

Histamine release in immediate-type hypersensitivity reactions in intact human skin measured by microdialysis

A preliminary study

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The purpose of this study was to evaluate the application of a microdialysis technique for measurement of interstitial histamine levels in intact human skin. Three allergic subjects were investigated. Single dialysis fibers were glued to nylon tubings and inserted in forearm skin by means of a fine cannula. Dialysis fibers were inserted in triplicate and perfused with isotonic saline at a rate of 3 µl/min. After a period of 2 h a 60-µl base-line period was established. Then the patients were skin prick tested (SPT) with allergen in duplicate and a single saline control. Dialysate was collected in consecutive 30 µl fractions. Histamine concentration in the dialysate was analyzed with a glass fiber fluorescence assay. Median base-line histamine level was 4 (range 4–7) ng/ml. Following allergen SPT, dialysate histamine concentration increased to 81 ng/ml (74–128), with maximum values 10–20 min after SPT. Intraindividual coefficient of variation on peak histamine levels was 18.9%. No histamine increase was seen following saline SPT. We consider microdialysis to be a valuable method for assessment of allergic mechanisms in intact human skin.

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Histamine is considered to be a major mediator in immediate-type hypersensitivity reactions in the skin, although other mediators also participate.

Until now, measurements of inflammatory mediators in the skin *in vivo* have been restricted to analysis of fluid from suction blister models (11). Meanwhile, keratinocytes are increasingly acknowledged as initiators of inflammation in the skin (2). Removal of the epidermis might therefore weaken the extrapolation to the true *in vivo* situation.

Analysis of blood from superficial veins draining a distally located skin area (4) and perfusion methods (10) can give valuable information. The techniques, however, measure mediators not only from the skin alone but also from the underlying tissues and from blood basophils. Until now, no method has allowed *in situ* measurement of mediators in intact human skin.

Microdialysis has been used for years for the assessment of metabolic events at the extracellular level in animal brain preparations; for review, see Robinson & Justice (8). The microdialysis method has only lately been used in peripheral tissues, including

subcutaneous adipose tissue (3) and skin (1, 6), to measure interstitial levels of, for example, glucose and lactate.

The aim of the present pilot study was to determine whether the microdialysis method is suitable for measurement of histamine levels in human skin.

Material and methods

The study included three nonmedicated patients with well-documented type I allergy. Median age was 27 (range 23–41) years. Two patients were allergic to hen's egg, and one had hay fever. All had positive skin prick tests (SPT). The subjects gave informed consent, and the study was approved by the local ethics committee (KF V 92-001).

The microdialysis probes, which are similar in principle to probes previously described (3, 6), were constructed with a single dialysis fiber (Gambro GFE 18, Gambro Dialysaten AG, Germany) glued to gas-tight nylon tubing and sterilized. The fibers (outer diameter 216 µm, wall thickness 8 µm, 2 kDa molecular mass cutoff) had a length of 20 mm; the

outlet of the nylon tubing had a standardized length of 30 mm.

By means of a 23G cannula, the dialysis probes were placed superficially in the skin on the ventral part of the forearm 10 cm below the cubital region. Three adjacent dialysis fibers were positioned 2–3 cm apart. An analgesic cream (EMLA, Astra AB) was applied under an occlusive dressing (Tegaderm, 3M) for 30 min before the implantation.

The inlets of the nylon tubings were connected to a microinjection pump with three syringes (CMA/100, Carnegie/Microdialysis, Sweden), and each fiber was perfused with isotonic saline at the rate of 3 μ l/min.

The experiments were started 2 h after implantation in order to ensure that the implantation trauma had dissipated (6). A 60- μ l base line (steady-state perfusion) was established before SPT. SPT was performed with the relevant allergen (raw hen's egg or commercially obtained grass pollen (Soluprick, ALK Laboratories)) in duplicate and a single saline control. A standard lancet was used (ALK Laboratories). Thereafter dialysates were collected in consecutive 30- μ l fractions. Histamine concentrations in the dialysates were analyzed with a glass fiber fluorescence assay (9). The technique has recently been reviewed (5).

Results

In all three patients, allergen SPT wheal diameters were greater than 5 mm; no wheals were seen following saline SPT. Exact wheal areas were not traced in this pilot study.

Median base-line histamine concentration in the dialysate of three fibers was 4 (range 4–7) ng/ml.

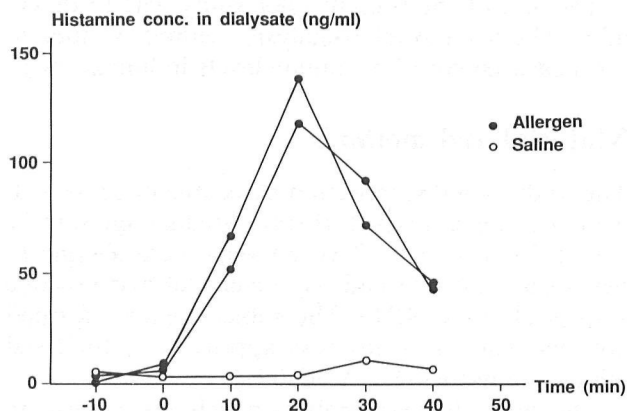


Fig. 1. Example of time course of skin histamine levels measured by microdialysis before and after SPT. A 60- μ l base line (two consecutive 30- μ l samples) was established before SPT. At time zero, SPT was performed with allergen in duplicate and a single saline control. Maximum histamine concentration in the dialysate was found in the second dialysis fraction (10–20 min) after SPT. Histamine level was virtually unchanged after saline SPT.

Intrafiber variation (SD/mean) on base-line histamine was 0% (0–8%). After allergen SPT, histamine levels increased to 52 (44–59) ng/ml in the first 10-min sample. A peak value of 81 (73–128) ng/ml was found in the second sample. The intraindividual coefficient of variation on peak histamine levels was 18.9%. During the next 10-min samples, histamine levels gradually declined towards base-line values. Forty minutes after allergen SPT, dialysate histamine concentration was 26 (19–44) ng/ml. Placebo SPT did not liberate histamine; the peak histamine level was 10 (9–11) ng/ml. An example is shown in Fig. 1.

Discussion

The results of this preliminary study demonstrate for the first time the usefulness of a microdialysis technique for *in situ* monitoring of allergen-induced histamine secretion in intact human skin.

We used EMLA cream to reduce the discomfort caused by the probe implantation. We assume that the EMLA cream did not interfere with the measurements, since histamine- and allergen-induced wheal reactions are unaffected by EMLA cream (7).

The microdialysis technique is widely used in animal brain research. In these studies several problems have been outlined, including choice of perfusion fluid, tissue damage, recovery and reproducibility, *in vivo* quantification of the substances in the tissue, and time resolution (8). Further studies to evaluate these parameters are in progress.

It was not possible to determine exact interstitial skin histamine concentrations from measurement of histamine in dialysates in this setup. For such purposes, knowledge of *in vivo* recovery of histamine is required. However, by a calibration technique previously described (6), quantitative measurements of skin histamine concentrations have been performed (unpublished observations). In the present setup, the method allows semiquantitative measurements, since the recovery of histamine is constant in a wide range of histamine concentrations (*in vitro* studies, data not shown). These *in vitro* measurements also showed that rapid change in histamine concentrations in the medium was recovered almost immediately in the dialysate (95% of a change was found in the subsequent 30- μ l dialysis fraction).

The use of microdialysis in peripheral tissues is scarcely described. Our preliminary data suggest that the technique is suitable for monitoring histamine levels in human skin. Therefore, we consider the technique of potential interest since it will be possible to monitor histamine as well as other mediators released during inflammatory reactions in the skin, and further to evaluate the influence of anti-inflammatory drugs.

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