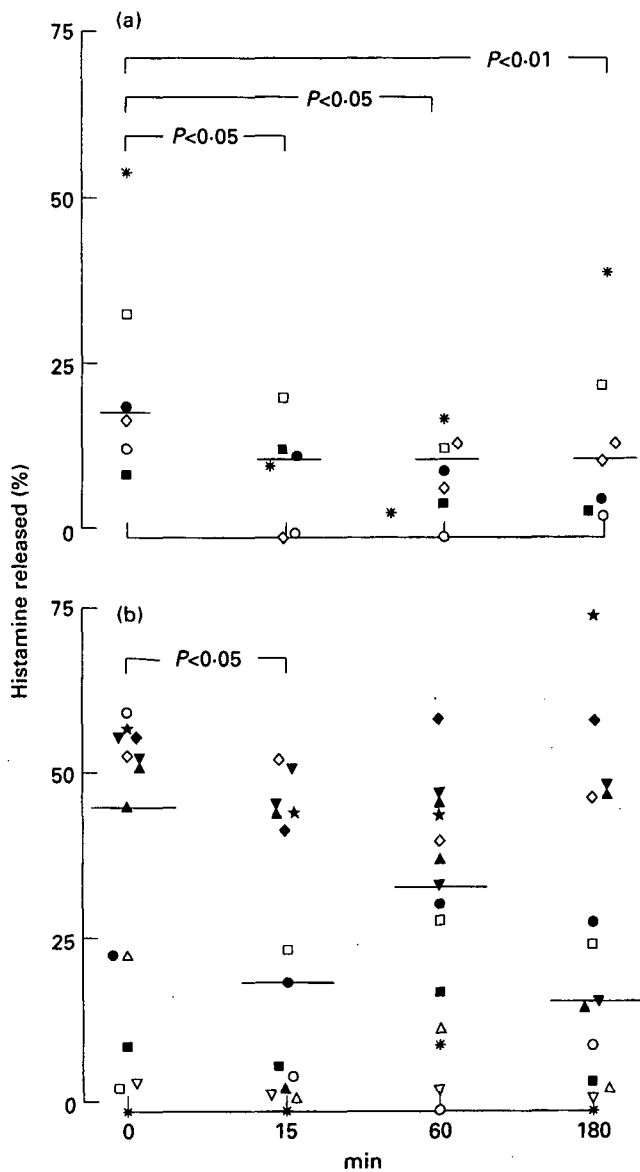


Fig. 3. Histamine release from peripheral blood leucocytes *in vitro* upon bee venom stimulation ($0.08 \mu\text{g/mL}$) before and after the bee sting challenge. Patients identification is according to Table 2. —. Median. Median. (a) Reactors. (b) Non-reactors.

Bee venom-specific IgG

Median bee venom-specific IgG was 56.5% in positive patients and 43.4% in negative patients before the sting challenge (no significant difference). These values were significantly decreased at 15 min and 180 min in positive patients (median 54.7%/49.5%; each $P < 0.05$) and in negative patients (median 42.4%/45.2%; each $P < 0.05$).

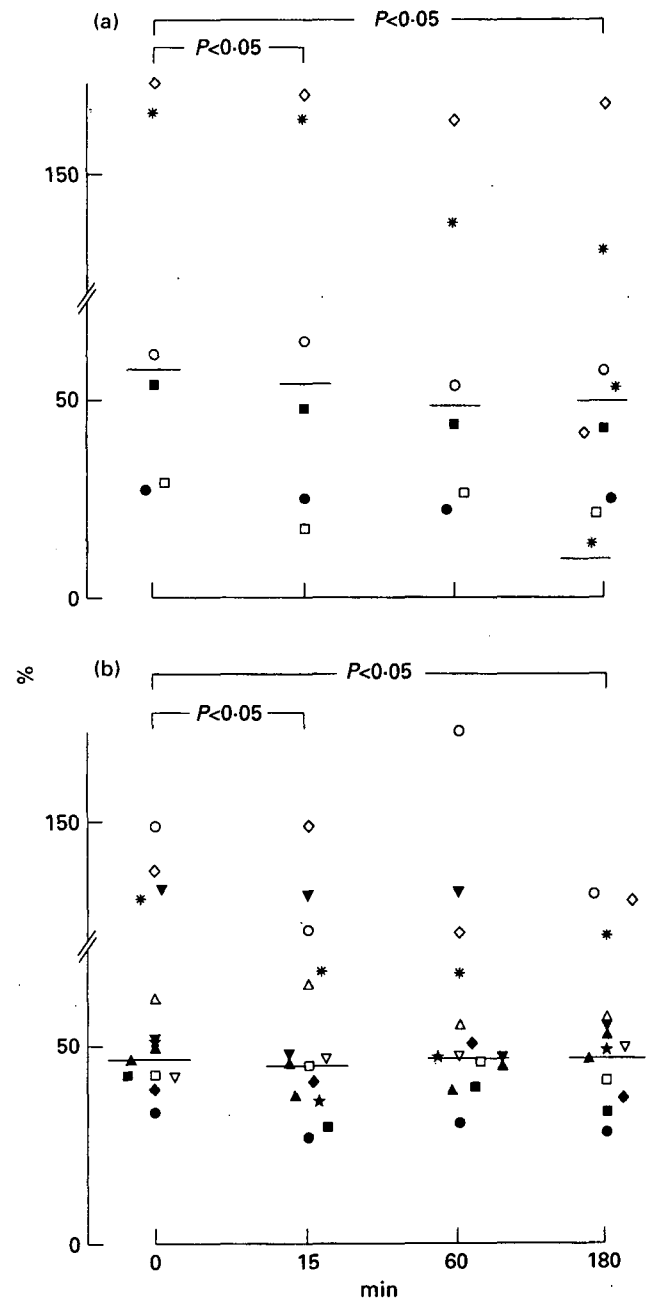


Fig. 4. Bee venom-specific IgG-antibodies of patients before and after the bee sting challenge. Patients identification is according to Table 2. —. Median. (a) Reactors. (b) Non-reactors.

The 60 min values did not differ from the baseline values. Bee venom-specific IgG antibody levels of reactors and non-reactors were not significantly different at 15 min, 60 min or 180 min. For details see Fig. 4.

Discussion

Mast cell tryptase is regarded to be a specific marker of mast cell activation [11]. Several studies have shown that tryptase can be recovered from the peripheral circulation in the course of immediate type hypersensitivity reactions such as bee sting anaphylaxis [5], anaphylactoid or anaphylactic reactions due to drugs [12], or aspirin-induced asthma [13].

Performing sting challenge tests in 19 bee venom-allergic hyposensitized patients, we found mast cell tryptase levels definitely above the detection limit in three individuals, who all had developed systemic symptoms due to the sting challenge. Another reactor had a borderline value of tryptase (2.1 U/L), and in two other reactors tryptase was not found. This is in accordance with other studies, in which tryptase could be demonstrated also in only a part of the symptomatic patients [14,15]. A positive association between the severity of reactions and the occurrence of tryptase in the circulation has been reported [14]. This was not that evident in our patients, but as their number was small and as they reacted at most moderately, it is not possible to draw from our results a definite conclusion regarding the association between the severity of reactions and the release of tryptase.

When mast cell tryptase was demonstrable, this was usually 15 min after the sting challenge and only in one case after 60 min as well. Similar results of tryptase peaking in the circulation at 15 min were obtained in another study, in which patients with a history of insect sting anaphylaxis were challenged without prior hyposensitization [14]. In another report three non-hyposensitized patients developing systemic symptoms due to a sting challenge had peak levels of tryptase 1–2 h after the sting [5]. This variation might be due to the different techniques used to determine mast cell tryptase. Our patients with systemic reactions due to the sting challenge had received antihistamines and/or glucocorticosteroids. Although an effect of systemically administered glucocorticosteroids on the antigen-induced cutaneous mast cell tryptase release was not demonstrable *in vivo* [16], effects on the release or metabolism of tryptase in the course of systemic reactions cannot be excluded. In the study producing similar results of tryptase kinetics after a sting challenge [14], treatment was also given to patients reacting systemically. Lastly, further studies are needed to characterize the kinetics of the release of mast

cell tryptase in the course of immediate-type hypersensitivity reactions.

It has been shown that peak levels of histamine are found 10–15 min after a sting challenge in patients developing anaphylactic symptoms [5]. This corresponds largely to the results obtained in the six patients who reacted, as in these a significant increase of peripheral histamine levels was found 15 min after the sting. Regarding individual kinetics, peak histamine levels were measured in four patients at 15 min, whereas in two others the levels peaked at 60 min or 180 min, respectively. Others have found histamine levels during anaphylactic sting reactions to be declined to essentially baseline levels by 30 to 40 min [5]. Also here it may be that treatment of our patients (no drugs were given in the aforementioned study) may have influenced the histamine levels.

A significant increase of the peripheral histamine levels was found also in patients not reacting clinically to the sting challenge. However, the median levels were well below those of the reactors at all time points of measurements. In contrast to patients reacting to the challenge, a significant, although slight increase of the histamine levels was seen at 60 min and 180 min. Others have demonstrated a significant change in histamine within 60 min after an insect sting challenge in one of eight volunteers without a history of systemic sting reactions, but in none of 22 patients not reacting allergic patients [14]. Peripheral histamine levels in the course of anaphylactic/anaphylactoid reactions have been determined in humans in several studies [5,12,14,17,18] yielding histamine levels of 1–12 800 nmol/L. These values are not directly comparable with the results of this study, as we have determined the serum histamine concentration, which usually is higher than that in the plasma [5]. Even though, it is apparent that histamine is unlikely to be the only (or even the most important) mediator of anaphylaxis. This opinion has been put forward already by others discussing different causative mediators [6]. The tolerance of patients not reacting to sometimes rather high histamine concentrations is remarkable. It can be speculated that this was due to 'desensitization' in the course of a delayed increase of histamine levels or, alternatively, due to unknown mechanisms related to hyposensitization.

The total histamine content of PBL, which can be considered as the histamine of the basophil fraction, was found to be significant lower in both groups at 15 and 60 min after the sting challenge than before. The changes were much more pronounced in patients who reacted than in those who did not. The release of histamine from basophils evidently contributed to the increase of serum histamine levels after the sting challenge. A decrease of the histamine content of PBL in the course of continuous

allergen exposure during bee venom hyposensitization has been reported [19].

The relative amount of histamine released from PBL upon stimulation with bee venom was decreased significantly in both groups after the sting challenge compared with the prechallenge values. A significant effect was seen in patients who reacted than in those who did not. A reduction of the reactivity of basophils on repeated allergenic stimulation is a known phenomenon [19]. The more pronounced refractory period of cells from reactors may be explained by the effects of endogenous, anti-anaphylactic regulation or by the administration of drugs, especially glucocorticosteroids. However, it has been reported that methyl-prednisolone given systemically led to a decrease of anti-IgE or allergen-induced histamine release from peripheral blood leucocytes after 24 h [21], but not after 4 h [22]. Remarkably, the prechallenge values of bee venom-induced histamine release were much higher in non-reacting patients than in those who reacted, although significant levels were not reached. This indicates at least that a low histamine release from PBL *in vitro* does not mean protection.

Whereas significant changes of the concentration of bee venom-specific IgE antibodies did not occur in relation to the sting challenge, allergen exposure led to a slight, but significant decrease of bee venom-specific IgG antibodies. This may be explained by the formation of immune complexes of specific IgG antibodies with bee venom components leading to occupation of the binding sites. This would interfere with the detection of bee venom-specific antibodies. Another possibility is that mediator-induced shifts of body fluids could have caused the changes of specific IgG concentrations. However, no monitoring of such shifts was done in this study.

The mechanisms of hyposensitization are still unknown [22,23]. Studies performed in patients with respiratory diseases have shown that hyposensitization has numerous effects on components of inflammatory reactions, e.g. eosinophil cell migration, production of histamine-releasing factor activity, generation of eosinophil or neutrophil chemotactic activity [25–30]. Hyposensitization in respiratory allergic diseases as well as in hymenoptera venom allergy also leads to a decrease of specific IgE antibodies and to an increase of specific IgG antibodies [31–36]. However, the relation of such effects to the mechanisms of hyposensitization and its actual outcome are not yet clear [37]. Our results indicate that, despite a beneficial clinical outcome, protected patients still react on a cellular level. These reactions seem attenuated in most, but not all patients who did not clinically react. Particularly, it has to be evaluated further why the release of still substantial amounts of histamine in some non-reactors is not associated with symptoms

and whether a pattern of mediator release different from that in reactors is related to protection. The latter possibility is of major interest, as it would indicate that the effect of hyposensitization is not primarily to abolish the hypersensitivity reaction, but rather to induce regulatory mechanisms interfering with the initiation of disease symptoms. To get more insight into these mechanisms, it would have been desirable to observe the sequels of a sting challenge in non-hyposensitized allergic patients also as well as in non-allergic patients. However, it is our opinion that such experiments cannot be performed for ethical reasons.

Taken together, our results indicate that the occurrence of mast cell tryptase in the circulation is a highly specific, but less sensitive marker of an immediate type hypersensitivity reaction. At least in IgE-mediated bee venom allergy, mast cells as well as basophils react upon allergen exposure *in vivo*. In some individuals even high peripheral levels of histamine are not sufficient to induce clinical symptoms. Successful bee venom hyposensitization seems not to induce a state of non-reactivity on the cellular level, but rather a beneficial modulation of the immunologically initiated reaction.

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