

Methacholine induces wheal-and-flare reactions in human skin but does not release histamine *in vivo* as assessed by the skin microdialysis technique

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A number of investigations have indicated that cholinergic agonists release histamine from isolated mast cells and suggested that cholinergic stimulation releases histamine *in vivo*. The purpose of this study was to investigate whether the cutaneous wheal-and-flare reaction induced by methacholine challenge in human skin involves histamine release as measured by the skin microdialysis technique. Five hollow dialysis fibers were inserted intradermally in forearm skin in eight healthy subjects. Each fiber was perfused with Kreb's-Ringer bicarbonate at a rate of 3 μ l/min. Dialysates were collected in 2-min fractions before skin challenge and for 20 min after intradermal injection of methacholine 10^{-3} – 10^{-1} M, the vehicle, and a positive control, codeine phosphate 0.3 mg/ml. Histamine was assayed spectrofluorometrically. Methacholine caused a statistically significant dose-related wheal-and-flare reaction, the flare reaction to methacholine 10^{-1} M being comparable with that seen with codeine 0.3 mg/ml. No significant histamine release was observed with methacholine, cumulative histamine release of 16 ± 8 nM by methacholine 10^{-1} M being similar to vehicle responses of 15 ± 9 nM. Histamine release by codeine was 2524 ± 435 nM. In conclusion, methacholine-induced wheal-and-flare reactions in human skin appeared not to involve histamine release from skin mast cells.

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There is considerable evidence that neural activity contributes to the control of the allergic reaction in various tissues, including the respiratory tract (3) and the skin (2, 6). The role of sensory neuropeptides in mast-cell activation has been extensively studied (15); in contrast, other parts of the nervous system, including the influence of cholinergic agonists and cholinergic nerve fiber activation on the allergic reaction, have not been investigated thoroughly.

In the human lung, methacholine and antigen induce bronchospasm, and both stimuli induce a concomitant release of histamine into the systemic circulation (1). Furthermore, nasal challenge with methacholine results in the release of histamine into the nasal cavity (9). Upon intradermal injection in man, methacholine elicits a wheal-and-flare reaction (7, 12, 29).

A number of investigations have been performed to determine whether cholinergic stimulation activates mast cells *in vitro*. Studies with mast cells from animals have revealed that cholinergic agonists release histamine (5, 13, 16), but the results are contradictory (11, 18, 19). Studies on human mast cells from the lung and the adenoids, as well as investigations on skin slices, have indicated that cholinergic agonists cause histamine release (10, 17, 26).

It remains to be determined whether the wheal-and-flare reaction to intradermal injection of cholinergic agonists involves histamine release from skin mast cells. The purpose of the present study was to use the microdialysis technique (20, 21) to study directly histamine release in intact human skin *in vivo* upon intradermal injection of methacholine, a potent synthetic choline ester.

Material and methods

Subjects

Eight nonmedicated, healthy subjects (three women and five men) without history of atopy or skin diseases took part in the study after giving informed consent, their mean age being 33 years (SD 12; range 20–48). The study was approved by the local ethics committee.

Microdialysis technique

Microdialysis probes were made as previously described (21, 24). In short, single hollow dialysis fibers with 216- μm outer diameter, 8- μm wall thickness, and 2-kDa molecular mass cutoff were glued to gas-tight nylon tubing and sterilized. The final dialysis probe had a length of 20 mm in the skin available for diffusion. The outlet of the probe was covered by a nylon tube with a standardized length of 30 mm. The volume of the outlet was approximately 1.2 μl .

An anesthetic cream (EMLA, Astra AB, Södertälje, Sweden) was applied under an occlusive dressing (Tegaderm, 3M, Leicestershire, UK) for 45 min and wiped off before insertion of the probes. Then, five 23-gauge cannulae were introduced intradermally in forearm skin. Dialysis fibers were introduced into the skin via the guide cannulae, which then were removed. The distance between each cannula was at least 5 cm. Previous studies have revealed that an interval of 2 h between removal of EMLA cream and skin challenge is sufficient for trauma-induced hyperemia to vanish and to restore an intact flare reaction to codeine, a mast-cell secretagogue (24).

After this 2-h interval, the inlet of each dialysis probe was connected to a syringe in a microinjection pump (CMA/100, CMA/Microdialysis AB, Stockholm, Sweden). The fibers were perfused at a rate of 3.0 $\mu\text{l}/\text{min}$ with isotonic, sterile Krebs'-Ringer solution. Dialysates were collected directly from the effluent tube into microtiter wells coated with a glass fiber matrix used for the determination of histamine. Dialysates from each fiber were collected at 2-min intervals (6- μl fractions), two samples were taken as baseline values before skin challenge, and 10 samples were sampled over 20 min after provocation.

Skin test reagents

Methacholine chloride was prepared as previously described (7). Methacholine was diluted in Krebs'-Ringer bicarbonate to final concentrations of 10^{-3} – 10^{-1} M. The vehicle was used as the negative control, and codeine 0.3 mg/ml served as a positive control (23). The substances were injected intrader-

mally in 25- μl aliquots with a 27-gauge needle, the injection being placed 1 mm from the center of midpoint of each fiber.

Histamine analysis

Dialysate histamine concentrations were determined by a spectrofluorometric glass fiber-based histamine method (27). This assay has been validated against an enzyme immunoassay for measurement of histamine in skin dialysate (24). The analytic sensitivity is approximately 25 nM (corresponding to 5 ng/ml of histamine dihydrochloride). Any histamine determinations below 25 nM histamine were set as 25 nM in the subsequent calculations. Methacholine did not interfere with the histamine analysis.

Skin responses

The wheal-and-flare reactions were outlined by a sharp pen and transferred to the record form. For logistic reasons, the recording was performed 20 min after injection. Determination of the wheal-and-flare areas was performed with a hand-held scanner connected to a personal computer (25).

Statistics

Friedman's analysis of variance and Wilcoxon's signed ranks test were used. *P* values (two-tailed) less than 5% were considered statistically significant. Values are given as mean $\pm$ SEM if not otherwise mentioned.

Results

For each individual experiment, peak and cumulative histamine release over 20 min above the limit of detection was calculated. Baseline histamine release was below the detection limit of 25 nM histamine.

Methacholine induced a statistically significant ($P < 0.001$) dose-related wheal-and-flare reaction as shown in Figs. 1 and 2. The wheal area of $80.1 \pm 11.5 \text{ mm}^2$ induced by methacholine 10^{-1} M was significantly smaller than that of $131.3 \pm 13.0 \text{ mm}^2$ induced by codeine 0.3 mg/ml, the positive control. However, the flare reaction caused by codeine of $1082.9 \pm 194.2 \text{ mm}^2$ was not significantly different from the flare reaction caused by methacholine 10^{-1} M ($860.4 \pm 116.8 \text{ mm}^2$).

The cumulative histamine release by methacholine and codeine is shown in Fig. 3. No significant histamine release was seen after skin challenge with methacholine whereas codeine released $2524 \pm 435 \text{ nM}$ histamine over the 20-min sampling period. The cumulative histamine releases for methacholine 10^{-1} , 10^{-2} , 10^{-3} M, and vehicle were 16 ± 8 ,

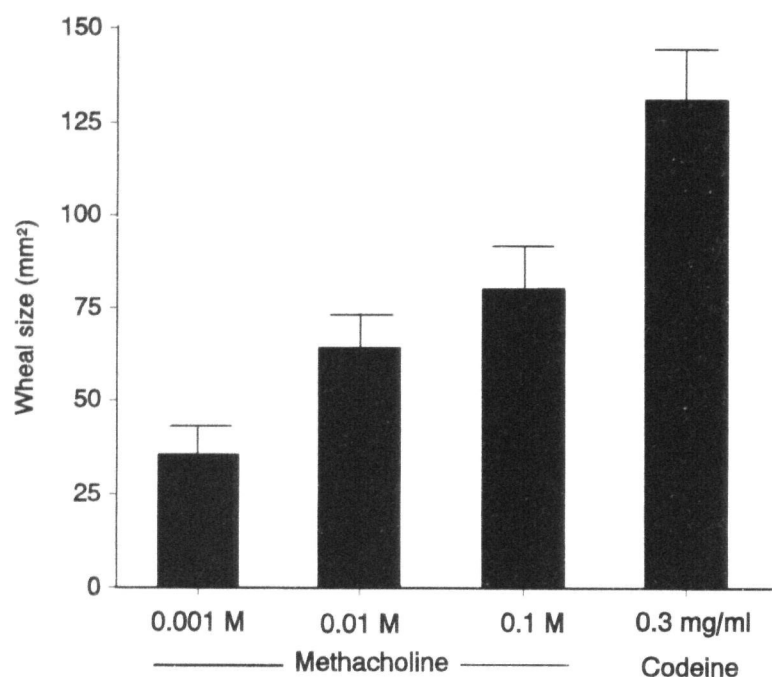


Fig. 1. Wheal areas after intradermal injection of methacholine 10^{-3} – 10^{-1} M and codeine 0.3 mg/ml. With methacholine, significant dose-response relationship was observed. Wheal induced by methacholine 10^{-1} M was significantly smaller than response with codeine 0.3 mg/ml. Values are given as mean $\pm$ SEM ($n = 8$).

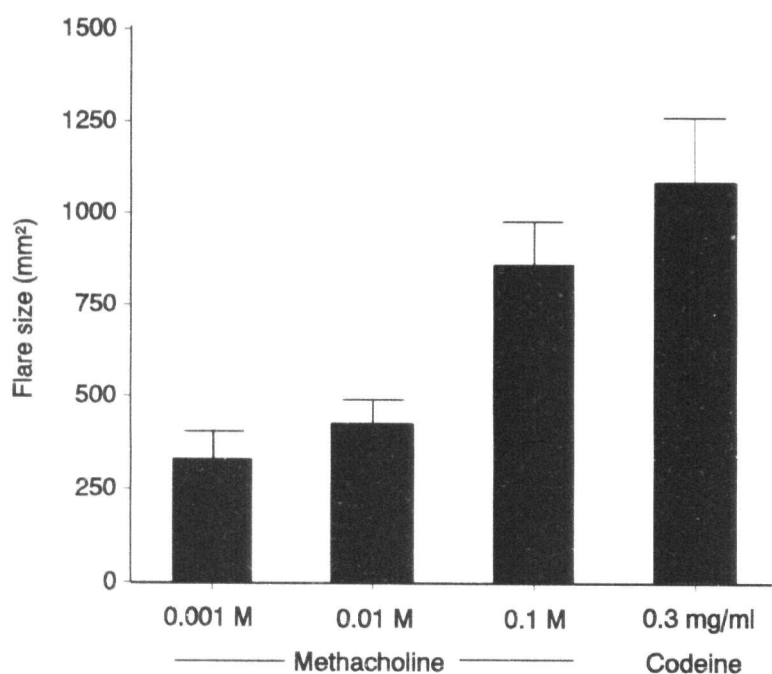


Fig. 2. Flare areas after intradermal injection of methacholine 10^{-3} – 10^{-1} M and codeine 0.3 mg/ml. Significant dose-dependent increase in flare areas with increasing concentrations of methacholine. Flare reaction with methacholine 10^{-1} M was not statistically significantly different from that with codeine 0.3 mg/ml. Values are given as mean $\pm$ SEM ($n = 8$).

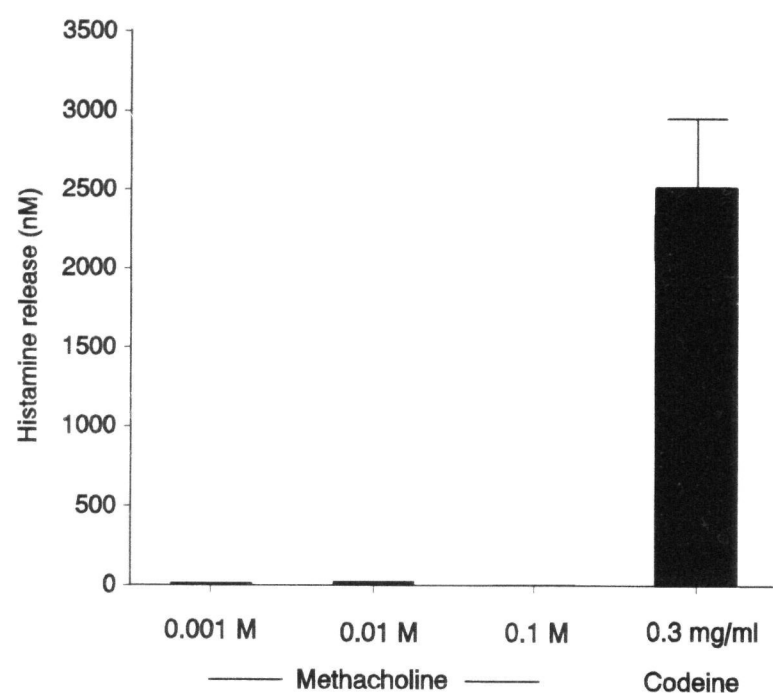


Fig. 3. Histamine release after intradermal injection of methacholine 10^{-3} – 10^{-1} M and codeine 0.3 mg/ml. No histamine release was seen with methacholine. In comparison, codeine 0.3 mg/ml caused significant histamine release. Values are given as mean $\pm$ SEM ($n = 8$), except for methacholine (mean values only).

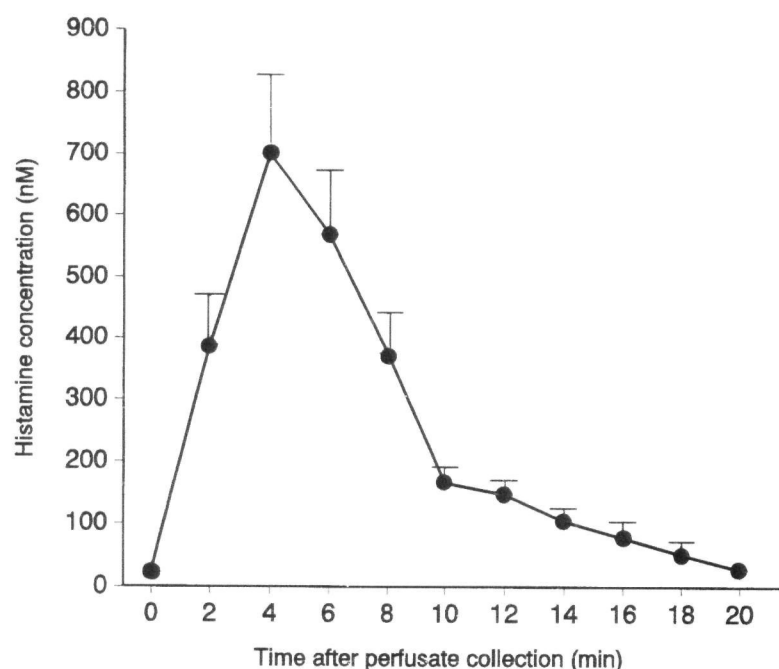


Fig. 4. Kinetics of histamine release induced by codeine. After establishment of baseline (labeled time 0), histamine concentrations are shown in consecutive 2-min samples for 20 min after intradermal injection of codeine 0.3 mg/ml. Mean peak histamine concentration of 700 nM was found in second sample (2–4 min) after skin challenge. After 20 min, dialysate histamine was virtually back to baseline values. Values are given as mean $\pm$ SEM ($n = 8$).

26 ± 21 , 6 ± 5 , and 15 ± 9 nM, respectively. Similar findings were observed with peak histamine release, peak release by methacholine 10^{-1} , 10^{-2} , and 10^{-3} M and vehicle being 17 ± 6 , 7 ± 4 , 3 ± 3 , and 7 ± 4 nM histamine, respectively. Fig. 4 shows the kinetics of histamine release induced by codeine 0.3 mg/ml. In all eight subjects, peak histamine release was found in the 2–4-min sample, showing a peak histamine release of 700 ± 118 nM. Thereafter, a fast decline was seen, and the dialysate histamine concentra-

tions approached baseline 20 min after skin challenge.

Discussion

Upon intradermal injection, methacholine induced wheal-and-flare reactions. However, no histamine release was observed within the center of the skin

reactions. These findings contrast with the observations after intradermal injection of codeine. This mast-cell secretagogue caused wheal-and-flare reactions similar to those seen with methacholine 10^{-1} M, but showed a significant histamine release. Therefore, the results indicate that intradermal injection of methacholine in concentrations causing clinically observable skin reactions does not cause histamine release in human skin *in vivo*.

In the respiratory tract, methacholine challenge elicits symptoms comparable with those seen after histamine challenge, but in these organs methacholine causes histamine release (1, 9). These symptoms may well be of neurogenic origin, and mast-cell degranulation may be minimal if it occurs at all. In the nose, provocation with methacholine causes histamine release but no concomitant release of the specific mast-cell enzyme, tryptase (H. H. Jacobi, personal communication). In addition, histamine is found in high concentrations in unchallenged nasal washings and washings from patients with the common cold. Therefore, the origin of histamine release caused by methacholine in the nose and lung remains unknown.

A number of reports have shown histamine release caused by cholinergic agonists *in vitro*. Meanwhile, evidence has accumulated to show a morphologic, biochemical, functional, and pharmacologic heterogeneity among mast cells from various tissues and from different species (4). This means that results obtained in rats or from dispersed human lung mast cells are not transferrable to mast cells in human skin. For example, neuropeptides and opiates are potent releasers of histamine from human skin mast cells but are without effect on other human mast-cell types (14). These findings may explain the conflicting studies on the effect of cholinergic stimulation on isolated mast cells from various tissues and animals.

Upon intradermal injection of cholinergic agonists in man, wheal-and-flare reactions develop (7, 8, 12, 29). We confirmed this observation with methacholine. Cetirizine, a highly selective H_1 -receptor antihistamine, was shown to block methacholine-induced skin reactions ((12), and J. P. Rihoux, personal communication), but conflicting results have been published (29). Atropine had no influence on the skin responses by methacholine in doses which caused systemic side-effects (29). The cholinergic agonist carbachol induced a flare reaction in human skin but did not release histamine from rat peritoneal mast cells (8). In contrast, acetylcholine released small amounts of histamine from human skin biopsies (26). It remains unexplained why different results are found. It seems unlikely that the conflicting results are caused by the agonist used. The cholinergic agonists differ mainly from acetyl-

choline by their reduced susceptibility to cholinesterase, the selectivity of action of methacholine being most comparable to acetylcholine (28). From the above-mentioned studies it remains to be determined whether cholinergic stimulation causes degranulation of skin mast cells *in vivo* at a level which causes the development of visible skin reactions. None of the studies so far have allowed such conclusions.

For the study of histamine release by methacholine in intact human skin, we used a novel microdialysis technique (20, 21). In this application, this technique possesses two advantages over other *in vivo* skin techniques. First, the levels of mediators are measured directly within the extracellular water space in intact human skin *in vivo*. Second, skin responses can be monitored at the same time as the biochemical events are followed. Using this technique, we have previously measured histamine release after intradermal injection of substance P, codeine, and allergen (22–24). In this study, we were unable to detect any significant histamine release induced by methacholine. Since the baseline histamine values were below the detection limit of 25 nM of histamine of the spectrofluorometric assay, we cannot rule out the possibility that methacholine caused a small histamine release not detected by our histamine analysis. However, from direct comparison with the skin responses and histamine release induced by codeine 0.3 mg/ml, we conclude that histamine release by methacholine plays only a minor role, if any role, in the clinical observations made.

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