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Involvement of TRPV4 in Serotonin-Evoked Scratching

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Several thermosensitive transient receptor potential channels (transient receptor potential vanilloid type-1, -3; transient receptor potential cation channel, subfamily A, member 1) have been implicated in itch. In contrast, the role of transient receptor potential vanilloid type-4 (TRPV4) in itch is unknown. Therefore, we investigated if TRPV4, a temperature-sensitive cation channel, plays an important role in acute itch in mice. Four different pruritogens, including serotonin (5-hydroxytryptamine [5-HT]), histamine, SLIGRL (protease-activated receptors 2/mas-related G-protein-coupled receptor C11 agonist), and chloroquine (mas-related G-protein-coupled receptor A3 agonist), were intradermally injected into mice and itch-related scratching behavior was assessed. TRPV4 knockout mice exhibited significantly fewer 5-HT-evoked scratching bouts compared with wild-type mice. Notably, no differences between TRPV4 knockout and wild-type mice were observed in the number of scratch bouts elicited by SLIGRL and histamine. Pretreatment with a TRPV4 antagonist significantly attenuated 5-HT-evoked scratching in vivo. Using calcium imaging in cultured primary murine dorsal root ganglion neurons, the response of neurons after 5-HT application, but not other pruritogens, was significantly lower in TRPV4 knockout compared with wild-type mice. A TRPV4 antagonist significantly suppressed 5-HT-evoked responses in dorsal root ganglion cells from wild-type mice. Approximately 90% of 5-HT-sensitive dorsal root ganglion neurons were immunoreactive for an antibody to TRPV4, as assessed by calcium imaging. These results indicate that 5-HT-induced itch is linked to TRPV4.

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INTRODUCTION

Itch can be elicited by a wide variety of chemical stimuli, including inflammatory mediators: amines, cytokines, proteases, neuropeptides, and mas-related G-protein-coupled receptor (Mrgpr) agonists (Akiyama and Carstens, 2014). Histamine, an inflammatory mediator, is the best-known itch inducer and is predominantly released by mast cells and basophils, and possibly keratinocytes (Dvorak, 1998; Inami et al., 2013). Histamine H1 and H4 receptors play a role in histamine-evoked itch (Bell et al., 2004). Serotonin (5-hydroxytryptamine [5-HT]), another inflammatory mediator, is released by mast cells, melanocytes, and platelets, to evoke itch (Kushnir-Sukhov et al., 2007; Slominski et al., 2003; Turetta et al., 2004). Whereas the intradermal injection of 5-HT elicits robust scratching behaviors in rodents (Nojima and Carstens, 2003; Yamaguchi et al., 1999), either the intradermal injection or the iontophoretic application of 5-HT elicits mild to moderate itch in humans (Hosogi et al., 2006; Weisshaar et al., 1997, 2004). Proteases such as trypsins, kallikreins, or tryptase exert pruritogenic effects through the activation of protease-activated receptors (PARs). PAR-2 is overexpressed in the skin of atopic dermatitis patients and its tethered ligand, SLIGRL, evokes itch-related behaviors in mice (Akiyama et al., 2009; Steinhoff et al., 2003). Mrgprs have recently been linked to chemically evoked itch (Han et al., 2013). Chloroquine, an agonist of MrgprA3, and bovine adrenal medullary peptide 8-22, an agonist of MrgprC11, both elicit itch. It has been reported that SLIGRL, a tethered ligand for PAR-2, in addition acts as an agonist of MrgprC11 (Liu et al., 2011).

Transient receptor potential (TRP) ion channels are involved in sensory physiology including itch and pain as well as vision, taste, olfaction, hearing, touch, and thermosensation. Recent studies have revealed that several thermosensitive TRP channels are implicated in itch (Akiyama and Carstens, 2013). Transient receptor potential vanilloid type-1 (TRPV1) is activated by noxious heat (≥43 °C) and is required for itch evoked by histamine and IL-31 (Cevikbas et al., 2014; Imamachi et al., 2009). Rodent transient receptor potential cation channel, subfamily A, member 1

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Abbreviations: DRG, dorsal root ganglion; 5-HT, 5-hydroxytryptamine (serotonin); Mrgpr, Mas-related G-protein-coupled receptor; PAR, protease-activated receptor; TRP, transient receptor potential; TRPV1, transient receptor potential vanilloid type-1; TRPV4, transient receptor potential vanilloid type-4; TRPV1KO, TRPV1 knockout; TRPV4KO, TRPV4 knockout; WT, wild-type

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(TRPA1) has been reported to respond to cold temperatures (below 17−18 °C; Chen et al., 2013a) and is required for itch evoked by chloroquine, IL-31, thymic stromal lymphopoietin, endothelin-1, and bile acids (Cevikbas et al., 2014; Kido-Nakahara et al., 2014; Lieu et al., 2014; Wilson et al., 2011, 2013). Transient receptor potential vanilloid type-3 is activated by warm temperatures (33−39 °C). Mice harboring a gain-of-function mutation in transient receptor potential vanilloid type-3 developed dermatitis accompanied by itch behavior (Yoshioka et al., 2009). TRPV4 is another TRP channel activated by moderately warm temperatures (27−34 °C) and is expressed in sensory neurons as well as keratinocytes in the skin. TRPV4 mRNA was upregulated in skin with itching burn scars (Yang et al., 2015) and with photodermatitis (Moore et al., 2013). However, the role of TRPV4 in itch is largely unknown. We tested if TRPV4 is required for certain types of itch in mice and, thus, demonstrated that TRPV4 is required for the transmission of 5-HT-induced itch, but not of three other tested pruritogens.

RESULTS

5-HT evoked itch is dependent on TRPV4 in vivo

Scratching elicited by 5-HT, but not the other pruritogens (histamine, SLIGRL, and chloroquine) was significantly reduced in the rostral back model (Figure 1a). Interestingly, chloroquine-evoked scratching was significantly enhanced in TRPV4 knockout (TRPV4KO) mice (Figure 1a). In this study, we did not further investigate the mechanisms underlying this enhancement. 5-HT-elicited scratching in TRPA1KO and TRPV1 knockout (TRPV1KO) mice was not significantly different compared with that in wild-type (WT) mice (Figure 1a). In the cheek model, 5-HT predominantly elicited scratching that was significantly diminished in TRPV4KO mice (Figure 1b). These findings suggest that 5-HT-elicted scratching requires TRPV4 in vivo.

To confirm the role of TRPV4 in 5-HT-evoked itch, a pharmacological approach was also used. The TRPV4 antagonist HC067047 significantly inhibited 5-HT-evoked scratching (Figure 2). 5-HT-evoked scratching was not significantly affected by pretreatment with the H1 histamine receptor antagonist terfenadine, but was significantly inhibited by pretreatment with the 5-HT2 antagonist, ketanserin (Figure 2). These results are consistent with previous reports (Akiyama et al., 2012; Yamaguchi et al., 1999).

Calcium imaging

To investigate whether 5-HT-responsive cells express TRPV4, calcium imaging of dorsal root ganglion (DRG) cells was performed, followed by immunostaining. The specificity of the TRPV4 antibody was confirmed by detecting immunoreactivity of TRPV4 in DRG cells of WT mice (Figure 3b), but not TRPV4KO mice (Figure 3c, n = 129). To investigate whether 5-HT-responsive cells express TRPV4, calcium imaging of DRG cells was performed, followed by immunostaining. Eighteen percent (206 of 1,131) of all DRG cells from WT mice were immunopositive for the TRPV4 antibody. Of the DRG cells shown by calcium imaging to respond to 5-HT, 93% (15 of 16) were immunopositive for TRPV4 (Figure 3a). Eighty-one percent of TRPV4-immunoreactive DRG cells were unresponsive to 5-HT.

We compared 5-HT-evoked responses in DRG neurons isolated from TRPV1KO, TRPA1KO, and TRPV4KO mice with those isolated from WT mice. DRG cells isolated from TRPV4KO mice showed a significant decrease in the proportion of those that were 5-HT sensitive (2 of 112). In contrast, there were no significant differences in the proportions of 5-HT-sensitive DRG neurons isolated from TRPA1KO mice (26 of 423) and TRPV1KO (9 of 205) compared with those from WT mice (10 of 202; Figure 4). DRG cells isolated from TRPV4KO mice showed no changes in the proportions of histamine-, chloroquine-, or SLIGRL-sensitive neurons compared with those from WT mice (Figure 4).

A pharmacological approach was again taken to confirm whether TRPV4 functions downstream of 5-HT receptor

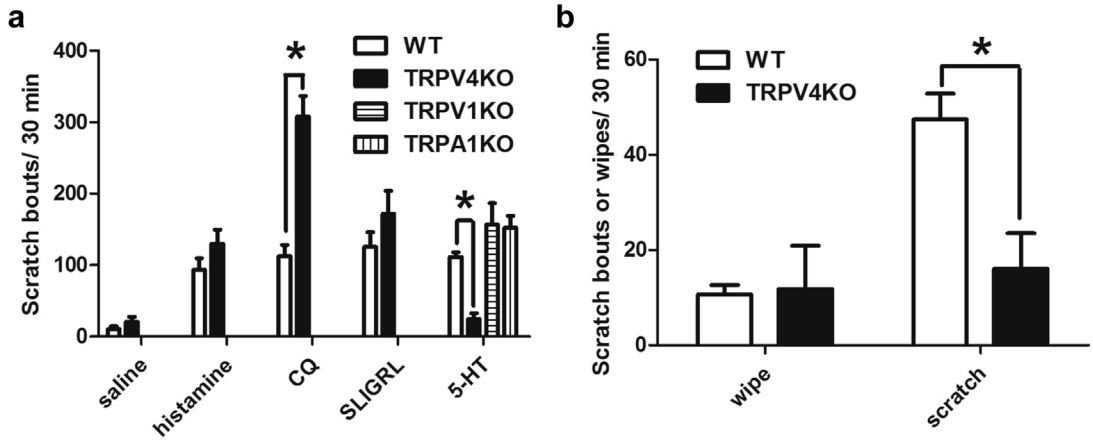

Figure 1. TRPV4KO mice are insensitive to 5-HT-mediated itch. (**a**) Saline, histamine (50 μg/10 μl), chloroquine (CQ, MrgprA3 agonist, 100 μg/10 μl), SLIGRL-NH2 (PAR-2/MrgprC11 agonist, 50 μg/10 μl), or 5-HT (10 μg/10 μl) were injected intradermally in the rostral back. Pruritogen-elicited scratch bouts were counted in WT (open bars) and TRPV4KO mice (black bars) over a 30-minute period. 5-HT-elicited scratching was significantly diminished in TRPV4KO mice, but not in TRPV1KO or TRPA1KO mice. Error bars are SEM. *$P < 0.05$, significant difference from the WT group (unpaired *t*-test or one-way analysis of variance followed by the Bonferroni test, $n = 6$ per group). (**b**) 5-HT (10 μg/10 μl) was injected intradermally into the cheek. Hindlimb scratch bouts and ipsilateral forelimb wipes directed to the cheek were counted over 30 minutes. *$P < 0.05$, significant difference from the WT group (unpaired *t*-test, $n = 4$ per group). 5-HT, 5-hydroxytryptamine (serotonin); PAR, protease-activated receptor; TRPV1KO, TRPV1 knockout; TRPV4KO, TRPV4 knockout; TRPA1KO, TRPA1 knockout; WT, wild-type.