

Histamine is released in the wheal but not the flare following challenge of human skin *in vivo*: a microdialysis study

L. J. PETERSEN, M. K. CHURCH* and P. STAHL SKOV**

Department of Dermatology, Bispebjerg University Hospital, **The Reference Laboratory, National University Hospital, Copenhagen, Denmark and *Immunopharmacology Group, Southampton General Hospital, Southampton, UK

Summary

Background The mediator mechanisms of the cutaneous wheal and flare response, which underlies allergic skin and urticarial conditions, are controversial. The wheal results primarily from a direct effect of histamine on the local vascular bed, but to what extent does histamine diffuse within the wheal? The flare is neurogenic in origin, being disseminated within the dermis by axon reflexes, but do the neuropeptides released from the nerve endings cause the vasodilatation directly or do they induce the further release of histamine which then transduces the flare?

Objective We have addressed these questions by inserting 216 μ m diameter microdialysis fibres into the dermis within the different areas of the wheal and flare to monitor changes in histamine levels provoked by intradermal injections of histamine, allergen, codeine and substance P. Twenty-one subjects participated in the investigations.

Results The histamine concentration in unprovoked skin was 10.5 ± 0.6 nM. As the dialysis efficacy was $\sim 50\%$, this equates to tissue concentrations of 20 nM. All provicants released large amounts of histamine at the injection site, maximum histamine levels being 337–1293 nM. Diffusion of histamine within the wheal was poor, levels at 2.3 mm and 3.7 mm from the site of injection being 4–22% and 0.2–3.7% respectively of those 1 mm from the injection site. No increased histamine levels were detected in the flare with any provicant. Atraumatic delivery to the skin of histamine in infusion concentrations of 30–10 000 nM caused concentration-related effects, at least 100 nM being necessary to induce a significant increase in skin blood flow, a threshold of 300–1000 being required to stimulate a visible flare and a measurable erythema, and 3000–10 000 nM being the minimum for induction of a wheal. Thus the skin blood vessels and nerves are responsive to histamine, but at relatively high concentrations.

Conclusions These data support the theory that the flare reaction to focal histamine injection or release is a neurogenic reflex not involving histamine release at its effector end.

Keywords: immediate hypersensitivity, skin test, neuropeptides, codeine, substance P, allergen, histamine, wheal, flare, blood flow, microdialysis

Clinical and Experimental Allergy, Vol. 27, pp. 284–295. Submitted 16 June 1995, revised 5 July 1996, accepted 25 July 1996.

Introduction

Although it is almost 70 years ago since Lewis described the triple response to the intradermal injection of histamine [1], its mediator mechanisms are still controversial. The wheal is caused by the actions of histamine on the local vascular bed,

causing vasodilatation initially and then leakage of plasma proteins from post-capillary venules [2]. Histamine also stimulates axon reflexes within the dermis [3]. The involvement of peptidergic nerves in transducing the flare is supported by the observations that capsaicin, which opens non-specific cation channels in C-fibre polymodal afferent nerve fibres [4], induces the release of sensory neuropeptides [5] and induces a flare on intradermal injection and

Correspondence: Dr L. J. Petersen, Department of Dermatology, Bispebjerg Hospital, DK-2400 Copenhagen, Denmark.

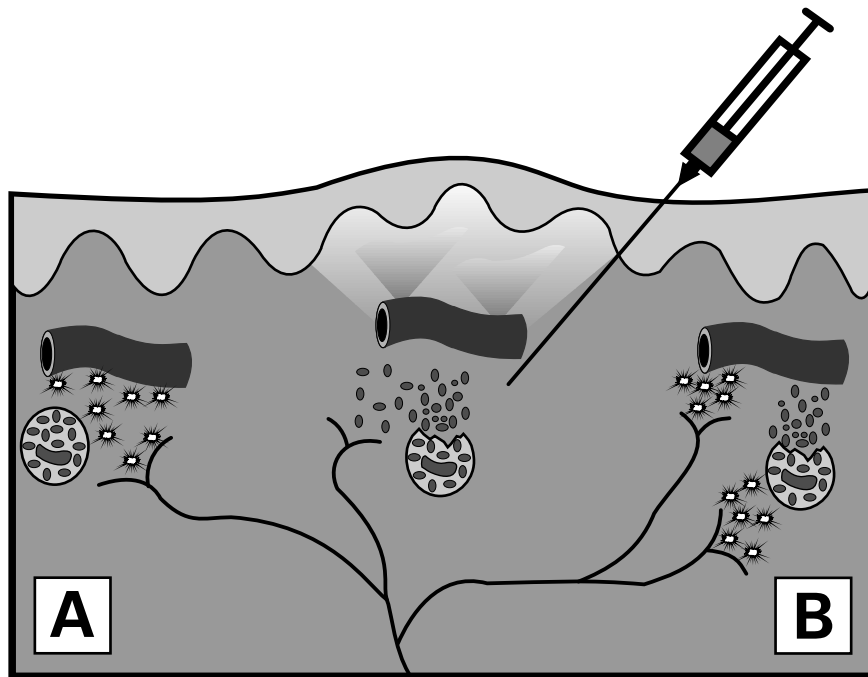


Fig. 1. Diagrammatic representation of the skin showing the wheal and the possible mechanisms of the flare response. Injection of allergen or a mast cell degranulating agent induces the release of mast cell mediators which cause a localized vasodilatation and increase of vascular permeability to produce the wheal. They also stimulate an axon reflex (arrowed) to release neuropeptides in the flare area. The vasodilatation in the flare area occurs by one of two mechanisms: **A**, by the direct vasodilator actions of sensory neuropeptides; or, **B**, by neuropeptide-induced release of mast cell mediators which then cause, or at least augment, the vasodilatation.

topical administration [6,7]. Furthermore, prolonged application of capsaicin reduces the flare response by depleting sensory neuropeptides from these nerves [7,8]. What is not clear from these studies, however, is the mechanism by which this neurogenic reflex induces vasodilation at its effector end. At present, two theories are proposed (Fig. 1). One states that, although histamine present in the wheal may initiate the axon reflex, the flare results solely from the actions on the blood vessels of neuropeptides released at the distal end of the reflex. The other theory proposes that the neuropeptides induce mast cell histamine release within the flare area and that histamine then causes or contributes to the observed vasodilatation.

The first theory assumes that, as several of the neuropeptides released in the flare area possess potent vasodilator properties [9,10], the presence of histamine is superfluous. Other supportive evidence for this theory includes reports showing that the antihistamine terfenadine does not reduce the flare response to intradermal capsaicin [11], and that rat paw inflammation, induced by electrical stimulation of the saphenous nerve, does not involve skin mast cells [12,13].

The theory that mast cell histamine is involved in producing the flare [14] is supported by several lines of circumstantial evidence. First, morphological studies have shown a close association between mast cells and sensory nerves in

the skin [15,16]. Second, the neuropeptides substance P, vasoactive intestinal peptide and somatostatin, all of which have been demonstrated in sensory nerves [17,18], all release histamine from human skin mast cells *in vitro* [19–21]. Third, neuropeptide-induced cutaneous inflammation is markedly reduced in mast cell deficient mice [22,23]. Fourth, intradermal injection of substance P into human skin *in vivo* induces a wheal and flare response [9,24] and releases histamine [11,25]. Fifth, the flare response to endothelin-1 and platelet activating factor, neither of which release histamine from human dispersed skin mast cells *in vitro* [26,27], is reduced by antihistamines [26,28,29].

In order to resolve this debate, it is necessary to demonstrate directly local changes in histamine levels in the skin *in vivo* during wheal and flare responses. The ability to do this has recently become feasible with the introduction of cutaneous microdialysis, a technique by which small molecular weight substances present in the extracellular compartment of intact human skin *in vivo* may be monitored continuously [30]. Using this technique, which involves the introduction of 216 μm diameter dialysis fibres into the upper dermis, we have already demonstrated histamine release in the wheal areas of cutaneous responses to substance P [25], codeine [31] and allergen [32,33]. We now

use this technique to determine the concentration of histamine in unstimulated skin and assess semi-quantitatively any changes which occur within wheal and flare following intradermal injection of histamine, allergen, codeine and substance P.

Materials and methods

Subjects

A total of 21 subjects, two women and 19 men, aged 19–44 yr, participated in the study. Six of the subjects were allergic to Timothy grass pollen, based upon seasonal symptoms, positive skin tests and the presence of allergen-specific antibodies to grass pollen (class >2, CAP-system, Pharmacia). The remainder were healthy, non-atopic volunteers. All subjects gave informed consent, and the study was approved by the local ethics committee.

Skin test reagents

Aqueous timothy grass pollen extract (ALK Laboratories, Hørsholm, Denmark), substance P (Sigma, St Louis, USA), codeine phosphate (Bispebjerg Hospital Pharmacy) and histamine dihydrochloride (SAD, Copenhagen, Denmark) were diluted to their final concentrations in Krebs's Ringer bicarbonate containing 0.02% human serum albumin. Final concentrations were as follows: histamine 50 μ M, codeine 2.5 mM, substance P 10 μ M, and grass pollen 1 or 10 SQ-U/ml, depending on the sensitivity of the subject. Fresh dilutions were made daily from the same batches of substances.

Insertion of dialysis fibres

Microdialysis fibres of 216 μ m outside diameter, 8 μ m wall thickness, and 2 kDa molecular weight cut-off were inserted into the dermis under topical local anaesthesia to give a diffusible length of 20 mm as previously described [31]. Ultrasound examinations have confirmed intradermal localization of the fibres, the position being approximately 0.6 mm from the skin surface [Petersen *et al.*, unpublished observations]. A recovery phase of at least 2 h was allowed to elapse between fibre insertion and skin challenge, a time which previously has been demonstrated to be sufficient for local anaesthesia to wear off, neurogenic vasodilation to be restored to normal, and the implantation-induced hyperaemia to resolve [31]. During the recovery phase, the fibres were not perfused. Each dialysis fibre was then perfused at 3.0 μ l/min with sterile, isotonic Krebs's Ringer solution, (1.0 mM MgCl_2 , 1.2 mM CaCl_2 , 0.4 mM NaH_2PO_4 , 25 mM NaHCO_3 , 85 mM NaCl, and 3.0 mM KCl) at pH 7.4 using CMA/100 perfusion pumps (CMA/Microdialysis, Stockholm, Sweden),

the stability of which was checked regularly. After the perfusion was established, the fibres were allowed to equilibrate for at least 10 min before collection of baseline samples.

Skin challenge procedure

To elicit wheal reactions covering the entire fibre, two 10 μ l intradermal injections of histamine, codeine, substance P or grass pollen, were made 5 mm from end of the fibre and exactly 1.0 mm to the side and parallel to the fibre. A 27 gauge needle attached to a 0.5 ml syringe was used to make each injection.

Assessment of the efficacy of dialysis

The efficacy of histamine diffusion across the microdialysis fibre *in vivo* was assessed using a modified 'no net flux method', in which a linear regression is established between infused and recovered solute concentrations [30]. Briefly, a single dialysis fibre was perfused with Krebs's Ringer bicarbonate containing 300 nM histamine and the concentration of histamine in the dialysate was measured at steady state by spectrofluorimetry. The *in vivo* recovery of histamine may then be calculated by the equation $((D_{300} - P_{300}) - (D_0 - P_0)) * 100 / (P_0 - P_{300})$, where P_{300} and P_0 are the concentrations of histamine infused (300 and 0 nM) and D_{300} and D_0 are the concentrations recovered in the respective dialysates. Based on data from study II, in which Krebs's Ringer bicarbonate containing no histamine was infused and approximately 10 nM histamine was recovered in the dialysate at steady state, the equation was simplified to $((D_{300} - P_{300}) - 10) * 100 / P_{300}$. The results showed the *in vivo* recovery of histamine during steady-state conditions to be $46.2 \pm 3.7\%$ ($n = 6$).

To assess whether increased skin blood flow altered the rate of clearance of histamine and, therefore, its recovery by dialysis, two separate fibres were perfused with 300 nM histamine in six subjects. An intense flare, which covered the entire diffusible length of one fibre, was then induced by injection of two aliquots of histamine 1.1 mM 15 mm from the centre of the fibre. The other fibre served as a control. The recovery of histamine in skin covered by a flare was similar to that of normal control skin, $48.4 \pm 3.7\%$ and $48.3 \pm 4.0\%$, respectively, indicating that increased blood flow in the flare area does not increase the elimination of tissue histamine.

Protocols for studies of histamine release

Study I Four groups of five dialysis fibres were inserted, two groups separated by at least 5 cm in each arm, into the forearms of six allergic subjects. Within each group, the fibres were inserted in parallel approximately 1, 2, 4, 6 and

Table 1. Histamine concentrations at various places within the wheal and flare reaction after skin challenge with histamine, allergen, codeine and substance P

	Fibre 1	Fibre 2	Fibre 3	Fibre 4	Fibre 5
<i>Histamine</i>					
Peak histamine concentration (nM)	1293 ± 249	52.4 ± 12.9	8.1 ± 4.7	0.0 ± 0.0	0.0 ± 0.0
Total histamine amount (pmoles/20 min)	20.22 ± 4.16	0.99 ± 0.35	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
Distance from injection site (mm)	1.0	2.4 ± 0.1	3.8 ± 0.2	6.4 ± 0.1	9.6 ± 0.2
<i>Allergen</i>					
Peak histamine concentration (nM)	337 ± 80	86.0 ± 38.3	9.5 ± 4.6	5.4 ± 2.8	0.0 ± 0.0
Total histamine amount (pmoles/20 min)	10.04 ± 2.37	2.26 ± 1.22	0.17 ± 0.10	0.08 ± 0.06	0.00 ± 0.00
Distance from injection site (mm)	1.0	2.3 ± 0.1	3.7 ± 0.2	6.3 ± 0.2	9.5 ± 0.2
<i>Codeine</i>					
Peak histamine concentration (nM)	772 ± 110	127 ± 32.2	27.0 ± 10.1	3.6 ± 3.6	0.0 ± 0.0
Total histamine amount (pmoles/20 min)	15.59 ± 2.34	2.65 ± 0.60	0.26 ± 0.13	0.04 ± 0.04	0.00 ± 0.00
Distance from injection site (mm)	1.0	2.3 ± 0.1	3.7 ± 0.2	6.5 ± 0.1	9.7 ± 0.2
<i>Substance P</i>					
Peak histamine concentration (nM)	372 ± 89.5	58.6 ± 14.3	13.5 ± 6.1	5.8 ± 2.3	3.1 ± 2.0
Total histamine amount (pmoles/20 min)	7.96 ± 1.98	1.06 ± 0.30	0.29 ± 0.18	0.05 ± 0.03	0.00 ± 0.00
Distance from injection site (mm)	1.0	2.3 ± 0.1	3.8 ± 0.1	6.1 ± 0.1	9.6 ± 0.1

After establishing baseline histamine levels, two 10 µl volumes of histamine (50 µM), codeine (2.5 mM), substance P (10 µM) and grass pollen (1 or 10 SQ units/ml) were injected intradermally and dialysate collected in 2 min samples for a total of 20 min thereafter. Histamine was assayed spectrofluorometrically. The mean wheal radii in these experiments were 4.4 ± 1.0, 5.4 ± 1.1, 5.3 ± 1.6 and 4.4 ± 0.8 mm for histamine, codeine, substance P and allergen, respectively. The corresponding flare radii were 16.3 ± 5.0, 22.2 ± 3.2, 18.0 ± 5.1 and 16.3 ± 6.2 mm. Fibres 1 and 2 were covered by wheals in all cases, fibre 3 in 22/24 cases and fibre 4 in 3/24 cases. In no case did a wheal extend to reach fibre 5. In all cases the flare reaction extended beyond fibre 5. Values are mean ± SEM of experiments in six subjects.

9 mm from the intended injection site, the exact locations being shown in Table 1. After equilibration, two 6 µl baseline samples were collected over a 4-min period before injection of histamine, allergen, codeine or substance P exactly 1 mm proximally to the most proximal fibre in each group. Further dialysate collections of 6 µl were made at 2-min intervals for a period of 20 min. The 2-min period was read as 6.0 µl as displayed on the perfusion pump. To minimize time-of-sampling errors, all sample collections within a single group of fibres were changed within 10 s of each other. Dialysate was collected directly into glass fibre coated microtitre wells used for histamine analysis as previously described [25]. Each group of fibres was challenged separately with an interval of approximately 30 min between them. The order of challenges with histamine, allergen, codeine and substance P were randomized. Twenty minutes after skin challenge, the contours of the wheal and the flare reaction above the fibres were outlined, and the mean wheal and flare radii measured at right angles to the path of the dialysis fibre. In all experiments, the number of fibres covered by wheal and/or flare reactions was recorded. Histamine was assayed spectrofluorometrically.

To observe whether or not the physical presence of dialysis fibres in the skin had any effect on the diffusion of histamine in the skin, three fibres were inserted into the forearms of each of four healthy volunteers at least 3 cm apart. Codeine, 2.5 mM, was injected 1, 2 or 3 mm from the centre of each fibre. Baseline and post-challenge dialysate samples were collected and assayed as above.

Study II The second series of experiments used a sensitive enzyme immunoassay for histamine to confirm the results obtained with the spectrofluorometric histamine assay. Two groups of four fibres were inserted into the forearms of each of five healthy volunteers, so that three fibres were positioned 1, 3 and 9 mm to one side of the intended challenge site and the fourth fibre was 9 mm to the other side (indicated as -9 mm). One group of fibres was challenged with codeine, 2.5 mM, and the other with substance P, 10 µM. In this series, dialysate was collected in 8-min samples, two baseline samples being collected over a 16-min period before challenge and two further samples collected for consecutive 8-min periods after challenge.

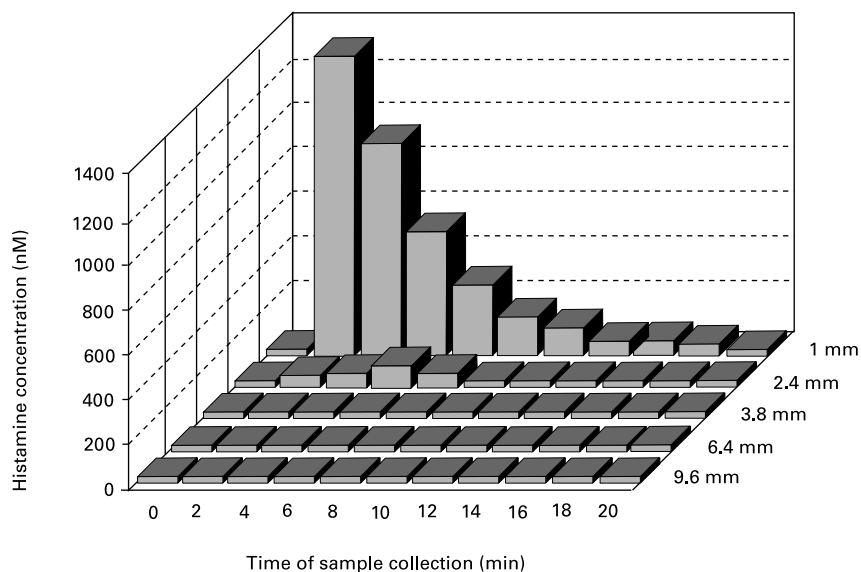


Fig. 2. An illustrative example of the pattern of detection of histamine following histamine injection taken from the first series of experiments. Five dialysis fibres were situated 1, 2.4, 3.8, 6.4 and 9.6 mm from the site of intradermal injection of two 10 μ l aliquots of histamine, 50 μ M. The mean value of two 6 μ l baseline samples (labelled 0 min) were collected over a 4-min period before injection of histamine. Thereafter, 6 μ l dialysate samples were collected from each fibre every 2 min for 20 min and the histamine concentrations were assayed spectrofluorometrically.

Kinetics of histamine diffusion To study the kinetics of histamine diffusion *in vivo*, individual fibres were perfused with Krebs's Ringer containing no histamine. Using a liquid switch system (CMA/110, CMA/Microdialysis, Stockholm, Sweden) [31] the infusion was then changed instantly from 0 to 300 nM histamine and 2-min replicates of dialysate collected for the next 20 min. Histamine was assayed spectrofluorometrically.

Assessment of the effects of histamine on skin blood flow and erythema To determine effects of histamine on the local vasculature, individual dialysis fibres were perfused first without histamine and then with various concentrations of histamine for 10 min. Skin blood flow above each fibre was measured every minute using laser Doppler flowmetry (Periflux PF 1d, Perimed AB, Stockholm, Sweden). The laser probe was fixed in a plastic holder with its head exactly on the top of the dialysis fibre in the skin. The parameters for the laser Doppler were: frequency limit 12 kHz, time constant 10 s, and gain 1 or 3. Skin erythema, and visible wheal and flare reactions were measured at 10–12 min. Skin erythema, estimated by the haemoglobin content, was measured by a hand-held spectrophotometer (Dermaspectrometer, Cortex Technology, Hadsund, Denmark) according to recent recommendations [34]. The opening head of this spectrophotometer was ~ 100 mm², the head being placed exactly on the same skin site as the laser Doppler recordings were performed. Data are mean values of 3–4 replicates. Wheal

and flare responses were quantified as described above. Laser Doppler values and skin erythema figures were corrected for pre-challenge baseline values. In all experiments, the subjects were placed in the supine position, the arms being kept at heart level. Inlet and mean outlet histamine concentrations over 10 min were analysed spectrofluorometrically.

Histamine assays

In most experiments, histamine was assayed spectrofluorometrically using microtitre plate wells coated with a glass fibre matrix [35]. The specificity of this assay for measuring histamine in skin dialysate after mast cell degranulation has previously been confirmed [25]. The lower detection limit is approximately 25 nM histamine (corresponding to 5 ng/ml histamine dihydrochloride), any histamine analysis below the limit of detection was set as 25 nM in the calculations. For the enzyme immunoassay for histamine (Immunotech S.A., Marseille, France), dialysate samples were diluted 10- to 100-fold before analysis, yielding a final sensitivity of 2–20 nM.

Statistics

Histamine concentrations in each 2-min sample and the total amount of histamine released over the 20-min sampling period were calculated for each fibre. Results are expressed as mean $\pm$ SEM if not otherwise mentioned. Variations in

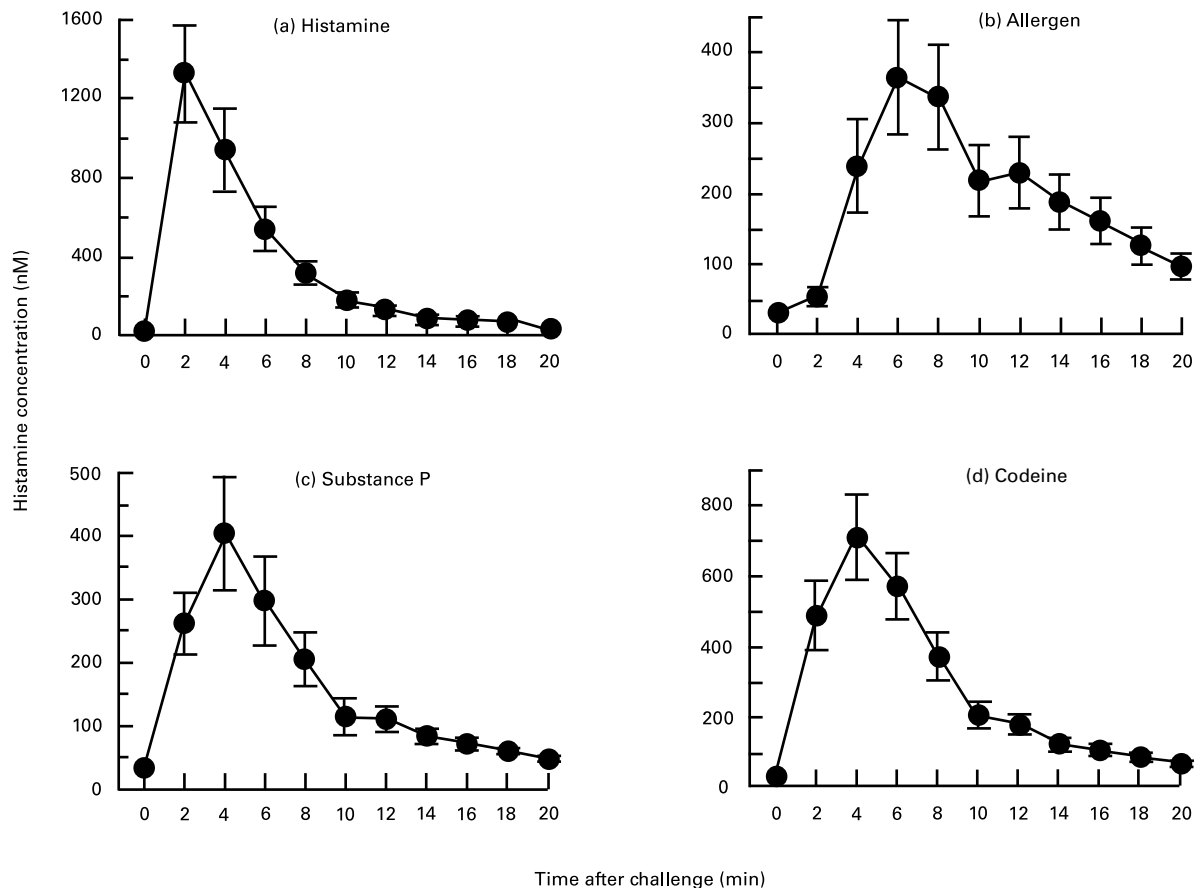


Fig. 3. Time course of histamine release induced by histamine, allergen, substance P and codeine. Two 10 μ l aliquots of (A) histamine, 50 μ M, (B) allergen, 1 or 10 SQ units/ml, (C) substance P, 10 μ M, and (D) codeine, 2.5 mM, were injected intradermally 1 mm from the dialysis fibres. Six microlitre dialysate samples were collected from each fibre every 2 min for 20 min and the histamine concentrations were assayed spectrofluorometrically. Results are expressed as mean $\pm$ SEM ($n = 6$).

histamine release within series of fibres were analysed using the Friedman test. Wilcoxon sign rank tests were used for comparison of single experiments (baseline versus post-challenge histamine levels). P values of <0.05 were considered to be statistically significant.

Results

Histamine concentrations in the perfusates of dialysis fibres in resting unchallenged skin in six atopics and four healthy subjects were below the detection limit of 25 nM histamine in 104 of 132 dialysis fibres (79%). A more accurate determination of baseline histamine levels was obtained by enzyme immunoassay of samples collected over 8 min in five individuals in study II. The observed histamine concentration in these samples was 10.5 ± 0.6 nM, which taking into account the recovery *in vivo* to be 50%, equates to tissue levels of approximately 20 nM.

In study I involving six atopic subjects (Table 1), large

amounts of histamine, 7.96–20.22 pmol over the 20-min collection period, were recovered in the dialysis fibres positioned 1 mm from sites of challenge, with peak levels of 337, 372, 722 and 1293 nM histamine being observed following injection of allergen, substance P, codeine and histamine respectively. A significant difference in histamine concentrations within fibres located in the different areas of the wheal and the flare was observed for all four compounds. At a distance of 2.3–2.4 mm from the challenge site, significant increases in the amounts of histamine release were detected, but they were only 4–22% of those detected at 1 mm. In the periphery of the wheal, 3.7–3.8 mm from the injection site, significant increases in histamine levels were seen only with codeine. In more distant fibres, positioned in the flare, histamine levels were not significantly greater than baseline. An illustrative example of the pattern of detection of histamine throughout the 20-min recording period following injection of 50 μ M histamine is shown in Fig. 2. The mean wheal and flare radii for allergen,

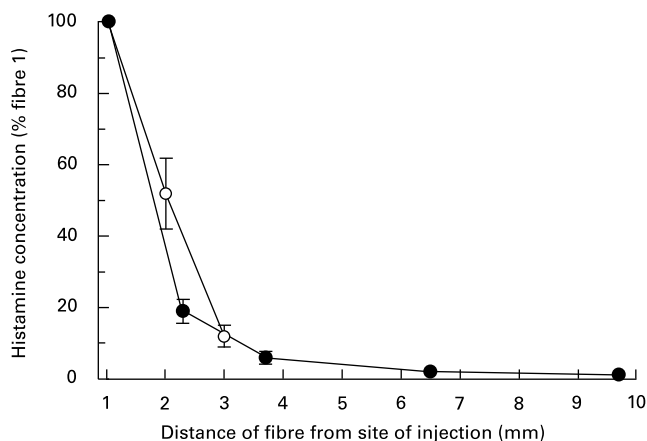


Fig. 4. The effect of dialysis fibres on the measurement of histamine levels at various distances from the site of injection. The solid circles represent the amounts of histamine recovered in more distant fibres, expressed relative to the amount detected at the 1 mm fibre, following the intradermal injection of codeine, 2.5 mM, into six atopic subjects. In these experiments, fibres were inserted in parallel at different distances from the site of injection. The open circles represent the amounts of histamine, calculated similarly to those above, following the intradermal injection of codeine, 2.5 mM, into four normal subjects. In these experiments, codeine was injected at various distances from individual fibres. All results are expressed as mean $\pm$ SEM.

substance P, codeine and histamine in these experiments are shown in Table 1. Thus, it may be seen from Table 1 that the amounts of histamine recovered by dialysis following provocation by all agents, declined rapidly from the centre to the periphery of the wheal with no significant histamine

release either in the most peripheral parts of the wheal or in any part of the flare area.

The short sampling intervals also allowed a detailed analysis of the kinetics of histamine release. In the fibre positioned 1.0 mm from the injection site, peak histamine levels were consistently found in the 0–2 min sample with histamine, in the 2–4 min sample with codeine and substance P, and in the 4–6 or 6–8 min samples with allergen (Fig. 3). After the injection of histamine, the time for peak histamine levels to be detected in the more distant fibres within the wheal was significantly later, the delay periods being 2.4 min at 2.3 mm and 6.0 min at 3.8 mm. No statistically significant delay was observed with codeine, substance P or allergen, even though a tendency for the later appearance of histamine was observed in most subjects. Maximum histamine levels were always found at the fibre positioned at 1 mm.

As the physical presence of dialysis fibres could conceivably affect the diffusion of histamine or interfere with nerve conduction within the skin, further experiments were performed in which codeine, 2.5 mM, was injected 1, 2 or 3 mm from the centre of individual fibres in four normal volunteers. Since the magnitude of histamine release varies widely between subjects, the concentrations detected in the 2 and 3 mm fibres are expressed as percentages of those observed at 1 mm for each individual. Comparison with the data obtained following codeine injection in the first part of study I (Fig. 4) showed that the presence of dialysis fibres *per se* did not significantly effect histamine levels in more distant fibres. The 95% confidence intervals (mean $\pm$ 2 SEM) overlapped between the two groups of data indicating no statistical difference.

Because the concentrations of histamine in the dialysis

Table 2. Histamine concentrations (nM) in dialysate from fibres within the wheal and flare reaction assayed by enzyme immunoassay

Distance from the site of challenge	–9 mm	+1 mm	+3 mm	+9 mm
<i>Codeine</i>				
Baseline	11.8 $\pm$ 1.4	9.2 $\pm$ 1.4	10.1 $\pm$ 1.2	9.4 $\pm$ 0.7
0–8 min	11.7 $\pm$ 1.9	1852 $\pm$ 694	224 $\pm$ 68	10.6 $\pm$ 1.6
8–16 min	11.0 $\pm$ 2.1	508 $\pm$ 84	75 $\pm$ 17	8.8 $\pm$ 0.6
<i>Substance P</i>				
Baseline	12.0 $\pm$ 1.2	12.0 $\pm$ 1.2	10.3 $\pm$ 1.2	10.8 $\pm$ 1.2
0–8 min	11.2 $\pm$ 1.7	648 $\pm$ 197	106 $\pm$ 52	9.5 $\pm$ 1.7
8–16 min	11.9 $\pm$ 2.0	196 $\pm$ 56	52 $\pm$ 19	10.4 $\pm$ 1.9

After establishing baseline levels, two 10 μ l volumes of codeine (2.5 mM) or substance P (10 μ M) were injected intradermally and dialysate collected in two 8-min samples thereafter. The mean wheal and flare radii in these experiments were 3.7 $\pm$ 0.6 and 24.2 $\pm$ 2.0 mm for codeine and 3.7 $\pm$ 0.5 and 23.5 $\pm$ 2.1 mm for substance P, respectively. Values are expressed as mean $\pm$ SEM (n=5).

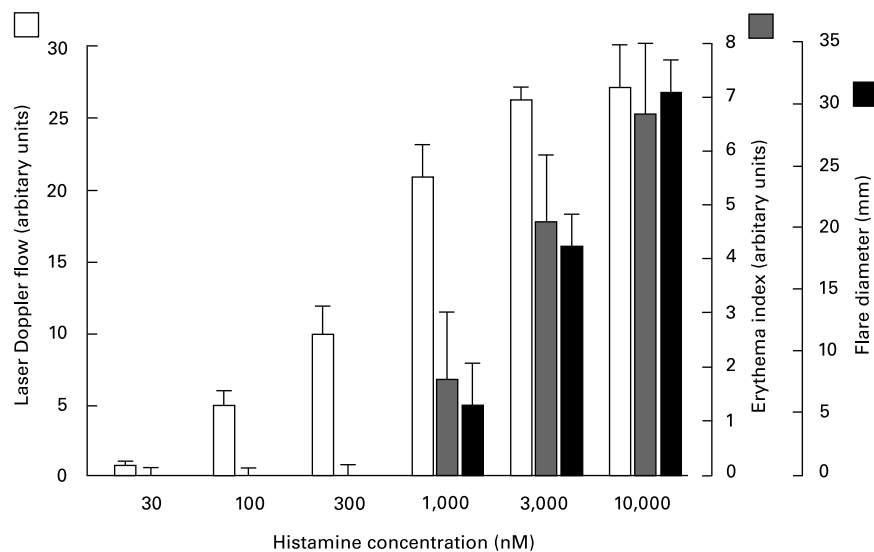


Fig. 5. The effect of histamine on skin blood flow, skin erythema and flare reactions. Various concentrations of histamine were administered atraumatically to the skin via dialysis fibres for 10 min. Approximately 50% of the histamine diffused from the inside of the dialysis fibre into the extracellular water compartment (see the Results section). Skin blood flow was measured every 1 min by laser Doppler flowmetry, the values being represented as maximum increase in blood flow corrected for baseline blood flow, expressed in arbitrary laser Doppler flux values. Skin erythema was measured at 10–12 min by a hand-held dermaspectrometer and flare diameter measured at 10–12 min by a ruler. Histamine caused a dose-related increase in skin blood flow, values from 100 nM and greater showing statistically significant increases compared to baseline values. No flare or increase in skin erythema were observed for histamine concentrations below 1000 nM.

fibres situated in the periphery of the wheal and flare were too small to be detected reliably by the spectrofluorometric histamine assay, we performed a second series of experiments (study II) in five normal volunteers in which histamine release provoked by codeine or substance P was assayed by the more sensitive enzyme immunoassay. The results (Table 2) show that for fibres in the center of the wheal, histamine concentrations in the dialysate increased 50–200 times above baseline values of 10–12 nM, while for those in the periphery of the wheal, histamine levels were 10–20 times above baseline. No increase in histamine concentrations above baseline was observed in dialysates from fibres within the flare reactions positioned 9 mm on either side of the challenge site. The mean wheal and flare radii induced by codeine and substance P in these experiments are shown in Table 2.

The diffusion of histamine from dialysis fibres into the dermis induced statistically significant increases in skin blood flow and skin erythema, and elicited flare reactions (Fig. 5). While blood flow at the site of challenge increased significantly at histamine concentrations at 100 nM and greater, no increase in skin erythema or induction of flare were observed for histamine concentrations below 1000 nM. Wheals were observed for all subjects at 10 000 nM ($P < 0.05$), in one subject at 3000 nM, but not at lower histamine concentrations. With infusion histamine concentrations of 100, 300, 1000, and 3000 nM, the concentrations in the outflows from

the dialysis fibres were $55.8 \pm 3.3\%$, $50.7 \pm 3.7\%$, $53.3 \pm 6.9\%$ and $55.2 \pm 5.1\%$ of those infused respectively, indicating a consistent transfer of approximately 50% of the infused histamine into the tissues.

The time course to reach steady-state recovery of histamine, together with the kinetics of increased skin blood flow assessed by laser Doppler flowmetry, after switching the

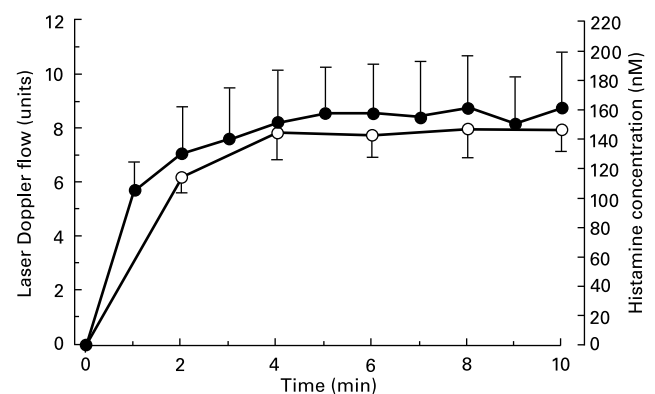


Fig. 6. The time course for dialysate histamine to reach steady-state after an increase in perfusate histamine concentrations from 0 to 300 nM and the kinetics of skin blood flow alterations. Dialysate was collected in 2 min fractions and assayed spectrofluorometrically for histamine (open circles). Changes in skin blood flow (solid circles) were assessed every 1 min by laser Doppler flowmetry.

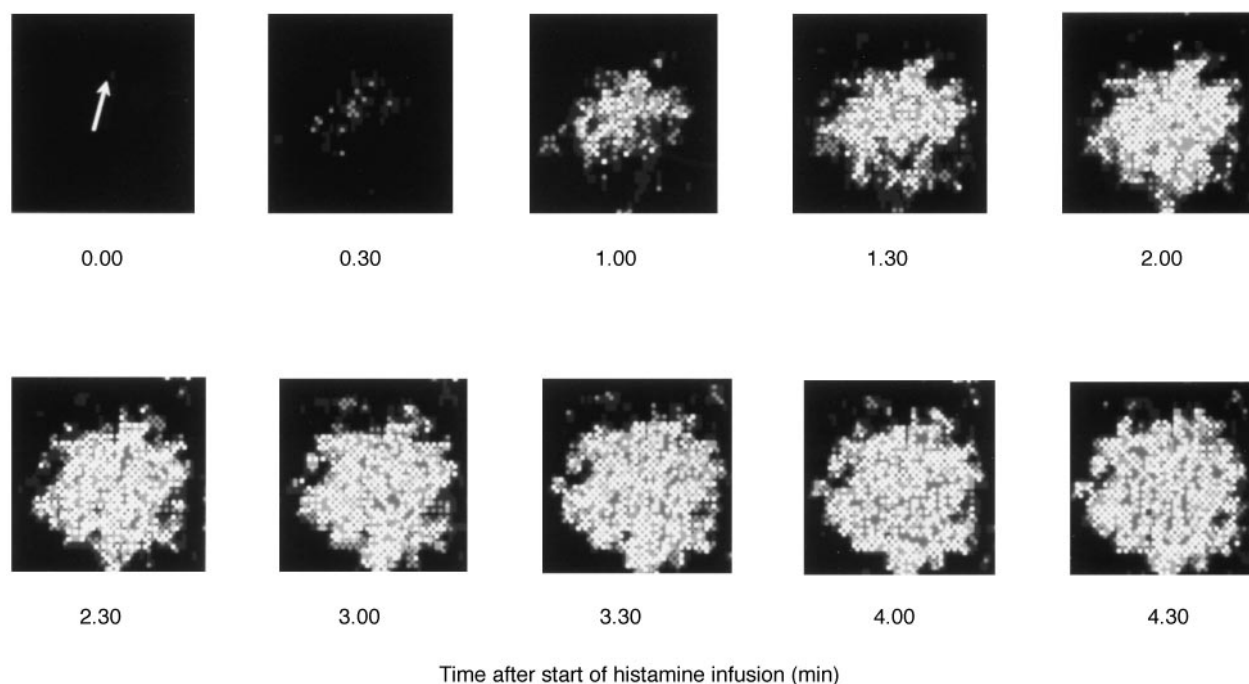


Fig. 7. Scanning laser Doppler images of the chronology of skin blood flow changes in the wheal and flare response to infusion of $1 \mu\text{M}$ histamine via the dialysis fibre in one subject. The direction of the dialysis fibre is from the upper right corner (arrow) to the lower left corner. Measurements of blood flow were made at 30 s intervals after the start of the infusion. The vasodilation developed quickly, apparently as a function of multiple points of vasodilation, to reach steady state at approximately 3 min.

infused histamine concentration from 0 to 300 nM is shown in Fig. 6. It is seen that histamine equilibrated rapidly, within 2–4 min, to reach a steady-state recovery of approximately 50% at 4 min, and that this was paralleled by an increase in skin blood flow.

The sequence of vasodilation in the wheal and flare response was studied in one subject using scanning laser Doppler imaging (Fig. 7). The images showed a homogeneous pattern of vasodilation with multiple points of increasing skin blood flow; steady state was reached at approximately 3 min. The impression of multiple points of vasodilation in the flare was supported by computerized videophotometric capillaroscopy in another subject (data not shown).

Discussion

Using cutaneous microdialysis, which has an approximately 50% efficacy in transferring histamine across its membrane, we have been able to describe in detail the distribution of histamine release in the wheal and flare reactions provoked by intradermal injection of histamine, allergen, codeine and substance P. Our major findings were the presence of large amounts of histamine only in the centre of the wheal, a steep gradient of histamine from the centre to the periphery of the wheal and no detectable histamine release in the flare response. These findings were reproduced using two

different histamine analyses. Analysis of the effects of exogenous histamine on cutaneous blood flow showed that infusion concentrations of at least 100 nM were necessary to induce a significant increase in local blood flow, the threshold infusion concentration necessary to stimulate a flare was 300–1000 nM, and infusion concentrations of 3000–10 000 nM were required to induce a wheal. From these results we conclude that the release of histamine in type I hypersensitivity reactions is a very localized process. We further conclude that histamine is not an effector substance in the flare area which surrounds the wheal.

The histamine concentration in the dialysis fluid draining unchallenged skin, determined using the sensitive enzyme immunoassay, was 10.5 ± 0.6 nM. However, dialysate histamine concentrations underestimate the histamine concentrations in the skin by approximately 50%. This *in vivo* estimate, which was relatively consistent throughout experiments assessing diffusion of histamine both into and out of dialysis fibres, is lower than that of 80% obtained *in vitro* [25], suggesting that physical contact of the tissue with the fibre wall creates a further barrier to diffusion. Thus, the true tissue histamine levels *in vivo* are likely to be about 20 nM, some 10–20 times higher than those of 0.5–2.5 nM found in plasma [36]. We consider it unlikely that these levels are related to the trauma of fibre insertion for the following reasons.

First, an interval of at least 2 h was interposed between insertion of fibres and collection of dialysate and, at the time of sampling, there were no signs of either oedema or vasodilation. Second, the concentrations of histamine recovered over a 30-min period were steady. Third, Anderson and colleagues [37] reported that trauma-induced increases in histamine levels has worn off by 40–60 min after insertion of a 16 gauge cannula. Also, studies by Petersen *et al.* (unpublished observations), using the smaller 23 gauge guide cannula employed in this study, suggested that tissue histamine levels returned to steady state low levels within 10–15 min. Thus, we conclude that the relatively high histamine levels in resting skin represent a continuous turnover of histamine, probably resulting from an incessant low level of mast cell degranulation. However, whether the continued presence of dialysis fibres in the skin has an impact on this process is not known.

Two interrelated questions, which may be readily addressed using microdialysis, are the diffusion of histamine within the wheal and the extent of histamine release in more distant parts of the wheal and flare. We used the injection of exogenous histamine as a tool to investigate the first of these questions. Time course studies showed that exogenous histamine was always found in the first 2-min fraction after intradermal injection, indicating that histamine diffuses very rapidly across the dialysis membrane *in vivo*, confirming previous observations *in vitro* [25]. A very limited diffusion within the wheal is suggested from the observations that the amount of histamine recovered by the fibre situated 2.4 mm from the site of injection was only ~4% of that at 1 mm, and histamine was below the levels of detection at the edge of the wheal. The half-life of exogenous histamine recovered in the dialysate from the fibre 1 mm from the injection site was 2–3 min, and the time taken for histamine to reach the 2.3 and 3.8 mm fibres was 2.4 and 6.0 min respectively. Thus, it appears that the lateral diffusion of histamine through the dermis is poor and most is either rapidly metabolized or removed by the blood.

With codeine or substance P, peak histamine release occurred in the 2–4 min sample, whereas with allergen it did not occur until the 4–6 or 6–8 min sample. These findings of a different temporal pattern of mediator release induced by IgE-dependent and non-immunological mast cell secretagogues are in accordance with previous studies *in vivo* [25,31,33] and *in vitro* [21]. The spread of histamine release, indicative of the extent of diffusion of codeine, substance P and allergen, is less easy to assess because the measured decay rates of histamine, after reaching peak levels, were 3–4 min with codeine and substance P and 6–8 min with allergen. This indicates some ongoing histamine release but does not indicate whether mast cells at the site of provocation are still releasing small amounts of histamine or mast cells at a more distant site have become

involved. However, the small amounts of histamine recovered in the periphery of the wheal suggest that the area of mast cell degranulation is limited.

Analysis of histamine levels in the flare area by spectrofluorometry in study I showed them to be below the levels of detection, while the use of the more sensitive enzyme immunoassay for histamine in study II confirmed that they were no higher than those in unchallenged skin, around 20 nM. Thus, as no rises in its concentrations were observed in the flare area, it is highly unlikely that histamine acts as the final local mediator in the flare. However, it may be argued that small localized rises in histamine concentrations are involved in the elicitation of the flare reaction and that such increases are not detectable by microdialysis due to the rapid metabolization of histamine or its increased washout by the blood during the period of vasodilation.

To help address these points, we examined the effects of exogenous histamine on the skin vasculature following its delivery by passive diffusion from indwelling microdialysis fibres. This technique is particularly appropriate for such studies as no injection or forcing of fluids into the tissue is involved. The salient points arising from this study are as follows. First, no localized erythema or flare was seen in challenged skin in which tissue histamine levels were around 20 nM. Second, infusion concentrations of at least 100 nM, equating to tissue levels of 50–60 nM, were necessary to induce a significant increase in local blood flow above the dialysis fibre. Third, infusion concentrations of 300–1000 nM, equating to tissue levels of approximately 150–500 nM, were required to stimulate a flare response. This concentration range probably represents the threshold for activating the axon reflex necessary to elicit the flare response. Fourth, it was not until infusion concentrations reached 3000–10 000 nM, equating to tissue levels of 1500–5000 nM, that a wheal was observed along the dialysis fibre. This concentration range probably reflects the amounts of histamine necessary to cause endothelial cell contraction in post-capillary venules of a sufficient magnitude for oedema to be obvious.

Taken together with the demonstration that the presence of increased blood flow in the flare area does not increase the clearance of tissue histamine, these findings illustrate that any increases in tissue histamine levels of sufficient magnitude to produce effects on the local skin vasculature would be readily detectable by dialysis. The possibility that vasodilation in the flare occurred via activation of a few mast cells does not seem valid, since scanning laser Doppler imaging and computerized videophotometric capillaroscopy supported the visual impression of a homogeneous vasodilation in the flare.

Thus, it would appear that a local erythema, a surrounding flare and a wheal are stimulated sequentially as a consequence of the rising histamine levels at the focus of

the skin challenge site. Also, the role of histamine in the flare is solely to stimulate axon reflexes which, by releasing neuropeptides distally, are responsible for the spread of this response many millimeters from the focus of histamine release. That the levels of neuropeptides occurring during this response are insufficient to cause histamine release in normal skin is supported by our findings that flare responses induced by the injection of capsaicin, which induces the release of neuropeptides [5], are not accompanied by increases in levels of tissue histamine (manuscript in preparation). This conclusion does, however, leave us with the apparent anomaly that flare responses provoked by intradermal injection of platelet-activating factor and endothelin-1, neither of which release histamine from human skin mast cells *in vitro* [26,27], are reduced by antihistamines [26,29]. However, this anomaly has recently been resolved by the findings using microdialysis that both platelet-activating factor [38] and endothelin-1 [39] do release histamine when injected into human skin.

In conclusion, the results presented in this manuscript support the hypothesis that axon reflex-mediated vasodilation does not involve the local release of histamine within the flare area in normal human skin. The possibilities that, in inflammatory skin diseases, cutaneous nerves may release endogenous neuropeptides to such an extent that they induce mast cell histamine release, or hyperresponsive dermal mast cells may degranulate in response to neuropeptides released by a normal axon reflex, cannot be excluded and must be the subject of further experimentation.

Acknowledgements

Dr Petersen holds a Senior Research Fellowship from the Weimann Foundation, University of Copenhagen and a 1995 Pharmacia Research Fellowship. The work was supported by the Novo Foundation Committee, the Aage Bang Foundation, and the Allergy Foundation of 1983. Dr H. Egekvist, Mrs Inge Aakilde and the staff at the Reference Laboratory are thanked for skillful technical assistance.

This paper was presented in part at the 51st annual meeting of the American Academy of Allergy and Immunology, New York, 24 February to 1 March 1995 (J Allergy Clin Immunol 1995; 95:255).

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