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Capsaicin Pretreatment Increases Mast Cell-Mediated But Not Histamine-Induced Wheal and Erythema Responses: A Proof-of-Concept Study

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ABSTRACT

Objective: Transient receptor potential (TRP) channels are crucial for the response of many different mediators, including histamine and substance P. Currently, it is not clear whether stimulation of TRP channels alter the degranulation thresholds of skin mast cells. Thus, this proof of concept study aimed to test whether mast cell-driven wheal and erythema responses are modified by short-term stimulation of TRP channels with capsaicin.

Materials and Methods: Skin prick testing was applied with histamine and grass pollen extract in sensitized individuals, as a model to explore the possible effects of capsaicin on IgE-mediated itch, wheal, and flare development.

Results: We showed that short-term TRP stimulation by topical capsaicin treatment resulted with a significant difference in maximum wheal diameter (12.5±3.7 mm vs. 7.9±3.4 mm, p=0.02), average wheal area (76.4±31.0 mm^2 vs 35.3±17.4 mm^2, p=0.01), maximum erythema diameter (38.5±13.9 mm vs. 28.8±14.5 mm, p=0.07) and average erythema area (973±709 mm^2 vs 556±499 mm^2 ; p=0.02) between capsaicin-pretreated areas compared to placebo-pretreated areas. No differences in itch, wheal and erythema responses between capsaicin-pretreated and placebo-pretreated areas were observed after skin prick testing with histamine.

Conclusion: This study showed that short-term TRP stimulation increases IgE-mediated but not histamine-induced wheal and erythema responses.

Keywords: Histamine, IgE, mast cells, transient receptor potential, capsaicin, chronic spontaneous urticaria

Abbreviations:
CSU: Chronic spontaneous urticaria
FcεRI: The high-affinity IgE receptor
IL: Interleukin
MRGPRX2: MAS-related G protein-coupled receptor-X2
NGF: Nerve growth factor
PAR2: Protease-activated receptor 2
SP: Substance P
SPT: Skin prick test
TLR: Toll-like receptors
TNF: Tumor necrosis factor
TPO: Thyroperoxidase
TRP: Transient receptor potential
TRPV1: Transient receptor potential vanilloid 1
TSLP: Thymic stromal lymphopoietin
VAS: Visual analog scale
VIP: Vasoactive intestinal peptide

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INTRODUCTION

Mast cells are innate immune cells that have a variety of surface and cytoplasmic receptors that allow them to react to various stimuli. They have a well-known role in type-I hypersensitivity reactions, which are triggered by IgE-mediated activation of the cell-surface high-affinity receptor (FcεRI) (1). IgE/FcεRI signaling is a primary mechanism through which mast cells are activated and degranulated. Several activating signals, such as thymic stromal lymphopoietin (TSLP) and interleukins (IL-4, IL-5, IL-13, IL-33), as well as complement C5a and nerve growth factor (NGF), upregulate the susceptibility of mast cells to activation. Conversely, inhibitory receptors like Siglec-8, CD200R, Allergin-1, and CD300a downregulate this susceptibility (2,3).

Chronic spontaneous urticaria (CSU) is a common and complex disease, characterized by itchy wheals and/or recurrent angioedema that result from the activation and degranulation of skin mast cells (4). We recently reported that nearly half of CSU patients reported worsening urticarial symptoms shortly after the intake of hot pepper (5). Therefore, we speculated that capsaicin, the natural pungent in hot pepper (*Capsicum spp*.), may aggravate CSU by promoting skin mast cell activation. Topical application of capsaicin to the skin results in the release of neuropeptides including substance P (SP) from sensory skin nerves, via the activation of the transient receptor potential vanilloid 1 (TRPV1) channel. This, in turn, induces itch, pain, and erythema, but not wheals. Currently, it remains unclear whether capsaicin pretreatment lowers the activation and degranulation thresholds for skin mast cells.

Transient receptor potential (TRP) channels are a large, functionally diverse family of cation-conducting channel proteins that are activated and controlled by multiple signals and mechanisms (6). TRP proteins in mammals include six main subtypes: TRPA (ankyrin), TRPV (vanilloid), TRPM (melastatin), TRPC (canonical), TRPML (mucolipin), and TRPP (polycystin). They contribute to cell depolarization and calcium entry upon activation and are involved in sensing of a variety of environmental stimuli such as temperature, pH, and osmolarity. TRP proteins are expressed in many excitable and non-excitable cells, including mast cells and sensory neurons. TRPV1, a member of TRPV family, was shown to be activated by vanilloid compounds, such as capsaicin found in hot chili and bell peppers (7). The involvement of the TRPV channel is crucial for the response of many different mediators, including histamine, serotonin, substance P, IL-31, IL-33, IL-4, IL-13, and TSLP (8). After binding to their corresponding receptor, all further activate the downstream TRP channels by secondary messenger molecules (9).

In CSU, skin mast cells exhibit a decreased threshold for activation (10), but the underlying priming mechanisms are unknown. Possible candidates included neuropeptides such as substance P, which are upregulated in CSU and linked with disease activity (11). The effects of SP released by capsaicin are mediated via activation of MAS-related G protein-coupled receptor-X2 (MRGPRX2), which is expressed at high levels in human skin mast cells (12), and SP is a potent activator of human skin mast cells *in vivo* and *in vitro* (2,13,14). In addition, SP can prime human mast cells, i.e., alter their response to subsequent activation, for example by increasing their expression of surface receptors (15, 16). It was also shown that mice lacking TRPA1 show reduced SP-evoked scratching behavior, suggesting that TRPA1 acts downstream of SP release in skin to elicit itch (17).

In many patients with CSU, skin mast cells are activated by autoantibodies, either IgE autoantibodies to thyroperoxidase (TPO), IL-24, and other autoantigens or IgG autoantibodies to IgE or its high-affinity receptor FcεRI (18,19). CSU patients with IgE to TPO exhibit positive skin prick test (SPT) responses to TPO, very similar to SPT responses to environmental allergens such as grass pollen in allergic patients (20). We, therefore, used SPT with grass pollen extract in sensitized individuals as a model to explore the possible effects of capsaicin on CSU wheal and flare development. In addition, we performed skin provocation testing with histamine to assess the effects of capsaicin on histamine-induced itch responses. The primary aim of this proof-of-concept study was to test whether mast cell-driven wheal and erythema responses are modified by short-term pretreatment with capsaicin.

MATERIAL and METHODS

Study Design and Implementation

Healthy volunteers were subjected to topical treatment with capsaicin, followed by skin prick testing with allergen and histamine. All procedures were performed at 20°C to neutralize the possible confounding effects of warm temperatures on TRP channels. Participants were in steady state, and skin application areas were not mechani-

cally stressed (e.g., no scratching or tight clothing) for 10 minutes before the procedures. None of the subjects were on any medication that interferes with skin prick test responses, and all provided written informed consent.

Assessment of Allergen-Induced vs. Histamine-Mediated Wheal and Flare Responses

Eight individuals, all with proven grass pollen sensitization (either with SPT or serum specific IgE measurements), were included. On the volar surface of the "study arm", we applied approximately 1/4 fingertip unit of capsaicin cream (Şanlı® kapsikumlu merhem) to two different areas separated by 5 cm. The "control arm" received equivalent vehicle cream (placebo) in corresponding areas. After 5 minutes, the pretreated areas were cleaned with a dry swab. We then performed skin prick testing with histamine (0.1%) at one of the pretreated sites of the capsaicin-pretreated arm (study arm) and at the corresponding site of the control arm and then with grass pollen extract (6-grass pollen mixture, Allergopharma GmbH & Co. KG, Reinbek, Germany) at the other capsaicin-pretreated site on the study arm and the corresponding site of the control arm (Figure 1). After 15 minutes, wheal and flare responses were assessed by a blinded, independent, and experienced nurse, and mean ± standard error of mean (SEM) wheal and flare areas were calculated from the maximum and midpoint orthogonal diameters, as measured with a transparent ruler (Figure 2).

Assessment of Histamine-Induced Itch Responses

Eight additional volunteers were investigated for their itch responses after skin provocation with histamine at capsaicin-pretreated and placebo-pretreated skin sites. We applied ~1/4 fingertip unit of capsaicin cream (Capsagamma Dolor Creme® [53 mg/100 g] with 0.05% capsaicin) to a 2 cm^2 skin area on one arm and the same amount of vehicle cream (placebo) to the corresponding area of the other arm. After 5 minutes, the pretreated areas were cleaned with a dry swab. We then performed SPTs with histamine (0.1%) at the pretreated sites of the study arm and the control arm. Subjects recorded their itch intensity every 1 min for 30 minutes or until it completely resolved using a 100-mm visual analog scale (VAS).

Statistical Analyses

Comparisons of the maximum diameters and average areas of wheal and erythema were performed using the Wilcoxon test. VAS scores from the itch intensity between capsaicin-pretreated and placebo-pretreated groups were compared with repeated measures of ANOVA. A *p-value* <0.05 was accepted as statistically significant.

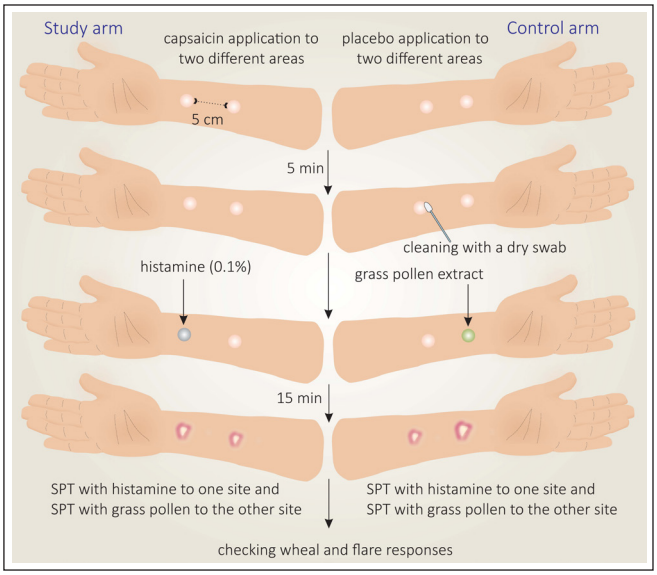

Figure 1. Schematic representation of the study design, illustrating the sequence of capsaicin and placebo pretreatments, skin prick tests (SPT) with grass pollen extract and histamine, and the evaluation of wheal and erythema responses.

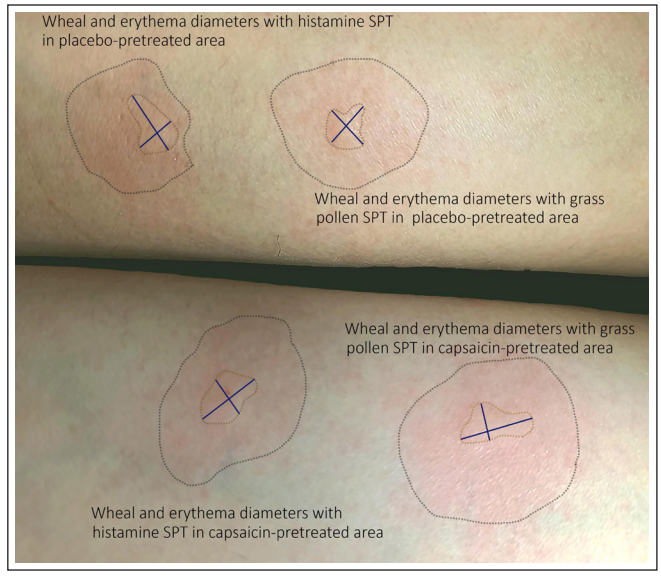

Figure 2. Photographic representation of wheal and erythema responses post-skin prick tests with grass pollen extract and histamine on capsaicin and placebo-pretreated areas. The lines indicate maximum and midpoint orthogonal wheal diameters, and the circles outline erythema margins.

RESULTS

Capsaicin Pretreatment Increases Allergen-Induced Mast Cell-Driven But Not Histamine-Mediated Wheal and Flare Responses

Our results revealed a significant difference between the maximum wheal diameter in the skin prick test with grass pollen extract between capsaicin and placebo pretreated areas (12.5±3.7 mm vs. 7.9±3.4 mm respectively, p=0.02). In contast, skin prick testing performed using histamine did not show a significant difference between capsaicin and placebo-pretreated areas (5.6±0.7 mm vs 5.3±1.3 mm respectively, p=0.43). (Figure 3A) The alteration in the average size of wheal area in skin prick tests with grass pollen extract showed a notable difference between capsaicin- and placebo-pretreated areas (76.4±31.0 mm^2 vs 35.3±17.4 mm^2 respectively, p=0.01) while no significant difference was seen for skin prick testing with histamine between capsaicin- and placebo-pretreated areas (23.4±5.0 mm^2 vs 20.6±7.9 mm^2 respectively, p=0.35). (Figure 3B).

As for the maximum erythema diameter, skin prick tests with grass pollen extract induced larger diameter lesions in both capsaicin and placebo-pretreated areas as compared to histamine skin prick tests. For grass pollen extract, the erythema diameter in capsaicin- pretreated areas was numerically larger (38.5±13.9 mm) than for placebo (28.8±14.5 mm), but this was not statistically significant (p=0.07). Similar results were obtained for histamine (30.9±7.3 mm [capsaicin-pretreated] vs. 26.3±8.7 mm [placebo-pretreated]; p=0.16). (Figure 3C)

For skin prick tests with the grass pollen extract, capsaicin-pretreatment also resulted in a significantly larger erythema area compared to placebo-pretreatment (973±709 mm^2 vs 556±499 mm^2 ; p=0.02). Skin prick testing with histamine did not produce erythema lesions of statistically significantly larger area size in capsaicin-pretreated skin (581±317 mm^2) as compared to placebo-pretreatment (424±260 mm^2, p=0.09) (Figure 3D)

Capsaicin Application Does Not Significantly Affect Histamine-Induced Itch Responses

In skin pretreated with capsaicin, histamine-induced itch responses initiated immediately after the skin prick test. These responses peaked within two minutes and rapidly declined to near-baseline levels within 30 minutes (Figure 4). Histamine-induced itch responses in placebo-pretreated skin were very similar in intensity and kinetics, and statistical analyses showed no significant difference in the course, maximum, or duration of histamine-induced itch as compared to capsaicin-pretreatment, as assessed by repeated measures of ANOVA (p=0.822).

DISCUSSION

Our results demonstrate that short-term stimulation of TRP channels facilitates IgE-mediated but not histamine-induced whealing, erythema development, or itch. Most previous studies on the effects of capsaicin on mast cell-mediated skin inflammation assessed the effects of long-term, rather than short-term application of capsaicin. Importantly, short-term capsaicin exposure causes an acute stimulating effect on TRPV1 channels, whereas long-term application of capsaicin has desensitizing effects. In contrast to our results, older studies that investigated the effects of long-term application of capsaicin found that capsaicin pretreatment has no effects on wheal and flare responses or even inhibited them. For example, Weisshaar et al. showed that pretreatment with capsaicin for 5 days caused a significant decrease, by 29%, in histamine-induced wheal responses (21). McCusker et al. found that long-term capsaicin pretreatment had no significant effect on histamine-induced or allergen-induced wheal volumes, but erythema reactions were markedly reduced (22).

The most important finding of our study is that short-term capsaicin-mediated TRP stimulation increases IgE-mediated but not histamine-induced wheal and erythema responses. This suggests that TRP stimulation has a direct effect on skin mast cells, rather than on the effects of mediators and mechanisms downstream of mast cell degranulation, for example on histamine receptors or vascular permeability. In support of this finding, Kimata reported that increased physical and physiological stress increases allergen-induced skin wheal responses (pollen and/or mite-induced) without any effect on wheal responses induced by histamine (23). Kimata also reported that relaxation reduces allergen-induced, but not histamine-induced skin wheal responses (24). In the referenced studies, increased stress elevated plasma levels of nerve growth factor (NGF), SP, and vasoactive intestinal peptide (VIP), while decreased stress lowered them.

How are our findings relevant to CSU? Mast cells are the primary effector cells in CSU (25), and they interact with sensory nerves in urticarial skin (4). For example, mast cell-derived NGF increases skin innervation. More

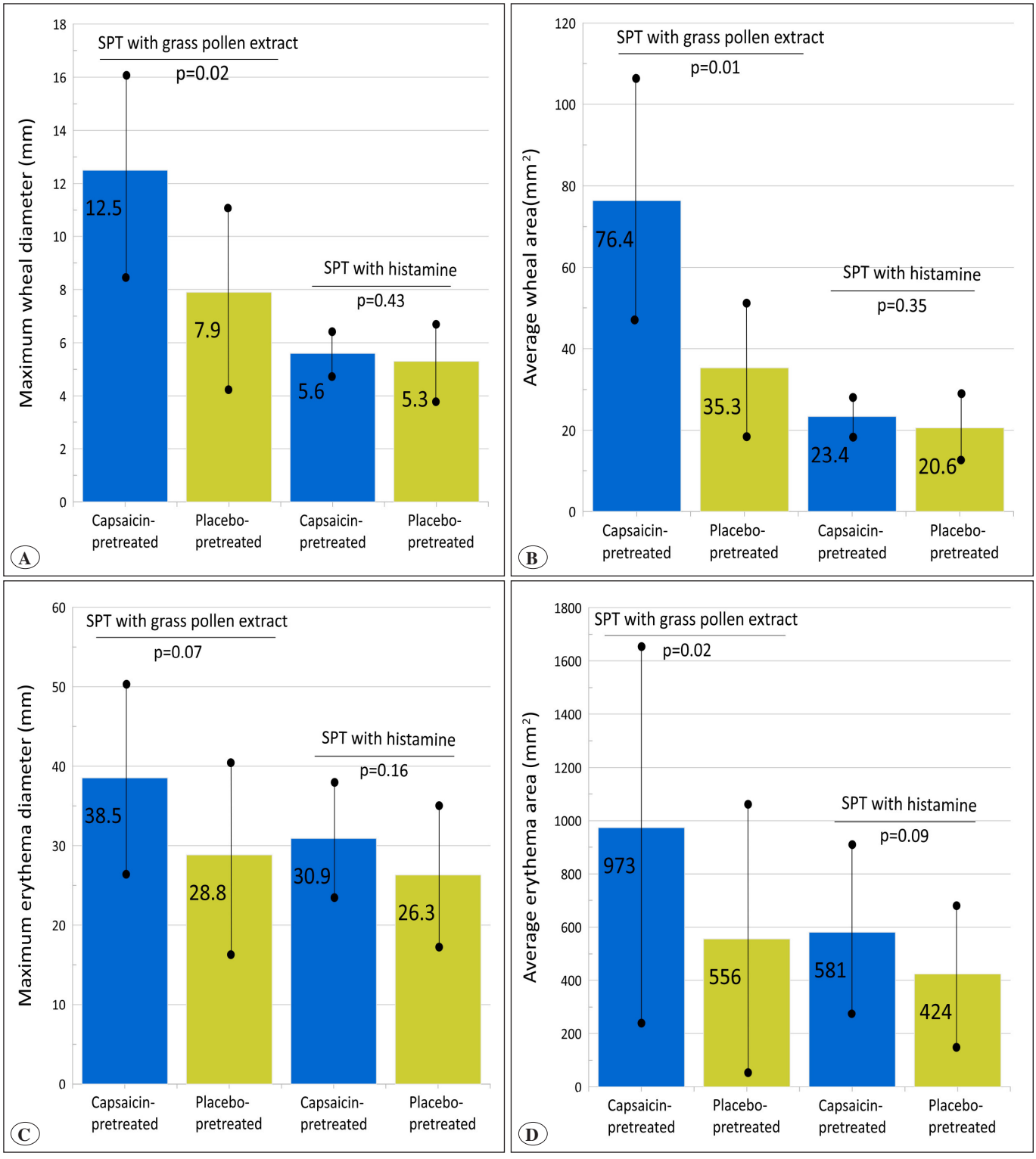

Figure 3. Comparison of wheal and erythema responses in capsaicin-pretreated skin following allergen and histamine skin prick tests. Maximum wheal diameter (**A**), average wheal area (**B**), maximum erythema diameter (**C**) and average erythema area (**D**) in capsaicin-pretreated areas were larger than the placebo-pretreated areas after skin prick testing with grass pollen extract. No differences in wheal and erythema responses between capsaicin-pretreated and placebo-pretreated areas were observed after skin prick testing with histamine.

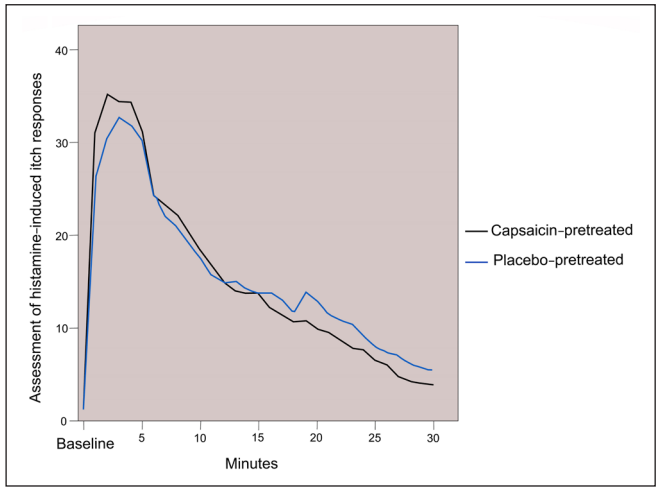

Figure 4. Itch intensity, assessed by 100-mm visual analog scale, after prick testing with histamine at capsaicin-pretreated and placebo-pretreated skin sites were very similar in intensity and kinetics.

importantly, sensory nerve-derived SP, VIP, and calcitonin gene-related peptide induce mast cell activation, and all of them are increased in CSU (4,24). The effects of sensory nerve-derived mediators on skin mast cells are largely driven by MRGPRX2, a receptor that mediates IgE-independent activation of mast cells (26). MRGPRX2 on skin mast cells is also responsible for their response to various endogenous stimuli such as neuropeptides and eosinophil granule proteins (26). Skin mast cells of patients with severe CSU were reported to overexpress MRGPRX2, compared to health controls, and this was correlated with disease severity (27). Increased skin mast cell-responses after capsaicin-pretreatment are likely driven, at least in part, by the release of SP from sensory nerves and its effects on mast cells via MRGPRX2, which include priming of mast cells and altering their response to subsequent activation, possibly by increasing their expression of surface receptors (Figure 5) (15,16). Incubation of mast cells with SP

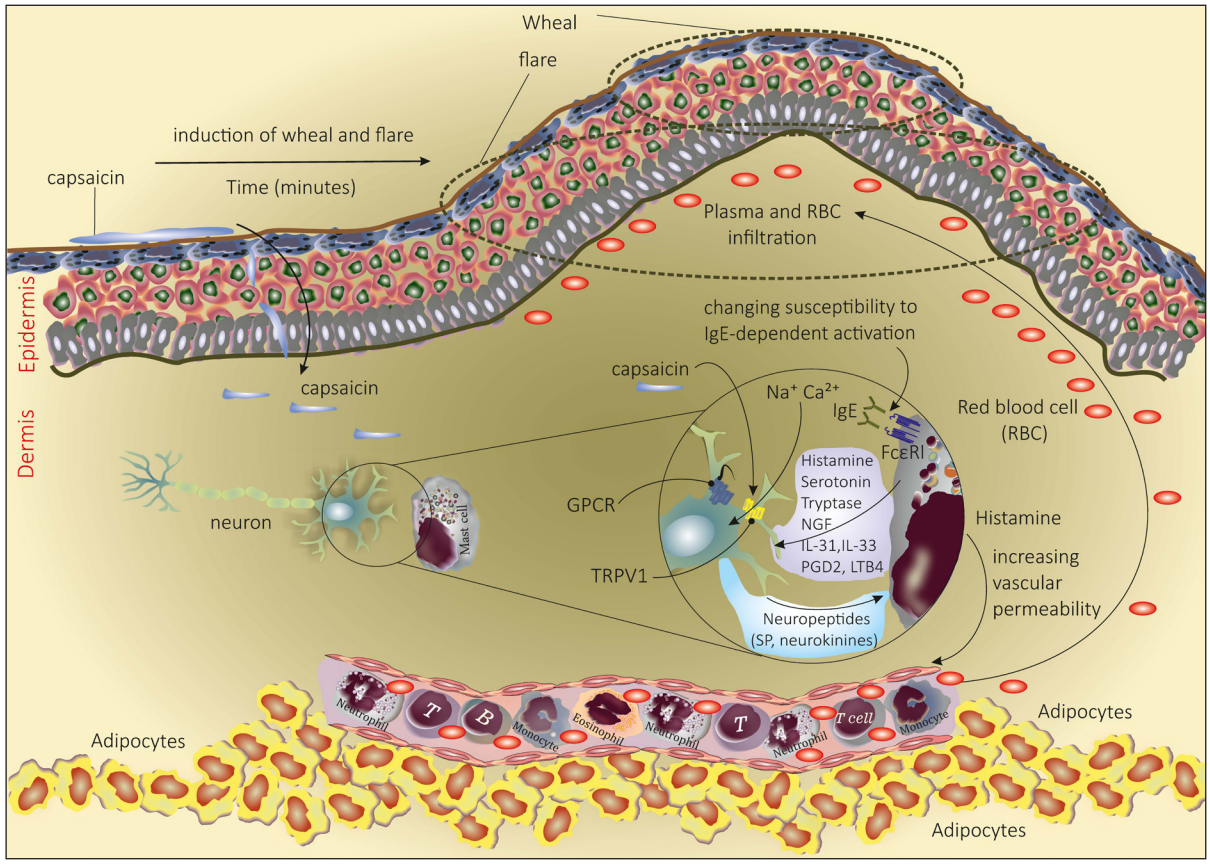

Figure 5. Illustration of the bidirectional communication between sensory nerves and mast cells, offering a probable explanation for our study findings. In the skin, there is a bidirectional interaction between sensory nerves and mast cells. Mast cell-derived NGF increases skin innervation and sensory nerve-derived neuropeptides induce mast cell activation. Short-term capsaicin application likely elevates the levels of neuropeptides in the skin and primes mast cells for subsequent activation via MRGPRX2, thereby increasing their susceptibility to IgE-mediated degranulation.

increased intracellular calcium levels, which was almost completely abolished by selective MRGPRX2 silencing (28). However, SP is not the only neuropeptide released from sensory nerves that exhibits MRGPRX2-mediated activating effects on mast cells. Cortistatin, for example, also activates skin mast cells via MRGPRX2 and induces itch, wheal, and flare reactions, which are concentration dependent and exhibit kinetics similar to those induced by substance P (29).

The activation of TRP channels by capsaicin is linked to many different inflammatory mechanisms and pathways that are independent of MRGPRX2, and some of them may be relevant for our findings. A specific subset of nociceptors is exclusively sensitive to capsaicin, and thereby defined as "capsaicin-sensitive" sensory neurons. These neurons comprise a subgroup of neurons defined as peptidergic because of their ability to produce calcitonin gene-related peptide, SP and neurokinin A, and cause inflammatory responses referred as neurogenic inflammation (30). SP has been shown to activate mast cells also via neurokinin receptors, with subsequent secretion of tumor necrosis factor (TNF) and IL-1, two potent pro-inflammatory cytokines. Capsaicin was also shown to stimulate expression of TNF-α (31). Taracanova et al. showed markedly increased TNF and IL-1 secretion from human mast cells when SP was administered in combination with IL-33 (32, 33). IL-33 synergizes with IgE-dependent and IgE-independent mast cell-activating signals, and mast cells express very high levels of IL-33 receptor (IL1RL1/ST2) (34). Interestingly, allergen-induced epithelial IL-33 secretion involves TRPV1 in human airways (35). Other than cytokines, innate immune responses also play a crucial role in mast cell responses, and toll-like receptors (TLR) were shown to modulate effector responses of mast cells (36). Lastly, pollen proteases may activate protease-activated receptor (PAR) 2, and TRP channels can regulate PAR2-dependent signaling (37,38).

Our study, a pilot and proof-of-concept study, has several limitations, which include the use of a topical capsaicin formulation that also contains menthol, camphor and methyl salicylate, whereas our vehicle control did not. Although their concentrations are low, these components stimulate different TRP channels, and it is not possible to pinpoint the TRP channel(s) responsible for our results. Future studies will need to address this by the stimulation of different single TRP channels. Also, we need to repeat a similar protocol on CSU patients' skin.

It is not possible to define the underlying mechanisms of our results by this proof-of-concept study. The most probable explanation is that short-term capsaicin application, similar to stress, increases neuropeptide and/or neurotrophin skin levels and primes mast cells, which results in their increased susceptibility to IgE-mediated degranulation (Figure 5). It is unlikely, but not impossible, that our results are explained by direct effects of capsaicin on skin mast cells. In support of this notion, Bíró et al measured serotonin release from rodent mast cell lines originally belonging to internal organs upon vanilloid treatment and found that mast cell degranulation was not directly coupled with the vanilloid receptor or the mediated calcium influx (39). Further studies should aim to confirm the results of our study and extend them to investigate other mast cell-activating pathways including skin mast cell degranulation by IgE and IgG autoantibodies involved in the pathogenesis of CSU.

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Institutional Review Board Statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board.

Informed Consent Statement

Informed consent was obtained from all subjects involved in the study.

Data Availability Statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to local regulations.

Conflict of Interest

The authors have no conflicts of interest to declare regarding any aspect of this study.

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