Modulation of Itch by Localized Skin Warming and Cooling

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Skin thermal changes modulate itch sensitivity. However, the mechanisms of this modulation are still unclear. Using mouse models of acute and chronic itch, we investigated whether local innocuous thermal stimulation of the skin alters itch sensitivity and if blockade of thermosensitive transient receptor potential (TRP) channels can reduce these changes. Localized thermal changes were achieved by placing a thermal probe in contact with the back skin for 30 s. Warming the skin significantly increased serotonin-evoked scratching and spontaneous scratching in the ovalbumin model of atopic dermatitis but decreased histamine-evoked scratching. These changes were blocked by a TRPV4 antagonist. Cooling the skin significantly increased serotonin-evoked scratching but reduced histamine-evoked scratching. The increase in serotonin-evoked scratching, but not the reduction of histamine-evoked scratching, was blocked by TRPM8 antagonism. Chloroquine-evoked scratching was unaffected by either warming or cooling. Our data indicate that different itch signaling pathways are differentially modulated by skin thermal changes.

Key words: temperature; chronic itch; atopic dermatitis; scratching; TRPV4; TRPM8.

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The perception of itch is modulated by skin temperature as affected either by the environment or by experimental local change. Human psychophysical studies consistently show that noxious thermal stimulation reduces histamine- and cowhage-evoked itch and spontaneous itch in patients with atopic dermatitis (AD) (1–6). Such noxious counter-stimulation can reduce itch when applied distally or contralaterally to the site of itch induction (1, 3, 7) and activates the periaqueductal gray, the control center for descending pain modulation (8). These results suggest that endogenous central inhibitory systems play a major role in the antipruritic effects of counter-stimulation. However, there has been comparatively limited research in the effects of innocuous thermal stimuli on itch and whether these stimuli are sufficient to drive central inhibitory systems.

Most human studies of innocuous temperature modulation of itch have been conducted using histamine. Innocuous cold stimulation has repeatedly been shown to significantly reduce histamine-evoked itch (1, 2, 9). In one study, innocuous warmth was reported to reduce histamine-evoked itch in a majority of subjects, but not all (4). Other studies consistently show an insignificant decrease in histamine-evoked itch by warming the skin (1–3). However, most types of chronic itch are not mediated by histamine, and thermal modulation appears to have different effects in the chronic itch state. As with histamine-induced itch, cooling of the skin is commonly reported to alleviate itch in AD (10, 11). In contrast, questionnaire data from patients with AD show that warmth is one of the major aggravating factors for itch in this disease (12, 13).

Itch is mediated by multiple neural pathways (14–17). Histamine, mainly released from mast cells and basophils in the skin, evokes itch through histamine receptors H1R and H4R (18). Chloroquine, an anti-malarial drug which activates Mas-related G protein-coupled receptor member A3, induces acute itch through a non-histaminergic pathway (19). Another histamine-independent itch mediator, serotonin, is released by platelets and activates 5-hydroxytryptamine type 2 (5-HT2) and 5-HT7 receptors to induce non-histaminergic itch (20, 21). Interestingly, serotonin-evoked itch, but not chloroquine-evoked itch, is enhanced by ambient warm temperature (22), implying that the effect of temperature on itch intensity differs among different types of pruritogens. However, the effects of temperature on different types of itch, especially histamine-independent itch, are poorly understood.

One possibility is that innocuous temperature changes modulate skin perfusion and thereby alter the concentration and distribution of pruritogens. While local cooling of the skin causes a localized vasoconstriction, local warming of the skin causes a localized vasodilation (23,
Skin surface temperature was measured using a microprobe thermometer (BAT-12; Physitemp Instruments, Inc., Clifton, NJ) following temperature probe contact and also following intradermal (i.d.) injection of histamine, serotonin, and chloroquine. None of the tested pruritogens themselves significantly altered skin temperature (Fig. S11), which is generally consistent with a previous study (29).

Behavioral tests

Mice were habituated twice to a Plexiglas recording arena for 60 min before testing. A thermal probe (0.5-inch diameter; NTE-2A; Physitemp Instruments, Inc.) set at a constant 18°C (innocuous cold), 33°C (control, baseline skin temperature), or 38°C (innocuous warmth) was placed in contact with the shaved skin surface of the rostral back for 30 s. For acute itch studies, immediately after thermal probe contact, mice received a 10-μl i.d. injection of either histamine (50 μg, Sigma-Aldrich), serotonin (10 μg, Sigma-Aldrich), or chloroquine (100 μg, Sigma-Aldrich) into the center of the thermal probe-contacted area, and scratching behavior was videotaped from above for 30 min. For OVA-treated mice, behavioral testing was conducted on Day 35 of the model. Following thermal probe application to the OVA-treated area of the back, spontaneous scratching was recorded for 30 min. The number of scratch bouts was counted by a trained observer blinded to the treatment condition. A scratch bout was defined as one or more rapid back-and-forth hind paw motions directed toward and contacting the treated area, ending with licking or biting of the toes or placement of the hind paw on the floor. Hind paw movements directed away from the treated area (e.g., ear-scratching) and grooming movements were not counted (28, 30, 31). The onset time of the first scratching behavior following either i.d. injection (acute itch experiments) or temperature probe removal (OVA experiments) was also recorded.

In TRP antagonist experiments, each animal received an i.p. injection of either vehicle (0.5% hydroxypropyl methylcellulose, 0.2% Tween-80, and 0.5% DMSO in saline), TRPV4 antagonist GSK205 (10 mg/kg; EMD Millipore, Billerica, MA), or TRPM8 antagonist AMTB hydrochloride (25 mg/kg; Alomone Labs, Jerusalem, Israel). GSK205 (or vehicle) was injected 10 min before temperature probe contact, and AMTB (or vehicle) was injected 30 min before temperature probe contact.

Cold plantar test

The cold plantar test was performed as described previously (32). Mice were acclimated to plastic chambers for 3 h. A dry ice pellet was then applied to the hind paw through a floor plate. The paw withdrawal latency was measured 4 times at an interval of at least 5 min, and the median latency of 4 trials was used for analysis. Mice were tested before and 30, 120, and 180 min after i.p. injection of TRPM8 antagonist or vehicle.

Skin perfusion imaging

Ears of anesthetized mice were positioned on a glass cover slip and imaged using a Leica DM6000B microscope (Leica, Inc., Exton, PA). Blood vessels were visualized using a photoplethysmographic technique under 488 nm excitation. To confirm effects of pruritogens on local perfusion and as a positive control for our system, images were taken at baseline and 5 min after i.d. injection of histamine or serotonin to the adjacent skin (approximately 1 cm from imaging site). To examine the effects of temperature stimulation on local perfusion, images were taken before and 5 min after a 30-s application of 18°C or 38°C temperature probe to the imaging site.
Data analysis
Between-group comparisons were made by one-way or two-way ANOVA with repeated measures followed by the Bonferroni post-test or by unpaired or paired t-test. In all cases, \( p < 0.05 \) was considered to be significant.

RESULTS

Effects of local warming of the skin on itch

To confirm that the temperature probe induced local skin warming, we measured skin surface temperature following contact of the thermal probe set at 33°C (control, baseline skin temperature) or 38°C. Following the 38°C probe contact, the mean ± standard error of mean (SEM) skin temperature increased to 35.2 ± 0.1°C and returned to baseline within 8 min (Fig. 1A). The same 38°C probe application did not change vasomotor tone (Fig. S21).

Warming the skin significantly delayed the onset of histamine-evoked scratching behavior and reduced the number of scratch bouts in the first and second 5-min bins (Fig. 1B, F). In contrast, warming the skin shortened the onset of serotonin-evoked scratching behavior and increased the number of scratch bouts in the first 5 min (Fig. 1C, F). Neither the onset or time course of chloroquine-evoked scratching behavior was changed by warming the skin (Fig. 1D, F). Finally, in the OVA-treated mouse model of AD, warming the skin significantly shortened the onset of scratching behavior and increased the number of spontaneous scratch bouts in the first 5 min of recording (Fig. 1E, 1F).

Effects of TRPV4 antagonist on warming-induced modulation of itch

To investigate the role of TRPV4 in modulation of itch by warming the skin, we pretreated mice with an i.p. injection of TRPV4 antagonist or vehicle control 10 min prior to warming the skin. Mice treated with the TRPV4 antagonist before skin warming showed significantly faster histamine-evoked scratching onset and increased number of scratch bouts compared to mice who received the vehicle injection (Fig. 2A). In contrast, the TRPV4 antagonist significantly decreased serotonin-evoked scratching behavior and spontaneous scratching in OVA-treated mice following warming and delayed the onset of scratching behavior (Fig. 2B, C). These results suggest that TRPV4 antagonist treatment reversed the effects of local skin warming on itch.

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Fig. 1. Effects of innocuous warmth stimulation on acute itch evoked by different pruritogens and on spontaneous itch in an atopic dermatitis (AD) model. (A) A thermal probe set at a constant 33°C (baseline skin temperature) or 38°C was placed in contact with the shaved rostral back skin for 30 s. Skin temperature was elevated following the 38°C stimulus and returned to baseline within 8 min (\( n = 6/\text{group} \)). Following a 30-s thermal probe application (33°C or 38°C), (B) histamine (\( n = 6/\text{group} \)) (C) serotonin (\( n = 6/\text{group} \)), (D) chloroquine (\( n = 10–12/\text{group} \)) was intradermally injected within the contacted area. Immediately after the injection, scratching behavior was videotaped for 30 min. The time course of scratching behavior is displayed in 5-min bins. *\( p < 0.05 \), significant difference from 33°C-treated group (two-way repeated measures ANOVA followed by Bonferroni post hoc test). (E) On Day 35 of the ovalbumin (OVA)-induced model of AD, the thermal probe set at either 33°C or 38°C was applied to the OVA-treated area for 30 s. Immediately after the application, spontaneous scratching behavior was videotaped for 30 min. The time course of scratching behavior is displayed in 5-min bins (\( n = 7/\text{group} \)). (F) Average onset time of first scratching behavior following an injection of pruritogen or thermal probe application in OVA-treated mice. *\( p < 0.05 \), significant difference from 33°C-treated group (unpaired t-test or paired t-test, \( n = 6–7/\text{group} \)). Error bars are standard error of mean.
Effects of local cooling of the skin on itch

To confirm that the temperature probe induced local skin cooling, we measured the skin surface temperature following contact of the thermal probe set at 33°C or 18°C. Following 18°C probe contact, the mean ± SEM skin temperature decreased to 31.9 ± 0.1°C and returned to baseline within 8 min (Fig. 3A). The same 18°C probe application did not change vasomotor tone (Fig. S21).

Cooling the skin significantly delayed the onset of histamine-evoked scratching behavior and reduced the number of scratch bouts in the second 5-min bin (Fig. 3B, F). In contrast, the same cooling treatment shortened the onset of serotonin-evoked scratching behavior and

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Fig. 2. Effects of TRPV4 antagonist on innocuous warmth modulation of itch. (A) TRPV4 antagonist or vehicle (n=6–7/group) and (B) serotonin (n=14/group) was intraperitoneally injected 10 min before temperature modulation. The thermal probe (38°C) was applied to the back skin for 30 s. Immediately after the application, the mouse received an intradermal histamine injection, and scratching behavior was videotaped. Bar graphs show the total number of scratch bouts from 0–5 min after the intradermal injection and the onset of scratching behavior. *p < 0.05, significant difference from vehicle-treated group (unpaired t-test). (C) In OVA-treated mice, TRPV4 antagonist or vehicle was intraperitoneally injected 10 min before thermal probe application. The thermal probe (38°C) was applied to the back skin for 30 s. Immediately after the application, spontaneous scratching was videotaped. *p < 0.05, significant difference from vehicle-treated group (paired t-test, n=6/group). Error bars are standard error of mean.

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Fig. 3. Effects of innocuous cold stimulation on acute itch evoked by different pruritogens and on spontaneous itch in an atopic dermatitis model. (A) A thermal probe set at a constant 33°C (baseline skin temperature) or 18°C was placed in contact with the shaved rostral back skin for 30 s. Skin temperature was reduced following the 18°C stimulus and returned to baseline within 8 min (n=6/group). Following a 30-s thermal probe application (33°C or 18°C), (B) histamine (n=9/group), (C) serotonin (n=6/group) and (D) chloroquine (n=8/group) was intradermally injected within the contacted area. Immediately after the injection, scratching behavior was videotaped for 30 min. The time course of scratching behavior is displayed in 5-min bins. *p < 0.05, significant difference from 33°C-treated group (two-way repeated measures ANOVA followed by Bonferroni post hoc test). (E) On Day 35 of the ovalbumin (OVA)-induced model of atopic dermatitis, the thermal probe set at either 33°C or 18°C was applied to the OVA-treated area for 30 s. Immediately after the application, spontaneous scratching was videotaped. *p < 0.05, significant difference from vehicle-treated group (paired t-test, n=6/group). Error bars are standard error of mean.

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increased the number of scratch bouts in the first 5-min bin (Fig. 3C, F). Neither the onset of chloroquine-evoked scratching nor the number of scratch bouts was changed by cooling the skin (Fig. 3D, F). Cooling the skin significantly delayed the onset of scratching behavior in the OVA-induced mouse model of AD (Fig. 3E, F).

**Effects of TRPM8 antagonist on cooling-induced modulation of itch**

First, we determined the timing of TRPM8 antagonist injection by using the cold plantar assay. The hind paw withdrawal latency from the cold stimulus was $2.6 \pm 0.2$ s before TRPM8 antagonist treatment (Fig. 4A). This latency was significantly increased compared to baseline 30 min after TRPM8 antagonist injection and returned to baseline within 3 h. Vehicle treatment did not change the withdrawal latency. At the mean withdrawal threshold, the skin surface temperature of the hind paw was reduced by $1.3^\circ$C (Fig. 4B), which is generally consistent with a previous study (32).

To investigate the role of TRPM8 in modulation of itch by cooling the skin, the TRPM8 antagonist was injected 30 min before cooling the skin. Neither the onset of nor the number of histamine-evoked scratch bouts was changed by TRPM8 antagonist pretreatment (Fig. 4C). However, the TRPM8 antagonist significantly delayed the onset of serotonin-evoked scratching behavior and decreased scratch counts in the first 5-min bin (Fig. 4D). These results indicate that while the TRPM8 antagonist reversed the cooling-induced enhancement of serotonin itch, it did not affect the cooling-induced suppression of histamine itch.

**DISCUSSION**

Innocuous thermal stimulation modulates itch, but evidence for the mechanisms of this modulation is limited. Here we show that: (i) Histamine-evoked scratching was significantly reduced by both innocuous warming and innocuous cooling stimulation, which is generally consistent with previous human psychophysical studies (1, 2, 4, 9, 33). (ii) Serotonin-evoked scratching was significantly enhanced by innocuous thermal stimulation. (iii) Chloroquine-evoked scratching was not modulated by innocuous thermal stimulation. (iv) Spontaneous scratching in the OVA-induced mouse model of AD was enhanced by warming the skin, while spontaneous scratching was slightly reduced by innocuous cold stimulation. These findings, summarized in Table I, indicate that innocuous thermal stimulation differentially modulates different types of itch.

The effects of temperature modulation on itch were confined to the first 10 min of recording, matching the timeline of skin temperature change. Regardless of temperature stimulation, scratching subsided within 30 min as in normal conditions. These results suggest that temperature modulation blunted or enhanced scratching and did not merely delay the time course of itch. However, the mechanisms for this modulation appear to be complex.

Local skin thermal changes affect vasomotor tone, which could potentially affect peripheral itch signaling. Indeed, pretreatment of the α2 agonist brimonidine reduced histamine-evoked vasodilation and increased the duration of itch without affecting itch peak latency or intensity (34). In this study, we successfully observed vasodilation or vasoconstriction following an injection
of histamine or serotonin, respectively. In contrast, innocuous warming or cooling was not sufficient to affect the vasomotor tone. As such, it appears that minor temperature changes can affect itch independently from changes in local blood perfusion. However, there may be more complex interactions of temperature, the vascular system, and itch. Cooling has been shown to potentiate endothelial nitric oxide production (35), and it is reported that nitric oxide mediates serotonin-evoked itch (36). Therefore, cold-induced nitric oxide may contribute to enhancement of serotonin-evoked itch.

Populations of sensory neurons expressing the warmth-sensitive TRP channel TRPV4 and/or the cold-sensitive TRP channel TRPM8 can directly detect local skin thermal changes. TRPV4 is expressed in most dorsal root ganglion (DRG) neurons expressing the chloroquine-activated receptor MrgrpA3 or the histamine H1 receptor and in most serotonin-responsive DRG neurons (21, 37). In this study, innocuous warming enhanced serotonin-evoked scratching, and this enhancement was inhibited by TRPV4 antagonist pre-treatment, suggesting that TRPV4 signaling contributes to this effect. This finding is in line with our previous report that calcium responses of DRG neurons to serotonin were significantly greater at a bath temperature of 35°C compared to lower temperatures (22).

In contrast to TRPV4, most TRPM8-expressing neurons do not express MrgrpA3 and largely do not overlap with the TRPV1-expressing neurons that play major roles in itch transmission (18, 38–41). As such, the mechanisms behind inhibition of itch by cold are thought to involve endogenous central inhibitory systems. However, the role of TRPM8 in inhibition of itch by cold is still debated (2, 5, 42–44). Some human psychophysical studies showed that the TRPM8 agonist menthol failed to inhibit histamine-evoked itch (43, 44), while other studies successfully inhibited histamine- and cowhage-evoked itch with menthol (2, 5). In the present study, TRPM8 antagonist treatment failed to block cold-induced inhibition of histamine-evoked itch. Additionally, cold did not inhibit either serotonin- or chloroquine-evoked itch. It appears that minor temperature changes are not sufficient to activate endogenous central inhibitory systems through TRPM8. It has been suggested that there are cold-sensitive channels other than TRPM8, and these could potentially contribute to cold inhibition of itch (45–47).

Our results suggest that innocuous temperature regulation of different pruritogens is complex and cannot be totally explained by vasomotor changes or TRP channel signaling in sensory neurons. Another way that temperature may modulate itch signaling is by affecting sodium channel kinetics that regulate neuronal excitability, though it is still unclear which sodium channels contribute to itch transduction. Cooling from 30°C to 10°C caused a slowing of activation kinetics and reduced peak values of both Nav1.7 and Nav1.8 sodium currents (48, 49). The decrease in current amplitudes on cooling was significantly stronger for Nav1.7 than for Nav1.8. Thus, cooling could reduce itch through inhibition of Nav1.7.

Finally, the regulation of itch by temperature is necessarily more complex in the setting of chronic itch, where there are many different itch mediators elevated in the skin (50). These mediators may all exhibit different responses to thermal stimuli that contribute to an overall effect. We report that warming the skin enhances spontaneous itch in the OVA-induced mouse model of AD. Additionally, cooling the skin delayed the onset of spontaneous scratching in OVA-induced mouse model of AD. These findings are consistent with previous human studies (4, 12). Interestingly, of the three itch mediators tested, only histamine itch was inhibited by cold stimulation. Because itch in the late phase of the OVA model, as in AD patients, is non-histaminergic (51), it remains unclear which itch mediator is responding to cold-induced itch inhibition. However, we showed that TRPV4 plays a role in warmth-enhanced itch in the OVA model. Therefore, inhibition of TRPV4 could be a potential treatment for one of the major exacerbating factors of itch in AD.

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