

Capsaicin-sensitive nerves and the cutaneous allergy reaction in man

Possible involvement of sensory neuropeptides in the flare reaction

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The effects of local capsaicin pretreatment on the cutaneous triple response reaction induced by allergen exposure or anti-IgE were studied in man. Acute exposure of the human skin to capsaicin caused a burning sensation and a clearcut flare reaction but no wheal response. Upon repeated administration these local reactions to capsaicin disappeared. The flare component and the subjective itching sensation of the cutaneous allergy reaction to rat antigen in sensitized persons or anti-IgE in non-allergic persons were then markedly reduced. Two weeks after capsaicin pretreatment the flare response to allergen was not significantly changed compared to the control reaction, suggesting a reversible effect of capsaicin treatment. The wheal component of the allergy or anti-IgE reaction was, however, not influenced by capsaicin pretreatment, indicating that the wheal and flare components are caused by different mechanisms. It is concluded that capsaicin sensitive sensory nerves are of importance for the human cutaneous triple response reaction induced by allergen exposure. Thus, secondary release of mediators, such as CGRP or tachykinins from sensory nerve branches, may contribute to the flare component of this reaction. Furthermore the itching sensation seems to be dependent to a large extent on capsaicin-sensitive nerves. However, sensory nerves seem to have less importance for the wheal reaction, i.e. the protein extravasation response.

Key words: allergy; skin; man; anti-IgE; capsaicin-sensory nerves; vasodilation; protein extravasation; calcitonin gene-related peptide; substance P.

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The cutaneous triple response reaction (17) upon allergen exposure in man is characterized by an itching sensation and concomitant vascular reactions consisting of a flare (vasodilation) and wheal (protein extravasation) response. It is well known that histamine release from mast cells is an important mediator of these effects (5) either by a direct action on blood vessels and/or indirectly via activation of sensory nerves. Capsaicin, the pungent principal of Hungarian red pepper, is known to induce vasodilation and protein extravasation due to an acute release of bioactive peptides, such as substance P (SP), neurokinin A (NKA) or calcitonin gene-related peptide

(CGRP) from sensory nerve branches (6, 13, 18, 19, 25). Repeated exposure to capsaicin results in a desensitization of chemosensitive sensory afferents (12), depletion of sensory peptides such as SP (14) and a loss of local tissue reactions upon chemical irritation (27). Also, in man, capsaicin pretreatment has been found to reduce the flare component in the skin upon chemical irritation (13) and local injection of histamine (1, 4). The close relationship between peripheral capsaicin-sensitive nerves and mast cells is further illustrated by the observation that the protein extravasation response seen upon antigen challenge in guinea pigs is reduced after capsaicin pretreat-

ment (24). Furthermore, SP injected locally into the human skin induces wheal and flare responses partly via histamine release from mast cells (4, 8). Also animal experiments have suggested a close relationship between sensory nerve activation, substance P and mast cells (16).

In the present study we have investigated whether capsaicin pretreatment can impair the cutaneous triple response reaction to mast cell degranulation induced by anti-IgE or antigen challenge in man.

MATERIAL AND METHODS

The skin of nine persons (3 males, 6 females, age 29–40) with an established allergy to rats, as well as six non-allergic persons (5 males, 1 female, age 24–48) was pretreated locally with capsaicin. A capsaicin solution (30 mM in 70 % alcohol) was applied to a 5 × 5 cm area of the volar surface of the forearm after the area had been previously abraded with fine emery paper. The procedure was repeated three times daily for 4 days and the skin was abraded once each day. The vehicle solution (70 % alcohol) was applied on the control forearm in a similar manner. On day 5 a cutaneous allergen skin test was performed in the center of the treated area by introducing a tubercule needle through a drop of allergen solution and lifting the most superficial layer of the epidermis (22). A 1:100 dilution of a rat allergen extract was used in six of the patients with an established allergy to rats. In the remaining three individuals the allergen extract (100 µl) was injected intracutaneously. Furthermore, in the non-allergic persons 100 µl antiserum, raised in sheep against Fc fragments from a human IgE myeloma protein, was injected intracutaneously. This antiserum contained 0.5 mg/ml of IgG against human IgE and the serum was diluted 1:10 (v/v) with Coca's solution (0.9 % NaCl + 0.3 % phenol) and dialysed against this solution overnight. The antiserum was passed through a Millipore filter (3.0 µm) and stored at +4°C until used. Aliquots were taken each day and further diluted to 1:100 with Coca's solution before intracutaneous injection. The areas

of the progressively developing wheal and flare reactions were outlined and traced onto a transparent plastic film 15 min as well as 1 and 2 h after allergen or anti-IgE injection. Reactions were only compared within the treated areas which were outlined by ink. The encompassed areas were cut out, weighed and compared with the control reaction on the contralateral vehicle-treated side. The subjective sensation of itching was recorded on a symptom score scale (1 – 5). The studies on human skin were approved by the Ethics Committee at the Karolinska Institute. All persons were volunteers and on no drug treatment. The statistical significance of changes compared to responses on the vehicle-treated side was analysed using the Mann-Whitney U-test. Values < 0.05 were considered as significant.

Drugs and chemicals

Rat allergen extract (Allergologisk Laboratorium (A/S Copenhagen, Denmark), Sheep antihuman myeloma IgE protein (Pharmacia, Uppsala, Sweden). Capsaicin (Merck, Darmstadt, FRG) was dissolved in 70 % ethanol to a concentration of 30 mM.

RESULTS

Capsaicin pretreatment produced acute burning sensation and flare reaction but no clearcut wheal response. These reactions subsided after 2 or 3 days and on day 4 virtually no irritation or flare reaction was observed. The sensation of touch was not impaired after the capsaicin pretreatment was completed, although the subjects experienced hyperesthesia from touch of clothing and cold water during the first 2 days of treatment.

Cutaneous skin test with rat allergen 1 day after completed treatment on the vehicle-treated skin areas resulted in an instant itching sensation followed by progressively developing flare and wheal reaction (Fig. 1) which was maximal after about 15 min and then gradually declined in magnitude (Figs. 2(a), 3(a)). In the capsaicin-treated skin area the subjective sensation

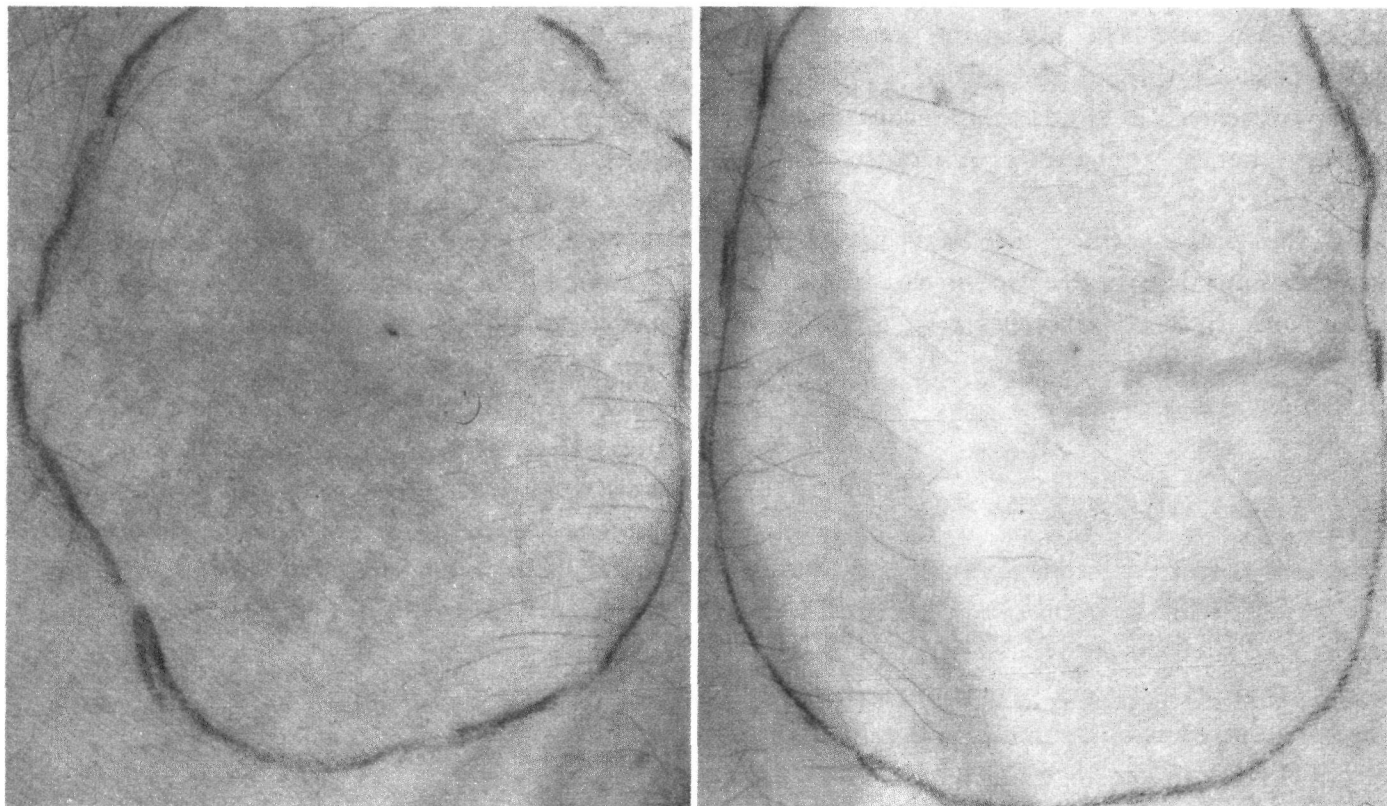
VEHICLE**CAPSAICIN**

Fig. 1. Photographs of cutaneous wheal and flare reactions 15 min after rat allergen exposure in a sensitized person. The effects in a vehicle (70% alcohol) - treated area on the control arm (left) are compared with the response 1 day after local pretreatment with capsaicin (right). Note the absence of the red flare reaction after capsaicin pretreatment while the white wheal response seems unchanged.

of itching was much less intense in all persons and delayed compared to the control side. The flare reaction on the capsaicin-pretreated side was virtually abolished (see Figs. 1, 2(a)) while the wheal response was not significantly changed from the vehicle-treated side during the 2 h observation period (Fig. 3(a)). Two weeks after completed capsaicin pretreatment the flare reaction upon rat allergen challenge had recovered and the response was no longer significantly different from the vehicle-treated side (Fig. 2(b)). The wheal reaction upon prick test with rat allergen was not significantly influenced 2 weeks after capsaicin pretreatment (Fig. 3(b)).

Local intracutaneous injection of rat antigen in three allergic persons caused a much stronger reaction on the control side which was within the treated area only at the 15-min observation period. However, the flare reaction on the capsaicin-treated side was $50 \pm 3\%$ smaller than

the response at the control side. The wheal reaction was not changed by capsaicin pretreatment.

Local intracutaneous injection of sheep anti-human IgE to non-allergic persons caused a triple response reaction which was progressively increasing during the observation period (Fig. 4). In the capsaicin-pretreated area the itching sensation upon anti-IgE injection was smaller (symptom score scale 1-2) compared to the control side (symptom score scale 4-5) in all persons. The touch sensation was similar on both sides (4-5) as revealed by touch of a cotton pin. The flare response was markedly reduced (by 88%) in the capsaicin-pretreated area after 15 min, while a considerable reaction developed progressively with time. Thus, after 2 h this response was 42% of the control side (Fig. 4(a)). The wheal response upon anti-IgE exposure was not significantly influenced by capsaicin pretreatment (Fig. 4(b)).

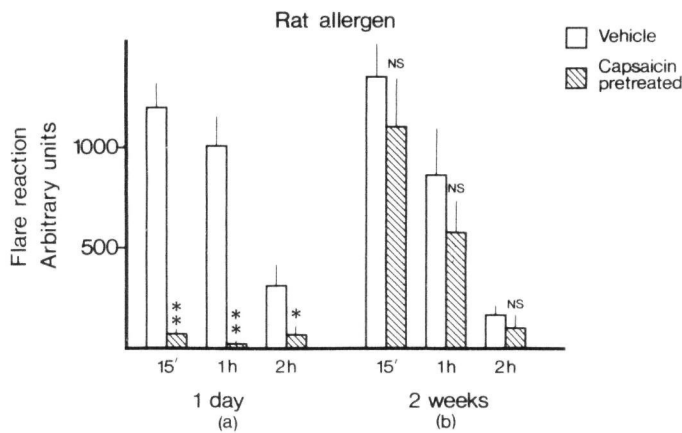


Fig. 2. Flare response in the skin, expressed in arbitrary units upon antigen challenge with rat allergen using prick test in sensitized persons ($n = 6$). The responses are expressed as means $\pm$ SEM and data have been obtained 15 min, 1 and 2 h after challenge. Open bars = control skin, hatched bars = capsaicin-treated skin. (a) Flare reaction 1 day after completed capsaicin pretreatment (b) Flare reaction 2 weeks after capsaicin pretreatment. * $P < 0.05$, ** $P < 0.01$ using Mann-Whitney U-test.

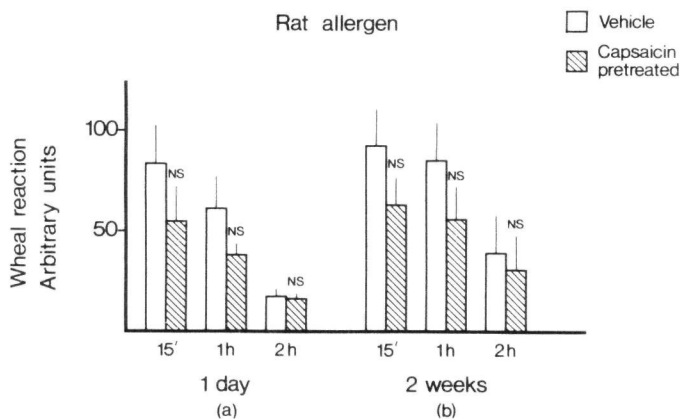


Fig. 3. Wheal response in the skin expressed in arbitrary units, upon antigen challenge with rat allergen using a prick test in sensitized persons ($n = 6$). Open bars = vehicle-treated skin, hatched bars = capsaicin-treated skin. The responses after 15 min, 1 and 2 h after challenge are expressed as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ using Mann-Whitney U-test. (a) Wheal reaction 1 day after completed capsaicin treatment. (b) Wheal response 2 weeks after capsaicin pretreatment.

DISCUSSION

In the present study capsaicin pretreatment of the human skin virtually abolished the acute flare component and reduced the itching sensation of the cutaneous allergy reaction. The acute skin response to allergen or anti-IgE is

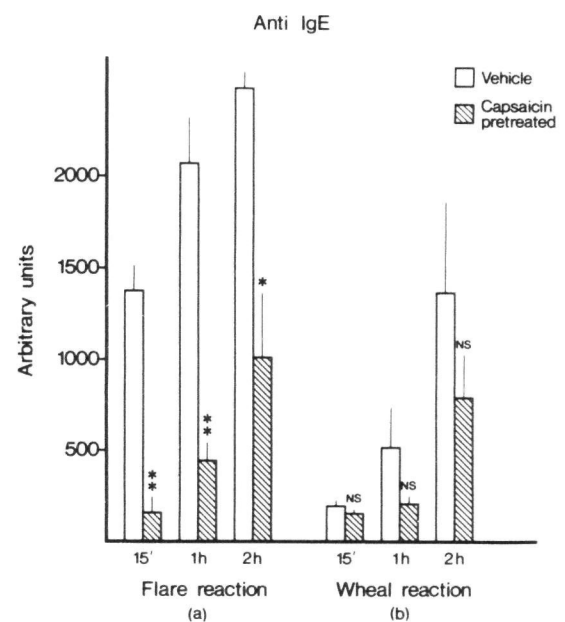


Fig. 4. (a) Flare and (b) wheal responses (expressed means $\pm$ SEM $n = 6$) in the human skin 15 min, 1 and 2 h after intracutaneous injections of anti-IgE. The response in the vehicle-treated area is compared with the effects 1 day after local capsaicin pretreatment (hatched bars). * $P < 0.05$, ** $P < 0.01$ using Mann-Whitney U-test.

known to a large extent to be dependent on histamine release from mast cells (7). Therefore the present data suggest that capsaicin-sensitive sensory nerve endings are activated by mediators such as histamine released upon mast cell degranulation (see Fig. 5). Sensory nerve activation then results in local release of vasoactive mediators, such as SP, NKA and CGRP which are present in peripheral branches of sensory nerves (10, 11, 23). After release these peptides are candidates to cause the flare response by inducing vasodilatation of cutaneous blood vessels. The present data are supported by earlier studies showing that histamine H1-receptors are present in certain primary afferent neurons (20) and that histamine can stimulate paravascular nociceptors (15). Furthermore, histamine has recently been demonstrated to release tachykinins from capsaicin-sensitive sensory nerves in the isolated guinea pig lung (26).

The wheal response to allergen challenge, however, was unchanged after capsaicin pretreatment. This suggests that activation of sensory nerves plays a major role for the flare and itching reaction but not for the wheal response,

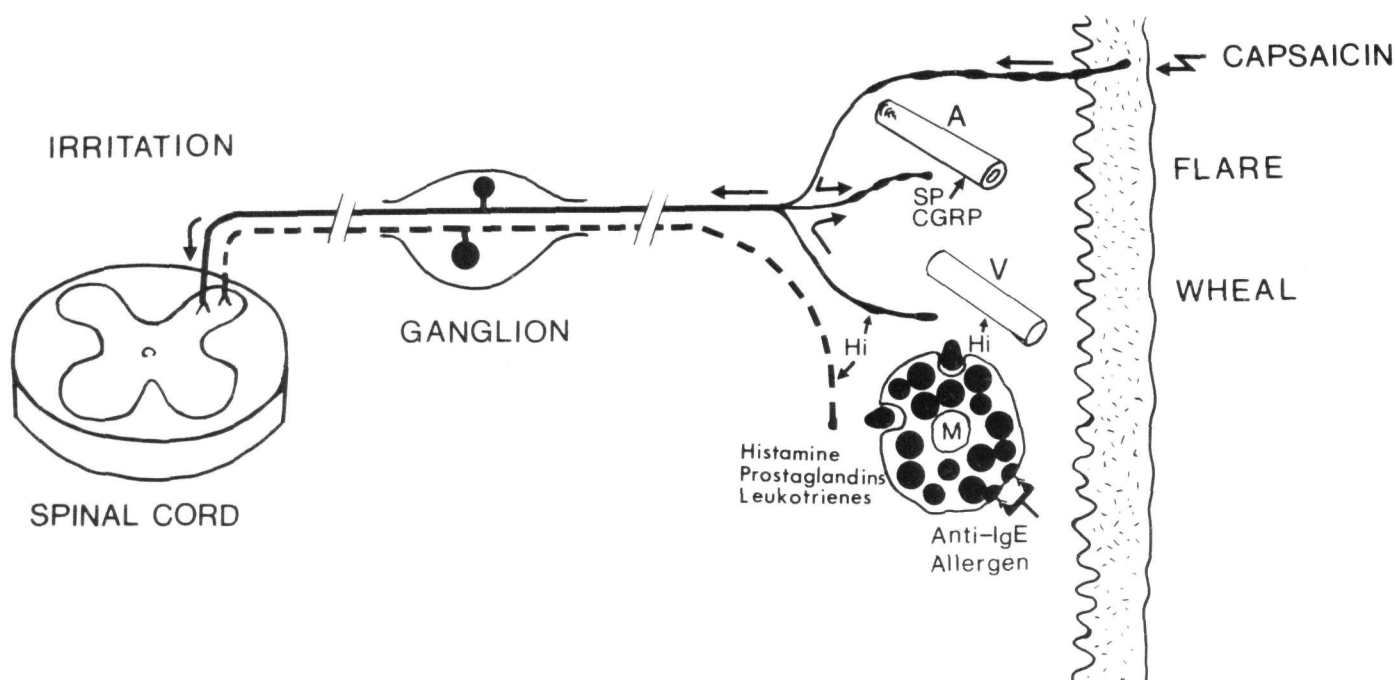


Fig. 5. Schematic drawing of possible involvement of sensory neuropeptides for the cutaneous wheal and flare reaction in the allergic response in man. Acute release of mediators such as histamine upon mast cell degranulation activates capsaicin-sensitive sensory nerve endings thus releasing vasoactive peptides such as SP, NKA and CGRP which could be of importance for the flare reaction. The subsequent wheal reaction seems to be independent of sensory nerves and may involve a variety of mediators including histamine, leukotrienes and prostaglandins (modified from 8).

which apparently to a major extent is a direct effect of histamine on postcapillary venules and independent of mediator release from capsaicin-sensitive nerves. Furthermore, the present data show that capsaicin *per se* does not induce any clearcut wheal reaction in the human skin in spite of a marked flare reaction (1, 13). This implies that mediators released from sensory nerves by capsaicin or antigen challenge do not cause any major protein extravasation in human skin (see Fig. 5), although merely visual observation of a wheal response may not be sensitive enough to elucidate such an effect. This is in contrast to results obtained in experimental animals such as rat and guinea pig, where tachykinins induce marked protein leakage as demonstrated by the Evans blue extravasation technique (24). The wheal response obtained upon local injections of high concentrations of SP (8) or CGRP (2) in the human skin may therefore largely depend on indirect effects, such as degranulation of mast cells and subsequent histamine release (4). Since a similar wheal reaction can not be elicited by capsaicin, peptides released from senso-

ry nerves do not seem to reach sufficiently high concentrations to degranulate mast cells in man. Thus, species differences may exist with regards both to the mechanisms for the protein extravasation effects of peptides such as SP and involvement of mast cells in this response.

In conclusion, mediator release from capsaicin-sensitive nerves seems to be of major importance for the flare component of the cutaneous IgE-mediated allergy response in man. It remains to be established whether this also applies to the human respiratory tract mucosa.

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