

Platelet-activating factor induces histamine release from human skin mast cells in vivo, which is reduced by local nerve blockade

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Background: Intradermal injection of platelet-activating factor (PAF) causes wheal and flare reactions, which are inhibited by antihistamines. However, PAF does not release histamine from human dispersed skin mast cells in vitro. The purpose of this study was to investigate the extent and possible mechanisms of PAF-induced histamine release in human skin in vivo with the use of dermal microdialysis.

Methods: Hollow dialysis fibers were inserted into the upper dermis in forearm skin and each fiber was perfused with Krebs-Ringer bicarbonate solution at a rate of 3.0 μ l/min.

PAF (4.5 to 36 μ mol/L), lyso-PAF (36 μ mol/L), vehicle (negative control), and codeine 750 or 250 μ mol/L (positive control) were injected intradermally above separate fibers.

Dialysate was collected in 2-minute fractions for 20 minutes and histamine analyzed spectrofluorometrically.

Results: PAF, but not lyso-PAF, caused statistically significant dose-related histamine release and wheal and flare reactions. Intradermal mepivacaine administration significantly abrogated flare reactions by PAF and codeine and inhibited histamine release and wheal reactions by PAF but not by codeine. Long-term topical capsaicin administration inhibited histamine release and wheal reactions by PAF but not by codeine. It inhibited flare reactions induced by both compounds. PAF did not release histamine from blood basophils.

Conclusions: These data suggest that PAF induced histamine release from mast cells in intact human skin indirectly via neurogenic activation. Further, on the intradermal injection of PAF histamine release and the skin responses, the wheal and the flare, are differentially regulated by neurogenic components. (*J Allergy Clin Immunol* 1997;99:640-7.)

Key words: Platelet-activating factor, histamine release, skin, human, microdialysis

Originally described as a proaggregatory basophil-derived mediator of anaphylaxis in the rabbit, platelet-activating factor (PAF) has been recognized to be an

Abbreviations used

KRB: Krebs-Ringer bicarbonate

PAF: Platelet-activating factor

important mediator of immediate hypersensitivity. After its de novo synthesis from membrane phospholipids, PAF acts on a variety of cells located in the intravascular and perivascular spaces, for example, neutrophils, eosinophils, monocytes, platelets, basophils, endothelial cells, fibroblasts, and mast cells.¹ Many cells that respond to PAF also synthesize PAF, which suggests that PAF possesses autocrine functions.

Data on the role of PAF as a pathogenetic mediator in allergic diseases stem primarily from studies in the lung and the skin. On inhalation PAF caused immediate and late bronchoconstriction, elicited airway hyperresponsiveness, and induced eosinophil accumulation and activation, all central elements in chronic asthma.² On intradermal injection, itch, flare, and wheal reactions developed in subjects with and without allergies.³⁻⁷ H₁-receptor antagonists (antihistamines) potently inhibited PAF-induced itch and flare,^{4-6, 8, 9} whereas they slightly but significantly reduced PAF-induced wheals.^{6, 8, 9} Similarly, injection of PAF caused an increase in plasma histamine levels in local venous drainage experiments.⁹ These experiments suggest that PAF-induced skin reactions occur, at least in part, as a consequence of histamine release from skin mast cells. These findings, however, contrast with the finding that PAF does not cause histamine release from dispersed human skin mast cells in vitro.^{10, 11}

To assess whether PAF induces histamine release in intact human skin and to what extent this histamine release contributes to the immediate skin reactions after intradermal injection of PAF, we used dermal microdialysis to assess directly histamine release in intact skin in vivo. The results showed that PAF induced histamine release in the skin and that this release was significantly reduced by local anesthetic and long-term topical capsaicin treatment, which suggests the mast cell activation mechanism in vivo is mediated by activation of peptidergic neurons in the skin.

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METHODS

Subjects

A total of 33 healthy nonmedicated subjects (23 men and 10 women; mean age 36 years, range 20 to 50 years) participated in the study. The atopic status of the subjects was not recorded, but wheal and flare reactions to PAF are similar in subjects with and without allergy.^{9, 12} The study was approved by the regional ethics committee, and the subjects gave oral and written informed consent before participation in the study.

Material

PAF C₁₈ (1- α -phosphatidylcholine, β -acetyl- γ -[octadec-9-cis-enyl]) and lyso-PAF (1-0-alkyl-sn-glycerol-3-phosphocholine) were purchased from Sigma Chemical Co. (St. Louis). Both were dissolved in 96% ethanol and diluted with isotonic Krebs-Ringer bicarbonate (KRB) solution containing 0.02% human serum albumin, which served as a phospholipid carrier, to final concentrations of 4.5, 9, 18, and 36 μ mol/L (the latter corresponding to 500 ng PAF per 25 μ l). Codeine phosphate was purchased from the institutional pharmacy and diluted in KRB solution to final concentrations of 250 to 750 μ mol/L (0.1 to 0.3 mg/ml). Mepivacaine hydrochloride 2%, a local anesthetic without vasoconstrictor activity, was purchased from Astra (Södertälje, Sweden). Capsaicin was purchased from Sigma, dissolved in 99% ethanol, and diluted to a final concentration of 10 μ mol/L in KRB solution containing 1.0% Tween 20 polysorbate. An ointment containing capsaicin 2% and a corresponding placebo ointment were prepared by the institutional pharmacy.

Microdialysis technique

Microdialysis fibers were obtained from an artificial kidney.^{13, 14} Each fiber had an outer diameter of 216 μ m, 2000 Dalton molecular weight cut-off, and length of 20 mm available for diffusion. Before insertion of fibers, the skin was treated with an anesthetic cream (EMLA, Astra) under an occlusive dressing (Tegaderm, 3M Health Care Ltd.) for 45 minutes; the creme was wiped off before implantation. A 23-gauge cannula (Terumo, Leuven, Belgium) was introduced into the upper dermis in the radial/ventral surface of each forearm at least 5 cm below the cubital region. The dialysis fiber was introduced into the skin via the guide cannula, which was then removed. A total of six to ten dialysis fibers were inserted in the ventral/volar aspects of both forearms in each subject, with the distance between each cannula being 3 to 5 cm.

After 2 hours, the inlet of each dialysis fiber was connected to a syringe in a microinjection pump (CMA/100, CMA/Microdialysis AB, Stockholm, Sweden). Each fiber was perfused at a rate of 3.0 μ l/min with KRB solution. Dialysate from individual fibers was collected directly from the efferent tube into microtiter wells coated with a glass fiber matrix used for determination of histamine. Two baseline fractions, each of 6 μ l collected over 4 minutes, were obtained before intradermal injections of 25 μ l aliquots of PAF, vehicle (KRB solution), or codeine (positive control). After skin challenge, 10 fractions, each of 6 μ l collected at 2-minute intervals, were obtained over 20 minutes. Each injection was made 1 mm away and parallel to the midpoint of the fiber as previously described.¹⁴

Wheal and flare reactions were read 20 minutes after skin challenge, after sampling of dialysate, by outlining the area of each with an ink pen and transferring the areas by adhesive tape to a record form. The areas were assessed with use of a hand-held scanner connected to a personal computer.

Local anesthetic and long-term topical capsaicin treatment

To study the role of local nerve blockade on histamine release, two separate series of experiments were done, each in six subjects. In the first, 50 μ l of mepivacaine 2% or isotonic saline solution was injected at the injection site 10 minutes before skin challenge. In the second, a 2% capsaicin ointment was applied to the volar aspect of the forearm twice daily for 3 consecutive days and vehicle ointment was applied on the opposite forearm.

Basophil histamine release

The effect of PAF on histamine release in blood basophils was studied in five subjects. Venous blood (10 ml) was drawn into tubes containing 0.2 mol/L of the anticoagulant ethylenediaminetetraacetic acid and mononuclear cells were separated from the erythrocytes by dextran sedimentation and washed twice in a piperazine-N,N'-bis[2-ethanesulfonic acid] buffer. Aliquots of 25 μ l of cells were incubated in glass fiber-prepared microtiter plates for 60 minutes at 37° C with 25 μ l of PAF (4.5 to 36 μ mol/L), lyso-PAF (4.5 to 36 μ mol/L), anti-IgE (400 U/ml), the calcium ionophore A32187 (1 μ mol/L), and KRB solution. Each experiment was done in duplicate. The plates were then washed twice with 0.4% sodium dodecylsulfate and the histamine content analyzed. Histamine release was expressed as a percentage of total histamine content.

Histamine measurement

Histamine concentrations were analyzed with use of a spectrofluorometric glass fiber-based histamine method. The analytic sensitivity of this assay is 25 nmol/L histamine. PAF, lyso-PAF, codeine, and all of the medications used did not interfere with the histamine analysis.

Statistics

Values are expressed as mean plus or minus the standard error of the mean unless otherwise stated. Nonparametric statistical methods were applied, comprising Friedman two-way analysis of variance test, Spearman rank correlation test, and Wilcoxon signed ranks test. Statistical significance was considered at a *p* value (two-tailed) <0.05.

RESULTS

For each individual experiment the peak histamine concentration and the total amount of histamine released over 20 minutes were calculated. Because peak and cumulative histamine releases were linearly correlated and all statistical analyses showed identical results with both measures, we present herein peak histamine data only. Spontaneous histamine release from unchallenged skin sites and, in most cases, histamine release to vehicle injection were less than the detection limit of 25 nmol/L of the spectrofluorometric histamine assay. Any histamine determinations less than the detection limit of 25 nmol/L were assigned a concentration of 25 nmol/L for further calculations.

Histamine release by PAF

PAF released histamine in a dose-dependent fashion in human skin in vivo. After injection of PAF, a rapid histamine release was observed; peak histamine release was observed at approximately 4 minutes, followed by a

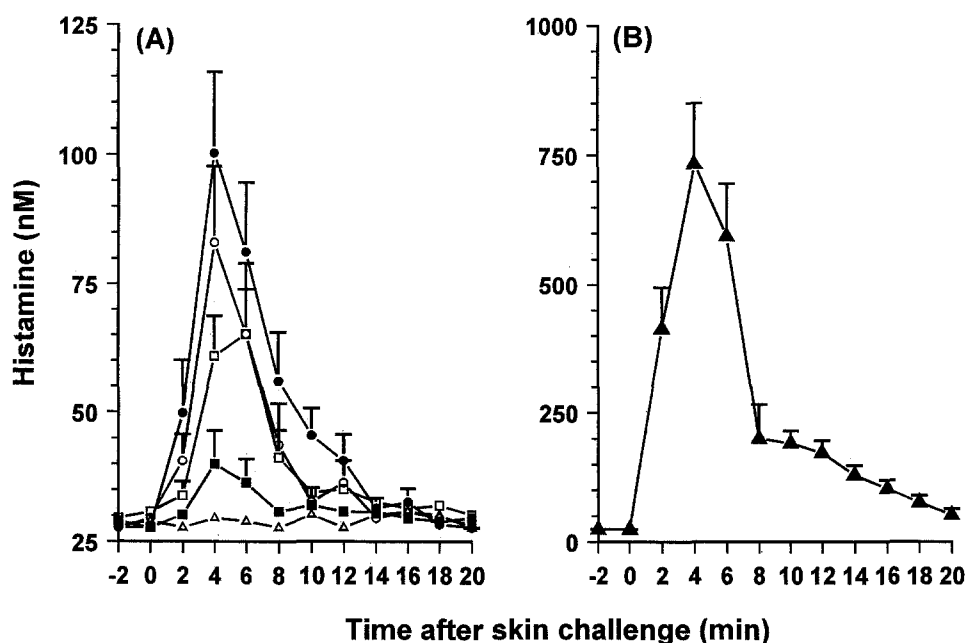


FIG. 1. Time course of histamine release in skin by (A) PAF and (B) codeine. Intradermal injection of 25 μ l of PAF 36 μ mol/L (solid circles), 18 μ mol/L (open circles), 9 μ mol/L (open squares), and 4.5 μ mol/L (solid squares) and vehicle (open triangles) and codeine 750 μ mol/L (solid triangles) were made into normal untreated skin. Dialysate was collected in 2-minute aliquot samples from nine subjects and assayed spectrofluorometrically.

fast decline (Fig. 1, A). In the same subjects, codeine (750 μ mol/L) induced a significant histamine release ($p < 0.01$, $n = 8$): the magnitude of histamine release was almost 10-fold greater than that with PAF 36 μ mol/L (Fig. 1, B). The kinetics of histamine release by PAF and codeine were similar.

PAF induced histamine release and wheal reactions in a dose-dependent fashion (Fig. 2, A, $p < 0.001$, $n = 9$). Flare reactions were not traced in these experiments. Logarithmically transformed peak histamine release correlated significantly with wheal sizes ($r = 0.65$, $p < 0.001$) as shown in Fig. 2, B. Codeine 750 μ mol/L induced wheal reactions of 145.0 ± 12.5 mm² ($p < 0.01$, $n = 8$).

In a separate series of experiments lyso-PAF was shown to be biologically inactive in this context. Histamine release and skin reactions to lyso-PAF (36 μ mol/L) were not significantly different from vehicle responses (data not shown, $n = 7$).

PAF-induced histamine release from blood basophils

Basophils did not release histamine in an amount different from the spontaneous histamine release of $8.3\% \pm 2.3\%$ when challenged with PAF (4.5 to 36 μ mol/L) or lyso-PAF (4.5 to 36 μ mol/L). The net amounts of histamine released at 36 μ mol/L of PAF and lyso-PAF were $1.3\% \pm 0.8\%$ and $2.0\% \pm 0.7\%$, respectively. In comparison, anti-IgE (400 U/ml) and A32187 (1 μ mol/L) resulted in net amounts of histamine release

of $52.3\% \pm 7.8\%$ and $29.9\% \pm 7.8\%$, respectively ($n = 5$).

Effect of local nerve blockade on PAF-induced inflammatory reactions

A series of experiments was done in six subjects to investigate whether PAF-induced wheal and flare reactions and histamine release depended on an intact skin nerve function. As shown in Fig. 3, local nerve blockade by mepivacaine significantly reduced PAF-induced histamine release and wheal and flare reactions. Histamine release was inhibited by 72% to 83% at 18 to 36 μ mol/L concentrations of PAF, and mepivacaine significantly reduced wheal reactions by 58% to 93% and flare reactions by 77% to 91%, as observed at all concentrations of PAF. Mepivacaine significantly reduced codeine-induced flare reactions by 36%, but it did not significantly inhibit wheal reactions or histamine release.

Role of capsaicin-sensitive nerves in PAF-induced inflammatory reactions

In another group of six subjects, capsaicin ointment was applied to forearm skin sites to investigate the role of capsaicin-sensitive nerves in PAF-induced skin reactions and histamine release. In these studies we used codeine 250 μ mol/L as the positive control. The results are shown in Fig. 4. Topical capsaicin significantly reduced the flare reactions by 57% to 65% at all concentrations of PAF, whereas no significant effect was seen on the wheal reactions. Histamine release was

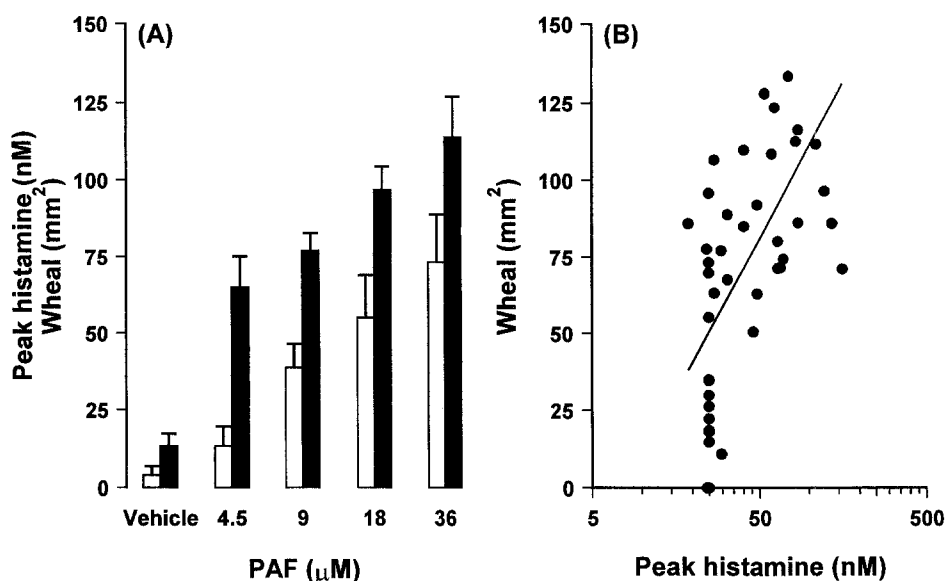


FIG. 2. Histamine release and wheal reactions in skin induced by various concentrations of PAF and vehicle. **A**, Histamine release (open bars) and wheal reactions (solid bars) increased significantly with increasing concentrations of PAF ($p < 0.01$) and histamine release and wheal reactions were significantly different from vehicle responses at PAF concentrations of 9 $\mu\text{mol/L}$ and greater. **B**, Histamine release by PAF correlated significantly with wheal reactions ($r = 0.65$, $p < 0.001$).

significantly inhibited by 44% to 57% with PAF 18 to 36 $\mu\text{mol/L}$. Capsaicin significantly inhibited codeine-induced flare reactions by 73%, but not wheal reactions or histamine release.

Injection of capsaicin alone did not induce wheal reactions to a significantly different extent from those induced by vehicle injections. Capsaicin 10 $\mu\text{mol/L}$ induced a statistically significant flare of 240.9 ± 38.1 mm² in vehicle-treated skin, which was reduced to 84.8 ± 34.0 mm² in capsaicin-treated skin ($p < 0.05$).

DISCUSSION

The main results of this study are (1) that intradermal injection of PAF induces histamine release in vivo, (2) that the magnitude of histamine release correlated with the extent of inflammatory skin reactions, and (3) that activation of peptidergic nerves is involved in PAF-induced histamine release, reflex vasodilation, and vascular permeability.

Circumstantial evidence suggests that PAF induces histamine release from skin mast cells in vivo, but direct evidence has not been documented. First, on intradermal injection of PAF wheal and flare reactions develop, and these reactions can be inhibited completely or partly by antihistamines. Second, histamine release has been demonstrated in venous effluents from skin sites after intradermal injection of PAF.⁹ Because PAF caused histamine release from human unprimed basophils in some studies^{11,15} but not in others,¹⁶ it remained to be determined whether PAF causes histamine release from mast cells. In fact, studies on dispersed human skin mast cells in vitro have failed to detect histamine release by

PAF,^{10,11} and histologically PAF in micromolar concentrations degranulates basophils but not skin mast cells.¹⁷ The results of this study directly evidenced that histamine is released at the site of intradermal injection of PAF. The histamine release was rapid, suggesting mast cells to be its source, with peak histamine release being observed by approximately 4 minutes, and the magnitude of histamine release was concentration dependent. Concomitant analysis of tryptase or chymase would have substantiated that mast cells are the histamine source, but the dialysis fibers used do not allow sampling of high molecular weight substances. However, mast cells were considered to be the main source of histamine for the following reasons. First, PAF in concentrations used for intradermal injection in this study did not release histamine from blood basophils in vitro. Second, the usual numbers of basophils present in uninflamed skin contain too little histamine to account for that released by PAF. In fact, intradermal injection of potent basophil histamine-releasing chemokines does not cause release of any histamine in intact human skin,¹⁸ which suggests no significant source of histamine from basophils in normal skin.

In comparison with codeine, a mast cell secretagogue that induces histamine release in intact human skin in vivo and in dispersed human skin mast cells in vitro,^{19,20} histamine appeared to play only a minor role in the plasma extravasation to PAF. In normal untreated skin, the wheal reactions, corrected for vehicle responses, to PAF 36 $\mu\text{mol/L}$ and codeine 750 $\mu\text{mol/L}$ were 100 and 130 mm², respectively. In comparison, peak histamine release was 69 and 696 nmol/L. Thus PAF released only

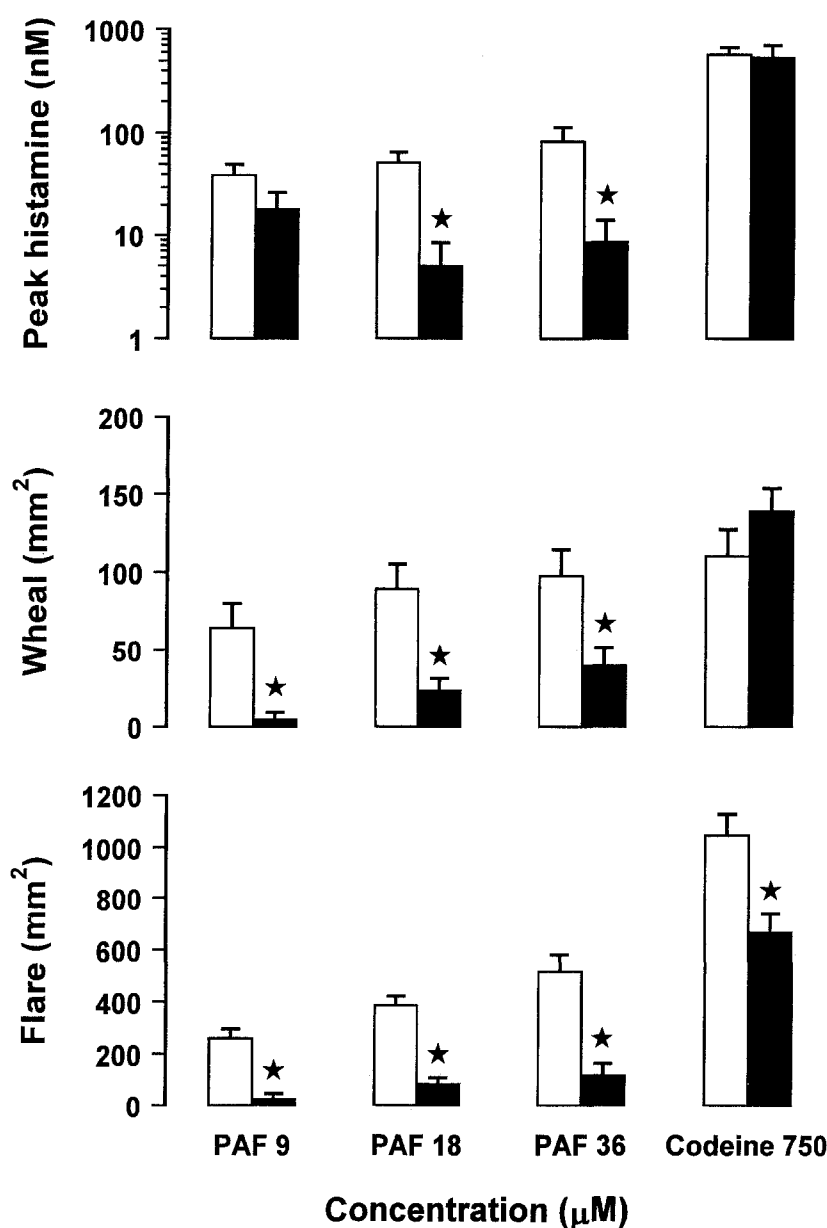


FIG. 3. Histamine release and skin reactions by PAF and effects on these of local nerve blockade by mepivacaine. Intradermal injection of PAF into skin sites preincubated 10 minutes before skin challenge with mepivacaine (solid bars) or vehicle (open bars) showed significant inhibition of histamine release and wheal to PAF and significant reduction in flare sizes to PAF and codeine. Mepivacaine did not affect histamine release or wheal reactions to codeine 750 $\mu\text{mol/L}$. Results are corrected for vehicle responses, which were mean histamine release of 16 nmol/L (higher than 25 nmol/L limit) and wheal and flare reactions of 35 and 60 mm², respectively. PAF concentrations given in micromolars per liter. Values are mean plus standard error of mean of results from six subjects. Stars indicate $p < 0.05$.

about 10% of the histamine observed with codeine at comparable wheal reactions, which indicates that histamine is not the main mediator in PAF-induced vascular leakage and protein extravasation. This observation is in accordance with findings that plasma extravasation to PAF is less sensitive to antihistamines than the reflex vasodilation, which suggests the involvement of histamine-independent components in its origin. Although PAF has an effect on the vascular endothelium independent from its aggregatory effects on platelets,²¹ it has

previously been shown that PAF and histamine prime the vascular effect of each compound.²²

The ability of PAF to release histamine from skin mast cells *in vivo* but not *in vitro*^{10,11} led us to perform studies to explore the role of secondary neurogenic activation of skin mast cells by PAF. PAF has been shown to activate neuronal cells in a variety of tissues,²³⁻²⁵ a finding that may have functional implications. For example, in the guinea pig PAF is suggested to release norepinephrine from the myocardium²⁶ and ac-

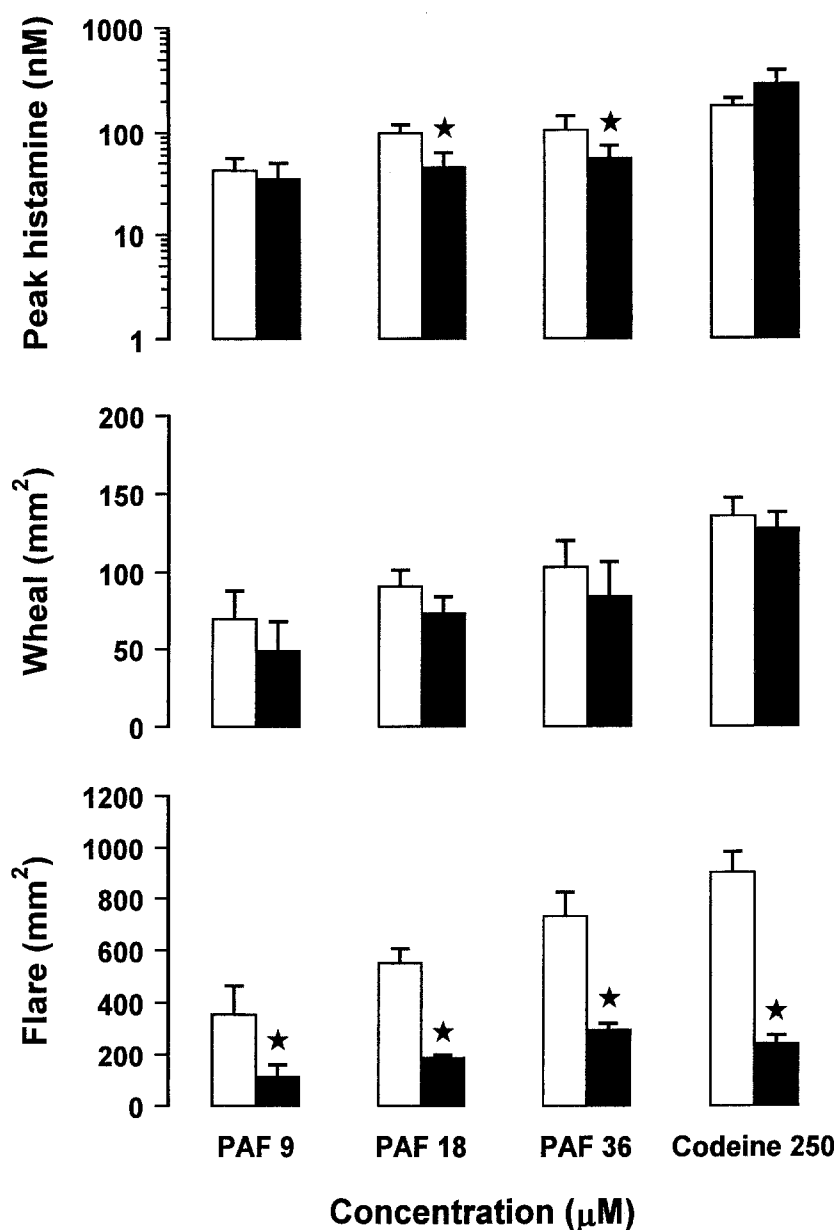


FIG. 4. Histamine release and wheal and flare reactions to PAF and effects on these of topical capsaicin treatment. Intradermal injection of PAF into skin sites treated twice daily for 3 days with capsaicin 2% ointment (solid bars) or corresponding vehicle (open bars) showed significant inhibition of histamine release to PAF, no change in wheal reactions, and significant reduction in flare sizes to PAF and codeine. Mepivacaine did not affect codeine-induced histamine release or wheal reactions. Results are corrected for vehicle responses, which were a mean histamine release of 9 nmol/L and wheal and flare reactions of 11 and 62 mm², respectively. PAF concentrations given in micromolars per liter. Values are mean plus or minus standard error of mean of results from six subjects. Stars indicate $p < 0.05$.

tivate sensory neurons in the lung.^{27, 28} The latter is demonstrated by the ability of topical capsaicin to abolish the immediate bronchoconstriction to PAF in some species and to reduce bronchial responsiveness induced by PAF. Capsaicin-sensitive sensory nerves contain neuropeptides, including substance P and vasoactive intestinal peptide, which when injected intradermally induce wheal and flare reactions largely as a consequence of their ability to release histamine from dermal mast

cells.^{14, 29-31} Thus it is possible that the activation of such neurons in the skin in vivo leads to the generation of the histamine detected by microdialysis after PAF injection. The hypothesis that PAF-induced dermal inflammation is mediated, either entirely or partly, by sensory nerves is supported by the effects of local anesthesia. Mepivacaine significantly inhibited the flare and also the wheal and histamine release after injection of PAF. Although it is possible that the effects of mepivacaine on histamine

release may be related to effects beyond that of simple anesthesia, for example, mast cell stabilization, two findings contradict this. First, local anesthetics did not inhibit histamine release by codeine in this study or, as observed in separate series of experiments, histamine release by vasoactive intestinal peptide.³¹ Second, in a preliminary study we showed that lidocaine 0.5%, when mixed with PAF before intradermal injection, significantly inhibited histamine release by PAF 36 $\mu\text{mol/L}$ by 58% but not histamine release by codeine (data not shown). Thus neither the type of local anesthetic nor its concentration seems to be of major importance. The inhibitory effects of mepivacaine also indicate that skin reactions and histamine release to PAF were not caused by a cytotoxic reaction.

Additional studies in capsaicin-treated skin showed a significant inhibition of the flare reactions by PAF, codeine, and capsaicin and a significant inhibition of histamine release by PAF but not by codeine. The failure of topical capsaicin to reduce significantly PAF-induced wheal reactions is in accordance with findings of previous studies.⁷ The data support the common theory that the flare is dependent on capsaicin-sensitive nerves,³² but they also suggest that such nerves are involved, in some part, in histamine release by PAF. However, the contribution of different types of nerves in PAF-induced histamine release needs further investigations.^{26, 33}

The concentrations of PAF used in this study are comparable with dose ranges previously used in human skin. It remains to be determined whether micromolar concentrations of PAF can be released endogenously in the skin, lung, or other organs. Although most PAF is retained intracellularly, the quantity of PAF released extracellularly is sufficient to be biologically active, and some metabolites of PAF found in such fluids have potent vasodilator activities.³⁴ PAF and lyso-PAF concentrations have been measured in abraded normal skin in nanomolar concentrations³⁵ and are increased on allergen skin challenge.^{36, 37} Thus micromolar concentrations of PAF at the cell surface in intact human skin in vivo may be possible.

In conclusion, this study indicates that PAF-induced histamine release in intact human skin in vivo is mediated via neurogenic activation. The mechanisms still remain to be fully elucidated, and the extent of neurogenic mast cell activation in normal and in particular in diseased skin requires further investigations.

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