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Human *In vivo* Cutaneous Microdialysis: Estimation of Histamine Release in Cold Urticaria

THOMAS ANDERSSON[1], KARIN WÅRDELL[2] and CHRIS ANDERSON[1]

Departments of [1]Dermatology and [2]Biomedical Engineering, University Hospital, Linköping, Sweden

A novel bioanalytical in vivo sampling technique, cutaneous microdialysis, was used to follow the chronology of skin histamine release in 3 patients with cold urticaria and in 2 healthy volunteers. Laser Doppler perfusion imaging was used simultaneously to monitor the skin circulatory response. Microdialysis samples were collected at 10-min intervals and analysed by radioimmunoassay technique. Fifty minutes after probe insertion, the ventral forearm skin in the area of the dialysis membrane was provoked for 5–15 min with a 25 × 40 mm ice cube covered with plastic foil. In the cold urticaria patients, an up to 80-fold increase of histamine was observed, with peak levels 20–30 min after challenge. Histamine levels then fell to reach "baseline" levels within 50 min. In the healthy subjects, the histamine increase was earlier, less pronounced and of shorter duration. Cutaneous microdialysis and laser Doppler imaging offer new possibilities for the chronological multiparameter assessment of inflammatory skin disorders in vivo. ***Key words: dermis; inflammatory mediators; laser Doppler perfusion imaging.***

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T. Andersson, Department of Dermatology, University Hospital, S-581 85 Linköping, Sweden.

Microdialysis is a sampling technique in which a probe is placed in a tissue and the interstitial level of endogenous or exogenous substances is monitored (1). The method is applicable to both animal and human studies (2). The first reports of the use of microdialysis, in vivo, in human skin concerned percutaneous absorption of ethanol (3), the monitoring of glucose levels (4, 5) and histamine release (6, 7). Cutaneous microdialysis can be performed, "sewing in" a loop of microdialysis tubing with subsequent gluing of afferent and efferent tubing (5) or using, as in our laboratory, manufactured probes. Insertion of the microdialysis probe causes histamine release (6) and a transient skin flare of variable extent (8). It is, therefore, usual to allow an equilibration period of 40 to 60 min after probe insertion prior to measurement or provocation of the test site. We have found the procedure to be well tolerated by subjects.

Histamine is involved, directly or indirectly, in a large number of cutaneous inflammatory reactions (9). Many reports of the role of histamine in the pathogenesis of physical urticarias have been published (10–13), but the at times poor effect of antihistamines would seem to indicate that histamine is not the only mediator involved. In previous studies of urticaria, pharmacological information has been obtained by examination of venous blood, urinary metabolites or suction blister fluid. Especially the latter technique is tissue-destructive, which raises questions about the origin of the inflammatory mediators measured and also interferes with the subsequent observation of the chronology of the reaction. Additionally, overall histamine liberation, as detected in venous blood, may be less relevant than local histamine concentrations. A continuous in vivo subcutaneous perfusion method (14, 15) has also been used for measurement of histamine release in urticaria pigmentosa and factitious urticaria (16). The technique involved insertion of two relatively large calibre needles, and the injection into and subsequent evacuation from the subcutaneous compartment of large volumes of fluid. This technique, however, never gained general use.

The main purpose of the present study was to evaluate the use of in vivo, human, microdialysis technique for the chronological study of histamine release in reactions provoked in the skin of patients with cold urticaria. An additional aim was to demonstrate that cutaneous microdialysis could be used in parallel with a newly developed technique, laser Doppler perfusion imaging (LDPI) (17), recording local skin circulatory changes during the provoked urticarial reactions.

MATERIALS AND METHODS

Subjects

Three patients with acquired cold urticaria (A, B, C) and 2 healthy volunteers (D, E) were examined. One of the patients with cold urticaria also had seasonal atopic rhinoconjunctivitis. The subjects, all duly informed of the experimental conditions, had not used any medication for 1 week prior to the experiments. Ice challenge time was previously determined individually for the cold urticaria patients and chosen empirically for the healthy subjects. The study was approved by the Regional Ethics Committee for Human Research, Linköping University Hospital.

Microdialysis

The microdialysis probe used (CMA/10, CMA/Microdialysis Research AB, Sweden) has a shaft length of 70 mm and a membrane length of 10 mm (3). The steel shaft of the probe has an outer diameter of 0.64 mm. The diameter at the tip of the probe, where the polyamide membrane is located, is 0.5 mm. The standard dialysis membrane (6) used in the present experiments has a molecular weight cut off point of 20 kD (a membrane permeable to molecules up to 100 kD can also be used). The outlet dead space volume is approximately 12 µl. In human in vivo experimentation, the probes are not reusable.

A site for insertion of the microdialysis probe on the ventral forearm was chosen so as to avoid obvious veins. The point of insertion was anaesthetized with local anesthetic (mepivacaine 5 mg/ml), injected intradermally to produce a bleb approximately 3 mm in diameter. The probe was inserted through a guide (Baxter arterial fistula needle 1.60 × 25 mm, Baxter Healthcare Ltd., UK), which was then withdrawn and taped in position. Thus, the dialysis membrane at the tip of the probe was 2.5 cm from the point of insertion. The probe was perfused at 3 µl/min with physiological Ringer solution at room temperature. The performance of the probes was controlled prior to and after experiments by determining the in vitro recovery of glucose in a standardized solution.

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Table I. *Histamine levels in the microdialysis perfusate after ice challenge experiments in 3 cold urticaria (CU) patients and 2 healthy (H) volunteers*

Subject B was challenged twice, 60 min apart (shown as B1 and B2). Microdialysis probes were perfused at a flow rate of 3 µl/min. Histamine analysis was performed using a RIA method. Probe depth (PD) and skin thickness (ST) were measured by ultrasound technique. To compensate for the dead space volume of 12 µl, the time intervals have been adjusted backwards by 4 min. The baseline level is the histamine value (nM) for the 10-min period ending 4 min prior to ice provocation. The following columns show the histamine levels in the perfusate, followed at 10-min intervals after commencement of ice challenge. ND = not detected.

Subject	Category	Age/sex	ST	PD	Challenge time (min)	Time after start of ice challenge (min)							
						Baseline	–4–6	6–16	16–26	26–36	36–46	46–56	56–66
A	CU	36/f	0.8	1.0	6	7.3	14.7	502.0	549.9	99.7	23.7	10.8	5.4
B1	CU	32/m	0.9	0.8	15	6.7	15.4	31.4	428.6	152.6	32.6	13.0	11.5
B2	CU	32/m	0.9	0.8	15	11.5	15.2	200.5	117.0	32.0	12.4	9.2	ND
C	CU	29/m	1.4	0.6	15	15.0	25.4	42.9	91.0	25.2	64.1	16.2	12.5
D	H	38/m	1.0	0.7	5	10.1	27.1	33.4	15.9	12.4	13.3	ND	ND
E	H	21/m	1.3	1.1	15	6.1	29.4	41.1	15.4	9.9	8.9	7.6	6.8

Analysis of histamine

Histamine was analysed using a radioimmunoassay (RIA) technique (Immunotech, France) (18). The lower detection limit for the RIA method was 0.5 nM, but since dilution of samples was performed (dilution factor 3.67), the real lower detection limit in the present experiments was 1.8 nM. The upper detection limit was 550 nM.

Laser Doppler perfusion imaging (LDPI)

LDPI (PIM 1.0 Lisca Development AB, Sweden, software version LDI 2.4) is a computer-controlled system that uses a low-power He-Ne laser to scan the tissue (17). At each site, moving blood cells give rise to Doppler shifts in the backscattered light. These Doppler components are detected and processed to generate an output signal (range 0-10 V), which is linearly proportional to tissue blood perfusion. The main proportion of the Doppler signal originates from the upper 200–300 µm of the skin. All measurement values are captured and stored by the system, and a colour-coded image is displayed on the monitor. Appropriate analysis and statistical calculations can then be performed on the captured data.

Recording of skin blood perfusion

The subjects were placed in a comfortable position with the forearm fixed. Reference points on the forearm skin were marked with black ink. During measurements the scanner head was positioned about 16 cm above the area of interest. At this distance, the laser beam was approximately 1.2 mm wide and the distance between two adjacent measurement sites was about 1.5 mm. With the LDPI system resolution set to "high" and the number of measurement sites to 64 × 64, inclusion of both the point of insertion and probe tip area was possible (approximately 100 cm^2). A recording of this size takes about 4.5 min.

In this study the collected LDPI data were analysed using the built-in statistical functions. Areas (8 × 8 sites) in the center of the reaction, close to the microdialysis probe, were analysed (expressed as mean ± SD). For the presentation, however, images were converted to TIFF-format and exported to PowerPoint® (Microsoft, USA) for printout. This processing results in a smoothened perfusion image.

Localisation of probes

Ultrasound measurement (19) (Dermascan A, Sonotron AB, Sweden) allowed the estimation of the actual probe depth as well as the skin thickness (epidermis + dermis) near the probe tip. In order to avoid possible interference caused by skin pressure, we performed the ultrasound measurement immediately after probe insertion.

Experimental design

Microdialysis probes were inserted in the ventral forearm skin of the subjects. After an equilibration period of 50 min, the skin area over the dialysis membrane was challenged with a 25 × 40 mm ice cube, covered with plastic foil. The ice cube was taped in place on the skin with its central point over the dialysis membrane for a period of 5–15 min. In one patient the ice challenge was repeated in the same manner 60 min after the start of the first challenge. Experiments were conducted at room temperature and without warming of the skin. Thirty-µl aliquots of the perfusate were collected at 10-min intervals during the experiments. The total duration of the experiments was 110–170 min. To compensate for a dead space volume of 12 µl in the probe and efferent tubing, the time intervals for the histamine results were adjusted backwards by 4 min. LDPI recordings were initiated prior to ice challenge and at 10-min intervals after challenge in subjects B-E.

RESULTS

The experimental milieu, including the probe insertion and the performance of LDPI measurements, was well tolerated by the subjects. Probe performance control after experiments, as assessed by in vitro glucose recovery, ranged from 14% to 25%, which was higher than pre-experiment values, and in accordance with normal probe variation.

Histamine values after probe insertion were high initially but fell to "baseline" levels, as previously reported, by 40 or 50 min (6). Probes were located in the dermis in all but one subject (A), in whom the probe was in the superficial subcutis. Table I is a summary of the raw data for the 5 subjects (subjects A-C with cold urticaria and subjects D-E healthy controls).

Ice provocation was performed after the initial skin flare associated with probe insertion had disappeared. Pronounced weal and flare reactions were seen in all 3 cold urticaria cases. The skin had returned to normal approximately 2 h after the end of the ice challenge.

In subject A, an almost 80-fold increase of histamine was detected in the perfusate after ice challenge. The peak level in the second 10-min sample after ice challenge was very close to the upper detection limit and might be a saturation value. The histamine values had reached resting levels by around 60 min after ice challenge.

In subject B, two 15-min ice provocations, 60 min apart, were performed (B1 and B2 in Table I). Fig. 1 shows the LDPI maps of the blood perfusion. Fig. 2a shows a numerical presentation of the LDPI data for the same subject using the mean blood

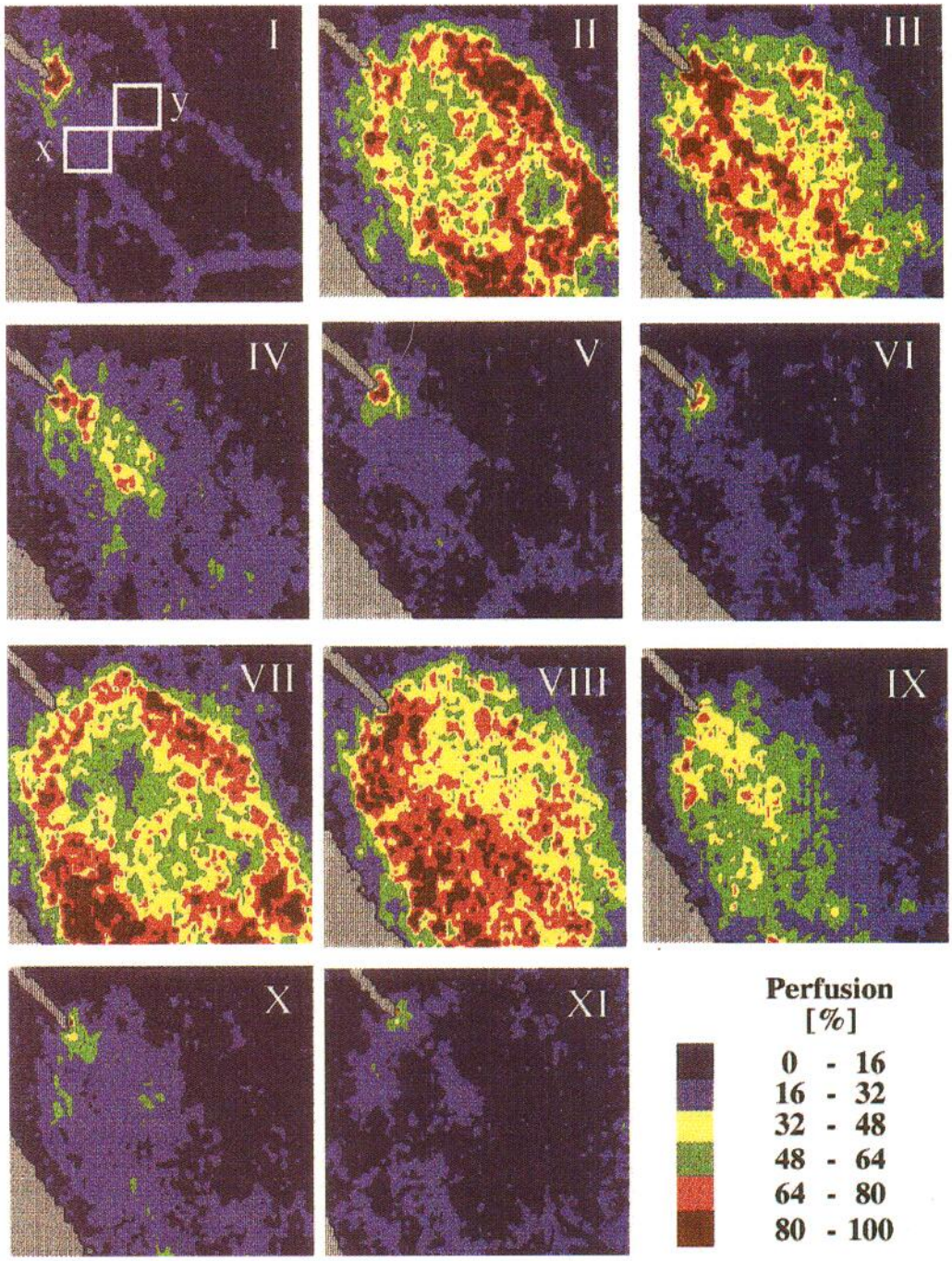

Fig. 1. LDPI maps of local skin blood flow in a cold urticaria patient (subject B) following two consecutive 15-min ice provocations, 60 min apart. The LDPI images were colour-coded within the same range. The white squares (x and y) in *I* show the areas used for the numerical calculations of skin blood perfusion data in Fig. 2a. *I.* 45 min after probe insertion and 6 min prior to first ice challenge. *II.* Immediately after end of first ice challenge. *III.* 10 minutes after first ice challenge. *IV.* 20 min after first ice challenge. *V.* 30 min after first ice challenge. *VI.* 40 min after first ice challenge and 5 min prior to second ice challenge. *VII.* 10 min after end of second ice challenge. *VIII.* 20 min after second ice challenge. *IX.* 30 min after second ice challenge. *X.* 40 min after second ice challenge. *XI.* 50 min after second ice challenge.

perfusion in two different 8 × 8 measurement point areas (outlined in Fig. 1) within the cold urticaria reaction. Fig. 2b shows the corresponding histamine values. Although the LDPI values had returned to near normal between challenges, the histamine "baseline" value prior to rechallenge was slightly higher than the first baseline value.

Subject C demonstrated a macroscopic reaction and LDPI results which were as pronounced as for the first 2 patients but which were not accompanied by the steep, high histamine peak seen in the first 2 patients.

Ice challenge in the 2 healthy subjects (D and E) without cold urticaria resulted in a reactive hyperemia in both cases. The duration of the hyperemia was shorter than for subjects A-C and weals were not seen. The histamine values (Table I) rose earlier, were less pronounced and were of shorter duration than for the cold urticaria subjects.

DISCUSSION

Histamine is well established as an inflammatory mediator of importance in cold urticaria. The exact role of histamine in the pathogenesis of vascular changes in cold urticaria is, however, not fully understood, and there are data indicating that other vasoactive mediators are involved (11). In this study, ice provo-

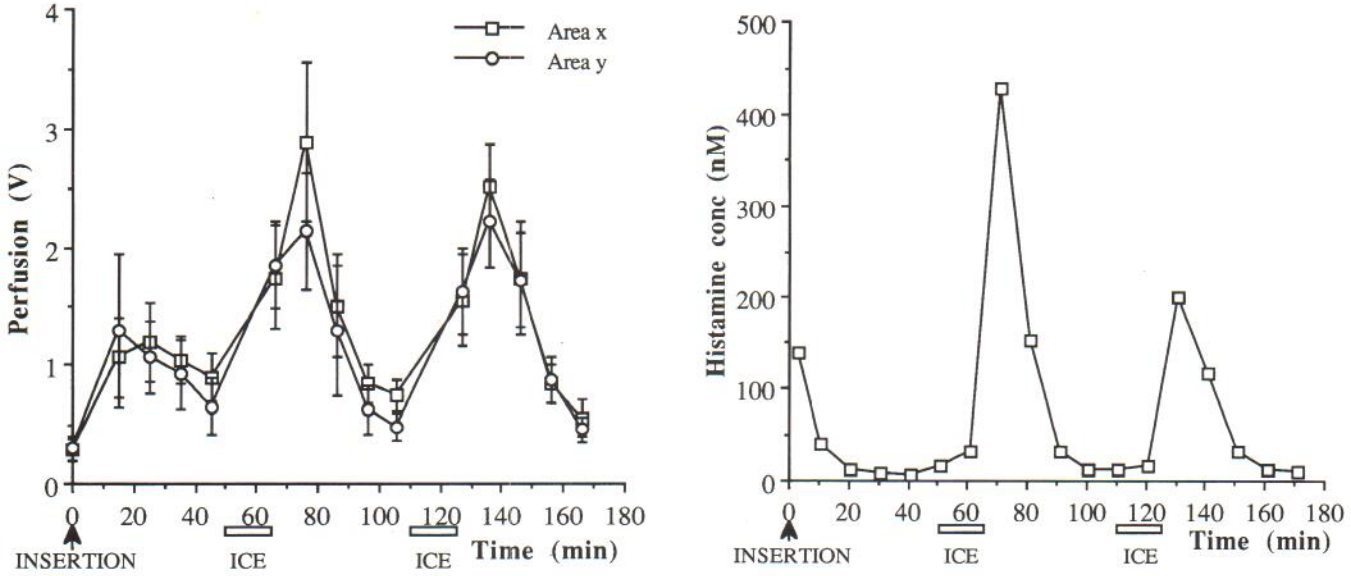

Fig. 2. Skin blood perfusion measured by LDPI (*a*) and skin histamine response measured by cutaneous microdialysis (*b*) after two separate 15-min ice challenges in a cold urticaria patient (Subject B). Fig. 2a shows the blood perfusion (mean ± SD) in two different 8 × 8 LDPI measurement site areas (*x* and *y*, shown in Fig. 1) within the urticarial reaction, followed at 10-min intervals. Fig. 2b shows histamine values obtained by cutaneous microdialysis technique at the same intervals.

cation gave increased histamine levels which correlated with the macroscopic response and the LDPI findings in two cold urticaria subjects. In the remaining subject (C), the increase in histamine levels was not commensurate with the LDPI values. There are several possible explanations for this discrepancy. The initial histamine levels, immediately after probe insertion, in subject C were very high, which may have depleted histamine stores. The skin reaction as assessed by naked eye and LDPI was, however, still prominent and in keeping with the patient's usual reaction pattern. Poor probe performance is another possible explanation, but this is unlikely since the probe detected the initial high histamine levels (and the post-experimental in vitro glucose recovery was normal). The possibility that the individual 26–36-min histamine value for patient C was incorrect needs also to be considered. The results suggest, however, that in this cold urticaria patient, histamine may not have been the critical inflammatory mediator or that the actual level of histamine was not as important as the susceptibility of the tissue to the histamine. In the cold provocation of normal subjects, histamine levels as high as those seen in subject C were noted, but no urtica. This may also support the notion that differences in tissue (end organ) susceptibility are more important than absolute histamine secretory levels.

The pre-challenge histamine levels in the present study were somewhat higher than the "baseline" levels in our previous study (6). The main explanation for this is the flow rate of 3 µl/min in the present study, which gives an almost doubled in vitro recovery of histamine in comparison to 5 µl/min, used in previous experiments. Differences in recovery characteristics of the present polyamide membrane and the previously used polycarbonate membrane and inter-individual differences may also have contributed. The experimental design and the interpretation of results do not, however, rely on absolute values, but rather changes within the individual experiment.

The area of cold-provoked skin was, in the present experiments, small in comparison to earlier human experimentation in cold urticaria, where the whole hand and forearm were often immersed in cold water. This is an important ethical and practical advantage in the design of experiments. Whilst our aim is to insert probes as superficially as possible, the actual placing in the skin may, for the estimation of histamine, not be too critical. In a regression analysis of histamine levels during the equilibration period in 24 uniform experiments (20), we found no correlation between probe depth and histamine levels obtained. Probes located in the high subcutis have shown similar histamine values to those placed in the dermis.

One of the main problems with microdialysis is the use of obtained concentrations in the dialysates for the estimation of absolute tissue concentrations. Several factors must be corrected for. The in vitro relative recovery of histamine has previously been shown to be approximately 30% at a flow rate of 3 µl/min (6). Based on this information, the real tissue concentrations of histamine could be extrapolated to be approximately 3-fold higher than those measured in the dialysate. This is, however, not fully adequate, since the recovery is not the same in vitro as in vivo. One way of calibrating the probes in vivo is to perfuse the microdialysis probe with different concentrations of the substance of interest, and record the net increase of the substance in the dialysate (21). By use of regression analysis, the concentration of the substance in the probe not resulting in any net influx of the substance in the perfusate can be calculated, reflecting the true tissue concentration. This method, however, is only valid under steady state conditions, not easy to obtain when monitoring biologically highly active substances, such as histamine. Another option is to use an "internal standard" for the correction of the in vivo recovery (22). The internal standard, however, must be as similar as possible in the physico-chemical sense to the substance of interest, which may be a difficult task as regards histamine. The present "single case study" design is not dependent on absolute values for histamine. The chronology

of the reaction can be adequately observed and interpreted using the obtained dialysate values.

The present paper shows how cutaneous microdialysis can be used in vivo to follow the biochemical chronology of provoked and reprovoked reactions in human skin in a setting which is acceptable to the patient and not too far from the clinical consulting room. The method can be used in parallel with naked eye and skin physiological methods to allow multiple parameter assessment of reactions over time. Future experiments will allow the study of inflammatory mediators other than histamine in cold urticaria and indeed many cutaneous inflammatory conditions.

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