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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SIGNIFICANCE

Many people report that temperature changes can increase or decrease itch. This study tests how gentle warm and cool temperature stimulation affects itch from 3 different chemicals and one atopic dermatitis model. Warming and cooling the skin decreased scratching in mice that received histamine but increased scratching from serotonin. Neither temperature change affected scratching from chloroquine. In the atopic dermatitis model, warming the skin increased scratching, and cooling slightly delayed scratching. In some cases, but not all, temperature-sensitive channels were involved in these effects. This study reveals that gentle temperature modulation of itch is complex and may involve multiple pathways.

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,

24). Vasoconstriction might slow the diffusion rate of pruritogens from the injection site and thereby increase the local pruritogen concentration, and vasodilation may have the opposite effect. These effects are compounded by the fact that itch mediators themselves influence vasomotor tone. For example, histamine is a potent vasodilator, while serotonin induces vasoconstriction (25, 26).

Another possibility is the direct influence of temperature on cutaneous sensory neurons. Temperature sensation is typically signaled via the transient receptor potential (TRP) family of ion channels. Each member of this family is activated by a specific range of temperatures. TRP vanilloid 4 (TRPV4), which responds to innocuous warming, has previously been implicated in modulating serotonin-induced itch (21). TRP melastatin 8 (TRPM8), which responds to innocuous cooling, has been studied in the context of itch inhibition. For example, itch-inhibitory spinal neurons receive direct input from sensory neurons that respond to menthol, a TRPM8 agonist (27). Therefore, these channels could potentially play a role in itch modulation by innocuous thermal stimuli.

We presently investigated whether innocuous warming or cooling stimulation to the back skin affects acute itch induced by either histamine, chloroquine, or serotonin as well as spontaneous itch in an ovalbumin (OVA)-induced AD mouse model. In addition, we measured the effects of this innocuous thermal stimulation on local perfusion and tested if antagonists for the warm-sensitive channel TRPV4 and the cold-sensitive channel TRPM8 could rescue itch.

MATERIALS AND METHODS

Animals

Experiments were performed using adult male C57BL/6J mice (23–31 g) under a protocol approved by the University of Miami Institutional Animal Care and Use Committee.

Ovalbumin sensitization

OVA sensitization was induced as previously described (28). Mice were given an intraperitoneal (i.p.) injection of OVA (100 µg; Sigma-Aldrich, St. Louis, MO), aluminum hydroxide (2.9 mg; Sigma-Aldrich), and pertussis toxin (300 ng Life Technologies, Grand Island, NY) on the first day. On Day 5, they received a subcutaneous injection of 50 µg of ovalbumin in saline. Fur on the rostral back was shaved with electric clippers. Then, local sensitization was performed once a day from Day 14 to Day 35 after the first systemic sensitization, as follows. Gauze (1 × 1 cm) soaked with 0.1% OVA (100 µl) in saline was applied to the shaved skin area. The treated skin area was covered with a patch (Tegaderm, 3M Health Care, St. Paul, MN). The next day, the patch was removed, and an identical piece of soaked gauze followed by Tegaderm patch was reapplied to the same skin area.

Skin temperature measurement

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. S1[1]), 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.

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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. 1**A). The same 38°C probe application did not change vasomotor tone (Fig. S2[1]).

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. 2**A). 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.

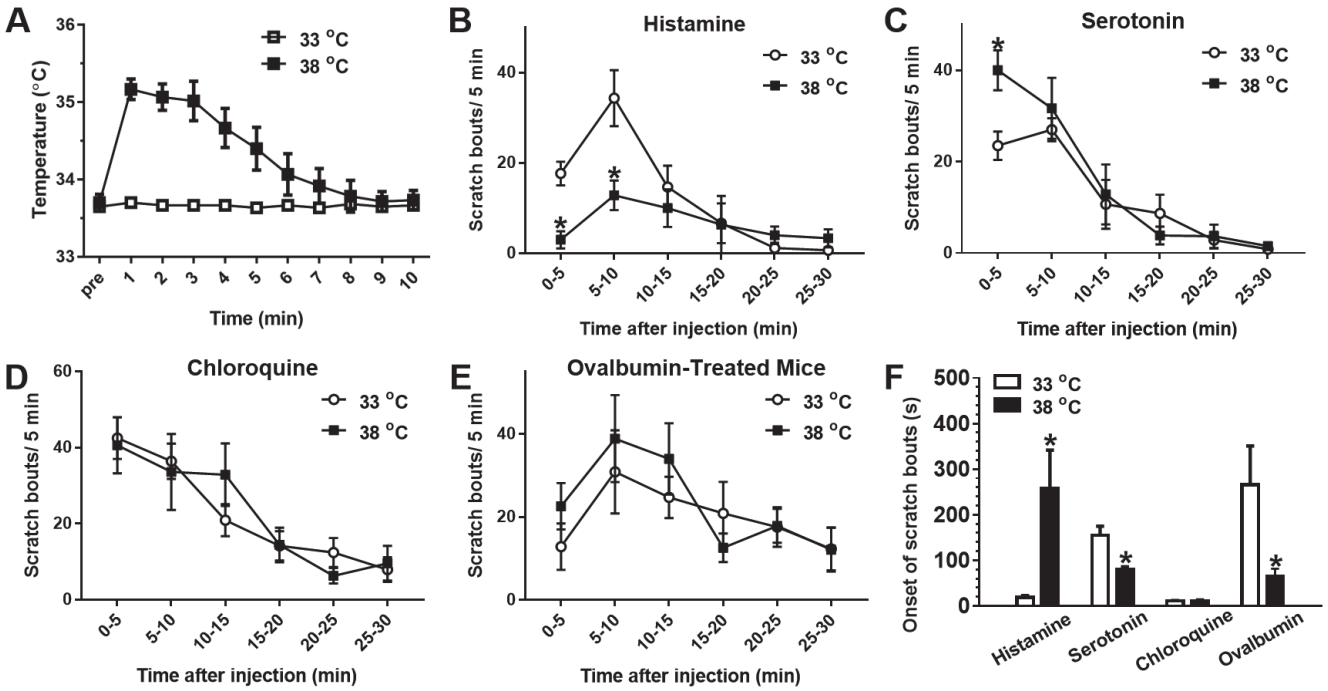

Fig. 1. Effects of innocuous warmth stimulation on acute itch evoked by different pruritogens and on spontaneous itch in an atopic dermatitis (AD) model. (A) A thermal probe set at a constant 33°C (baseline skin temperature) or 38°C was placed in contact with the shaved rostral back skin for 30 s. Skin temperature was elevated following the 38°C stimulus and returned to baseline within 8 min ($n = 6$/group). Following a 30-s thermal probe application (33°C or 38°C), (B) histamine ($n = 6$/group) (C) serotonin ($n = 6$/group), (D) chloroquine ($n = 10–12$/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 ($n = 7$/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, $n = 6–7$/group). Error bars are standard error of mean.

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.

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. 3**A). The same 18°C probe application did not change vasomotor tone (Fig. S2[1]).

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

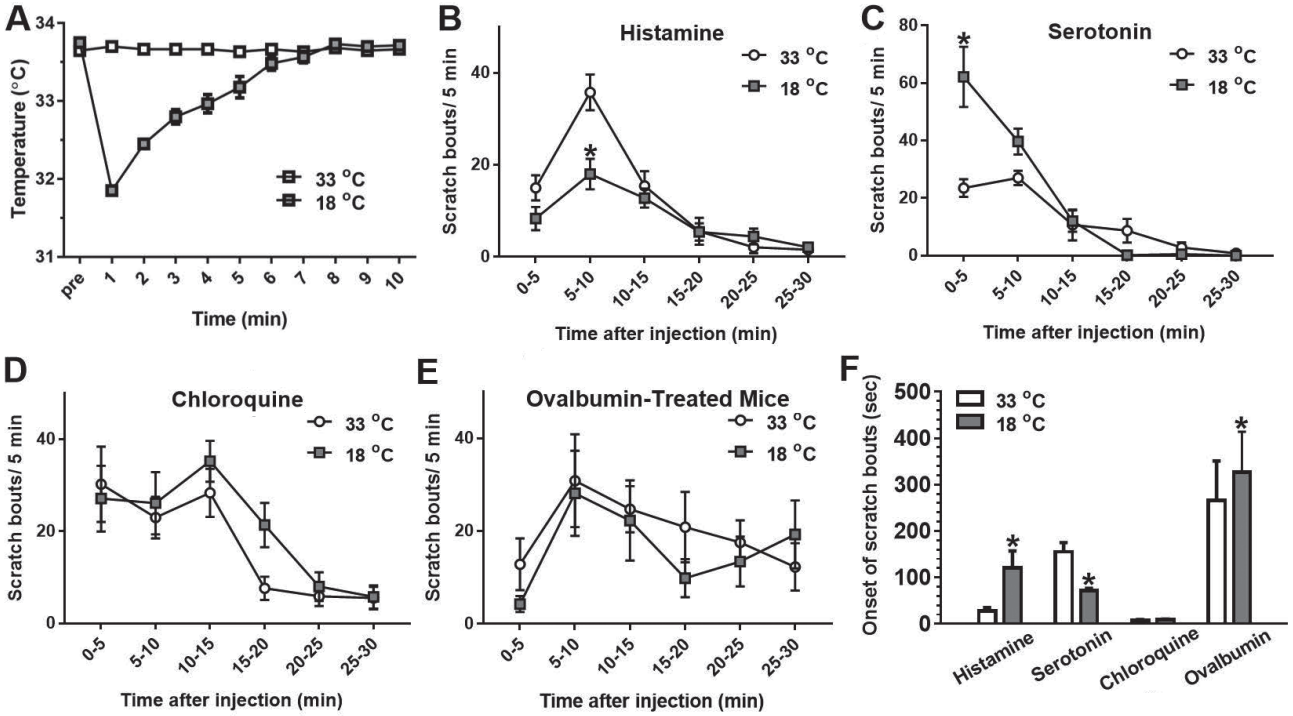

Fig. 3. Effects of innocuous cold stimulation on acute itch evoked by different pruritogens and on spontaneous itch in an atopic dermatitis model. (A) A thermal probe set at a constant 33°C (baseline skin temperature) or 18°C was placed in contact with the shaved rostral back skin for 30 s. Skin temperature 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 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, $n = 7$/group). (F) Average onset time of first scratching behavior following 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, $n = 6–7$/group).

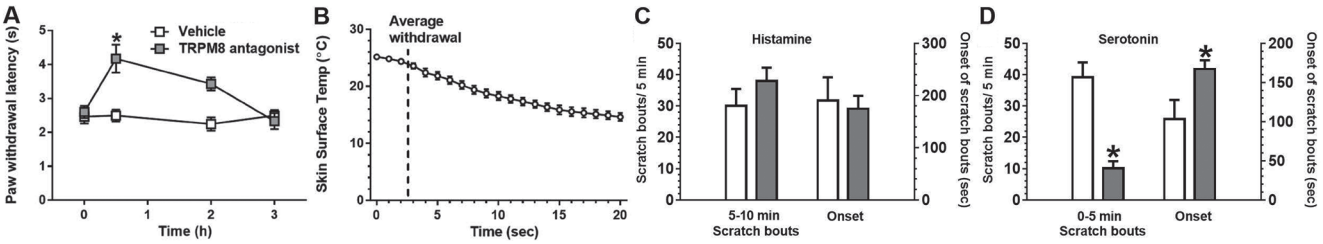

Fig. 4. Effects of TRPM8 antagonist on innocuous cold modulation of itch. (A) The cold plantar test was used to confirm TRPM8 antagonist timing. A dry ice pellet was applied to the hind paw through the floor. Withdrawal latencies were measured before and 30, 120, and 180 min after intraperitoneal injection of TRPM8 antagonist or vehicle. *$p <$ 0.05, significant difference from time 0 (one-way repeated measures ANOVA followed by Bonferroni test, $n = 8$/group). (B) Skin surface temperature was measured following dry ice pellet application. The dotted line indicates average withdrawal latency ($n = 8$/group). (C) TRPM8 antagonist or vehicle ($n = 8$/group) and (D) serotonin ($n = 7$/group) was intraperitoneally injected 30 min before temperature modulation. The thermal probe (18°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 (C) 5 to 10 min and (D) from 0 to 5 min after the intradermal injection and the onset of scratching behavior. *$p < 0.05$, significant difference from vehicle-treated group (unpaired *t*-test). Error bars are standard error of mean.

increased the number of scratch bouts in the first 5-min bin (Fig. 3C, F). Neither the onset of chloroquine-evoked scratching behavior 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 ± 0.2 s before TRPM8 antagonist treatment (**Fig. 4**A). 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°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

Table I. Summary of effects of innocuous temperature stimulation on itch and involvement of transient receptor potential (TRP) channels

	Innocuous warmth (38°C Probe)	Innocuous cold (18°C Probe)
Histamine	↓ scratch bouts	↓ scratch bouts
	↑ scratch onset	↑ scratch onset
	TRPV4-sensitive	Not TRPM8-sensitive
Serotonin	↑ scratch bouts	↑ scratch bouts
	↓ scratch onset	↓ scratch onset
	TRPV4-sensitive	TRPM8-sensitive
Chloroquine	No change in scratch bouts	No change in scratch bouts
	No change in scratch onset	No change in scratch onset
Ovalbumin model	No change in scratch bouts	No change in scratch bouts
	↓ scratch onset	↑ scratch onset
	TRPV4-sensitive	–

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 MrgprA3 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 MrgprA3 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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The authors have no conflicts of interest to declare.

REFERENCES

1. Yosipovitch G, Fast K, Bernhard JD. Noxious heat and scratching decrease histamine-induced itch and skin blood flow. J Invest Dermatol 2005; 125: 1268–1272.
2. Andersen HH, Melholt C, Hilborg SD, Jerwiarz A, Randers A, Simoni A, et al. Antipruritic Effect of Cold-induced and Transient Receptor Potential-agonist-induced Counter-irritation on Histaminergic Itch in Humans. Acta Derm Venereol 2017; 97: 63–67.
3. Ward L, Wright E, McMahon SB. A comparison of the effects of noxious and innocuous counterstimuli on experimentally induced itch and pain. Pain 1996; 64: 129–138.
4. Fruhstorfer H, Hermanns M, Latzke L. The effects of thermal stimulation on clinical and experimental itch. Pain 1986; 24: 259–269.
5. Bromm B, Scharein E, Darsow U, Ring J. Effects of menthol and cold on histamine-induced itch and skin reactions in man. Neurosci Lett 1995; 187: 157–160.
6. Murray FS, Weaver MM. Effects of ipsilateral and contralateral counterirritation on experimentally produced itch in human beings. J Comp Physiol Psychol 1975; 89: 819–826.
7. Andersen HH, van Laarhoven AIM, Elberling J, Arendt-Nielsen L. Modulation of Itch by Conditioning Itch and Pain Stimulation in Healthy Humans. J Pain 2017; 18: 1437–1450.
8. Mochizuki H, Tashiro M, Kano M, Sakurada Y, Itoh M, Yanai K. Imaging of central itch modulation in the human brain using positron emission tomography. Pain 2003; 105: 339–346.
9. Cormia FE, Kuykendall V. Experimental histamine pruritus. II. Nature; physical and environmental factors influencing development and severity. J Invest Dermatol 1953; 20: 429–446.
10. Brenaut E, Garlantezec R, Talour K, Misery L. Itch charac-

teristics in five dermatoses: non-atopic eczema, atopic dermatitis, urticaria, psoriasis and scabies. Acta Derm Venereol 2013; 93: 573–574.
11. Yosipovitch G, Goon AT, Wee J, Chan YH, Zucker I, Goh CL. Itch characteristics in Chinese patients with atopic dermatitis using a new questionnaire for the assessment of pruritus. Int J Dermatol 2002; 41: 212–216.
12. Darsow U, Scharein E, Simon D, Walter G, Bromm B, Ring J. New aspects of itch pathophysiology: component analysis of atopic itch using the 'Eppendorf Itch Questionnaire'. Int Arch Allergy Immunol 2001; 124: 326–331.
13. Wahlgren CF. Itch and atopic dermatitis: clinical and experimental studies. Acta Derm Venereol 1991; Suppl 165: 1–53.
14. Wilson SR, Bautista DM. Role of Transient Receptor Potential Channels in Acute and Chronic Itch. In: Carstens E, Akiyama T, editors. Itch: Mechanisms and Treatment. Boca Raton (FL); 2014.
15. Akiyama T, Carstens E. Neural processing of itch. Neuroscience 2013; 250: 697–714.
16. Sun S, Dong X. Trp channels and itch. Semin Immunopathol 2016; 38: 293–307.
17. Moore C, Gupta R, Jordt SE, Chen Y, Liedtke WB. Regulation of Pain and Itch by TRP Channels. Neurosci Bull 2018; 34: 120–142.
18. Imamachi N, Park GH, Lee H, Anderson DJ, Simon MI, Basbaum AI, et al. TRPV1-expressing primary afferents generate behavioral responses to pruritogens via multiple mechanisms. Proc Natl Acad Sci U S A 2009; 106: 11330–11335.
19. Liu Q, Tang Z, Surdenikova L, Kim S, Patel KN, Kim A, et al. Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. Cell 2009; 139: 1353–1365.
20. Morita T, McClain SP, Batia LM, Pellegrino M, Wilson SR, Kienzler MA, et al. HTR7 Mediates Serotonergic Acute and Chronic Itch. Neuron 2015; 87: 124–138.
21. Akiyama T, Ivanov M, Nagamine M, Davoodi A, Carstens MI, Ikoma A, et al. Involvement of TRPV4 in Serotonin-Evoked Scratching. J Invest Dermatol 2016; 136: 154–160.
22. Akiyama T, Nagamine M, Davoodi A, Ivanov M, Carstens MI, Carstens E. Innocuous warming enhances peripheral serotonergic itch signaling and evokes enhanced responses in serotonin-responsive dorsal horn neurons in the mouse. J Neurophysiol 2017; 117: 251–259.
23. Pergola PE, Kellogg DL, Jr., Johnson JM, Kosiba WA, Solomon DE. Role of sympathetic nerves in the vascular effects of local temperature in human forearm skin. Am J Physiol 1993; 265: H785–792.
24. Taylor WF, Johnson JM, O'Leary DS, Park MK. Modification of the cutaneous vascular response to exercise by local skin temperature. J Appl Physiol Respir Environ Exerc Physiol 1984; 57: 1878–1884.
25. Schoeffter P, Godfraind T. Histamine receptors in the smooth muscle of human internal mammary artery and saphenous vein. Pharmacol Toxicol 1989; 64: 64–71.
26. Dobbins DE, Adamski SW, Lokhandwala MF, Grega GJ. Evidence that serotonin receptors mediate the cutaneous vasoconstriction produced by 5-hydroxytryptamine in canine forelimbs. Circ Res 1983; 53: 473–481.
27. Kardon AP, Polgar E, Hachisuka J, Snyder LM, Cameron D, Savage S, et al. Dynorphin acts as a neuromodulator to inhibit itch in the dorsal horn of the spinal cord. Neuron 2014; 82: 573–586.
28. Akiyama T, Nguyen T, Curtis E, Nishida K, Devireddy J, Delahanty J, et al. A central role for spinal dorsal horn neurons that express neurokinin-1 receptors in chronic itch. Pain 2015; 156: 1240–1246.
29. Kaliner M, Sigler R, Summers R, Shelhamer JH. Effects of infused histamine: analysis of the effects of H-1 and H-2 histamine receptor antagonists on cardiovascular and pulmonary responses. J Allergy Clin Immunol 1981; 68: 365–371.
30. Akiyama T, Merrill AW, Zanotto K, Carstens MI, Carstens E. Scratching behavior and Fos expression in superficial dorsal horn elicited by protease-activated receptor agonists and other itch mediators in mice. J Pharmacol Exp Ther 2009; 329: 945–951.
31. Akiyama T, Carstens MI, Carstens E. Enhanced scratching evoked by PAR-2 agonist and 5-HT but not histamine in a mouse model of chronic dry skin itch. Pain 2010; 151: 378–383.
32. Brenner DS, Golden JP, Gereau RWt. A novel behavioral assay for measuring cold sensation in mice. PLoS One 2012; 7: e39765.
33. Halkier-Sorensen L, Thestrup-Pedersen K. The relevance of low skin temperature inhibiting histamine-induced itch to the location of contact urticarial symptoms in the fish processing industry. Contact Dermatitis 1989; 21: 179–183.
34. Andersen HH, Elberling J, Lo Vecchio S, Arendt-Nielsen L. Topography of itch: evidence of distinct coding for pruriception in the trigeminal nerve. Itch 2017; 2: 1–10.
35. Fernandez N, Monge L, Garcia-Villalon AL, Garcia JL, Gomez B, Dieguez G. Cooling effects on nitric oxide production by rabbit ear and femoral arteries during cholinergic stimulation. Br J Pharmacol 1994; 113: 550–554.
36. Ostadhadi S, Haj-Mirzaian A, Azimi E, Mansouri P, Dehpour AR. Involvement of nitric oxide in serotonin-induced scratching in mice. Clin Exp Dermatol 2015; 40: 647–652.
37. Kim S, Barry DM, Liu XY, Yin S, Munanairi A, Meng QT, et al. Facilitation of TRPV4 by TRPV1 is required for itch transmission in some sensory neuron populations. Sci Signal 2016; 9: ra71.
38. Dhaka A, Earley TJ, Watson J, Patapoutian A. Visualizing cold spots: TRPM8-expressing sensory neurons and their projections. J Neurosci 2008; 28: 566–575.
39. Jankowski MP, Rau KK, Koerber HR. Cutaneous TRPM8-expressing sensory afferents are a small population of neurons with unique firing properties. Physiol Rep 2017; 5.
40. McKemy DD, Neuhausser WM, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 2002; 416: 52–58.
41. Morenilla-Palao C, Luis E, Fernandez-Pena C, Quintero E, Weaver JL, Bayliss DA, et al. Ion channel profile of TRPM8 cold receptors reveals a role of TASK-3 potassium channels in thermosensation. Cell Rep 2014; 8: 1571–1582.
42. Palkar R, Ongun S, Catich E, Li N, Borad N, Sarkisian A, et al. Cooling relief of acute and chronic itch requires TRPM8 channels and neurons. J Invest Dermatol 2018; 138: 1391–1399.
43. Yosipovitch G, Szolar C, Hui XY, Maibach H. Effect of topically applied menthol on thermal, pain and itch sensations and biophysical properties of the skin. Arch Dermatol Res 1996; 288: 245–248.
44. Melton FM, Shelley WB. The effect of topical antipruritic therapy on experimentally induced pruritus in man. J Invest Dermatol 1950; 15: 325–332.
45. Alloui A, Zimmermann K, Mamet J, Duprat F, Noel J, Chemin J, et al. TREK-1, a K+ channel involved in polymodal pain perception. EMBO J 2006; 25: 2368–2376.
46. Munns C, AlQatari M, Koltzenburg M. Many cold sensitive peripheral neurons of the mouse do not express TRPM8 or TRPA1. Cell Calcium 2007; 41: 331–342.
47. Zimmermann K, Lennerz JK, Hein A, Link AS, Kaczmarek JS, Delling M, et al. Transient receptor potential cation channel, subfamily C, member 5 (TRPC5) is a cold-transducer in the peripheral nervous system. Proc Natl Acad Sci U S A 2011; 108: 18114–18119.
48. Sarria I, Ling J, Gu JG. Thermal sensitivity of voltage-gated Na+ channels and A-type K+ channels contributes to somatosensory neuron excitability at cooling temperatures. J Neurochem 2012; 122: 1145–1154.
49. Zimmermann K, Leffler A, Babes A, Cendan CM, Carr RW, Kobayashi J, et al. Sensory neuron sodium channel Nav1.8 is essential for pain at low temperatures. Nature 2007; 447: 855–858.
50. Nattkemper LA, Tey HL, Valdes-Rodriguez R, Lee H, Mollanazar NK, Albornoz C, et al. The genetics of chronic itch: gene expression in the skin of patients with atopic dermatitis and psoriasis with severe itch. J Invest Dermatol 2018; 138: 1311–1317.
51. Yatsuzuka R, Inoue T, Jiang S, Nakano Y, Kamei C. Development of new atopic dermatitis models characterized by not only itching but also inflammatory skin in mice. Eur J Pharmacol 2007; 565: 225–231.