Targeting TRP Ion Channels for Itch Relief

Xuming Zhang

Rowett Institute of Nutrition and Health & Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, United Kingdom

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Corresponding author: Dr. Xuming Zhang, Rowett Institute of Nutrition and Health & Institute of Medical Sciences, University of Aberdeen, Foresterhill, Aberdeen AB25 2ZD, United Kingdom

Tel: +44 1224 437366; E-mail: x.zhang@abdn.ac.uk

Abstract:
Acute itch (pruritus) is unpleasant and acts as an alerting mechanism for removing irritants. However, severe chronic itch is debilitating and impairs the quality of life. Rapid progress has been made in recent years in our understanding of the fundamental neurobiology of itch. Notably, several temperature-sensitive Transient Receptor Potential (thermo-TRP) ion channels have emerged as critical players in many types of itch, in addition to pain. They serve as markers that define the itch neural pathway. Thermo-TRP ion channels are thus becoming attractive targets for developing effective anti-pruritic therapies.

1. Introduction

Itch is classically defined as an unpleasant cutaneous sensation which triggers the desire to scratch. It is often caused in the local affected skin by itch-producing agents (pruritogens) including amines, proteases, neuropeptides, cytokines, inflammatory metabolites and some drugs (Akiyama and Carstens, 2013;Ross et al., 2010), and can also be a widespread symptom associated with systemic diseases, such as autoimmune diseases, liver diseases, metabolic diseases and malignant cancers. Itch can be acute and transient (e.g., by biting insects). Acute itch is considered to be a defence mechanism that alerts the body to remove irritants; however, in some cases acute itch can progress to become persistent and chronic. Chronic itch is pathological and debilitating to the well-being of humans. Itch was classified into four main categories: pruritoceptive (caused by pruritogenic agents), neurogenic, neuropathic (e.g. lesions in the nervous sytem) and psychogenic, according to the pathophysiology of itch (Yosipovitch *et al.*, 2003;Greaves, 2007).

Itch is historically linked to pain because of their close relationship and the fact that pain inhibits itch. However, compared to extensive research on pain, itch has received less attention and is still an unmet clinical need. The lack of effective itch therapies requires a deeper insight into the molecular underpinnings of itch. A growing body of recent evidence reveals TRP ion channels as key players of both acute and chronic itch elicited by a diversity of pruritogens. TRP ion channels are thus becoming alternative targets for itch treatment.

TRP ion channels are a large family of nonselective cation ion channels comprising seven subfamilies: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin) and TRPN (NOMPC-like), TRPP (polycystin) and TRPML (mucolipin) (Venkatachalam and Montell, 2007;Nilius and Owsianik, 2011). They are widely expressed in the skin, sensory neurons and a number of other tissues, and implicated in widespread functions ranging from sensory physiology (e.g., phototransduction, chemo-, thermo-, and mechano-sensation) to disorders in many systems such as the cardiovascular and respiratory systems (Nilius *et al.*, 2007). Of note, several temperature-sensitive ion channels (known as thermo-TRP ion channels), such as TRPV1, TRPA1, TRPM8 and TRPV3, have been demonstrated to be essential for the transduction of pain (Julius, 2013;Brederson *et al.*, 2013;Huang *et al.*, 2006). Interestingly, these same TRP ion channels also participate in the sensation of itch. The role of thermo-TRP ion channels in itch sensation is the focus of the review. The concomitant

involvement of Thermo-TRP ion channels in transducing both pain and itch may underlie the intimate and promiscuous relationship between pain and itch.

2. Neural mechanisms of itch

Itch has long been considered as a submodality of pain, with low intensity of stimuli eliciting itch and high intensity of stimuli causing pain (McMahon and Koltzenburg, 1992). Indeed both itch and pain are mainly transduced by the Aδ and C polymodal nociceptive fibers innervated in peripheral tissues such as skin (Ringkamp *et al.*, 2011). However, recent research lends support to the population coding based selectivity model. According to this model, pain and itch are transduced separately by two subpopulations of sensory neurons: pain-selective (nociceptive) and itch-selective (pruriceptive), though pruriceptive neurons are polymodal and can also be activated by nociceptive stimuli (algogens) (Lamotte et al., 2014;Patel and Dong, 2010). Therefore, a selective activation of pruriceptive neurons causes itch, regardless of the nature of stimuli, nociceptive or pruriceptive; in contrast, a broad activation of both nociceptive and pruriceptive neurons by nociceptive stimuli causes pain, instead of itch, because the dominant activation of nociceptive neurons inhibits and overrides the activity of the pruriceptive pathway(Roberson et al., 2013;Han et al., 2013;Lagerstrom et al., 2010;Liu et al., 2010b), an event which maybe mediated by the release of dynorphin, a kappa opioid, from the central inhibitory Bhlhb5^{+} interneurons (Ross *et al.*, 2010;Kardon *et al.*, 2014). In support of this model, selective ablation of a subpopulation of the itch-specific Dorsal Root Ganglion (DRG) neurons from mice significantly reduced itch-associated behaviours elicited by various pruritogens in mice, but spared pain behaviours (Han *et al.*, 2013). On the other hand, pain behaviours were markedly impaired, but itching responses were even enhanced in mice in which the nociceptive neuron transmission has been selectively silenced (Liu *et al.*, 2010b;Lagerstrom *et al.*, 2010), suggesting that the nociceptive pathway constitutively inhibits the itch transducing pathway.

3. The involvement of Thermo-TRP ion channels in itch

There are two types of itch according to the category of pruritogens: histamine-dependent (histaminergic) and histamine-independent (non-histaminergic) (Table 1). The histaminergic itch is caused by histamine, and thus can be effectively treated by anti-histamines. However, the majority of itch are non-histaminergic, and cannot be effectively treated by the anti-histamines. For example, chloroquine, a drug used to treat malaria, can cause intolerable itch resistant to the anti-histamine therapy.

3.1 Histaminergic itch and TRPV1

It has long been recognized that histamine provokes itching by acting on the histamine receptor H1R on sensory nerve endings (Greaves and Davies, 1982). But it was not known how activation of H1R causes the excitation of pruriceptive sensory neurons until 2007, when Shim et al discovered that activation of H1R opens TRPV1, a heat gated ion channel that also responds to capsaicin and acid, leading to the excitation of a subpopulation of sensory neurons (Shim *et al.*, 2007). TRPV1 was then implicated in transducing histaminergic itch. Consistent with this idea, the majority of histamine-responding DRG neurons co-expressed TRPV1 (TRPV1^{+}) (Figure 1), and histaminergic itch was significantly reduced by deleting

the TRPV1 gene (Shim *et al.*, 2007). Furthermore, histamine-induced itching response (scratching) was eliminated after prior silencing the $TRPV1^+$ pruriceptive neurons through prior co-injection of histamine with the membrane impermeable lidocaine derivative QX-314, a sodium channel blocker which acts by inactivating sodium currents after entering neurons through the large pore of opened TRPV1 (Roberson *et al.*, 2013). The same manipulation, however, did not affect capsaicin-evoked pain behaviour (wiping)(Roberson et al., 2013). These findings suggest that there is a subpopulation of $TRPV1^+$ neurons that specifically mediates itch, but is dispensable for pain, and that TRPV1 is a main downstream coupling ion channel of H1R, mediating histaminergic itch.

It should be noted that histamine-induced scratching was only partially reduced by deleting the TRPV1 gene (Shim *et al.*, 2007). Strikingly, the histaminergic itch was completely abolished by deleting the $TRPV1^+$ neurons (Imamachi *et al.*, 2009), implying that other transducers, in addition to TRPV1, contained within the $TRPV1^+$ neurons are also involved. Consistent with this suggestion, histamine still induced intracellular calcium ($[Ca^{2+}]_i$) increases in the DRG neurons lacking TRPV1(Shim *et al.*, 2007), and a proportion of histamine-excited neurons (11.4%) did not respond to capsaicin and thus did not co-express TRPV1(Figure 1). One possibility for mediating TRPV1-independent responses is the histamine receptor H4R, because it has been shown that selective activation of H4R is sufficient to trigger $[Ca^{2+}]_i$ increases in the DRG neurons and itching responses in animals (Bell *et al.*, 2004;Rossbach *et al.*, 2011). However, downstream coupling ion channels and signalling mechanisms activated by H4R remain to be established.

3.2 Non-histaminergic itch and TRPA1

The mechanisms of the histaminergic itch had been sought for centuries. By contrast, the non-histaminergic itch has received attention just in recent years. It is caused by a diversity of pruritogens (Table 1), such as chloroquine (CQ), serotonin, endothelin-1 (ET-1) and cowhage etc., of which CQ has been exploited as a primary chemical tool towards our understanding of the non-histaminergic itch.

In contrast to TRPV1-mediated histaminergic itch, CQ induces itching by exciting the TRPA1 ion channel after binding to the CQ receptor MrgprA3, a Mas related G protein coupled receptor (Wilson *et al.*, 2011;Han *et al.*, 2013;Liu *et al.*, 2009). In support of this concept, a strong itching deficit caused by CQ was observed either by deleting the TRPA1 gene from mice or by electrically silencing the $TRPA1^+$ neurons or by ablating the $MrgprA3^+$ neurons (Wilson et al., 2011;Han et al., 2013;Roberson et al., 2013). Although TRPA1 was initially identified as a transducer for pain (Julius, 2013), a specific subpopulation of $TRPA1^+$ neurons is indispensable for mediating itch, not for pain. This conclusion is based on the fact that TRPA1-mediated pain behaviour induced by injection of AITC, an agonist to TRPA1, is not altered after selective silencing the $TRPA1^+$ pruriceptive neurons by prior co-injection of CQ and QX-314, a process, however, does abolish CQ-elicited itching (Roberson *et al.*, 2013). This subpopulation of the itch-specific $TRPA1^+$ neurons seems to also underlie the actions of many other non-histaminergic pruritogens, such as BAM8-22(Wilson *et al.*, 2011), SLIGRL (Roberson *et al.*, 2013;Liu *et al.*, 2011), IL-31(Cevikbas *et al.*, 2014) and oxidative

stress (Liu and Ji, 2012). As with CQ, these non-histaminergic pruritogens co-excited $TRPA1^+$ neurons, and their elicited itching behaviours were also correspondingly reduced in mice lacking TRPA1. However, we have recently found that TRPA1 mediates responses from only a subpopulation of CQ-sensitive neurons (Than *et al.*, 2013). More than half of CQ-sensitive neurons did not co-express TRPA1, and their responses were not prevented by blocking TRPA1, instead were blocked by an antagonist to the TRPC3 ion channel, suggesting that TRPC3 mediates the actions of CQ in this subpopulation of CQ-sensitive neurons (Than *et al.*, 2013). It remains to be determined whether this population of $TRPC3^+$ neurons contributes to itch.

In addition to acute itch, TRPA1 is involved in chronic itch such as atopic dermatitis and other inflammation associated dermatitis. The expression of TRPA1 was increased in skin lesions from patients with chronic itch (Oh *et al.*, 2013;Yang *et al.*, 2014). Behaviourally the itching response was significantly diminished in mice with chronic itch either by blocking TRPA1 pharmacologically or by deleting TRPA1 genetically (Wilson *et al.*, 2013a;Liu *et al.*, 2013;Wilson *et al.*, 2013b;Oh *et al.*, 2013). Furthermore, TRPA1 deficient mice exhibited less pathological changes in the affected skin in a mice dry-skin model (Wilson *et al.*, 2013a;Liu *et al.*, 2013), presumably caused by reduced neurogenic inflammation provoked by the activity of TRPA1(Bautista *et al.*, 2013). Interestingly, thymic stromal lymphopoietin (TSLP) was demonstrated as an important cytokine that directly excites TRPA1, resulting in enhanced scratching behaviours associated with atopic dermatitis in animals (Wilson *et al.*, 2013b;Jariwala *et al.*, 2011). Given the prominent role of TRPA1 in itch, it is promising to combat acute and chronic itch by targeting the TRPA1 ion channel.

3.3 Relationship between TRPV1- and TRPA1-mediated itch

It has been reported that TRPA1 is largely contained within a subpopulation of TRPV1-expressing neurons (Story *et al.*, 2003). However, it was not known whether the $TRPV1^+$ pruriceptive neurons that mediate histaminergic itch, overlap with the $TRPA1^+$ pruriceptive neurons that mediate non-histaminergic itch. Answer to this question has been suggested by the following behavioural and cellular evidence. First, non-histaminergic itching induced by CQ was not affected by either deleting the TRPV1 gene or by specifically silencing histamine activated $TRPV1^+$ pruriceptive neurons, but substantially reduced by either deleting the TRPA1 gene or by silencing $TRPA1^+$ pruriceptive neurons (Wilson *et al.*, 2011;Roberson *et al.*, 2013); on the other hand, histamine-elicited itching was reduced by deleting or silencing $TRPV1^+$ neurons, but not by silencing $TRPA1^+$ pruriceptive neurons (Shim *et al.*, 2007;Imamachi *et al.*, 2009;Roberson *et al.*, 2013). Therefore, the histaminergic itch and CQ-induced itch are transduced by two largely separate and non-overlapping populations of $TRPV1^+$ and $TRPA1^+$ pruriceptive neurons, respectively. It also suggests that histamine and CQ-activated pruriceptive pathways divide at the outset in the peripheral DRG neurons. Consistent with behavioural studies, histamine and CQ excited two largely separate populations of DRG neurons (Roberson *et al.*, 2013). However, for some non-histaminergic pruritogens such as IL-31 and leukotriene B-4 (LTB-4), both TRPV1 and TRPA1 are required for mediating the excitation of sensory neurons and itching behaviour in mice (Cevikbas et al., 2014;Rabenhorst and Hartmann, 2014;Fernandes et al., 2013), presumably

pruritic receptors for these cytokines are expressed in subsets of both $TRPA1^+$ and $TRPV1^+$ pruriceptive neurons.

In summary, the $TRPV1^+$ and $TRPA1^+$ pruriceptive neurons represent two discrete subpopulations of itch-specific neurons, in which TRPV1 and TRPA1 are the main downstream coupling ion channels that mediate the excitation of pruriceptive neurons evoked, respectively, by histamine and non-histaminergic pruritogens including CQ, BAM8-22, SLIGRL and TSLP (Table 1).

3.4 Possible role of other TRP ion channels in mediating non-histaminergic itch

Histamine and CQ have served as powerful fishing tools in disclosing the two subpopulations of itch-specific $TRPV1^+$ and $TRPA1^+$ neurons in the itch neural pathway. Accumulating evidence also suggests that there exist other types of itch-transducing neurons independently of either TRPV1 or TRPA1 (see below). In these cases, other non-histaminergic pruritogens (Table 1) will provide further impetus in searching for molecules and ion channels that are responsible for other itch transducing mechanisms.

ET-1, serotonin and imiquimod: ET-1 induces both nociceptive and itching behaviour when injected intradermally. These effects are mediated by the ETAR receptor (Liang *et al.*, 2010;Kido-Nakahara *et al.*, 2014;Trentin *et al.*, 2006;Gomes *et al.*, 2012). $TRPV1^+$ pruriceptive neurons were found to be critical for mediating itching responses induced by ET-1, because ET-1 elicited scratching was substantially reduced after selective killing the central terminals of TRPV1-expressing nociceptors through intrathecal injection of capsaicin (Imamachi *et al.*, 2009). With the same approach, $TRPV1^+$ neurons were also found to be essential for itching elicited by serotonin and imiquimod (Imamachi et al., 2009;Kim et al., 2011). However, itching behaviours induced by all these pruritogens were not significantly different between TRPV1 knockout mice and wild type littermates (Imamachi *et al.*, 2009;Liu *et al.*, 2010a;Kim *et al.*, 2011), suggesting that the TRPV1 ion channel *per se* is not required for ET-1-, serotonin- and imiquimod-induced itching, and that other unknown ion channels expressed within the $TRPV1^+$ neurons are involved. Consistent with the behavioural studies, the percentage of DRG neurons responding to ET-1 was not significantly reduced by either deleting TRPV1 or TRPA1(Kido-Nakahara *et al.*, 2014). Taken together, $TRPV1^+$ pruriceptive neurons comprise functionally diversified subpopulations and contain, in addition to TRPV1, other unknown, potentially TRP ion channels that mediate scratching responses caused by ET-1, serotonin and imiquimod.

β-alanine: β-alanine is an β amino acid. It causes an itching and tingling sensation after consumption. Interestingly, β-alanine induced itching by activating a subpopulation of neurons expressing Mrgprd, a G protein coupled receptor (Liu et al., 2012). Furthermore, β-alanine excited neurons did not respond to either histamine or CQ (Liu *et al.*, 2012), suggesting that the $Mrgprd^+$ neurons define a separate subpopulation of pruriceptive neurons, distinct from the $TRPV1^+$ and $TRPA1^+$ pruriceptive neurons. However, ion channels downstream of Mrgprd activation remain to be established.

Cowhage: Cowhage spicules have long been recognized to be able to induce a strong itching sensation. The underlying mechanisms, however, remain obscure. It appears that cowhage-induced itch is not correlated with histamine-induced itch, because cowhage and histamine activate largely separate sets of peripheral afferent C fibres and also two largely independent populations of spinothalamic tract neurons (STT) neurons (Johanek *et al.*, 2007;Davidson *et al.*, 2007;Davidson *et al.*, 2012;Papoiu *et al.*, 2011;Johanek *et al.*, 2008). The active component of cowhage has been identified as mucunain, a cysteine protease (SHELLEY and ARTHUR, 1955). It acts on protease-activated receptor (PAR) 2 and 4 on DRG neurons to trigger calcium influx(Reddy *et al.*, 2008). However, downstream ion channels coupled to PAR2 and PAR4 remain undetermined. Several TRP ion channels have been suggested including TRPV1, TRPV4 and TRPA1(Nakagawa and Hiura, 2013), because the PAR receptors were co-expressed with these TRP ion channels in DRG neurons(Amadesi *et al.*, 2004;Dai *et al.*, 2007;Grant *et al.*, 2007;Dai *et al.*, 2004).

Cholestatic pruritus: Cholestatic liver diseases are often accompanied with a severe and intractable itching sensation. It has recently been demonstrated that increased bile acids (BAs) in the circulation, at least in part, are responsible for cholestatic pruritus (Alemi *et al.*, 2013). BAs increased the excitability of a subset of DRG neurons by acting on the G protein coupled receptor TGR5, leading to the release of pruritogenic gastrin-releasing peptide (GRP) (Alemi *et al.*, 2013). This process was recently demonstrated to be mediated by activating and sensitizing TRPA1 following TGR5 activation (Lieu et al., 2014). Apart from BAs, lysophosphatidic acid (LPA) was also identified as pruritogenic agents from cholestatic patients (Kremer *et al.*, 2010). It will be interesting to determine on which subpopulations of pruriceptive neurons ($TRPV1^+$ or $TRPA1^+$ or others) LPA act in the future.

4. Itch signalling mechanisms

Most painful stimuli (algogens) excite nociceptive neurons often by directly targeting ion channels on sensory neurons. For example, capsaicin and mustard oil act on TRPV1 and TRPA1, respectively to produce nociception (Julius, 2013). By contrast, nearly all pruritogens elicit itch by acting on pruritic receptors on sensory neurons, most of which are GPCRs. Activated pruritic receptors then trigger the activation of a downstream ion channel, often a TRP ion channel, through an intracellular signalling coupling mechanism, leading to consequent excitation of pruriceptive neurons (Fig. 2). Thereby, pruritogens excite pruriceptive neurons through an indirect intracellular signalling coupling mechanism.

4.1 $TRPV1^+$ pruriceptive neurons: Itch induced by histamine is mediated by the histamine receptor H1R, which is then coupled to TRPV1. How TRPV1 is opened by H1R-evoked signalling? H1R is a Gq coupled G-protein Coupled Receptor (GPCR). Activated Gq activates PLCβ and PLA2 resulting in increases in $[Ca^{2+}]_i$ and the liberation of arachidonic acid. Arachidonic acid is ultimately metabolized via lipooxygenase into various products, of which 12-hydroperoxyeicosatetraenoic acid (12-HPETE) was found to be critical for activating TRPV1 (Shim *et al.*, 2007). Notably, PLCβ3 is specifically required for H1R-mediated $[Ca^{2+}]_i$ increases in the DRG neurons and histamine elicited itching in mice,

because both events were prevented by deleting PLCβ3 (Imamachi *et al.*, 2009;Han *et al.*, 2006).

Although histamine-elicited itch signalling mechanisms are well-defined, it is poorly understood regarding the itch signalling mechanisms evoked by serotonin and ET-1, both of which act also by activating the $TRPV1^+$ pruriceptive neurons (Imamachi et al., 2009). This is probably due to unknown downstream ion channels activated by serotonin and ET-1. Nevertheless, PLCβ3 has been found to be essential for the itch signalling elicited by serotonin, but not by ET-1 (Imamachi *et al.*, 2009). Imiquimod is another pruritogen that elicits itch by activating the $TRPV1^+$ pruriceptive neurons (Kim *et al.*, 2011). However, the signalling mechanisms underlying the actions of imiquimod are controversial. One group reported that the Toll-like receptor 7 (TLR7) is required for imiquimod to elicit intracellular signalling in DRG neurons (Liu *et al.*, 2010a); another group found that imiquimod excites $TRPV1^+$ neurons independently of TLR7, instead by inhibiting the background K(2P) channel and the voltage gated Kv1.1 and Kv1.2 channels (Kim *et al.*, 2011;Lee *et al.*, 2012). Altogether, $TRPV1^+$ pruriceptive neurons seem to employ diverse intracellular signalling mechanisms to mediate the pruritogenic actions of histamine, serotonin, ET-1 and imiquimod.

4.2 $TRPA1^+$ pruriceptive neurons: Several pruritogens, such as CQ, BAM8-22 and TSLP, activate intracellular signalling that couples to the TRPA1 ion channel, leading to the excitation of the $TRPA1^+$ pruriceptive DRG neurons. BAM8-22 acts on MrgprC11, a Gq coupled GPCR, and TSLP binds to the TSLP receptor consisting of the IL7 receptor alpha (IL7Rα) chain and a TSLP-specific receptor chain (TSLPR). Although the two pruritogens act on different types of receptors, both appear to require Phospholipiase C (PLC) to excite TRPA1 (Wilson *et al.*, 2011;Wilson *et al.*, 2013b). By contrast, even though CQ and BAM8-22 bind to a similar type of Gq coupled GPCRs, CQ excites TRPA1 through released Gβγ without the involvement of PLC, while BAM8-22 activates TRPA1 via the PLC pathway without the need of Gβγ (Wilson *et al.*, 2011). It remains uncertain how Gβγ activates TRPA1 and what are the detailed intracellular signalling mechanisms mobilized by PLC, leading to the excitation of TRPA1.

5. Itch modulation

TRPV1 and TRPA1 are the two main ion channels that mediate itching responses induced by a number of pruritogens. Agents or factors capable of enhancing activities of TRPV1 and TRPA1 should increase the intensity of the itch sensation (i.e. hyperkinesis) (Ikoma *et al.*, 2006;Huang *et al.*, 2006). For example, CQ not only directly excited TRPA1, but also potently enhanced TRPV1 responses through the PLC-PKC pathway (Than *et al.*, 2013), suggesting that CQ contributes to itching not only by directly exciting TRPA1, but may also by indirectly facilitating TRPV1 mediated itching responses. Furthermore, the basal sensitivity of TRPV1 is enhanced in a subpopulation of $TRPV1^+$ neurons co-expressing PKCβII(Li *et al.*, 2014). Enhanced functions of TRPV1 in the subpopulation of $TRPV1^+$/$PKC\beta II^+$ neurons could result in a higher sensitivity to either algogens or

pruritogens or both, depending on whether this neuron population is pruriceptive or nociceptive, which remains to be elucidated.

5.1 Modulation of itch by inflammatory mediators

Pain sensitization caused by inflammation is often accompanied with increased sensation of itch. Inflammation is a typical feature of chronic itch such as atopic dermatitis.
The mechanisms underlying the sensitization of TRPV1 and TRPA1-mediated pain by various inflammatory mediators have been extensively studied in the past. These mechanisms may also apply to $TRPV1^+$ and/or $TRPA1^+$ pruriceptors -mediated itch. For example, the inflammatory mediator bradykinin enhances pain at least through potentiating TRPV1 and/or TRPA1 (Mizumura *et al.*, 2009;Zhang *et al.*, 2008). Interestingly, bradykinin also increased inflammation-evoked itch (hyperkinesis) and induced allokiness (touch-evoked itch) (Liang *et al.*, 2012;Feng *et al.*, 2014;Hosogi *et al.*, 2006), suggesting that bradykinin sensitizes itch. However, it was not established whether bradykinin-elicited pruritus is mediated by TRPV1 or TRPA1. Another example is neurotrophic factors such as NGF and artemin, which are known to sensitize nociceptors and promote thermal and mechanical hyperalgesia (Pezet and McMahon, 2006;Malin et al., 2011;Malin et al., 2006;Zhang et al., 2005). NGF and artemin have also been recently shown to sensitize pruriceptors and thus enhance itching upon injected intradermally (Rukwied *et al.*, 2013;Murota *et al.*, 2012). Consistent with their role in regulating itch, the level of NGF and artemin was significantly elevated in the skin from patients with atopic dermatitis (Yamaguchi *et al.*, 2009;Murota *et al.*, 2012). Intriguingly, NGF sensitized only cowhage-induced itch, but hot histamine-induced itch(Rukwied *et al.*, 2013), which is mainly mediated by TRPV1. It is therefore tempting to speculate that the NGF receptor TrkA is not expressed in the $TRPV1^+$ pruriceptive neurons, but present in pruriceptive neurons that mediate the action of cowhage. It also suggests that cowhage and histamine elicit itch by activating distinct populations of pruriceptive neurons, consistent with previous reports (Davidson et al., 2007;Johanek et al., 2008). In addition to neurotrophic factors, several other inflammatory mediators including the PAR-2 agonist tryptase (Steinhoff *et al.*, 2003;Akiyama *et al.*, 2010), PGE2 (Belghiti *et al.*, 2013) and 5-HT (Akiyama *et al.*, 2010) were increased in chronic itch conditions. Scratching behaviour was attenuated by either blocking the action of these factors with antibodies or by blocking their acting receptors, showing that these inflammatory agents are involved in chronic itch. In a recent study of a mice model of cholestatic pruritus, there was a significant increase in the expression of the PAR-2 receptor and prostaglandin PGE2, leading to the sensitization of TRPV1 and consequently enhanced pain and itch (Belghiti *et al.*, 2013), suggesting that tryptase and PGE2 enhance itching at least by targeting TRPV1.

5.2 Modulation of itch by TRP ion channels

TRPV1 and TRPA1 are directly involved in the itch transduction. This process can also be influenced by the actions of several other Thermo-TRP ion channels such as TRPV3 and TRPM8.

TRPV3: TRPV3 is a warm temperature-sensitive ion channel with an activation threshold of over 33°C (Peier *et al.*, 2002;Moqrich *et al.*, 2005;Xu *et al.*, 2002;Smith *et al.*, 2002). In

contrast to TRPV1 and TRPA1, which are mainly expressed in peripheral sensory neurons and directly mediate pruritogens-induced excitation of neurons, TRPV3 is instead abundantly expressed in the epidermal keratinocytes in the skin with little expression in DRG (Peier *et al.*, 2002;Xu *et al.*, 2002). Consistent with its unique expression profile, TRPV3 was involved in skin barrier formation and hair morphogenesis (Cheng *et al.*, 2010;Yamamoto-Kasai *et al.*, 2012). Interestingly, activation of TRPV3 (e.g. by warm temperature) in keratinocytes led to the release of a diversity of factors including ATP (Mandadi *et al.*, 2009), PGE2 (Huang *et al.*, 2008), IL-1α (Xu *et al.*, 2006), nitric oxide (Miyamoto *et al.*, 2011), TGF-α (Cheng *et al.*, 2010) and NGF (Yoshioka *et al.*, 2009). These factors aggravated inflammatory processes occurred in atopic dermatitis and altered skin structure (e.g. increased density of epidermal C-fibres) (Nilius and Biro, 2013;Yamamoto-Kasai et al., 2012). The released inflammatory factors may also sensitize pruriceptive fibres innervated in the skin, collectively resulting in an outcome of heightened itching responses. In further support of a critical role of TRPV3 in itch, overexpression of the highly active TRPV3 mutant G573S in mice caused even spontaneous dermatitis and scratching behaviour (Yoshioka *et al.*, 2009). Increased scratching behaviour induced by AEW (acetone/ether mixture and water) in a dry skin mice model was absent in TRPV3 deficient mice (Yamamoto-Kasai *et al.*, 2012). Furthermore, Both TRPV3 and TSLP expression were increased in skins from DS-Nh mice, an atopic dermatitis model (Yamamoto-Kasai et al., 2013). Interestingly, TSLP secretion was dependent on TRPV3 (Yamamoto-Kasai et al., 2013). Since it has recently been shown that Ca^{2+} is critical for TSLP secretion (Wilson et al., 2013b), it is very likely that TRPV3 promotes TSLP secretion by increasing Ca^{2+}, and secreted TSLP, in turn, activates TRPA1 to elicit itching (Wilson et al., 2013b). Although it seems to be an appealing mechanism by which TRPV3 promotes itching, it remains to be validated in the future. Collectively, these findings implicate TRPV3 in the modulation of various types of itch. Furthermore, activation of TRPV3 by warm temperatures may provide a mechanistic explanation for the commonly known experience that warmth facilitates the itch sensation.

TRPV4: As with TRPV3, TRPV4 was also found to be expressed in keratinocytes and sensitive to warm temperatures (>27°C) (Chung *et al.*, 2003;Guler *et al.*, 2002;Mihara *et al.*, 2011;Chung *et al.*, 2004). These features make TRPV4 another candidate that may play a role in itch associated with dermatitis. Indeed, the expression of TRPV4, together with TRPV3 and TRPA1, was increased in skin from patients with post-burn pruritus(Yang *et al.*, 2014). However, the exact role of TRPV4 in dermatic pruritus needs more investigation.

TRPM8: TRPM8 is an ion channel that can be activated by cold (<26°C) and cooling compounds such as menthol and icilin (McKemy *et al.*, 2002;Chuang *et al.*, 2004). It is mainly expressed in neurons from DRG and the trigeminal ganglia, consistent with its predominant role in the detection of cold in mice (Colburn *et al.*, 2007;Dhaka *et al.*, 2007;Bautista *et al.*, 2007).

Cooling has a marked effect on the itch sensation. Researchers observed that cooling almost abolished itching in atopic dermatitis patients and histamine-induced itching in healthy

subjects(Fruhstorfer *et al.*, 1986). Skin cooling also diminished responses of spinal dorsal horn neurons elicited by intracutaneous microinjection of histamine (Carstens and Jinks, 1998). Furthermore, topical application of the TRPM8 agonist icilin alleviated pruritus (Han *et al.*, 2012). However, whether the inhibitory effect of cooling or cooling compound on itching is mediated by TRPM8 has not been tested. TRPM8 knockout mice will help to resolve this question in the future. It is also unknown how activation of the cold thermoreceptors inhibits the itch sensation. It is likely to be mediated through a central mechanism, for example, by acting on the inhibitory mGluRII/III receptors in the dorsal horn, which also underlies the analgesic actions of icilin on neuropathic pain (Proudfoot *et al.*, 2006).

Concluding remarks:

Peripheral sensory neurons contain multiple subpopulations of specialized pruriceptive neurons responsible for transducing various types of itch. $TRPV1^+$ and $TRPA1^+$ pruriceptive neurons represent two main distinct subpopulations of sensory neurons that respond to histamine and a diversity of other non-histaminergic pruritogens, respectively. These pruriceptive neurons employ diverse intracellular signalling mechanisms that couple the activation of pruritic receptors to the excitation of downstream ion channels (e.g. TRPV1, TRPA1 and other unknown TRP ion channels). Of note, different pruritogens activate different downstream ion channels even within the same type of pruriceptive neurons, highlighting the heterogeneity and diversity of pruriceptive neurons. Future efforts should be directed to identify other unknown downstream ion channels that are activated by various pruritogens. In addition to directly mediating the excitation of pruriceptive neurons (TRPV1, TRPA1), thermo-TRP ion channels (e.g. TRPV3 and TRPM8) are also involved in the modulation of itch through both peripheral and central mechanisms. A full understanding of the role of TRP ion channels in the itch transduction will be invaluable in developing effective anti-pruritic therapies by targeting these ion channels.

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Table 1: Pruritogens, pruritic receptors and their acting downstream TRP ion channels

Pruritogens		Pruritic receptors	Excited ion channels	References
Histaminergic	Histamine	H1R, H3R and H4R	TRPV1, others?	(Shim et al., 2007;Roberson et al., 2013;Bell et al., 2004;Rossbach et al., 2011;Imamachi et al., 2009)
Non-histaminergic	Chloroquine	MrgprA3	TRPA1, TRPC3	(Wilson et al., 2011;Liu et al., 2009;Than et al., 2013)
	SLIGRL, BAM8-22	MrgprC11	TRPA1	(Wilson et al., 2011;Liu et al., 2011;Roberson et al., 2013;Liu et al., 2009)
	5-HT	5-HT2A	?	(Imamachi et al., 2009;Akiyama et al., 2010)
	ET-1	ETAR	?	(Liang et al., 2010;Kido-Nakahara et al., 2014;Trentin et al., 2006;Gomes et al., 2012)
	Cowhage	PAR2 and PAR4	TRPA1?	(Reddy et al., 2008)
	IL-31	IL-31RA	TRPV1, TRPA1	(Cevikbas et al., 2014)
	TSLP	TSLPR	TRPA1	(Wilson et al., 2013b)
	LTB4	BLT1	TRPV1, TRPA1	(Fernandes et al., 2013)
	β-alanine	MrgprD	?	(Liu et al., 2012)
	Bile acids	TGR5	?	(Alemi et al., 2013)
	LPA	LPA4?	?	(Kremer et al., 2010)

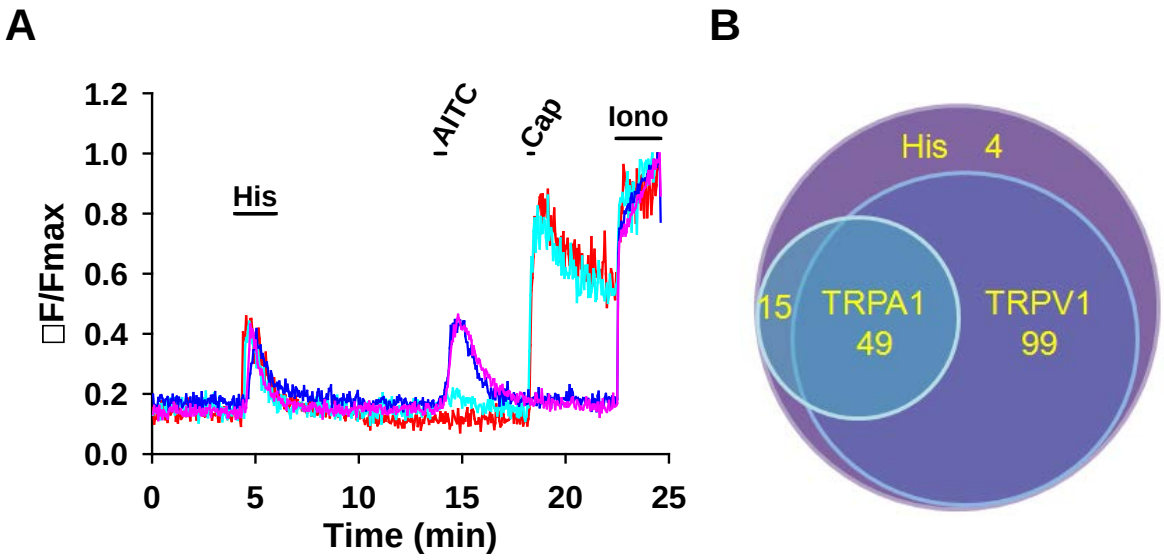

Figure 1. Responses of DRG neurons elicited by histamine. (A) Examples of responses of $[Ca^{2+}]_i$ from 4 DRG neurons stimulated with histamine (His, 100μM), AITC(100μM), an agonist to TRPA1, and capsaicin (Cap, 0.5μM), an agonist to TRPV1. Ionomycin (Iono, 10μM) was added at the end of the experiments. **(B)** The Venn diagram summary of different neuron populations co-expressing TRPV1 and/or TRPA1 within histamine excited neurons from experiments similar to those in A. The number of neurons is shown for each population and was obtained from a total of 2693 neonatal DRG neurons, as before (Than et al., 2013)

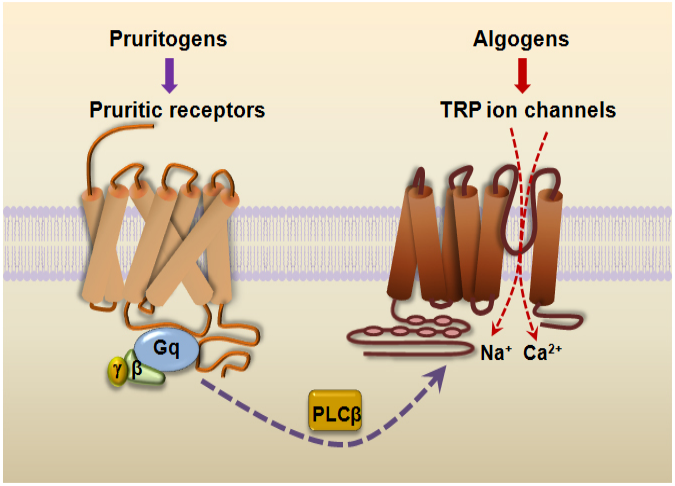

Figure 2. A schematic diagram depicting the excitation of sensory neurons elicited by pruritogens through intracellular signalling coupling mechanisms. Following binding to corresponding pruritic receptors (mostly G protein coupled receptors), pruritogens excites pruriceptive neurons by activating intracellular signalling messengers (e.g. PLCβ), which are then coupled to a downstream ion channel (e.g. TRP ion channels), leading to the influx of cation ions and subsequent neuron excitation. Noxious stimuli (algogens), however, directly open TRP ion channels resulting in neuron excitation.