

## DIRECT RECOVERY OF HISTAMINE FROM CUTANEOUS ANAPHYLAXIS IN MAN

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**Abstract.** Using a continuous in vivo perfusion method, pharmacological substances released in human skin during cutaneous anaphylaxis have been studied. Nine patients with respiratory allergy were studied and in 7 of these histamine was recovered in the perfusate following pricking of specific antigen into the perfused skin. In some experiments histamine continued to be released although the inflammatory reaction in the overlying skin had faded. Kinin activity was detected in 4 of the 9 patients but its incidence and concentration was no greater than in a comparable control group. No other pharmacological activity was found.

The association between histamine and immediate hypersensitivity reactions [Type I, (6)], is well established both in animal tissues (3, 5, 7, 8), in human lung (2, 18, 19), and human leucocytes (1, 16). Direct evidence that histamine mediates cutaneous wheal-and-flare reactions induced by challenge with antigen in sensitised human subjects is scanty and conflicting. Katz (13), using a cantharidin blister technique, demonstrated histamine release at the site of human cutaneous anaphylactic reactions. By contrast, Michel et al. (17) were unable to demonstrate histamine in cutaneous anaphylaxis using a skin perfusion method.

In this report we describe experiments using a skin perfusion method to examine pharmacological substances released in immediate hypersensitivity reactions.

### METHODS

**Patients.** Nine patients with respiratory allergy (hay fever, asthma) were studied, all of whom showed positive immediate wheal-and-flare reactions following challenge of the skin by specific antigen<sup>1</sup> using the prick test technique.

<sup>1</sup> Bencard Allergy Unit, Beecham Research Labs., Great West Road, Brentford, Middlesex.

Results in normal skin were obtained in 16 control subjects. These were patients with various non-inflammatory localised skin conditions sparing the arms.

**Skin perfusion.** This was carried out by the method of Greaves & Søndergaard (9).

Two wide bore needles with 8 holes perforated along the shaft were inserted immediately subdermally in parallel 10 mm apart in opposite directions along the flexor aspect of the forearm. Warm sterile Tyrode solution was infused through one needle and recovered through the other into siliconised glass tubes in an ice-packed fraction collector. Perfusate was collected in successive 15 min aliquots.

Assay for smooth muscle-contracting agents was carried out using an automatic bioassay apparatus (4). Histamine was assayed biologically against the guinea pig ileum, its presence being confirmed using the specific antagonist pyrilamine. The guinea pig ileum responded to 1-2.5 ng per ml standard histamine solution in all experiments. Kinin activity was assayed by contraction of the oestrus rat uterus and relaxation of the rat duodenum, both preparations being mounted in the same organ bath. Synthetic bradykinin (Sandoz) was used as a standard, a response to as little as 0.1 ng being regularly obtained with both preparations. In both bioassays activity due to acetyl choline and 5-hydroxytryptamine was excluded by adding atropine  $2 \times 10^{-6}$  and bromlysergic acid diethylamide  $5 \times 10^{-7}$  to the organ baths. Bradykinin was assayed in the presence of pyrilamine  $10^{-6}$ . 5-hydroxytryptamine was extracted by the method of Wise (20) and measured fluorimetrically. The lower limit of detection of 5-hydroxytryptamine was 10 ng per ml.

### RESULTS

**Normal skin.** Perfusion of normal skin was carried out in 16 control subjects for 15-75 min. In 5 subjects kinin activity was detected of concentration 0.4-4 ng/ml. Neither histamine nor other smooth muscle-contracting activity was detected.

**Cutaneous anaphylaxis.** In 9 allergic subjects perfusion was carried out and continued for 15-30 min. Specific antigen was then pricked into the

Table I

Patients	Diagnosis	Histamine <sup>a</sup> (ng/ml)	Kinin <sup>a</sup> (ng/ml)
T. H.	Asthma	5	0.9
A. S.	Asthma	6.5	0.2
A. A.	Asthma	12	0.7
J. R.	Asthma	0	0
B. P.	Asthma	11	0.4
W. P.	Hay fever	56	0
E. H.	Asthma	6	0
J. M.	Hay fever	5	0
S. H.	Hay fever	0	0

<sup>a</sup> Maximum concentration recovered in perfusate.

perfused skin and perfusion of the inflamed skin was continued for a further 30–60 min.

The results are summarised in Table I.

When perfusates recovered before challenge with antigen were added to the isolated guinea pig ileum no activity was detected. By contrast, perfusates collected after injection of specific antigen caused contraction of the guinea pig ileum in 7 of 9 subjects, the activity ranging from 5–56 ng/ml in terms of histamine-equivalents. In 2 patients activity was detected as long as 45–60 min after challenge with antigen even though by this time the inflammatory reaction had subsided.

That this smooth muscle-contracting activity was due to histamine was confirmed since in each patient it could be completely antagonised by the specific competitive histamine antagonist pyrilamine. The kymograph tracing of results from a typical experiment is shown in Fig. 1. No attempt was made to correlate magnitude of the

visible skin reaction with results of assay on the corresponding perfusate.

Kinin activity was found in 5 of the 9 subjects, the highest concentration obtained being 0.9 ng/ml. No serotonin or other smooth muscle-contracting activity was detected.

## DISCUSSION

The results of the present paper suggest that histamine mediates the cutaneous wheal-and-flare response to antigen in subjects with Type 1 hypersensitivity.

Histamine was recovered from perfusate of 7 of 9 subjects. Failure to detect histamine in the remaining 2 may be related to the dose of antigen or to lack of control of variables in the techniques used. The prick test method precludes accuracy of antigen dosage and results in variation in magnitude of the ensuing hypersensitivity reactions.

The semi-quantitative nature of the skin perfusion technique has been discussed in a previous publication (9).

Visible evidence of cutaneous whealing reactions diminishes from about 30 min onwards but this was not always accompanied by a decline in histamine concentration in the perfusate (Fig. 1). That histamine release can persist for as long as 60 min is not surprising and is probably due to the mast cell-degranulating properties of histamine itself (14). The failure of the wheal to persist in the presence of histamine is explained by development of tachyphylaxis (10).

Kinin activity was found in perfusates from 5 subjects. The frequency and concentrations were,

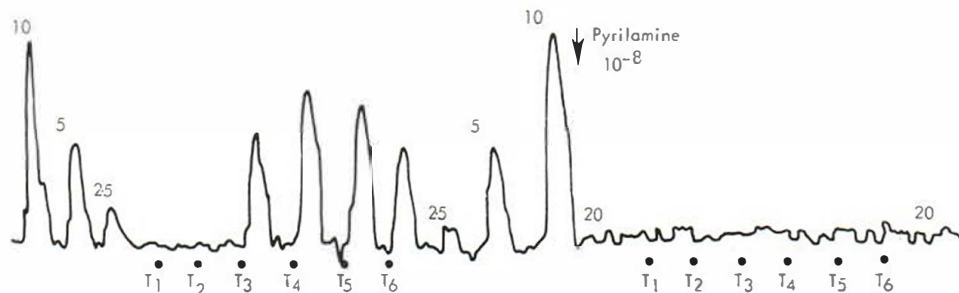


Fig. 1. Kymograph tracing of histamine activity in perfusate obtained from a single experiment. Contractions of isolated guinea pig ileum preparation occur in response to 2.5, 5 and 10 ng/ml doses of standard histamine solutions and to successive 15 min aliquots of perfusate ob-

tained after injection of perfused skin by specific antigen (T3, 4, 5, 6). Addition of pyrilamine 10<sup>-8</sup> inhibits the response to the perfusate samples and to a dose of 20 ng/ml standard histamine. Perfusates obtained before challenge by antigen (T1, 2) contain no activity.

however, no higher than in the control group.

Our results differ from those of Michel et al. (17) who concluded, using a skin perfusion technique, that kinins are specifically associated with cutaneous anaphylaxis. Although they were unable to detect any histamine, they found an agent which contracted the isolated rat uterus in the perfusates of 6 allergic subjects during antigen-induced wheal-and-flare reactions. Their conclusion that this activity was due to kinins was based on the results of gel filtration of a single perfusate sample, which indicated that the agent was of approximately the same molecular size as bradykinin. Possible reasons for failure to detect histamine in perfusates using the skin perfusion technique have been discussed in detail in a previous paper (9).

Our own results support the traditional view originally proposed by Lewis (15) that histamine mediates short-lived cutaneous wheal-and-flare reactions. This simple situation contrasts with our results in delayed cutaneous inflammation due to allergic contact eczema (11), and exposure to ultraviolet irradiation (12), where the findings are more complex.

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