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Neuropeptide- and Capsaicin-induced Histamine Release in Skin Monitored with the Microdialysis Technique

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Mast cells are thought to be involved in neurogenic inflammation in skin, and numerous neuropeptides are known to degranulate mast cells. We monitored histamine release in skin *in situ* with the microdialysis method after skin challenge with neuropeptide injections (10 μM substance P, vasoactive intestinal peptide and calcitonin gene-related peptide), capsaicin injection (30 μM) and 0.1% capsaicin cream with a moist compress. Fractions were collected for 15 min each at 3.0 μl/min. One hour after insertion of the probe, the baseline histamine level was 4.5±4.5 nM (mean±SD, $n=20$). Substance P (250 pmol) induced histamine release peak (66.1±52.5 nM, $n=8$) in the 0–15 or 15–30 min fraction. Thereafter, the histamine concentration declined steadily and rapidly and no second rise was observed. A single substance P injection was sufficient to induce major histamine release in three out of four experiments; and the release kinetics of the second injection (1 h later) mimicked that of the first injection. Vasoactive intestinal peptide (100 and 250 pmol) induced a rapid release of histamine in 4 subjects comparable to substance P, whereas calcitonin gene-related peptide (250 pmol) did not release detectable amounts of histamine in 2 subjects tested. Capsaicin induced a low and rather non-significant release of histamine in 4 out of 5 patients who received capsaicin injection and in 2 out of 5 who were treated with capsaicin cream.

The present study shows that neuropeptides substance P and vasoactive intestinal peptide, but not calcitonin gene-related peptide, can induce activation of mast cells and release of histamine into the extracellular space. The low release of histamine by capsaicin suggests low levels of neuropeptides or infrequent morphological contacts between mast cells and sensory nerves in normal human skin. The microdialysis method can be used for studying skin inflammatory reactions involving mast cells. *Key word: mast cells.*

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Microdialysis is a unique sampling technique that enables the measurement of small molecules in the extracellular space. It has been applied to some preliminary studies to monitor allergen- and substance P (SP)-induced release of histamine in skin (1–3). Histamine is the principal mediator of Type I allergic reaction. Upon activation of the mast cells, histamine is released together with numerous other mediators (4), resulting in intense weal and flare reactions of the skin.

Unmyelinated sensory nerve fibers richly innervate the skin. These sensory fibers contain neuropeptides such as SP, vasoactive intestinal peptide (VIP), calcitonin gene-related peptide (CGRP) and neuropeptide Y (5). Mast cells are found to be in direct physical contact with sensory nerves containing neuropeptides (6, 7). Furthermore, increasing evidence suggests that the secretory function of mast cells is regulated by the nervous system. SP has been demonstrated to release histamine from rat peritoneal mast cells (8) and from human skin mast cells *in vitro* (9). VIP has been demonstrated to release histamine from human skin mast cells as well; and as in the case of SP, the histamine release is rapid, reaching completion in 10–20 s. Compared to SP and VIP, the ability of CGRP to release histamine from human skin mast cells *in vitro* is weak (10).

Intradermal injection of SP produces a dose-related flare and weal response and itching. The flare induced by SP is inhibited by compound 48/80, which depletes histamine from mast cells or H_1-histamine antagonists (11), but the weal response is only partly inhibited (12). It appears, therefore, that the weal is partly a direct effect of the peptide, but the flare is likely to be mediated indirectly through histamine release from mast cells. These observations indicate that the dermal responses induced by SP are at least partly mediated by histamine. The maximum flare response is obtained within the first 3 min of injection, whereas the weal response is at its maximum about 12 min after injection (12). Like SP, somatostatin and VIP also produce dose-related weal and flare reactions in human skin, and these three neuropeptides are about equally potent in producing their characteristic responses (13). CGRP can also produce weal and flare reactions in human skin, although these reactions are much weaker than those produced by SP (14). Intradermal injection of CGRP also produces a third type of response in the form of a slowly developing, long-lasting and intense erythema at the site of injection. The erythema develops at much lower doses of CGRP than is needed for inducing flare and weal (15). This vasodilatation does not involve the release of histamine in skin (14, 16).

Capsaicin can excite C-fibers and release neuropeptides such as SP, VIP, neurokinin A and CGRP (17). When injected intradermally, capsaicin evokes flare and pain. This flare is reduced by local anaesthetic but not by compound 48/80 or antihistamine (18). Thus, it appears that histamine is not the final mediator of vasodilatation in capsaicin-induced flare.

In the present study, histamine release in human skin caused by SP, VIP, CGRP and capsaicin was studied further. The microdialysis technique was used to measure histamine levels in skin before and after provocation of skin with neuropeptide and capsaicin injections, and capsaicin cream.

MATERIAL AND METHODS

Patients

Ten healthy volunteers of both sexes, aged 21–67 years (mean 38.2 years), and 10 patients at the Department of Dermatology (Kuopio University Hospital), aged 40–70 years (mean 56.6 years) participated in the study. These patients suffered from localized skin diseases, e.g.

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leg ulcers. All experiments were performed on normal-appearing skin. None of the patients were administered any oral or topical anti-allergic, corticosteroidal or non-steroidal anti-inflammatory medication for at least 1 week prior to the study. Some subjects participated twice in the study. All subjects gave their consent after receiving information about the study protocol. The methods used in this study were approved by the Ethics Committee of Kuopio University Hospital, Kuopio, Finland.

Microdialysis equipment

The basic microdialysis equipment, which included a pump (CMA/100), a probe (CMA/20) and a fraction collector (CMA/140), was purchased from CMA Microdialysis (Stockholm, Sweden).

The perfusate (Ringersteril®, Orion Corporation/Medipolar, Oulu, Finland) was conducted through the system by a microinjection pump at a perfusion rate of 3.0 µl/min. In the present study, a probe with a shaft length of 25 mm and a membrane length of 10 mm was selected. The diameter of the membraneous part of the probe was 0.5 mm. The semipermeable polycarbonate membrane allows passive diffusion of small molecular size substances up to 20,000 Da from the extracellular space into the streaming isotonic saline solution. Plastic tubing carries the perfusate to the microfraction collector. The tubing from the microdialysis probe to the microfraction collector was 1 m long, and its dead volume was 12 µl. In this study, 45-µl samples were collected at 15-min intervals. Before analysis, the samples were stored at −20°C for less than 4 months.

The relative recovery is the concentration of histamine in the microdialysis perfusate compared to the concentration of histamine outside the dialysis membrane of the probe, and it is expressed as a percentage. In this study, under the experimental conditions *in vitro* using 100 nM histamine standard and the flow rate of 3.0 µl/min, the relative recovery was determined to be 33%.

To allow reuse of probes up to 3 times, they were cleaned by perfusing with and soaking in 70% ethanol overnight, followed by soaking for 1 h in 1.3% hypochlorite, which contained 0.2% NaOH (17% Erikloori®, Erisan hospital hygiene, Orion, Helsinki, Finland). No effect on the relative recovery of histamine could be observed by repeating this procedure up to 3 times.

Insertion of probes

The probe was inserted into the dermal skin of the dorsal aspect of the forearm as superficially as possible. A needle (Terumo 0.8 × 38 mm) was inserted with a plastic guiding cannula, provided by the manufacturer, intradermally parallel to the surface of the skin. The needle was withdrawn and the probe was inserted into the guiding cannula, which was then withdrawn. The probe was then taped in position. No local anaesthetic was used, because it might have influenced the results by affecting the sensory nerves. One probe was inserted into the skin of 17 patients and two probes were inserted into the skin of 3 patients. In experiments where two probes were used simultaneously, the tips of the probes were placed at least 3 cm apart.

Provocation of skin with neuropeptides and capsaicin

SP, VIP and CGRP were obtained from Cambridge Research Biochemicals (Cambridge, U.K.), and capsaicin was purchased from Sigma (St. Louis, MO, U.S.A.). The neuropeptides and capsaicin were dissolved in sterile physiological saline. Capsaicin cream was prepared to a concentration of 0.1% by using Novalan® emollient (Orion Corporation, Espoo, Finland).

Skin challenging was begun 1 h after the probe insertion. Previous reports have confirmed that the implantation trauma dissipates in 40 min and that it does not influence the histamine levels in the skin (2). In all experiments the baseline sample was collected for 15 min before the skin was provoked. SP and CGRP (10 µM) were injected intradermally as a dose of 250 pmol in a volume of 25 µl, and VIP (10 µM) was injected as a dose of 100 or 250 pmol in a volume of 10 or 25 µl, respectively. The peptides were injected about 5 mm apart from the microdialysis probe. Samples were collected at 15-min intervals for up to 2 h. In cases where a second peptide injection was given 1 h after the first one, it was given at exactly the same site. SP and VIP injected intradermally produced weal and flare reactions (Fig. 1); CGRP produced a prominent long-lasting, indurated erythema with pseudopodia.

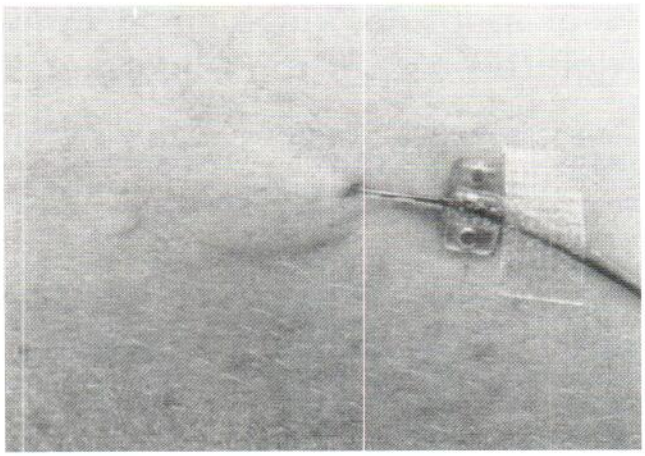


Fig. 1. The microdialysis probe in the dorsal skin of forearm. The photograph was taken 5 min after substance P injection (250 pmol in 25 µl). Neuropeptide-induced weal surrounds the microdialysis probe entirely.

Capsaicin was injected intradermally at a concentration of 30 µM and a volume of 25 µl in close proximity to the probe, as described above. Samples were collected at 15-min intervals for up to 75 min. Capsaicin cream (0.1%) was administered topically on the probe and was covered with a compress moistened with isotonic saline solution. The compress was removed after 1 h. Samples were collected at 15-min intervals for up to 2 h.

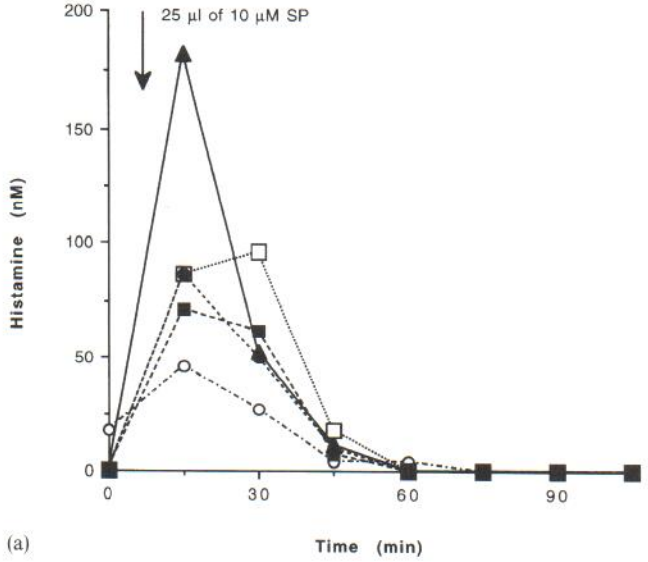
Analysis of histamine

Histamine was analysed in duplicate by using the radio enzyme assay (19), with a detection limit of 0.5–1.0 nM. Histamine-N-methyltransferase was purified from rat kidneys as described previously (20). ^{3}H-Methyl-S-adenosyl-L-methionine (15 Ci/mmol) and ACS® aqueous counting scintillant were obtained from Amersham (Amersham, U.K.). K_2-EDTA was purchased from Fluka (Buchs, Switzerland). All other chemicals were of analytical grade from Sigma or E. Merck (Darmstadt, Germany). Deionized water was used throughout the study. Radioactivity was measured using an LKB 1215 Rackbeta liquid scintillation counter with automatic quench correction (Wallac, Turku, Finland).

RESULTS

Histamine release by SP

The baseline concentration of histamine was 4.7 ± 6.6 nM (mean ± SD, range 0.0–17.4 nM, $n = 8$). A single SP injection (250 pmol) was given to 5 patients (Fig. 2a). Three other patients received a second SP injection 1 h after the first one (Fig. 2b); one subject participated in this experiment twice. In each case, the histamine release after the first SP injection was rapid and reached its maximum either in the first 0–15 min fraction (60.0 ± 55.6 nM) or in the subsequent 15–30 min fraction (43.7 ± 26.7 nM) after skin challenge. Thereafter, histamine levels declined steadily and reached the baseline 60–75 min after the single SP injection, and no second rise was observed (Fig. 2a) within the time course of the experiment. The histamine concentration varied greatly from 7.0 to



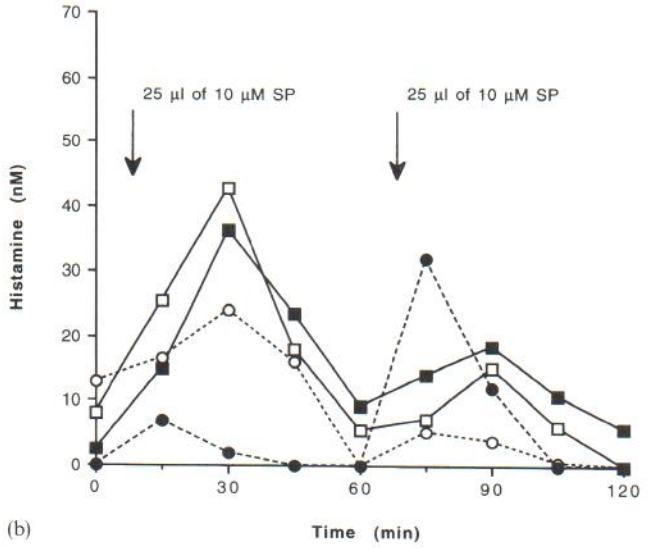


Fig. 2. The release of histamine by substance P: (*a*) Five individual subjects received a single SP injection (250 pmol in 25 µl), and (*b*) three other subjects received two consecutive SP injections (250 pmol). One subject, indicated with open and closed square symbols (male, aged 66 years), participated twice in this experiment, with a 3-week interval; note the close similarity of the release curves.

183 nM in the 0–15 min peak fractions (Fig. 2a, b). In the 15–30 min fractions, the histamine concentration varied between 2.0 and 96.6 nM. In patients who received two consecutive SP injections, the histamine release following the second injection was weaker than that after the first injection in all but one subject (Fig. 2b). Thus, the first SP injection was sufficient to liberate most of the releaseable histamine at the injection site.

Histamine release by VIP

The results obtained for VIP were essentially the same (Fig. 3). The baseline concentration of histamine was 4.8 ± 1.9 nM ($n = 4$). Two consecutive VIP injections were given to 4 patients, who received either 100 pmol or 250 pmol VIP (Fig. 3). As

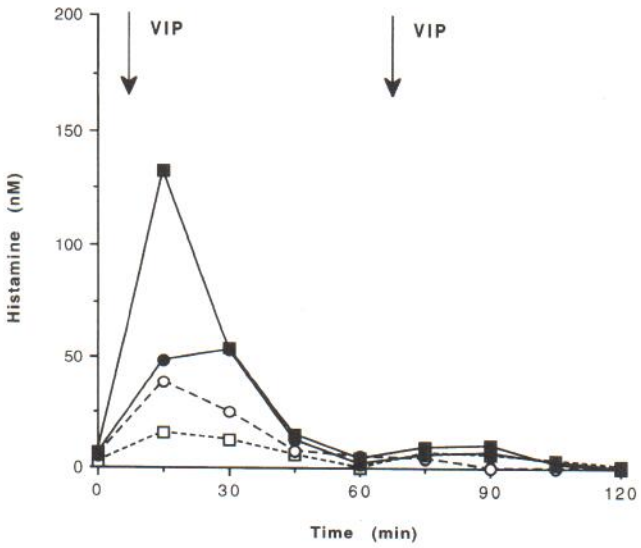


Fig. 3. The release of histamine after repeated VIP injections. Two subjects, indicated with open squares and circles, received 100 pmol (10 µl) and two other subjects, indicated with closed squares and circles, received 250 pmol (25 µl) VIP repeatedly.

observed in the case of SP, the first injection released most of the histamine reservoir, and the second histamine rise was clearly lower. The amount and rapidity of histamine release induced by 250 pmol VIP (Fig. 3) was comparable to that induced by 250 pmol SP (Fig. 2a, b). In all but one subject, the maximum histamine concentration after the first VIP injection was reached in the first 0–15 min fraction following skin provocation.

The weal and flare reactions induced by SP and VIP were seen briefly within 5–10 min after the injection, which is in accordance with the observed rapid release of histamine.

Histamine release by CGRP and capsaicin

CGRP (250 pmol) was injected into 2 patients who had two microdialysis probes simultaneously. SP (250 pmol) was injected as a control adjacent to the second probe. No increase in histamine concentration was observed after the CGRP injection up to 105 min following skin provocation. By this time the erythema induced by CGRP was clearly developed. In contrast, the control injections of SP showed high histamine release (maximum concentrations 183 and 71.8 nM), as shown in Fig. 2a. No clear weal or flare reactions were observed at the injection site of CGRP.

Capsaicin injection (30 µM) induced a very weak and rather non-significant histamine release in most subjects (Fig. 4a). The baseline histamine concentration was 3.4 ± 3.1 nM ($n = 5$). The curve for histamine release was broad and of relatively long duration. The maximum histamine concentration (5.7 ± 3.8 nM) was reached within 0–15 min after the injection. In every case, capsaicin evoked flare and intense pain but not weal. The pain subsided quickly after the injection. Application of 0.1% capsaicin cream resulted in a release of histamine in 2 out of 5 patients (Fig. 4b) and caused a burning or itching sensation in 30 min and erythema in 60–90 min.

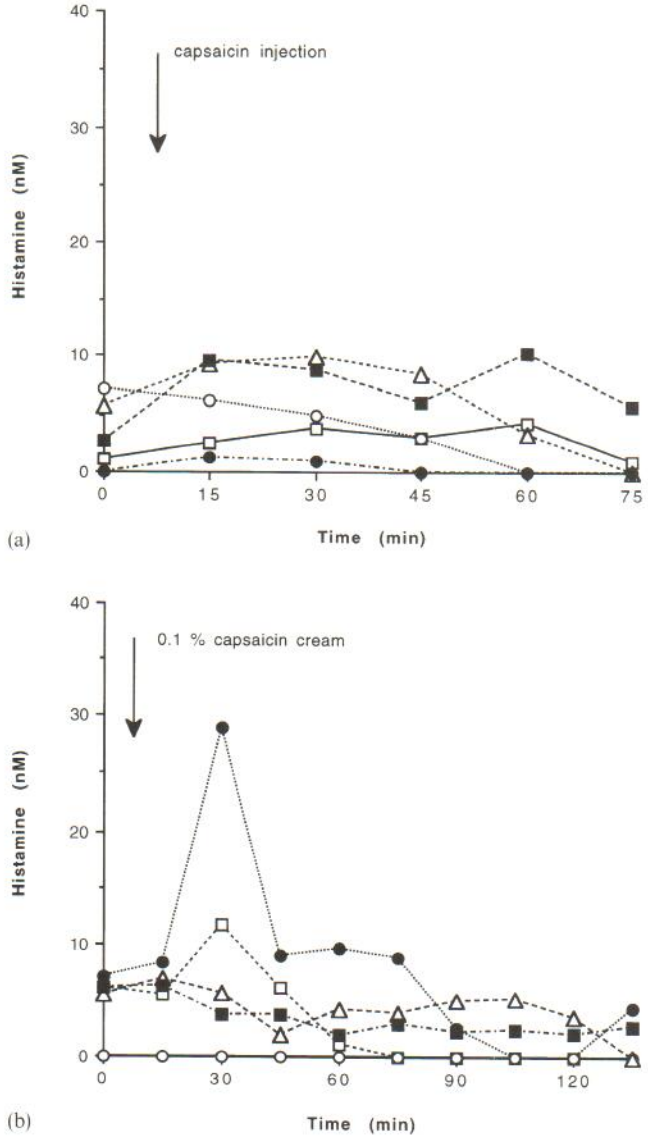


Fig. 4. The release of histamine by capsaicin: (*a*) Five subjects received 25 µl of 30 µM capsaicin injected intradermally, and (*b*) five other subjects were treated with 0.1% capsaicin cream, which was covered with wet saline compress for 1 h.

DISCUSSION

The neuropeptides SP, VIP and somatostatin have been found to release histamine from isolated mast cells of human skin. So far, only sparse information on the direct *in situ* effects of neuropeptides in the skin has been available other than observations of the clinical flare, weal and itch reactions they induce, and histological data.

In this study, we used the microdialysis technique to monitor histamine release following skin challenge with the neuropeptides SP, VIP and CGRP, and capsaicin. SP and VIP are known to release histamine rapidly from mast cells of human skin (9, 10). Previously, SP has been stated to be one of the most potent histamine-releasing agents in human skin (11). On the other hand, VIP has been shown to have similar potency to SP in inducing itch and flare in human skin (21). Our findings concerning the ability of SP and VIP to release histamine from skin are consistent with both the *in vitro* findings concerning their effects on isolated mast cells and their skin reactions at comparable concentrations. We found that SP and VIP in equipotent doses (10 µM) caused comparable histamine release after single and repeated injections, whereas CGRP in the same concentration did not release detectable amounts of histamine. Capsaicin induced very weak liberation of histamine.

SP and VIP induced a relatively rapid release of histamine. After the neuropeptide injections, the peak concentration of histamine was detected in the first 0–15 min or in the subsequent 15–30 min fraction. Previously, histamine release from human skin mast cells induced by SP *in vitro* has been shown to be rapid, being complete within 20 s. On the other hand, IgE-dependent activation of skin mast cells is relatively slow, reaching completion within 6 min after the challenge (22). Our group has used the microdialysis technique under the same experimental conditions to measure allergen-induced histamine release following multiple skin prick-tests around the probe (23). The peak concentration of histamine was measured invariably in the 15–30 min fraction, and thus, histamine release during prick-test challenge is slower than during neuropeptide challenge. However, this method, using 15-min sampling time, cannot monitor the kinetics of histamine release accurately in a very short time period.

CGRP is considered to be a poor releaser of histamine from mast cells. Human CGRP releases histamine from mast cells of rat peritoneum at a concentration of 10 µM, although it is a much weaker histamine-liberating agent than SP (14). CGRP is also much less potent than SP in inducing both weal and flare reactions in human skin (24). At high doses (250 pmol), however, CGRP has been reported to induce a weal and flare response comparable to that induced by 500 pmol histamine (15). In our experiments, CGRP did not release measurable amounts of histamine, nor did it induce weal and flare responses.

Capsaicin is used in the treatment of various cutaneous disorders that involve pain, pruritus and inflammation, for example, psoriasis and postherpetic neuralgia. In clinical studies, capsaicin has been used topically in concentrations from 0.01% up to 0.075% (25). Capsaicin is known to exite C-fibers and to release neuropeptides. Capsaicin pretreatment of human skin markedly reduces the flare response of intradermally injected histamine but does not affect the weal response (12). Although capsaicin itself does not release histamine from mast cells *in vitro*, capsaicin treatment has been shown to cause apparent mast cell degranulation observed histologically *in vivo* (26). On the basis of these observations, it is probable that capsaicin liberates histamine from mast cells by releasing neuropeptides from neurones.

Topical administration of capsaicin onto human skin induces erythema at the site of application, accompanied by sensations of itching, stinging and burning. Previously, also a weal formation after intradermal injection of capsaicin has been reported (18). In the present study, neither capsaicin injection at comparable concentrations nor capsaicin cream caused any clear weal formation, which could be due to few neuropeptide-containing nerves or a low number of contacts between C-fibers and mast cells in healthy skin. The skin effects of capsaicin might be different in psoriatic lesions, where both neuropeptide-containing nerves and their contacts with mast cells are increased (27, 28). In this study, we applied capsaicin as a

0.1% cream covered with moist saline compress, to enhance penetration into the skin, and as a 30-μM injection. Capsaicin injection induced a very weak, and rather non-significant, histamine release in most of the patients, and capsaicin cream under occlusion resulted in a detectable histamine liberation in 2 out of 5 patients. The observed release of histamine could be a consequence of the ability of capsaicin to release neuropeptides from sensory nerve endings.

Microdialysis is a unique sampling technique that allows monitoring of mast cell activation in human skin *in situ*. The present results provide direct evidence that mast cells in skin readily activate when they are challenged with neuropeptides SP and VIP, which supports earlier *in vitro* observations and clinical experiments.

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