Invited review article

The molecular and cellular mechanisms of itch and the involvement of TRP channels in the peripheral sensory nervous system and skin

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A B S T R A C T

Itch is an unpleasant cutaneous sensation that can arise following insect bites, exposure to plant ingredients, and some diseases. Itch can also have idio pathic causes. Itch sensations are thought to protect against external insults and toxic substances. Although itch is not directly lethal, chronic and long lasting itch in certain diseases can worsen quality of life. Therefore, the mechanisms responsible for chronic itch require careful investigation. There is a significant amount of basic research concerning itch, and the effect of various itch mediators on primary sensory neurons have been studied. Interestingly, many mediators of itch involve signaling related to transient receptor potential (TRP) channels. TRP channels, especially thermosensitive TRP channels, are expressed by primary sensory neurons and skin keratinocytes, which receive multimodal stimuli, including those that cause itch sensations. Here we review the molecular and cellular mechanisms of itch and the involvement of TRP channels in mediating itch sensations.

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Introduction

Itch is an unpleasant cutaneous sensation that evokes the desire to scratch. Among different forms of pain that lead to avoidance of noxious stimuli, itch is primarily thought to be a means for eliminating exogenous compounds such as parasites and plant particles.
Itch sensation and scratching behaviors are conserved across a broad range of species, from rodents and birds to humans. In humans, environmental substances such as allergens, mosquito bites, and some chemical compounds can cause itch, but chronic itch can accompany systemic diseases including atopic dermatitis (AD), kidney failure, cholestasis, and neuronal lesions.

For the past two decades, significant effort has been devoted to elucidate the molecular mechanisms of itch. Several studies presented evidence showing that the peripheral nervous system in particular transduces cutaneous sensory stimuli into electrical signals and transmits them to the central nervous system. Two calcium-permeable ion channels, transient receptor potential vanilloid 1 (TRPV1) and ankyrin 1 (TRPA1), which are both well expressed in primary afferent sensory neurons, were found to play crucial roles in detecting pruritogens and nociceptive stimuli.

This review focuses on the TRP channels that detect pruritogens in the periphery and discusses the molecular and cellular mechanisms of itch that involve TRP channels.

Numerous basic experiments have been conducted to investigate the molecular mechanisms of itch sensation. Among them, the simplest and most quantitative method to evaluate itch sensation, especially in rodents, is counting scratching behaviors directed towards the sites of compound injection. The most famous method reported more than 20 years ago is counting scratching at the nape of the back to which chemicals were applied. Later, the cheek injection model was developed to evaluate itch sensation by counting scratching with the ipsilateral hindpaw at the cheek to which the chemical was applied. The cheek injection model allows itch- and pain-related behaviors to be distinguished because with pain the animals wipe the cheek with the ipsilateral forepaw. These models facilitated progress in itch research, and here we cite those works that focus on TRP channels and itch sensation.

**TRP channels involved in itch**

TRP channels are non-selective calcium-permeable cation channels that compose the TRP ion channel superfamily. TRP channels were first described in *Drosophila*, in which photoreceptors carrying *trp* gene mutations exhibited an abnormal transient responsiveness to continuous light. In mammals, TRP channels comprise six related protein families (TRPC, TRPV, TRPM, TRPA, TRPML, TRPP) (Fig. 1). In general, TRP channels are ubiquitously expressed, indicating that most cells have several TRP channel proteins. Although the physiological functions of most TRP channels are unknown, their wide distribution indicates that the biological functions and activation mechanisms for these channels are diverse. As such, TRP channels are best recognized for their contributions to sensory transduction, response to temperature, nociceptive stimuli, touch, osmolarity, pheromones and other stimuli from both within and outside the cell. Several kinds of TRP channels are expressed exclusively in subsets of sensory neurons, suggesting their involvement in detecting nociceptive stimuli and itch-producing stimuli.

Among the large TRP ion channel superfamily proteins, TRPV1, TRPV2, TRPV4, TRPM (melastatin) 2, TRPM3, TRPM8, and TRPA1 channels are expressed in sensory neurons such as dorsal root ganglion (DRG) neurons and trigeminal ganglion (TG) neurons. Meanwhile, TRPV3 and TRPV4 are expressed by skin keratinocytes. Some of these TRP channels are reportedly involved in itch sensation. Interestingly, they are all so-called thermo-sensitive TRP channels and show sensitivity to multimodal stimuli that include acid, alkaline, osmolarity, artificial compounds, and phytochemicals. We review these multimodal TRP channels with regard to their involvement in itch sensation.

![Fig. 1. A phylogenetic tree of human TRP channels. A phylogenetic tree was made with minimum evolution principle upon expecting amino acid substitutions using a JTT model. There are 27 TRP channels in 6 subfamilies (shown in red) in human. The TRP channels shown in orange circles indicate channels described in this review. Numbers at each branch indicate statistical bootstrap values. Mouse Trpc2 gene was used because human TRPC2 gene is a pseudogene. Scale: genetic distance (recombination rate).](image-url)
TRPV1 and itch

TRPV1 and histamine-induced itch

The first report that provided pharmacological evidence for the involvement of TRP channels in itch caused by the pruritogen histamine was done by Kim et al. Histamine H1 receptor activation induces membrane-delimited increases in intracellular calcium concentrations ([Ca\(^{2+}\)]\(_{i}\)) that are dependent on TRPV1 channels in mouse DRG neurons (Fig. 2). In the former pathway, H1-mediated TRPV1 currents were recorded and one LOX product, 12(S)-HETE, which is produced following H1 engagement and PLA2 activation, was suggested as an endogenous TRPV1 agonist. The study showed that histamine-induced scratching behaviors by TRPV1KO mice were decreased but not abolished, which suggests the involvement of other molecules in the itch response. For the PLC\(^{b}3\) pathway, Gq/11 and Plc\(^{b}3\) mRNAs are highly expressed in mouse DRG neurons, and PLC\(^{b}3\)KO mice did not show H1 and H4-selective agonist-induced scratching behaviors. Additionally, TRPV1KO mice showed loss of histaminergic itch, whereas itch caused by non-histaminergic pruritogens such as α-1H3T and endothelin 1 (ET-1) was not affected. Although TRPV1 is involved in histaminergic itch, it remains unclear which PLA2 and LOX subtypes are involved or how they are activated following histaminergic receptor activation. In the latter pathway, whether TRPV1 is activated by endogenous agonists in vitro is also unclear.

TRPV1 and PAR2-mediated itch

In contrast to histaminergic itch, the relationship between non-histaminergic itch and TRP channels is less clear. Costa et al. showed that trypsin induced scratching behaviors, whereas the selective histamine H1 receptor antagonist pyrilamine did not decrease behaviors that require protease activity. The authors supplied pharmacological evidence to show the in vivo involvement of PAR2 receptors, which are expressed in peripheral tissues such as DRG neurons, mast cells, and keratinocytes. Trypsin-induced itch was diminished in TRPV1KO mice, although the TRPV1 antagonist SB366791 did not completely inhibit trypsin-induced scratching behaviors. This result suggests the involvement of other TRP channels or molecules in histaminergic itching (Fig. 2).

TRPV1 and VGLUT2-expressing DRG neurons

Glutamate is the most abundant excitatory neurotransmitter both in the peripheral and central nervous system, and most DRG neurons express glutaminase, a critical mitochondrial enzyme that catalyzes the conversion of glutamine into glutamate. Synthesized glutamate is packaged into synaptic vesicles by vesicular glutamate transporters (VGLUTs). Among the three VGLUTs (VGLUT 1–3), VGLUT2 mRNA and protein are the most broadly expressed in DRG neurons. Mice having conditional ablation of Vglut2 in a subset of primary afferent neurons showed spontaneous scratching of back skin lesions. The Kullander group also established that Trpv1-Cre-driven VGlut2KO mice had similar phenotypes that were characterized by spontaneous scratching behaviors, low thermal

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**Fig. 2.** Molecular mechanism of itch signaling in the peripheral nervous system. Various endogenous and exogenous itch mediators, including histamine, imiquimod, Poly(I:C), tryptase, IL-31 LPA, BAM8-22, chloroquine, bile acids, serotonin, and TSLP activated their respective receptors expressed in DRG neurons, such as H1 receptor TLR7, TLR3, PAR2, IL-31R, LPA\(_{6}\), MrgrPC11, MrgprA3, TGR5, 5-HT\(_{3}\), 5-HT\(_{2}\), and TSLPR. This metabotropic receptor activation couples with TRPV1, TRPA1, and TRPV4 channels to generate action potentials in DRG neurons. H2O2 might cause itch by directly activating TRPA1. Pruritogens might activate TRPA1 and enhance TRPV1 channel activity. H1 receptor signaling is mediated by the PLA2/LOX and Gq/11/PLCβ3 pathways. LPA\(_{6}\) receptor signaling is mediated by the PLA2/PLD pathway. TRPA1 is activated by various mediators, including PLC, PKC, adenylyl cyclase, and G\(_{q}\). TSLP is produced in keratinocytes through STIM1/Orai1/NFAT signaling following PAR2 activation by tryptase. TRPV3 and TRPV4 expressed in keratinocytes suggest the release of itch mediators to activate DRG neurons. Electric signals generated in the peripheral nerve endings are transmitted to the brain through the spinal cord.
pain sensitivity, and no changes in mechanical and formalin-induced pain, suggesting that glutamate release from TRPV1-expressing DRG neurons in the dorsal horn is required for itch suppression under normal conditions and thermal pain sensation.40

TRPV1-lineage sensory neurons and itch

Although the findings described above regarding TRPV1 and itch revealed crucial mechanisms of TRPV1 in itch sensation in vitro and in vivo, there is still debate about the use of TRPV1KO mice for itch assays42,43 because after the E14.5 developmental stage TRPV1-lineage neurons in mice differentiate into diverse subsets of DRG neurons, which could affect TRPV1, TRPA1, and TRPM8 expression.44 Therefore, the physiological roles of TRPV1-lineage sensory neurons must be investigated by ablating TRPV1-expressing neurons in vivo. Mishra et al. developed TRPV1-DTA mice to show that TRPV1-lineage neurons play roles in both histaminergic and non-histaminergic itch, as well as nociception, thermosensation, and thermoregulation.45 The overall phenotypes regarding thermal and mechanical pain in TRPV1-DTA and Vglut2f/f;TRPV1-Cre mice were similar. Furthermore, transcriptome analysis of TRPV1-DTA mice revealed the expression of a new crucial role for natriuretic polypeptide b (Nppb) in DRG neurons, in that Nppb is required for itch sensation without affecting nociception.46

TRPV1 and Toll-like receptor-mediated itch

A major side effect of imiquimod, a Toll-like receptor 7 (TLR7) agonist used to treat cutaneous infectious diseases such as genital warts, is itch, and its analogues were found to be pruritogens in the cheek injection model.47 DRG neurons showing TRPV1-mediated currents responded to TLR7 agonists and DRG neurons expressing TLR7 mRNA also expressed TRPV1 mRNA.48 The imiquimod-induced calcium signaling is 2-aminoethoxydiphenylborane-sensitive, suggesting that d-myo-inositol 1,4,5-trisphosphate receptor is involved.49 Although the mechanisms by which imiquimod causes action potentials in DRG neurons remain to be elucidated, it is clear that TRPV1-expressing DRG neurons play a pivotal role in TLR7-mediated itch. Surprisingly, the TLR7 agonist let-7b microRNA induced pain that was dependent on TRPA1.50

In humans, the TLR3 receptor detects double-stranded viral RNA. Similar to TLR7, TLR3 is expressed in a small-diameter TRPV1-expressing subpopulation of DRG neurons. In contrast to TLR7KO mice that show decreased sensitivity to non-histaminergic pruri-
togen, TLR3KO mice were insensitive to both histaminergic and non-histaminergic pruritogens.51 A synthetic double strand RNA Poly(I:C) (PIC) induced itch in a TLR3-dependent manner and DRG neurons responding to PIC express TRPV1. Intriguingly, TRPV1KO mice showed no decrease in PIC-induced itch, indicating that a TRPV1-independent mechanism involving endogenous TLR3 ago-

The phosphoinositide interacting regulator of TRP channels Pirt and itch

The phosphoinositide interacting regulator of TRP channels Pirt, is expressed specifically in the peripheral nervous system. PirtKO mice show not only decreased nociception for heat and capsaicin, but also decreased capsaicin-activated currents in DRG neurons.51 Pirt binding to TRPV1 does not affect plasma membrane expres-
sion of TRPV1, suggesting that Pirt binding to TRPV1 could change its channel gating to contribute to TRPV1-mediated nociception. Additionally, PirtKO mice showed decreased scratching behaviors caused by histamine, 5α-Me-5HT, SLIGRL, and ET-1, suggesting that Pirt interacts with TRPV1 in TRPV1-expressing neurons and transmits histaminergic and non-histaminergic itch signals as well as TRPV1-mediated pain signals.52

Genetic analysis of a subpopulation of sensory neurons transmitting non-histaminergic itch

MrgprA3 (Mas-related G protein-coupled receptor A3) is the receptor for the anti-malarial drug chloroquine (CQ) and is important for non-histaminergic and TRPV1-independent itch.53 The ability of a small subpopulation of MrgprA3-expressing DRG neurons to transmit itch signals was examined using transgenic mice. Dong’s group first generated mice lacking MrgprA3-expressing sensory neurons and showed acute histamine- and CQ-induced itching in both the neck and cheek injection models, but also that chronic, dry skin-induced and allergic itch was decreased in mice in which MrgprA3-neurons were ablated.54 The authors also generated transgenic mice in which TRPV1 was expressed under the control of the MrgprA3 promoter in the TRPV1KO background and showed that capsaicin activates only the MrgprA3-expressing small subpopulation of DRG neurons.55 The Trpv1fl/−; MrgprA3GFP-cr; Rosa26°TRPV1 mice surprisingly showed capsaicin-induced scratching behaviors in the cheek injection model without changes in AIFC sensitivity as well as equivalent responsiveness to heat and mechanical stimulation in TRPV1KO mice.56

TRPA1 and itch

TRPA1 and endothelin-1-induced itch

Endothelin-1 (ET-1) is a 21-amino acid vasoconstricting peptide that is known to cause pain-related behaviors.55 Scratching behaviors caused by ET-1 in back injection models are sensitive to the TRPA1 antagonist AP-18, but not the TRPV1 antagonist cap-
sazepine.56 This result suggests that ET-1-induced itch is exclu-
sively dependent on TRPA1, which is consistent with the finding that ET-1 caused scratching behaviors in TRPV1KO mice and PLCβ3KO mice.57 Further investigation will be needed to define the mechanism of ET-1-induced itch.

TRPA1 and chloroquine-induced itch

Itching is a common side effect of the anti-malarial drug chloro-
quine (CQ).57 MrgprA3 is specific for CQ, and Mrgpr-cluster deletion mice showed normal nociception and histaminergic pru-
rispception but did not exhibit CQ-induced itch.58 Bovine adrenal medulla peptide (BAM) 8–22 also acts as a pruritogen and a se-
lective agonist for MrgprC11. In studies of calcium signaling induced by CQ and BAM, CQ-induced [Ca2+]i increases in DRG neurons were suggested to be caused by TRP channel activation. On the other hand, CQ- or BAM-induced [Ca2+]i increases in HEK293 cells that heterologously express MrgprA3 or MrgprC11, respectively, were found to be due to calcium influx.59 This newly identified non-histaminergic mechanism of itch, including CQ- and BAM-induced calcium signaling, was further investigated using TRPV1KO and TRPA1KO mice and antagonists for TRPV1 and TRPA1.60 CQ-induced [Ca2+]i increases were later found to be mediated by calcium influx through TRPA1 channels in DRG neurons that was independent of TRPV1 activity. On the other hand, BAM-induced [Ca2+]i increases were suggested to be due to calcium release from the ER, which disappeared in TRPA1KO DRG neurons, but not in TRPV1KO DRG neurons. In calcium-imaging experiments using a heterologous expression system with NG108 cells, CQ-
induced [Ca2+]i increases were observed when MrgprA3 was expressed with TRPA1, whereas BAM caused additional [Ca2+]i in-
creases when MrgprC11 was expressed with either TRPV1 or TRPA1. Further pharmacological experiments to examine GPCR-mediated [Ca2+]i increases focused on Gq/11-coupled signaling in the presence of galanin (a Gαq, inhibitor) or U73122 (a phospholipase C (PLC) inhibitor), and showed that CQ signaling is mediated by Gαq, but not PLC, whereas BAM signaling was dependent on PLC, but not Gαq. Notably, U73122 directly activates PLCβ5,60 TRPA1, and DRG
TRPA1 and hydrogen peroxide-induced itch

Hydrogen peroxide (H$_2$O$_2$) is an oxidant produced during oxidative stress, and causes scratching behaviors in the cheek injection model. Pharmacological and genetic experiments revealed that H$_2$O$_2$-induced scratching behaviors are non-histaminergic and TRPV1-independent, but TRPA1-dependent based on results from assays using antagonists for the H1 receptor in TRPV1- and TRPA1-KO mice. H$_2$O$_2$ directly activates TRPA1 channels and if H$_2$O$_2$ activates TRPA1-expressing DRG neurons similarly to AITC, pain should be induced, but in these mice was not (Fig. 2). As such, the underlying molecular mechanism of TRPA1 agonists that cause pain and itch remains unclear.

TRPA1 and chronic itch model

As discussed above, various molecular mechanisms of acute itch involving TRPV1 and TRPA1 are known. To evaluate chronic itch, a dry-skin chronic itch model was developed that uses treatment with acetone, ether and water (AEW). Compared with WT mice, scratching behaviors were decreased in resiniferatoxin-treated mice and TRPA1KO mice, but not TRPV1KO mice, suggesting that a small population of TRPA1-expressing sensory neurons, especially those without TRPV1 expression, is required for the pathogenesis of itch in the AEW model. The pathogenesis of chronic contact dermatitis models sensitized by oxazolone and urushiol was also reported to be dependent on TRPA1. However, the relationship between the phenotype and TRPA1 functions was not completely explained, suggesting that additional mechanisms may be involved in chronic itch pathogenesis.

TRPA1 and thymic stromal lymphopoietin-induced itch

Thymic stromal lymphopoietin (TSLP) is a well-known cytokine produced by epithelial cells. Overexpression of TSLP in keratinocytes is known to cause an atopic dermatitis (AD)-like phenotype, although how TSLP production in skin evokes scratching behaviors is unclear. Investigation of neuronal mechanisms involving TSLP showed that expression of the TSLP receptor and its dimerization partner interleukin-17 receptor (IL-17R) in DRG neurons initiates itch signaling, and that U73122-sensitive, TRPA1-expressing neurons respond to TSLP. Further examination of the missing link between non-neuronal, PAR2-dependent signals and sensory neurons showed that TSLP production in keratinocytes triggered by tryptase could contribute to the integration of neuronal signals for itch. According to this report, [Ca$^{2+}$]$_i$ increases mediated by STIM1 and ORAI1 following PAR2 activation in keratinocytes lead to nuclear translocation of NFAT and de novo TSLP production, followed by activation of sensory neurons. The authors also found that TSLP-induced itch is non-histaminergic and CQ- and BAM-independent, suggesting the existence of another TRPA1-dependent itch mechanism (Fig. 2).

TRPA1 and interleukin-13-induced itch

Since TRPA1 is reported to mediate non-histaminergic itch and AD is thought to be resistant to antihistamines, the involvement of TRPA1 in AD pathogenesis was investigated from a dermatological and histological perspective using a transgenic mouse model of AD, K5-Tg- interleukin-13 (IL-13), wherein IL-13 expression is selectively upregulated in the skin. IL-13 is a Th2 cytokine that is thought to be involved in AD pathogenesis. This induced AD model showed increases in the number of dermal mast cells and TRPA1-expressing afferent neurons, as well as TRPA1- and transgene-dependent and histamine-independent scratching behaviors. In addition, in the vicinity of afferent fibers, TRPA1-expressing mast cell function was enhanced, which was correlated to the induced scratching behaviors. These data suggest that IL-13-dependent transcriptional changes in TRPA1 expression may underlie the observed phenomena. Detailed cellular and molecular mechanisms that link the increased numbers of TRPA1-expressing afferent neurons with increased numbers of mast cells in this AD model are very intriguing and await elucidation.

TRPA1 and bile acid-induced itch

Cholestatic itch is a symptom of several liver diseases. Various candidate pruritogens related to cholestasis, such as histamine and tryptase, were examined in humans, as was whether increases in these molecules that occur during cholestasis correlate to itch intensity. In cholestatic patients bile acid (BA) levels can increase in the circulation and induce pruritus, but BA increases are not thought to be associated with cholestatic itch in humans. Although the molecular mechanism of BA-induced itch is unclear, the receptor for BA, TGR5, was reported to be involved in BA-induced itch. Although TGR5 is widely expressed in various tissues or organs, including the nervous system, TGR5 is expressed in small-diameter DRG neurons that also express TRPV1, TRPA1, CGRP, SP, and GRP, suggesting that a small subpopulation of peptidergic DRG neurons transmit BA-evoked itch signals (Fig. 2). In addition, further examinations to detail the molecular signaling of BA-induced itch in mice focused on TRPV1 and TRPA1. Since TRPA1 and TRPV1 are thought to be regulated by C$_\text{rg}$, PKA, and PKC, respectively, a pharmacological approach was used to evaluate the involvement of these channels in itch sensations using a calcium-imaging method in DRG neurons. These studies showed that C$_\text{rg}$, PKA, and PKC, but not PKA, are required for BA-induced [Ca$^{2+}$]$_i$ increases mediated through TRPA1 (Fig. 2). The involvement of TRPV1 in BA-induced itch was excluded by results from experiments using the TRPV1 antagonist AMG 9810 and TRPV1KO mice.

TRPA1 and serotonin-induced itch

Although itch induced by serotonin (5-hydroxytryptamine, 5-HT) has been extensively studied, the 5-HT receptor subtype that is involved is unclear, as are the molecular mechanisms and signaling pathways. The Bautista group compared the relationship between scratching behaviors evoked by CQ acting as a representative non-histaminergic pruritogen, and gene expression patterns in DRG neurons from mice produced from a cross of C57BL/6 and DBA/2 mice, to show different behaviors in histaminergic and non-histaminergic itch. Through this screening of candidate 5-HT receptors, the authors identified HTR7 as a new itch receptor. Using the HTR7-selective agonist LP44, the LP44-responding subpopulation of DRG neurons was also found to respond to capsaicin, AITC, and 5-HT, and the majority were in the CQ-responding population. Further examination using mice lacking Htr7, Trpa1, or Trpv1 revealed that HTR7 and TRPA1, but not TRPV1, were required for LP44 action in cellular and behavioral levels, prompting the clarification of the coupling mechanism between HTR7 and TRPA1 (Fig. 2). Because HTR7 is a G$_\text{q}$-coupled receptor.
that activates adenylyl cyclase (AC) through both G_{\alpha} and G_{\beta\gamma}, the involvement of AC, as well as G_{\beta\gamma} and PLC, which was examined previously,27,30 was investigated at pharmacological and electrophysiological levels. These studies showed that AC and G_{\beta\gamma}, but not PLC, were required for the HTR7-TRPA1 coupling (Fig. 2). Serotoninergic signaling is known to be involved in not only acute but also chronic itch such as that which occurs in AD.95 Therefore, the requirement of HTR7-TRPA1 signaling for AD was also explored, and showed that both HTR7 and TRPA1 are necessary for AD pathogenesis and itch persistence in AD model mice. Although several other serotonin receptors such as 5-HT_{2c} could be involved in both itch and pain, this study was among the first to demonstrate a neuronal molecular mechanism for serotonergic itch.

**Itch signaling involving both TRPV1 and TRPA1**

**TRPV1/TRPA1 and leukotriene B_{4}-induced itch**

Leukotriene B_{4} (LTB_{4}) released from leukocytes is a potent chemotactic factor for leukocytes, and increases in LTB_{4} levels are related to AD85 and psoriasis.96 LTB_{4}-induced scratching behaviors in mice47 and both TRPV1 and TRPA1 were shown to be involved in LTB_{4}-induced scratching behaviors using pharmacological tools and KO mice.88 Among the known LTB_{4} receptors, B_{4} mRNA was expressed in DRG neurons;85 but the molecular mechanism underlying LTB_{4}-induced itch awaits further investigation.

**Silencing itch fibers expressing TRPV1 and TRPA1**

To investigate which sensory neurons are activated in mice showing scratching behaviors, a unique activity-dependent silencing of sensory neurons was conducted using the charged, membrane-impermeable ligand derivative N-ethyl-lidocaine (QX-314, a sodium channel blocker).90 QX-314 permeates activated hH1 receptors and MrgrpA3, respectively, and inhibits sodium channels from the intracellular side of DRG neurons.91 Co-application of QX-314 and a pruritogen in advance of stimuli application can inhibit the activity of sensory neurons in a pruritogen-dependent manner. Histamine-induced scratching was inhibited after co-application of QX-314 with capsaicin, but not with AITC, indicating that histaminergic itch is TRPV1-dependent and TRPA1-independent. On the other hand, non-histaminergic SLIGRL- and CQ-induced scratching were decreased by co-application of QX-314 with capsaicin and AITC, respectively, suggesting that TRPV1 and TRPA1 are involved in SLIGRL- and CQ-induced itch, respectively. Capsaicin- and AITC-evoked scratching behaviors also occurred following silencing of AITC- and capsaicin-responsive neurons with QX-314.90 These paradoxical, algogen-induced scratching behaviors were explained by the finding that a subset of sensory neurons expressing both TRPV1 and TRPA1, which normally play an inhibitory role, might be silenced by capsaicin or AITC.

**TRPV1/TRPA1 and interleukin-31-induced itch**

Both IL-31 and IL-13 are involved in itch sensation. Increases in IL-31 levels were observed in patients with AD94 and cutaneous T-cell lymphoma,42 which both have severe pruritus as a symptom. The IL-31 receptor (IL-31R) is highly expressed in DRG neurons44 and exists as a heterodimer consisting of an IL-31-specific subunit, IL-31 receptor alpha (IL-31R{alpha}), and oncostatin M receptor beta (OSMR{beta}). Therefore, Civylkas et al. used the cheek injection model and a calcium-imaging method with TRPV1KO and TRPA1KO mice to ask whether IL-31 induces itch through IL-31R expressed in DRG neurons, and found that IL-31 indeed induced itch by directly activating a subpopulation of primary sensory neurons expressing IL-31R, TRPV1, and TRPA1 (Fig. 2).95 They also showed the major source of increased IL-31 levels in Th2 cells from both AD patients and AD model mice.

**TRPV1/TRPA1 and lysophosphatidic acid-induced itch**

The phospholipid lysophosphatidic acid (LPA) is reported to be associated with the extent of cholestatic pruritus,74 but the mechanism by which LPA causes itch was unknown. Kittaka and Tominaga observed that LPA caused scratching behaviors without significant increases in pain-related wiping behaviors using the cheek injection model. In addition, they found that LPA re-produced downstream of LPA{alpha} activation through calcium-independent PL{alpha}2 and PLD signaling activates TRPA1 and TRPV1 from the intracellular side (unpublished observation) (Fig. 2).

**TRPV3 and itch**

TRPV3, a warmth-activated TRP channel,17,24,25 is expressed in keratinocytes, but not in DRG neurons or the spinal cord.17 TRPV3 is activated between 31 and 33 °C17,24 and also by the chemical agonist camphor, a phytochemical that affects temperature sensation on the skin. In the context of TRPV3 involvement in itch, spontaneous mutant mouse strains carrying Glys73Ser and Glys73Cys mutations isolated from DS-Nh mice and WBN/Kob-H rats, respectively, are hairless and have AD-like dermatitis with spontaneous scratching.96,97 The involvement of TRPV3 in itch was confirmed in the phenotype of TRPV3 Gly73Ser transgenic DS mice.98 The Glys73 missense mutation was also found in patients with Olmsted syndrome, a rare congenital disorder characterized by skin hyperplasia with severe itching.99 Glys73Ser, Glys73Cys, Glys73Ala,100 and Glys73Val101 mutations are located in the S4–S5 linker of TRPV3, whereas Trp692Gly99 and Trp692Cys102 are within the TRP box. When expressed in HEK293 cells, TRPV3 channels having mutations at both sites showed gain-of-function properties in which all mutant TRPV3 channels represented spontaneously activated currents with gained and strong inward rectification, resulting in apoptosis that was also observed in biopsy sections from patients.99 Although TRPV3KO mice exhibit skin-related loss-of-function phenotypes, including wavy hair, curly whiskers, misaligned hair follicles, and a thin stratum corneum,103 interestingly, they show no itch-related scratching behaviors. Although TRPV3 is not expressed in primary sensory neurons, TRPV3 is indeed involved in skin homeostasis and defects in this channel can cause itch. However, the neuroscientific evidence is currently insufficient to make clear conclusions about the involvement of TRPV3 in itch. Therefore, how itch signaling proceeds in TRPV3-derived skin lesions will be important to investigate in future studies (Fig. 2).

**TRPV4 and itch**

TRPV4 is mainly expressed in keratinocytes, and at much higher levels than those seen in DRG neurons. Skin barrier formation and recovery is very important for preventing skin dehydration, while [Ca^{2+}] increases promote development of cell–cell junctions. TRPV4 plays an important role in skin barrier recovery,104 and contributes to intercellular junction formation in keratinocytes,105 as well as increases in [Ca^{2+}], that are crucial for accelerating barrier recovery after stratum corneum disruption.106,107 As described above, there is no clear scientific evidence for the involvement of TRPV4 in itch sensation, despite long held implications of a role for TRPV4 in itch. Recently, TRPV4 was reported to be involved in serotonin-induced itch23 in which scratching behaviors induced by histamine, SLIGRL, or CQ were not reduced in TRPV4KO mice. In contrast, CQ increased scratching behaviors in TRPV4KO mice through a mechanism that awaits characterization. Scratching
behaviors induced by 5-HT were inhibited by antagonists for both the 5-HT_{2} receptor and TRPV4, but not for the H1 receptor. Moreover, no reduction in 5-HT-induced behaviors was observed in TRPA1KO and TRPV1KO mice, which is an apparently opposite result from the finding that 5-HT_{7} and TRPA1 are required for serotonergic itch.\(^5\)\(^6\) This difference not only in physiological effects between 5-HT and the selective 5-HT_{7} agonist LP44, but also in the pathways of 5-HT-TRPA1 and 5-HT_{2}-TRPV4,\(^1\)\(^0\) could explain this apparent discrepancy (Fig. 2). More recently, Liedtke’s group showed that TRPV4 agonist-induced scratching behaviors were decreased in keratinocyte-specific TRPV4KO mice to levels seen for WT mice\(^1\)\(^0\) (Fig. 2). As such, more detailed examinations, especially to address the question of how TRPV4 is involved in itch sensation, are needed.

**Other TRP channels in pruritogen signaling**

CQ induces non-histaminergic itch sensation that is dependent on TRPA1, but not TRPV1\(^5\)\(^7\) as described above. However, one in vitro study showed that around half of CQ-responsive DRG neurons did not express TRPA1 and suggested that CQ-evoked calcium responses in a few neurons are mediated by TRPC3.\(^1\)\(^0\) Nonetheless, the in vivo significance of the involvement of TRP channels other than TRPA1 has not been established.

**Conclusions and future perspectives**

Since the TRPV1 channel was cloned, research on TRP channels expressed by primary sensory neurons has contributed significantly to elucidating the mechanism of both itch and pain. Although several key molecules such as Bhlhb5,\(^1\)\(^1\) GRPR,\(^1\)\(^2\)\(^1\)\(^1\)\(^2\)\(^1\)\(^0\) Nppb\(^1\)\(^0\) and neuropeptide Y\(^1\)\(^1\)\(^5\)\(^6\) are involved in central mechanisms of itch, some mechanisms that could involve TRP channels remain uncharacterized. In addition, the involvement of the immune system in itch could be an intriguing theme in relation to chronic itch that occurs in AD, and the relationship between TRP channel function and immune system is under exploration. We hope that this review covering recent basic research on itch and TRP channels will contribute to the future clarification of itch mechanisms.

**Conflict of interest**

The authors have no conflict of interest to declare.

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