

The use of cutaneous microdialysis to measure substance P-induced histamine release in intact human skin in vivo

Lars J. Petersen, MD,^a Lars K. Poulsen, PhD,^b Jørgen Søndergaard, PhD,^a and Per S. Skov, PhD^c Copenhagen, Denmark

Background: The purpose of this study was to introduce a microdialysis technique, which makes it possible to measure the release of small inflammatory mediators into the extracellular water space in intact human skin in vivo. Using this technique, we have studied the histamine releasing properties of substance P, a putative skin mast cell releasing agent.

Methods: Small hollow fibers were inserted into the upper dermis of nine healthy subjects. Each fiber was perfused with Kreb's Ringer bicarbonate buffer at a rate of 3.0 $\mu\text{L}/\text{min}$. After establishment of a baseline, each fiber was challenged intracutaneously with substance P (0 to 4 $\mu\text{mol}/\text{L}$). Samples were collected at 2-minute intervals for 18 minutes. Histamine was measured by a fluorometric method, which correlated with an enzyme immunoassay ($r = 0.96$).

Results: Baseline dialysate histamine concentration was 1.7 ± 0.3 ng/mL. Peak histamine release after injection of vehicle, 0.5, 1, 2, and 4 $\mu\text{mol}/\text{L}$ substance P was 0.0, 1.0, 6.0, 44.5, and 88.5 ng/mL, respectively ($p = 0.00002$). Statistically significant histamine release was demonstrated with 1.0 $\mu\text{mol}/\text{L}$ substance P and greater. Most peak values were seen 2 to 4 minutes after injection. The histamine elimination showed a monoexponential decline; dialysate histamine half-life was 3.81 ± 0.28 minutes.

Conclusions: This study showed that substance P releases histamine in a dose-dependent manner from intact human skin in normal subjects. We suggest that microdialysis may be a promising technique for the evaluation of mediator levels in intact human skin after intradermal injection of an inflammatory or allergenic stimulus. (*J ALLERGY CLIN IMMUNOL* 1994;94:773-83.)

Key words: Substance P, histamine release, microdialysis, skin, mast cell

The measurement of inflammatory mediators released into the extracellular environment after intradermal provocation has been limited by lack of appropriate techniques. Up to the present, investigators have mainly been restricted to mea-

Abbreviations used

CV:	Coefficient of variation (SD/mean $\times$ 100)
EIA:	Enzyme immunoassay
FHR:	Fluorometric glass fiber-based histamine (method)
Pipes:	Piperazine-N,N'-bis[2-ethanesulfonic acid]

From ^athe Department of Dermatology, Bispebjerg Hospital, University of Copenhagen; ^bLaboratory of Medical Allergology, National University Hospital; and ^cthe Reference Laboratory, Copenhagen.

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Reprint requests: Lars Jelstrup Petersen, MD, Department of Dermatology, Bispebjerg Hospital, Bispebjerg Bakke 23, DK-2400 Copenhagen, NV, Denmark.

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surement of mediators in the venous effluent from the challenged area^{1, 2} and in the exudate partitioning into a skin chamber or suction blister.³⁻⁵ None of these techniques can provide a quantitative and kinetic measurement of mediator release directly into the target tissue within intact skin. Without epidermis, it is not possible to monitor wheal, flare, and itch reactions; and removal of the epidermis may be disputable in some situations.⁶

Microdialysis, a technique originally developed for the *in vivo* monitoring of biochemical reactions in the extracellular compartment of the brains of animals,^{7, 8} has been adapted for the use in peripheral tissues, including subcutaneous adipose,⁹ eye,¹⁰ liver,¹¹ and muscle¹² tissue. More recently, the use of microdialysis technique to measure low molecular weight substances, including histamine, in the extracellular water compartment of the dermis has been described.¹³⁻¹⁶

In this study we describe the use of small microdialysis fibers introduced into the upper layers of the dermis, the site where the majority of cutaneous mast cells are found,¹⁷ to monitor the time course of histamine release and elimination after intradermal provocation. As a stimulus, we have used the putative histamine-releasing neuropeptide substance P. This neuropeptide, which is found in cutaneous sensory neurons,¹⁸ on intradermal injection elicits a wheal and flare response¹⁹⁻²¹ induces histamine release in venous effluent from the challenged area,²² and induces histamine release from both skin fragments²³ and dispersed skin mast cells *in vitro*.²⁴⁻²⁶

METHODS

Subjects

The study included nine nonmedicated, healthy subjects (five women and four men). Mean age was 33 years (SD, 16 years; range, 20 to 64 years). All subjects gave informed consent, and the study was approved by the local ethics committee.

Microdialysis technique and histamine measurement

The microdialysis probes, which are similar to probes previously described,^{9, 15} were constructed with a single dialysis fiber (Gambro model GFE 18; Gambro Dialysaten AG, Hechingen, Germany) glued to gas-tight nylon tubing and sterilized. The fibers (outer diameter = 216 μm , wall thickness = 8 μm , molecular weight cutoff = 2000 d) have a length of 20 mm available for diffusion; the outlet of the nylon tubing had a standardized length of 30 mm. The volume of the outlet tube is approximately 1.2 μL .

In all experiments the inlet of each dialysis probe was connected to separate syringes in a micro-injection pump (CMA/100; CMA/Microdialysis, Stockholm, Sweden). Each dialysis fiber was perfused at a rate of 3.0 $\mu\text{L}/\text{min}$ with an isotonic, sterile Krebs' Ringer solution, which consisted of 1.0 mmol/L MgCl_2 , 1.2 mmol/L CaCl_2 , 0.4 mmol/L NaH_2PO_4 , 25 mmol/L NaHCO_3 , 85 mmol/L NaCl , and 3.0 mmol/L KCl , adjusted to pH 7.4 with 0.1 mmol/L HCl .

Dialysates were collected directly from the efferent tube into microtiter wells coated with a glass fiber

matrix used for determination of histamine. Dialysate was collected at 2-minute intervals (6 μL fractions).

Dialysate histamine concentrations were determined with a fluorometric glass fiber-based histamine (FHR) method as previously described.²⁷ In previous studies the FHR method has been shown to measure histamine with high selectivity and affinity; other possible interfering substances, including methylhistamine, are not measured. The assay determines histamine concentrations without decimals from 0 ng/ml; the sensitivity for measuring histamine concentrations in skin dialysates is 6 ng/ml. The fluorescence output is linear, with histamine concentrations up to 1000 ng/ml (Skov, PS. Unpublished observations). Further studies on the use of the FHR histamine assay for measuring histamine in dialysate are described below.

The microtiter plates were kept at room temperature in the dark until analysis was performed on the following day. Because the dialysates in the microtiter wells evaporate before analysis, the samples were resuspended in 6 μL Pipes buffer before analysis. The Pipes buffer was a solution of pH 7.4 and consisted of 10 mmol/L piperazine- $\text{N,N}'$ -bis[2-ethanesulfonic acid], 140 mmol/L sodium acetate, 5 mmol/L potassium acetate, 0.6 mmol/L CaCl_2 , and 1.1 mmol/L MgCl_2 .

Control experiments showed that no degradation of histamine occurs between sampling and analysis: histamine, in various concentrations, was added to either pipes buffer or skin dialysate. Samples were evaporated and resuspended as described and compared with native samples. Identical results were obtained.

Substance P in concentrations greater than 100-fold of those used in this study did not interfere with the FHR histamine assay alone or in combination with histamine.

Validation of the technique *in vitro*

At a perfusion rate of 3.0 $\mu\text{L}/\text{min}$, as used throughout this study, the relative recovery of histamine (dialysate/medium exchange ratio) at 37° C was about 80%. Because flux across the dialysis membrane occurs according to the principle of diffusion, the recovery of histamine is independent of the histamine concentration in the surrounding medium. Recovery of histamine in various solutions containing 200, 100, 50, and 25 ng/ml histamine was $80.4\% \pm 1.9\%$, $84.2\% \pm 2.9\%$, $82.2\% \pm 2.7\%$, and $80.0\% \pm 4.9\%$, respectively ($n = 5$). The recovery of histamine seemed to be strongly dependent on the temperature because recovery was about 35% at room temperature.

The ability of the dialysis probe to detect rapid changes in histamine concentrations in the surrounding medium was evaluated *in vitro*. Dialysis fibers were placed in separate vials containing a known histamine concentration. Three consecutive 6 μL dialysates were collected from each fiber (0 to 6 minutes). Then the outer medium was immediately changed from a low to a high histamine concentration in the medium (10 to 25, 10 to 50, 10 to 75, and 10 to 100 ng/ml histamine,

respectively), and seven consecutive dialysates were collected (6 to 20 minutes). Experiments with high to low histamine concentrations were also performed (25 to 10, 50 to 10, 75 to 10, and 100 to 10 ng/ml histamine, respectively). In each experiment the mean value of the first three consecutive dialysates (0 to 6 minutes) was used as baseline I. After a sudden change, seven consecutive dialysates were collected; the last three dialysates (14 to 20 minutes) were used as baseline II. The ability of the fibers to detect the relative difference between baseline I and baseline II was calculated. The experiments were performed in triplicate.

In vivo experiments

Three 23-gauge (0.6 mm) cannulas were introduced into the upper dermis in the radial/ventral surface of each subject's forearm at least 5 cm below the cubital region. Dialysis fibers were introduced into the skin through the guide cannulas, which were then removed. The distance between each cannula was 3 cm.

To reduce the discomfort during implantation, an anesthetic cream containing prilocaine and lidocaine (EMLA; Astra, Södertälje, Sweden) was applied under an occlusive dressing (Tegaderm; 3M Health Care Ltd., Loughborough, Leicestershire, U.K.) for 45 minutes before the implantation. EMLA cream does not interfere with mast cell degranulation.²⁸ The cream was wiped off before implantation. Because the local anesthetic cream may interfere with sensory nerve conduction or the cutaneous vasculature, an interval of 2 hours was interposed between implantation and the measurements. Two hours after implantation, skin blood flow had returned to normal,¹⁵ and the flare response was similar to that seen in untreated skin, indicating a fully functional sensory neuron network.

Preliminary ultrasound scanning of the fibers confirmed the intradermal localization.¹⁵ Later studies on 30 fibers have shown that the dialysis probe is placed intradermally with a constant distance (0.6 mm) from the skin surface (Petersen et al. Unpublished observations).

Before the injection of substance P or vehicle, two baseline dialysates of 6 μ l were collected over 4 minutes. Fifty-microliter replicates of substance P, dissolved in water and diluted with isotonic Krebs' Ringer bicarbonate buffer containing 0.02% human serum albumin to concentrations of 0.5, 1, 2, and 4 mmol/L or vehicle, were injected intradermally with a 27-gauge needle attached to a 0.5 ml syringe (Insupak; ASIK A/S, Rødby, Denmark). After injection, dialysate was collected at 2-minute intervals (6 μ l) for 18 minutes. Each fiber was challenged separately, with an interval of approximately 30 minutes between each challenge. The injection was placed in the midpoint of the 20 mm long dialysis fiber, 1 mm proximal to the center of each probe, to avoid puncture of the fiber. The injection point was marked with an ink pen during the implantation procedure to ensure identical distance from the injection point to the fiber in each experiment. In all

cases the injection-induced wheal covered the center of the fiber. To rule out possible systematic errors in histamine release caused by regional differences in skin reactivity to substance P, intradermal injections were performed according to a randomized Latin square design. The reproducibility of the microdialysis technique to detect histamine release by substance P was tested in all subjects by injection of a single substance P concentration (range, 0 to 4 μ mol/L) in duplicate. Mean values of duplicate measurements were used in the analyses.

Wheal sizes were determined 15 minutes after injection of substance P or vehicle.^{20, 21} Perpendicular diameters were measured in all subjects, and area was determined by planimetry in the last six subjects studied. Because the results obtained by these two methods were strongly correlated ($r = 0.97$), the use of perpendicular diameters for estimation of wheal areas introduced no systematic errors. Consequently, this technique was used for all analyses. Because of the short distance between the fibers, flare reactions were not measured. However, no wheal reaction was seen without a concomitant flare reaction.

Verification of histamine determinations

For determination of histamine levels in nasal lavage fluids, the FHR assay has previously been demonstrated to correlate closely with radioimmunoassays of histamine.²⁹ To validate the specificity of the FHR assay to measure histamine concentrations in dialysate, we performed parallel determinations with this method and an immunoassay technique for histamine.

This study included 13 healthy subjects (nine women and four men) with a mean age of 50 years (SD, 16 years; range, 27 to 73 years). Six fibers (range, 3 to 9) were inserted in forearm skin and perfused as described above. Two hours after insertion, each fiber was challenged with 50 μ l aliquots of substance P (4.0 μ mol/L). Each separate fiber was challenged for one to six times at 6-minute intervals. The distance between the injections on each fiber was 3 mm along the 20 mm fiber. Thus dialysate from a single subject contained the cumulative histamine release from one to six challenges. Dialysate from each fiber was collected in a 40-minute (120 μ l) fraction.

Dialysate from each fiber was then divided into two parts, 30 μ l to be assayed by an enzyme immunoassay (EIA) technique (Immunotech International, Marseilles, France) and 90 μ l for histamine determination by the FHR method. Samples were also measured after incubation with diamine oxidase (Behringwerke AG, Marburg, Germany) and after spiking with exogenous histamine.

The EIA histamine technique is based on the competition for a solid-phase-bound antibody between the histamine to be assayed and a histamine-enzyme conjugate used as a tracer. Both preparations were derivatized to increase the binding to the antibody. The assay has a sensitivity of 0.2 nmol/L (~ 0.022 ng/ml), and

TABLE I. The ability of microdialysis probes to detect rapid changes in the histamine concentration in the outer medium in vitro

	Histamine concentration (ng/ml) in the outer medium			
	10-25	10-100	25-10	100-10
Baseline I (samples 1-3)	0.00	0.00	1.00	1.00
Sample 4	0.72 ± 0.12	0.77 ± 0.04	0.30 ± 0.01	0.20 ± 0.01
Sample 5	1.19 ± 0.08	1.03 ± 0.03	0.01 ± 0.01	0.03 ± 0.00
Sample 6	1.08 ± 0.10	0.98 ± 0.01	0.15 ± 0.05	0.01 ± 0.01
Sample 7	1.06 ± 0.10	0.97 ± 0.05	0.00 ± 0.10	0.00 ± 0.03
Baseline II (samples 8 to 10)	1.00	1.00	0.00	0.00

Three fibers were placed in separate vials with known concentrations of histamine. The mean value of three 6 μ l (2-minute) fractions (samples 1 to 3) was used as baseline I. Then the histamine concentration in the outer medium was suddenly changed as indicated above. Seven consecutive dialysates were collected, and the last three were used as baseline II. The relative delay and carryover effect was calculated. Identical results were found with the intermediate histamine concentrations—10 to 50, 10 to 75, 50 to 10, and 75 to 10 ng/ml, respectively.

because the samples were diluted 10 times, the detection limit was 0.2 ng/ml.

Histamine was measured in the FHR assay as described above with 10 μ l samples in duplicate. Coefficient of variation (CV) on duplicate determinations was 18.7%.

After the histamine concentrations were measured by the two methods, the residual dialysate was combined into four pools with estimated histamine concentrations ranging from 10 to 100 ng/ml. The samples were analyzed before and after treatment with diamine oxidase (0.05 U/ml) incubated for 3 hours at 37° C. Pooled dialysate was compared with histamine solutions with concentrations similar to the pooled dialysate. Furthermore, dialysates were added to exogenous histamine 50 ng/ml, and recovery was calculated.

Statistics

Descriptive statistics included calculation of mean $\pm$ SEM, if not otherwise stated. CV was calculated (SD/mean $\times$ 100). Because the responses (peak histamine and cumulative histamine release) to substance P differed within subjects, nonparametric statistical analysis was used, (i.e., Friedman analysis of variance by ranks and Wilcoxon tests). Probability (*p*) values of less than 0.05 were considered significant.

RESULTS

The dialyzing properties of a microdialysis probe are expressed as its recovery of a particular substance. Because of several factors, including the surface area of the dialysis membrane, flow rate, molecular weight of the substance studied, and the temperature in the dialyzed medium, the dialysate represents only a fraction of the substance concentration in the outer medium. In vitro recovery of histamine at 37° C was about 80% under steady-state conditions in our setup.

The results of the in vitro dynamic studies are presented in Table I. These studies indicate that a change in the histamine concentration in the outer medium is not detected instantly in the dialysate but is delayed for up to 2 minutes. The delay and carryover effects were independent of the concentration. However, because the diffusion characteristics of histamine most likely differ in vitro and in vivo,⁸ the correction factor may not be true for in vivo data. Consequently, the in vivo data were not corrected for the delay and carryover effect found in the in vitro studies.

Baseline dialysate histamine concentration was 1.7 $\pm$ 0.3 ng/ml (SD = 2.2 ng/ml). After injection of 0 to 4 μ mol/L substance P, a rapid dose-dependent increase in histamine concentration was seen, followed by a fast decline. Analysis of the time of the histamine peak after injection of substance P showed that it occurred at 0 to 2 minutes in 8.5% of the cases, at 2 to 4 minutes in 83% of the cases, and at 4 to 6 minutes in 8.5% of the cases. An example of dialysate histamine kinetics after injection of different concentrations of substance P is presented in Fig. 1, and all the peak histamine values are presented in Fig. 2. From Fig. 2 it may be seen that there was a wide variability in the extent of histamine peak heights between individuals; peak releases in response to 4 μ mol/L substance P ranged from 34 to 232 ng/ml. Dialysate peak values and cumulative histamine release are presented in Table II. All values in Table II are histamine releases above baseline.

Statistical analyses were performed on both individual peak heights and the total histamine recovered over the 18-minute observation period.

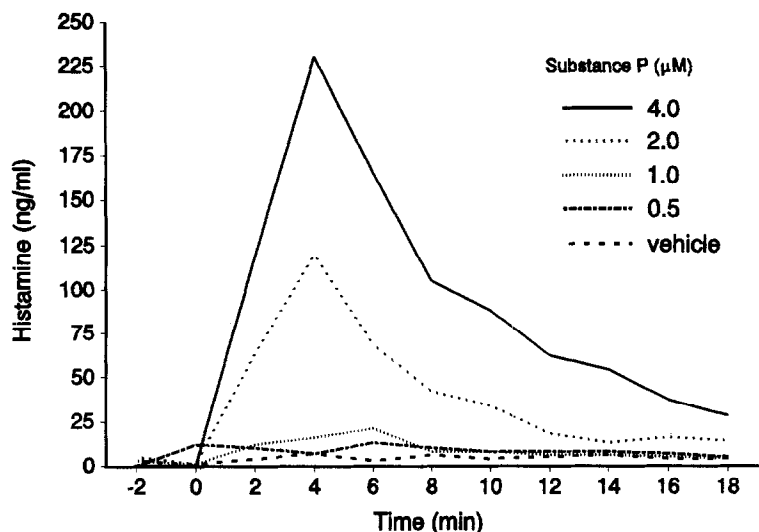


FIG. 1. An example of dialysate histamine kinetics after intradermal injection of substance P (50 μ l) in a single subject. Peak heights with 2 and 4 μ mol/L substance P showed a pronounced histamine release, whereas peak heights with vehicle and 0.5 and 1 μ mol/L substance P were only minor.

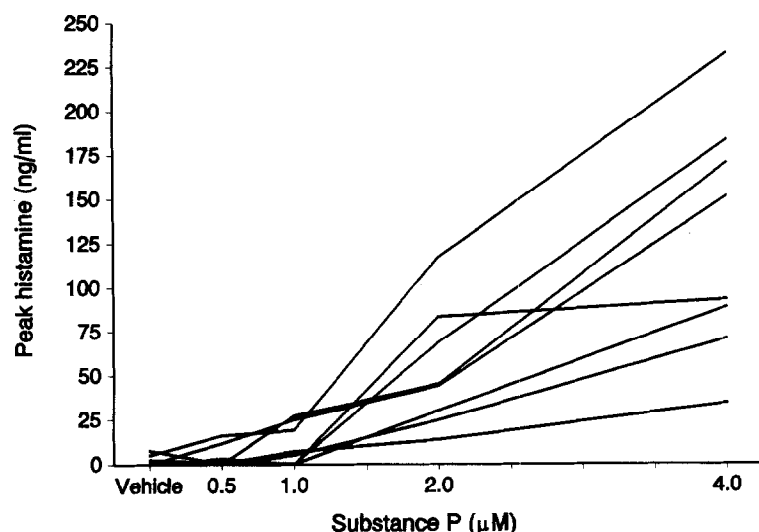


FIG. 2. Individual peak dialysate histamine values in eight subjects obtained with different substance P concentrations are shown. A dose-response relationship was demonstrated ($p = 0.00002$) in the group as a whole. Data from a 2 μ mol/L substance P experiment was lost in one subject; therefore, only data for eight subjects are presented.

Analysis of variance showed a statistically significant histamine release with both methods ($p = 0.00002$ and $p < 0.00001$, respectively). Analysis of single doses of substance P with the Wilcoxon test showed, with both methods, statistically significant histamine releases with 1, 2, and 4 μ mol/L substance P. No significant differences were seen between vehicle and 0.5 μ mol/L substance P.

The interindividual CV on peak histamine release is shown in Table II. CV decreased with increasing concentrations of the stimuli. Intraindividual variation in peak histamine release was studied with a single substance P concentration in duplicate in each subject. The intraindividual variation in peak histamine release obtained with substance P concentrations, which yielded statistically significant histamine release, was

TABLE II. Peak and cumulative dialysate histamine concentrations (median and total range) and wheal sizes after intradermal injections of different substance P concentrations in nine subjects

	Substance P				
	Vehicle	0.5 $\mu\text{mol/L}$	1 $\mu\text{mol/L}$	2 $\mu\text{mol/L}$	4 $\mu\text{mol/L}$
Peak histamine (ng/ml)	0.0 (0.0-5.0)	1.0 (0.0-12.5)	6.0 (0.0-27.0)	44.5 (13.5-117.0)	88.5 (34.0-232.0)
CV	146.5%	134.6%	108.5%	66.6%	55.3%
Cumulative histamine (ng/ml)	0.0 (0.0-30.0)	6.0 (0.0-97.0)	38.0 (0.0-123.0)	137.0 (72.0-368.0)	374.5 (140.0-949.0)
CV	155.4%	141.6%	99.1%	62.9%	55.9%
Wheal size (mm ²)	—	64.1 $\pm$ 10.6	134.9 $\pm$ 8.4	149.9 $\pm$ 8.8	187.3 $\pm$ 14.1

Data from a 2 $\mu\text{mol/L}$ substance P experiment was missing; therefore, data on this concentration of neuropeptide represents eight subjects.

44.2% $\pm$ 5.4% ($n = 2$), 19.7% $\pm$ 2.0% ($n = 2$), and 56.9% $\pm$ 9.8% ($n = 2$) for substance P 1.0, 2.0, and 4.0 $\mu\text{mol/L}$, respectively. For histamine release greater than 10 ng/ml, mean CV was 35.0%.

Wheal sizes are presented in Table II. No visible wheal reaction was seen 15 minutes after injection of vehicle in any subject. To examine the relationship between histamine and wheals, wheal size was plotted against logarithmically transformed peak dialysate histamine concentrations. There was a statistically significant but fairly modest correlation ($r = 0.67$; $p < 0.00001$). Fig. 3 also shows that in five cases there was a measurable wheal in the absence of demonstrable rise in histamine levels, and in six cases some histamine release was observed in the absence of demonstrable wheal. Most cases occurred with 0 to 0.5 $\mu\text{mol/L}$ substance P. For histamine values greater than the sensitivity of the FHR assay (6 ng/ml), the correlation was improved ($r = 0.78$, $p < 0.00001$).

The ability to assess the time course of histamine release and disappearance, as shown in Fig. 1, suggests that our microdialysis technique may also be used to monitor histamine elimination. We therefore calculated dialysate histamine half-life. Peak histamine was set to time 0. Then, only data sets that fulfilled the following criteria were taken into consideration: (1) at least 4 data points on the elimination curve above baseline + 2 SD of the fiber in question (21 of 54 injections) and (2) r value of the regression analysis of at least 0.80 (19 of 21 injections). The r value of 0.8 was chosen to ensure appropriate linearity; there were no

other reasons for excluding elimination curves. The 19 elimination curves fulfilling these criteria are shown in Fig. 4. The graphic presentation of the logarithmically transformed dialysate histamine concentrations versus time indicated that the elimination of histamine concentration in the dialysate followed a monoexponential course. Median number of data points was 6 (interquartile range = 5 to 8). Mean r value of the regression lines was 0.95 ± 0.01 . Mean dialysate histamine half-life was 3.81 ± 0.28 minutes. There was no correlation between peak dialysate histamine concentration and dialysate histamine half-life ($r = 0.02$). Intraindividual variation in dialysate histamine half-life in duplicate measurements was 26.7% ($n = 4$). Taking into account the carryover effect shown in the in vitro experiments, histamine half-life was slightly underestimated.

To ensure the validity of the histamine results, we investigated the specificity of the FHR for measuring histamine concentration in skin dialysate. The results of the specificity studies are presented in Fig. 5 and Table III. From a total of 79 dialysates, a comparison between the FHR and EIA histamine assay was possible in 71 cases; in eight cases the histamine concentration in the dialysate was too high to be analyzed with the EIA technique even after 10-fold dilutions were made. The results showed an excellent correlation between the two methods ($r = 0.96$, $p < 0.00001$).

The results obtained with the FHR method before and after diamine oxidase treatment and spiking with exogenous histamine are presented in Table III. Diamine oxidase treatment of pooled dialysate showed that most of the histamine was

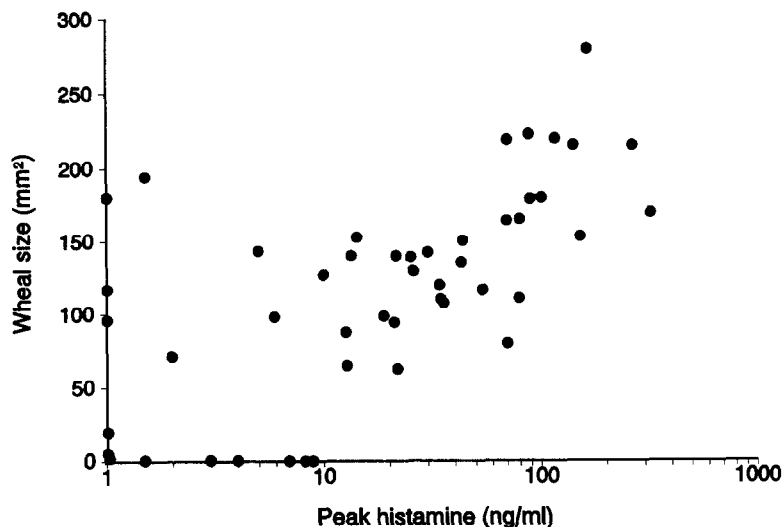


FIG. 3. Correlation of wheal size with logarithmically transformed histamine peak levels. A statistically significant correlation was found ($r = 0.67$, $p < 0.00001$). For histamine values greater than the sensitivity of the FHR assay (6 ng/ml), the correlation was improved ($r = 0.78$, $p < 0.00001$).

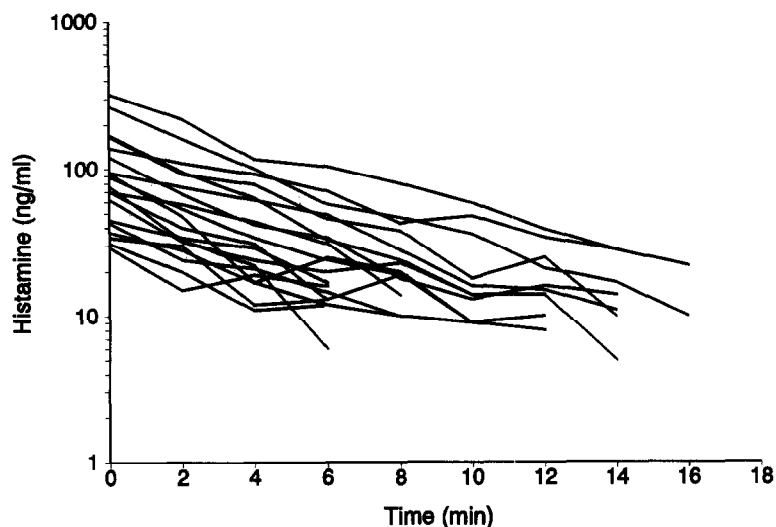


FIG. 4. Dialysate histamine elimination curves for 19 dialysate histamine elimination curves fulfilling the criteria mentioned in the Results section. Dialysate histamine half-life was 3.81 ± 0.28 minutes. Mean r value of the regression lines was 0.95 ± 0.01 .

eliminated after 3 hours of incubation. However, the residual histamine concentration ranged from 3.5 to 10.5 ng/ml, depending on the histamine concentration in the native sample. Because the period of 3 hours may be too short to eliminate the total histamine content in the samples, comparative studies with histamine solutions were performed. The residual histamine in these samples after 3 hours of incubation with diamine oxidase ranged from 1.0 to 6.3 ng/ml. We there-

fore consider that the predominant fluorescence seen with the FHR method originates from histamine.

The cosmetic consequences after insertion of microdialysis probes in forearm skin are minimal and completely reversible. In some cases there may be minor hemorrhage at the site of insertion of the guide cannula in the skin. In all cases in this study hemorrhage had completely vanished within 1 week.

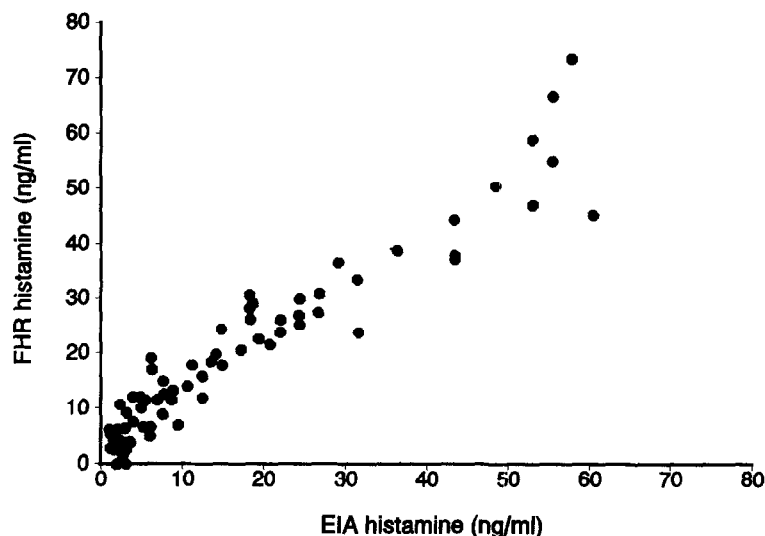


FIG. 5. Correlation between histamine determinations obtained with the EIA method and the FHR technique is shown. The r value of the correlation was 0.96. $p < 0.00001$.

TABLE III. Histamine determinations on pooled dialysates and matched histamine solutions before and after treatment with diamine oxidase (mean $\pm$ SD of triplicate measurements)

	Native sample	Samples after incubation with diamine oxidase	Recovery of spiked histamine (%)
Dialysate pool			
No. 1	9.0 $\pm$ 1.2	3.5 $\pm$ 0.6	95.3
No. 2	22.0 $\pm$ 2.4	7.5 $\pm$ 1.8	106.3
No. 3	48.5 $\pm$ 1.8	6.5 $\pm$ 1.8	116.8
No. 4	101.5 $\pm$ 6.6	10.5 $\pm$ 1.8	96.8
Histamine solution			
No. 1	10.7 $\pm$ 1.3	6.3 $\pm$ 1.0	ND
No. 2	21.3 $\pm$ 5.0	1.0 $\pm$ 0.0	ND
No. 3	51.3 $\pm$ 2.8	3.0 $\pm$ 3.0	ND
No. 4	104.0 $\pm$ 4.5	5.7 $\pm$ 2.6	ND

Recovery of exogenous histamine with pooled dialysates is also shown.
ND, Not done.

DISCUSSION

We have used substance P to evaluate the use of the microdialysis technique to monitor histamine release from skin mast cells after intradermal provocation. Our results show a median peak histamine release of 88.5 ng/ml 2 to 4 minutes after injection of 4 μ mol/L substance P. Both histamine peak levels and the total histamine release over the 18-minute observation period showed a statistically significant dose-response relationship and correlated with wheal size. Furthermore, analysis of the rate of decrease in histamine levels after the peak allows an estimation of the half-life of histamine in the dermis. Substance P did not release histamine from cor-

responding basophils in the same individuals (data not shown).

Peak histamine levels measured by microdialysis ranged between 34 and 232 ng/ml. However, this is likely to be an underestimation of the levels in vivo because the recovery of in vitro experiments was about 80%. This recovery factor may not be extrapolated to the in vivo situation because diffusion characteristics in vivo differ from those in vitro.⁸ For example, in vivo recovery of glucose in human skin is about 20% compared with 60% in vitro¹⁵ when fibers identical to those in this study are used. The in vitro experiments indicated a small delay in the measurement of changes in histamine concentrations, such that

the first aliquot collected 0 to 2 minutes after a change was approximately 25% different from the following samples. We presume that most of the delay can be explained by the 1.2 μ l of dead space in the efferent tube. Because this was independent of concentration, it may be considered a consistent effect. Because of the potential inaccuracy of artificially adjusting our results for recovery and delay, we present our raw data knowing that they closely reflect the changes occurring *in vivo*.

Our results showed a dose-dependent release of histamine after intradermal injection of substance P; statistically significant median peak levels of 6.0, 44.5, and 88.5 ng/ml were observed with 1, 2, and 4 μ mol/L neuropeptide, respectively. Any possible changes in histamine levels occurring after injection of 0.5 μ mol/L substance P were below the detection limit of the FHR histamine assay. The intraindividual and interindividual variations in peak and cumulative histamine releases showed modest to pronounced variations. The mean intraindividual variation in peak histamine concentration was 48.8%, whereas the variation in wheal size was 13.2%. CV in histamine determinations with 10 μ l replicates of dialysate was 18.7%. Thus a great portion of the variation seems to arise from the sampling technique itself. Variations in histamine release caused by the small sampling volume may be pronounced, but *in vitro* studies showed that the variation, at least expressed as CV, declined with increasing histamine concentrations. Meanwhile, other sources of errors must be considered. Because the injections followed a randomized Latin square schedule, regional variations in histamine release may cause some variation. The variation may also arise from irregularity in the intradermal injections. In some cases a lateral extension of the wheal reaction suggested that the injected volume spread along water space surrounding the dialysis probe. Therefore more mast cells close to the dialysis membrane are stimulated compared with localized injections. A reduction in injection volume may minimize this problem.

The histamine release findings confirm the *in vitro* observations in skin fragments and human dispersed skin mast cells, which show substance P to be a directly active histamine releaser.²³⁻²⁵ Two comparisons may be made with dispersed mast cell experiments. First, it appears that substance P is a more potent histamine releaser *in vivo* where there is a comparatively steep dose-response curve between 0.5 and 4 μ mol/L; *in vitro* a shallow

curve from 0.3 to 30 μ mol/L is seen, with histamine release of 12% to 16% at the highest concentration.^{24, 25} However, this statement may be reconsidered because the total histamine content in the skin is unknown, and the substance P concentration was not increased to elaborate maximal histamine release in this model. Second are the kinetics of histamine release. The *in vitro* studies indicate that substance P causes histamine release from skin mast cells within 10 to 15 seconds,²⁴ whereas while our *in vivo* studies show that in most experiments maximal release does not occur until 2 to 4 minutes after injection. This difference cannot be explained by the diffusion rate of histamine because control experiments have shown that injected histamine is maximal in the first (0 to 2 minutes) sample of dialysate. A more likely explanation is that the delay *in vivo* reflects the relatively slow diffusion of substance P from its site of injection, thus causing a sequential activation of mast cells from the site of injection to the outer parts of the wheal.

Our results with the microdialysis technique indicated that histamine was rapidly eliminated in the skin. The elimination curve seemed to be linear in semilogarithmic plots, but the data do not allow us to go into detail on elimination by different routes. Further studies are needed to explore this issue. Meanwhile, the results suggest a role of skin microdialysis for monitoring skin histamine metabolism, which may be pathologic in some skin disorders (e.g., urticaria).^{30, 31}

The complexity of the factors that influence recovery in microdialysis experiments have been studied in detail in animal brains.^{7, 8} However, in a previous study we found that recovery may be affected by changes in the composition of the extracellular water space in human skin. After the entire fiber was covered with histamine-induced wheal reactions, recovery of glucose was increased by about 50%.¹⁵ We attributed this increase in recovery to leaking of plasma into the extracellular water space, thereby increasing the aqueous surface area of the dialysis membrane probe for diffusion. Similar phenomena may be active in this study. It is not clear whether increased recovery is directly correlated with wheal size or wheal thickness. Preliminary data from an ongoing study suggest that the size of the wheal is of minor importance. Because the difference in wheal size between the different substance P concentrations used in this study was moderate, we consider the conclusions on the histamine release data to be valid.

We realize that our knowledge of the complexity and magnitude of factors influencing the flux of substances from the site of release to the sampling position in skin microdialysis experiments is inadequately described. However, such considerations are not pertinent to microdialysis studies only. Similar issues may be seen with nasal, conjunctival, and bronchial lavage techniques and with the skin window technique. The magnitude of such events on recovery of mediators with these techniques is sketchily described.

The comparison between the FHR method and the EIA technique for measuring histamine concentrations in skin dialysate showed an excellent correlation between the two methods. The sensitivity of the FHR assay of 6 ng/ml histamine in this application and the results of the diamine oxidase experiments suggest to us that the EIA technique is preferable for measurement of baseline histamine concentrations in normal subjects. However, in release studies we consider the FHR technique to be superior to the EIA method for several reasons. The dialysate need not be diluted, because the FHR method measures histamine concentrations up to about 1000 ng/ml. We can sample directly into the microtiter wells used for the histamine analysis, and the cost of the histamine analysis is much less with the FHR method compared with the EIA technique.²⁹

We consider the microdialysis technique of potential interest in clinical and experimental medicine because it offers the opportunity to monitor biochemical events in intact human skin together with clinical observations (i.e. wheal, flare, and itch responses). The method allows measurement of baseline mediator values in different skin diseases, releasability by various stimuli, and effects of immunopharmacologic interventions.

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