Histamine released locally after intradermal antigen challenge in man

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- 1 Plasma histamine concentration was measured in venous blood draining the site of antigen-induced wheal and flare responses in the forearm skin of seven atopic volunteers. Concentrations were measured using a double isotope radioenzymatic method.
- 2 The mean $\pm$ s.e. mean resting plasma concentration was 0.18 ± 0.01 ng/ml. In all subjects an increase was detected in local plasma histamine concentration after intradermal antigen challenge.
- 3 The peak histamine concentration occurred between 2 and 15 min after challenge, and represented an increase of three- to 20-fold above the resting concentration.
- 4 Plasma histamine concentration remained significantly elevated for at least 60 min after challenge.
- 5 A 30 min incubation at 37°C of blood taken at the time of peak plasma concentration caused a fall in histamine concentration. This suggests that histamine release from basophils during the sampling procedure was not a significant problem.
- 6 This method is less invasive than skin chamber or blister techniques for the demonstration of mediator release in cutaneous inflammation and allows a simultaneous assessment of tissue response.

Keywords histamine skin antigen

Introduction

Histamine is one of the most abundant pre-formed mediators released from mast cells on immunological challenge. Other mediators are synthesized at the time of degranulation either by mast cells themselves or by other cell types. These products include prostaglandins and leukotrienes (for review see Wasserman, 1983). The relative contribution of each mediator type to the overall inflammatory response remains obscure, and is unlikely to be determined easily from *in vitro* studies. An *in vivo* method, applicable to man, is required in which mediator production associated with inflammation cannot only be detected but also any

contribution to the overall response assessed. Although skin chamber and blister techniques have been used to measure production of mediators following cutaneous inflammation (Ting *et al.*, 1980), they do not allow an assessment of tissue response. The methods are in themselves likely to produce inflammation and so may influence subsequent mediator production. By using the double isotope radioenzymatic method for measurement of plasma histamine (Brown *et al.*, 1982), we have now demonstrated local increases in plasma histamine concentration following intradermal antigen challenge in atopic subjects.

Methods

Seven volunteers (six male) took part in the main study. They were selected on the basis of a positive wheal and flare response to intradermal injection with an extract of *D. pteronyssinus*, but were non-asthmatic. Ages ranged from 26 to 38 years, and all had abstained from medication including antihistamines and non-steroidal anti-inflammatory drugs for at least 2 weeks prior to the study. None had smoked or taken caffeine containing products for 6 h before challenge. All intradermal injections were given in a volume of 50 μ l using a no. 27 gauge needle. The dose of antigen required to produce a wheal of approximately 1.5 cm² was determined by previous construction of dose response curves in the skin of the back. Antigen (Bencard SDV) was supplied in 0.9% saline with 0.5% phenol (wt/vol.) as a preservative. All further dilutions of antigen were made in 0.9% saline and control injections of vehicle alone contained the appropriate concentration of phenol in 0.9% saline.

Each subject was seen on two occasions at the same time of day at least 1 week apart, and received intradermal injection of either antigen or vehicle into the skin of the ventral aspect of one forearm. Order of injection was random-

ized. Blood was sampled from a large vein in the antecubital fossa approximately 10 cm proximal to the challenge site via a no. 19 gauge butterfly (Abbott). Patency of the cannula was maintained by slow intravenous infusion of 0.9% saline. Blood samples (5 ml) were drawn over a period of 10–12 s and collected into polystyrene tubes containing 50 μ l 0.5 M ethylenediaminetetraacetate (sodium salt) pH 7.4. The samples were centrifuged at 2000 g for 10 min, the upper 1 ml of plasma carefully aspirated and stored in polypropylene tubes at -80°C . A double isotope radioenzymatic method was used to determine plasma histamine concentrations (Brown *et al.*, 1982). Histamine concentration was measured at intervals before and after intradermal injection. The sites of injection and sampling were recorded carefully, and were re-used on the second visit. An independent observer recorded the areas of flare and wheal at 9 and 16 min, respectively, post-challenge. Areas were marked on the skin using light pressure from a ball pen and, at the end of the study period, transferred using clear adhesive tape applied over the skin onto plain paper. The areas were measured using a digitising pad, and calculated using the trapezoidal rule.

One subject was challenged with the same

Figure 1 Local plasma histamine concentration after intradermal antigen challenge in seven atopic subjects. The amount of antigen was determined from previous dose-response curves and produced wheals of 1.77 ± 0.09 cm² (mean $\pm$ s.e. mean). All injections were made into the skin of ventral aspect of the forearm and blood sampled from an antecubital fossa vein approx. 10 cm proximal. The mean values $\pm$ s.e. mean are shown after antigen ($\circ$) and vehicle (Δ) injection. Results at each time point were compared using Student's *t*-test. * = $P < 0.05$; ** = $P < 0.01$.

dose of antigen on four occasions. Three challenges were at least 2 weeks apart but one was only 1 week after the preceding injection.

In the second part of the study three subjects (two of whom had taken part in the main study) were challenged with antigen in the same manner. Blood was sampled at intervals before and for 15 min after challenge both locally and in the contralateral forearm. Each sample of 10 ml was divided into two aliquots. One was handled in the usual manner, and one incubated for 30 min at 37°C before centrifugation to allow any basophil degranulation occurring in the sample to be completed.

All subjects gave written informed consent. The protocol was seen and approved by the Ethics Committee of the Hammersmith Hospital and Royal Postgraduate Medical School.

Statistical analysis was performed using Student's paired *t*-test. Significance was taken as $P < 0.05$. Results are expressed as mean $\pm$ s.e. mean. Plasma histamine concentration is expressed as ng/ml (to convert to nmol/l multiply figures by 9.01).

Results

Injection of antigen produced a well defined response in all subjects with wheal and flare areas of 1.77 ± 0.09 and 35.2 ± 5.1 cm² respectively. At 9 min after injection, there was no detectable flare with vehicle, and at 16 min

an ill defined induration of 0.52 ± 0.09 cm² was observed.

Basal plasma histamine concentration was 0.18 ± 0.01 ng/ml. All subjects showed a rise after antigen but not vehicle injection. The peak concentration achieved after antigen varied from 0.53 to 4.17 ng/ml and represented between three- and 21-fold rise above resting concentration (mean 7.5). Time to reach peak plasma histamine concentration varied from 2 to 15 min after challenge, and plasma concentration was significantly greater than that following vehicle injection from 5 to 60 min (Figure 1).

Table 1 shows the results from simultaneous remote and local sampling at the time of peak concentration in three subjects. There was

Table 1 Plasma histamine concentration in blood taken from antecubital fossa veins following antigen induced wheal and flare response in one forearm. The plasma concentration in the unchallenged arm (remote) at the time of peak concentration in the challenged arm (local) is shown.

Subject	Plasma histamine concentration (ng/ml)	
	Local	Remote
1	1.92	0.12
2	2.90	0.14
3	0.97	0.21

Figure 2 Plasma histamine concentration after intradermal injection of antigen. One subject challenged on four occasions. Sites of injection and sampling were identical. Injection of vehicle (0.125% phenol in 0.9% saline) alone (---) produced no rise in plasma histamine concentration.

no increase in histamine concentration in blood from the remote arm at the time of the local increase. The increase in plasma histamine concentration is most probably due to histamine which has diffused from the site of wheal formation. It is possible however that antigen leaking into blood initiated degranulation of circulating basophils and so contributed to the rise in plasma histamine concentration. Little degranulation is likely to have occurred in the short circulation time from the site of injection to sampling and should have been inhibited in the presence of 10 mM EDTA once the sample was drawn. The possibility of continued degranulation of basophils within the sample was examined in three subjects by incubating an aliquot of blood at 37°C for 30 min so allowing any degranulation to be completed. Following incubation there was actually a reduction rather than an increase in plasma histamine concentration. Thus, plasma concentrations in blood taken at the time of peak concentration and incubated at 37°C showed a mean fall of 0.48 ng/ml (range 0.8–0.25 ng/ml). This was a drop of 27% (range 26–28%).

The results from the subject challenged on four occasions are shown in Figure 2. The time taken to reach peak concentration was the same on each challenge (2 min). Peak histamine concentrations were similar (4.17, 3.56, 3.55 ng/ml) following the three injections given at least 2 weeks apart, but was lower (0.68 ng/ml) when the challenge was made after only 1 week.

Discussion

The investigation of cutaneous inflammation in man requires a method that is minimally invasive and which permits an assessment of tissue response. Progress in determining mechanisms of inflammation is likely to lie in the ability to assess the production and interactions of mediators such as prostanoids and leukotrienes which are newly synthesised at the time of inflammation. Current methods employed to demonstrate histamine release in skin include skin blister and chamber techniques (Ting *et al.*, 1980). Not only are these invasive and likely to provoke mediator production on their own, but they do not allow a simultaneous assessment of tissue response. Hence changes in mediator release cannot be correlated with changes in

tissue response. The measurement of histamine released into blood following mast cell degranulation *in vivo* in man has been restricted previously to disease states in which large areas of skin are involved (Soter *et al.*, 1976; Kaplan *et al.*, 1975; Atkins & Zweiman, 1981) in systemic mastocytosis (Roberts *et al.*, 1980) and anaphylaxis (Smith *et al.*, 1980). There have been conflicting reports of increases in plasma histamine concentration during attacks of asthma. This probably reflects the difficulties in the measurement of low concentrations in plasma (for review see Ind *et al.*, 1983).

We have described a method using a simple intradermal injection and local venous sampling in which increases of up to 20-fold in plasma histamine concentration can be produced. This release has been shown to be local and not accompanied by an increase in plasma histamine concentration at a remote site. Incubation of the blood sample (collected into 10 mM ethylene diaminetetra-acetate) at 37°C has shown that these increases are unlikely to be due to basophil degranulation occurring within the blood sample. Thus the rise in local plasma histamine concentration appears to reflect degranulation of mast cells and possibly basophils at the site of injection. The fall on incubation may be due to histaminase activity.

Large interindividual variation in the changes in plasma histamine concentration were seen for similarly sized wheals. This is probably due, in part, to anatomical differences in venous drainage. However, the dose-response curve of wheal area against amount of antigen injected is rather shallow, so that even in the same individual similar wheal areas may represent widely different degrees of degranulation. In the subject examined four times similar changes in local histamine concentration were seen when the challenges were at least 2 weeks apart.

The technique that we have described allows simultaneous measurement of histamine release and wheal and flare response following cutaneous mast cell degranulation in man. The non-invasive nature of the method should make it useful in the investigation of cutaneous inflammation.

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