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Molecular and Cellular Mechanisms that Initiate Pain and Itch

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Abstract

Somatosensory neurons mediate our sense of touch. They are critically involved in transducing pain and itch sensations under physiological and pathological conditions, along with other skin resident cells. Tissue damage and inflammation can produce a localized or systemic sensitization of our senses of pain and itch, which can facilitate our detection of threats in the environment. Although acute pain and itch protect us from further damage, persistent pain and itch are debilitating. Recent exciting discoveries have significantly advanced our knowledge of the roles of membrane-bound G protein-coupled receptors and ion channels in the encoding of information leading to pain and itch sensations. This review focuses on molecular and cellular events that are important in early stages of the biological processing that culminates in our senses of pain and itch.

Keywords

allodynia; alloknesis; atopic dermatitis; BAM8-22; B cells; chemokines; chloroquine; cytokines; dendritic cells; dorsal root ganglia; endothelins; gastrin-releasing peptide; glutamate; G protein-coupled receptors; histamine; hyperalgesia; hyperknesis; inflammatory pain; IL-13; IL-31; ion channels; keratinocytes; Langerhans cells; macrophages; mas-related G-protein-coupled receptors; mast cells; MrgprA3; MrgprC11; MrgprD; natriuretic polypeptide receptor subtype A; neutrophils; neuropathic pain; nociceptors; nodose ganglia; opioids; proteinase-activated receptor; pruritogens; pruritus; thymic stromal lymphopoietin; pruriceptors; serotonin; skin-resident cells; SLIGRL; spared nerve injury; substance P; tachykinins; T cells; toll-like receptors; transient receptor potential cation channels; trigeminal ganglia; TRPM3; TRPM8; TRPV1; TRPA1; TRPV3; TRPV4; Wallerian degeneration

1. Introduction

Pain and itch are closely related. Both are aversive but are associated with distinct behaviors: pain elicits a withdrawal response and itch elicits reflexive scratching as well as the desire to scratch. Although both itch and pain are initiated by activation of primary sensory neurons and involve some of the same ion channels, there is convincing evidence that distinct subpopulations of sensory neurons and spinal circuits are involved in

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transduction of pain and itch stimuli [1–3]. Furthermore, activation of pain-related sensory fibers inhibits the scratching response in rodents, suggesting the existence of crosstalk between the pain and itch signaling pathways [4, 5].

Transduction and generation of pain and itch sensations involve many molecules, cells, and neural circuits at numerous levels from the periphery to spinal cord and brain [1, 6]. Dynamic interactions among skin resident cells (keratinocytes, Merkel cells, innate and adaptive immune cells, etc.) and sensory nerve endings strongly influence our senses of touch, pain and itch [7, 8] (Figure 1). Spinal interneurons and descending excitatory and inhibitory inputs also work together to fine tune the transmission of pain and itch in the central nervous system (CNS) [9, 10]. The activity of primary and higher order sensory cells in the periphery and CNS is largely controlled by a delicate balance between excitatory and inhibitory events involving activation of membrane-bound ion channels, G protein-coupled receptors (GPCRs), cytokine receptors and other tyrosine kinase receptors.

Under pathological conditions such as tissue damage and nerve injury, our sensation of pain and itch can increase markedly, such that a normally insignificant stimulus can produce painful (allodynia) or itching (alloknesis) sensations. Moreover, a normally pruritic stimulus can elicit a greater than normal duration and/or magnitude of itch (hyperknesis). Similarly, a normally painful stimulus such as the prick of a needle can be perceived as even more painful (hyperalgesia). These abnormal sensory states have been associated with increased expression and function of pain- and/or itch-related ion channels and GPCRs in the pain and itch pathways [11, 12]. Here we present an overview of the molecular and cellular signaling events in the periphery and spinal cord that are essential for our senses of pain and itch. Our focus is on neuronal and non-neuronal cells that are critically involved in the signaling pathways of pain and itch under normal and pathological conditions. We do not consider the complementary roles of P2X receptors, acid-sensing ion channels (ASICs), hyperpolarization- activated cyclic nucleotide-gated (HCN) channels, mechanically gated piezo channels, or voltage-gated Na^+, Ca^{2+}, and K^+ channels in the initiation of touch, pain, and itch sensations, as these have been covered recently in several excellent reviews [11, 13–18].

2. The cellular basis of itch and pain

Our somatosensory system includes sensors in the skin, cutaneous receptors, that inform us about surface temperature (thermoreceptors), pressure and texture (mechanoreceptors), itch (pruriceptors) and actual or potential injury (nociceptors). The activity of cutaneous receptors is influenced by numerous surrounding cells including keratinocytes and both innate and adaptive immune cells. Subsets of the primary sensory neurons act as cellular sensors for itchy and painful stimuli and promote scratching or withdrawal behaviors upon activation.

2.1. Keratinocytes

Many specialized skin resident cells contribute to different sensory modalities, adding to the great diversity in structure and function of sensory nerve endings. Keratinocytes, the predominant cell type in the skin, form the protective barrier between the body and the

external environment. Although direct involvement of keratinocytes in pain sensation remains controversial, emerging evidence indicates paracrine communication from keratinocytes to sensory nerve terminals. Paracrine mediators released by keratinocytes include thymic stromal lymphopoietin (TSLP), ATP, endothelins, prostaglandins, histamine, nitric oxide (NO), and serotonin [19–25] (Figure 1). These mediators can directly activate or sensitize primary sensory neurons to initiate pain or itch processing.

In addition, keratinocytes produce and secrete various biologically active chemokines, cytokines, and trophic factors [26, 27], all of which can regulate the expression and function of many ion channels in sensory neurons, suggesting that they may promote hyperexcitability of sensory terminals. Indeed, the production and release of these cytokines and trophic factors are increased in injured tissues. Radtke et al. showed that the excitability of DRG neurons with axons in ligated sciatic nerve is markedly increased when keratinocytes are transplanted to the site of the nerve ligation [28]. Animals with keratinocyte transplants display a rare behavior, autotomy (scratching and biting their denervated paws) that is thought to reflect severe pain. The role of keratinocytes in pain activation is further supported by the finding that conditional knockout of the TRPV4 (transient receptor potential cation channel subfamily V member 4) channel from epidermal keratinocytes reduces the release of skin-derived endothelins, which mediate both thermal and mechanical hypersensitivity induced by UVB exposure in mice [23].

2.2. Innate immune cells

These cells are secretory immunocytes strategically located in defensive positions in various tissues of our body, where they can release a versatile ensemble of inflammatory mediators [29].

2.2.1. Mast cells—In the skin, mast cells are in close proximity to unmyelinated nerve fibers and establish dynamic interactions with itch-activating pruriceptors and/or pain-activating nociceptors [30] (Figure 1). Increased numbers of epidermal and dermal sensory fibers expressing PGP 9.5 and calcitonin gene related peptide (CGRP) are found in contact eczema or atopic dermatitis (AD). These alternations, including the probable release of tumor necrosis factor (TNF) from mast cells in the lesions, are greatly attenuated in mast cell-deficient c-*kit* mutant mice or TNF-deficient mice [31]. The expression of cell adhesion molecule-1 (CADM1) is also increased by mast cells in AD, which promotes adhesion and communication between the mast cells and sensory neurites [32].

Activation of mast cells releases histamines, serotonin, proteases, lipid mediators, neuropeptides, and various cytokines, all of which are important mediators of itch and pain signaling [33] (Figure 1). These mediators either act directly on pruriceptors and nociceptors or stimulate the production of secondary mediators such as substance P (SP), leukotrienes and prostanoids that act on sensory neurons [33]. Compound 48/80, a canonical basic secretagogue, induces hyperalgesia in mice [34] by activating Mrgprb2 on mast cells to trigger release of histamine [35]. Inhibition of mast cell degranulation by a mast cell stabilizer suppresses compound 48/80-induced hyperalgesia and postoperative pain in mice [34, 36, 37], and nerve injury-induced hyperalgesia in rats [36]. Furthermore, mast cell-

deficient mice have attenuated pelvic pain behavior, suggesting that mast cells are likely involved in the pathogenesis chronic pelvic pain [38]. Mast cell-derived histamine is well known as a key mediator of allergic pruritus (also see 3.3) [39]. However, chronic itch often is insensitive to antihistamines, suggesting engagement of non-histaminergic mechanisms in chronic itch [40].

Both mast cells and neurons secrete NGF and SP that bind to trkA and NK-1, respectively, and induce nociceptive and itch signaling [30]. Furthermore, mast cells interact with sensory neurons by releasing tryptases, which bind to proteinase- activated receptor 2 (PAR2) on neurons and initiate a signaling cascade involving TRPV1/4 activation via phospholipase C (PLC) to evoke CGRP release from neurons, which in turn activates the CGRP receptor on mast cells and promotes histamine release [41].

2.2.2. Neutrophils—A hallmark of acute inflammation is neutrophil infiltration toward the site of injury in response to various chemoattractants. Although it remains unclear if neutrophils contribute to the genesis of pruritus, they have complex effects on pain sensation. Pharmacological inhibition or genetic ablation of the function of chemoattractive factors including C5a receptor [42] and keratinocyte-derived chemokine [43] reduces or prevents behavioral hypersensitivity in neuropathic pain models. Inhibition of leukocyte adhesion and migration also reduces mechanical hyperalgesia caused by partial sciatic nerve ligation [44, 45]. Furthermore, peripheral nerve injury resulting from transection of sciatic nerve promotes endoneurial infiltration of neutrophils and depletion of circulating neutrophils at the time of nerve injury can attenuate the induction of hyperalgesia [46].

Neutrophils are critical mediators of thermal hyperalgesia induced by intraplantar injection of carrageenan, which involves activation of the sphingosine 1-phosphate (S1P) receptor signaling pathway [47]. Acute pain responses elicited by IL-17 or TNF-α also require the presence of neutrophils [48]. Although it was reported that depletion of neutrophils by vinblastine sulfate or anti-neutrophil antibody reduces mechanical hyperalgesia induced by paw incision in mice [49], other studies show that antibody-mediated depletion of neutrophils significantly reduces or abolishes edema formation but has no effect on mechanical hypersensitivity in mouse models of hind paw incision or zymosan-induced inflammation [50, 51]. The discrepancy in these observations might result from differential effects of functionally distinct inflammatory mediators released by activated neutrophils. For instance, PGE2 is the major excitatory inflammatory mediator released by neutrophils and sensitizes primary sensory neurons to enhance pain sensation [44, 52]. Granulocyte-derived chemokine Bv8/prokineticin 2 might also contribute to the generation of inflammatory pain [53]. On the other hand, neutrophils also release opioid peptides which bind to opioid receptors on primary sensory neurons, potentially reducing inflammatory pain in both acute and chronic inflammatory pain models [54, 55].

2.2.3. Macrophages—Macrophages have house-keeping functions, removing worn out cells and other cellular debris through phagocytosis, and also have immune effector functions in both innate and adaptive immune responses [56]. Injury to peripheral tissues or nerves activates resident macrophages and stimulates in ltration of blood-borne macrophages into peripheral tissues, and activates microglia (often considered resident

macrophages in the CNS) [57]. Peripherally activated macrophages express specific surface markers, secrete cytokines/chemokines and mitogenic factors, and are involved in removing myelin debris as part of the Wallerian degeneration process [58] (Figure 1). Interestingly, nerve injury also promotes activation of resident macrophages and an increased in ltration of blood-borne macrophages into DRG, which produces various inflammatory mediators and enhances regenerative capacity of DRG neurons [59]. These degeneration and subsequent regeneration processes in injured peripheral nerves are frequently associated with neuropathic pain.

Although it is not yet clear if the cellular and molecular changes in Wallerian degeneration are directly involved in the induction and maintenance of neuropathic pain, many studies suggest that macrophages are important contributors to the pathogenesis of neuropathic pain following peripheral nerve injury [57, 59]. Depletion of macrophages using liposome-encapsulated clodronate significantly reduces or prevents the progression of mechanical hyperalgesia and allodynia after traumatic or metabolic nerve injury in diabetic rats [60]. A broad-spectrum tetracycline antibiotic, minocycline, also reverses the activation of macrophages by retarding their migration to the injury sites after spared nerve injury (SNI), which results in a reduction of neuropathic pain [61]. Depletion of peripheral monocytes/macrophages also delays resolution of intraplantar interleukin-1 beta (IL-1β)- and carrageenan-induced inflammatory hyperalgesia, which might be caused by reduced release of IL-10 from macrophages [62]. Macrophages are also implicated in pruritus precipitated by hydroxyethyl starch [63] and a pruritic inflammatory skin disease in mice lacking heterogenous nuclear ribonuclear protein D (Hnrnpd), a regulator of inflammatory cytokine mRNA stability [64].

2.2.4. Dendritic cells—Skin dendritic cells (DCs) are antigen-presenting cells (APCs) that initiate and shape the adaptive immune response against invading pathogens by presenting antigens to naïve T cells in lymph nodes (LNs) [65]. Skin DCs can be divided into two major populations: epidermal Langerhans cells (LCs) and dermal dendritic cells. LCs reside in the basal and suprabasal epidermis, where they form a network between keratinocytes and contribute to the generation of painful hypersensitivity after peripheral tissue or nerve injury [66]. On the other hand, LC-deficient mice also display enhanced contact hypersensitivity [67], suggesting different roles of LCs in neuropathic pain and contact hypersensitivity.

Compared to epidermal LCs, dermal DCs are a more heterogeneous population that is developmentally and functionally distinct from LCs, and is critical to both innate and adaptive immunity in the skin [68]. Recent studies showed that the dermal DCs rather than LCs are involved in antigen presentation and T cell activation, and LCs are dispensable in T cell immunity in response to haptens [69]. Intraplantar or intraneural injection of recombinant IL-17 increases infiltration of DCs and neutrophils, which might contribute to neuropathic pain following peripheral nerve injury [70]. Moreover, overexpression of cysteine protease cathepsin S increases PAR-2 expression in DCs and induces spontaneous scratching which is also associated with increased expression of Type 1 helper T (Th1) cell-related cytokines [71].

2.3. Adaptive immune cells

The interplay between adaptive immune system and nervous system also plays a critical role in the pathogenesis of pain and itch. T lymphocytes, the central players in cell-mediated immunity, and antibody-producing B lymphocytes are the major cellular components in the adaptive immune response. T cell-deficient Rag1 mice exhibit a significant reduction in mechanical hyperalgesia after peripheral nerve injury and T lymphocyte-deficient nude mice recover faster and display less thermal hyperalgesia after nerve injury compared to their heterozygous littermates [72, 73]. Adoptive transfer of Th1 cells into the athymic nude rats, which lack mature T cells, restores their pain sensitivity in response to chronic constriction injury (CCI) to the peripheral nerve to a level comparable to that of the heterozygous rats. Conversely, passive transfer of Type 2 helper T (Th2) cells into heterozygous rats significantly reduces their pain sensitivity [74], suggesting that Th1 cytokines, interferon γ (IFN-γ) and IL-2, and IL-17, derived from the T helper 17 (Th17) cells, are important for the generation and maintenance of increased pain sensitivity, whereas Th2-derived cytokines, IL-4, IL-10, and IL-13 may have a pain-soothing effect [75]. The injured sciatic nerve and spinal cord dorsal horn are likely the major sites of actions for T cells because most of the infiltrated T cells are found in these locations [72–75]. Furthermore, up-regulation of gene expression of inflammatory cytokines is also detected in the spinal dorsal horn, which is correlated with neuropathic hypersensitivity [72]. However, adaptive immune responses may also excite DRG neurons via direct effects of serum IgG immune complexes [76].

Severe spontaneous scratching is evident in atopy-like dermatitis skin of an IL-31 transgenic mouse model of chronic AD [77]. Further studies showed that the IL-31 receptors are expressed by DRG neurons co-expressing TRPV1 and TRPA1, two critical itch-evoking ion channels. Application of the Th2 cell-derived IL-31, a candidate pruritogen in inflammatory and lymphoma-associated itch, directly activates sensory neurons and produces a robust scratching response that requires the function of both TRPV1 and TRPA1. Thus, IL-31 is a critical neuroimmune link between Th2 cells and sensory neurons for the generation of T cell-mediated itch [78] (Figure 1). Consistent with this finding, an anti-IL-31 receptor neutralizing antibody can reduce ear swelling and chronic pruritus-inducing dermatitis in an AD-like mouse model [79].

Although immune cells play critical roles in sensitizing nociceptors through release of cytokines and chemokines which facilitate the activation of nociceptors [7], recent studies have demonstrated that Nav1.8-positive nociceptors are directly activated by N-formylated peptides and the pore-forming toxin α-haemolysin isolated from bacteria [80]. Furthermore, bacterial lipopolysaccharide (LPS) excites both somatic and visceral nociceptors via a TRPA1-dependent but TLR4-independent mechanism, which can readily explain the early phase of LPS-induced acute pain and neurogenic in ammation [81]. Thus, direct activation of nociceptive sensory neurons by bacterial products may be a more general pain signaling mechanism than previously recognized.

2.4. Sensory neurons

The primary sensory neurons initiate the neural events that culminate in our senses of pain and itch. They transduce noxious or threatening stimuli through activation of specialized

membrane-bound ion channels and receptors. In addition, long-lasting sensitization of primary sensory neurons accounts for hypersensitivity in many chronic itch and pain conditions. Primary sensory neurons have their cell bodies in dorsal root ganglia (DRG), nodose ganglia, trigeminal ganglia (TG), jugular and petrosal ganglia and relay sensory signals from skin, muscle, joints, and viscera to the CNS [82]. These neurons are highly diverse in somal sizes, axon diameters, expression of different ion channels and receptors, electrophysiological properties, and innervation territories.

The slowly conducting unmyelinated C ber nociceptors and a subset of myelinated Aδ bers are most important for mediating itch and pain information, although Aβ nociceptors also exist [83]. The C nociceptors can be subdivided into an NGF (nerve growth factor)/TrkA-dependent, peptidergic population and a GDNF (glial cell-derived neurotrophic factor)/cRet-dependent, nonpeptidergic subset [84]. The segregation of the TrkA- versus cRet-expressing neurons is controlled by two critical transcription factors, Tlx3 (T-cell leukemia homeobox 3) and Runx1 (Runt-related transcription factor 1), which are essential to the development of a cohort of nociceptors, thermoreceptors, and pruriceptors in mice [85]. Many of the peptidergic neurons express SP and calcitonin gene-related peptide (CGRP) as well as the capsaicin receptor TRPV1, which confers the heat pain sensitivity [86]. The non-peptidergic population of C fibers is defined anatomically by high binding affinity for the lectin, IB4, and expression of many Mas-related G protein-coupled receptors (Mrgprs) [87]. The peptidergic and nonpeptidergic sensory neurons target different spinal cord areas to process and relay information for pain and/or itch.

Nociceptive sensory neurons are tuned specifically to detect tissue injury and potential damage, protecting the organism by initiating pain and eliciting defensive behaviors. The recent finding that MrgprA3, expressed by a unique subset of IB4-binding, CGRP-expressing small-diameter sensory neurons, is an itch-specific receptor that fails to initiate pain strongly supports the labeled-line theory in which the itch fibers are separate from the pain fibers at the level of primary sensory neurons [87]. Interestingly, there is also convincing evidence that itch signaling can be suppressed by painful stimuli, which involves vesicular glutamate transporter type 2 (VGLUT2)-dependent synaptic glutamate release from sensory neurons expressing Nav1.8, a marker for nociceptors (also see 3.7) [4, 5]. Furthermore, Prescott et al. argued that natural stimuli often activate more than one type of afferent and when multiple types of afferents interact in spinal cord microcircuits they can generate a perception that is different from the single modality expected to be generated by either type of afferent alone, suggesting the neural code for pain is at least partly combinatorial [82].

3. The molecular basis of pain and itch

3.1. Thymic stromal lymphopoietin (TSLP)

Thymic stromal lymphopoietin (TSLP) belongs to the cytokine family and initially was recognized as a stimulator of myeloid cells for the maturation of T cells [88]. TLSP is mainly produced in epithelial cells including epidermal keratinocytes [89]. The expression of TSLP is elevated in the keratinocytes from patients with AD and in mice with AD-like syndrome resulting from ablation of retinoid X receptors [90, 91]. In addition, keratinocyte-

specific overexpression of TSLP results in an AD-like phenotype in transgenic mice [91, 92]. Further studies have shown that Orai1-mediated cytosolic Ca^{2+} increase in keratinocytes controls the release of endogenous TSLP [25].

TSLP mainly acts on immune cells, such as T cells, B cells, dendritic cells, and mast cells by activating a heteromeric receptor complex composed of the TSLP receptor chain and the IL-7 receptor α chain, leading to the release of inflammatory cytokines [93]. Interestingly, Wilson et al. showed that direct application of TSLP induces robust itch behavior in mice, which is significantly reduced by ablation of the TRPV1-expressing primary sensory neurons with resiniferatoxin, but not affected by ablation of lymphocytes or mast cells [25]. Further studies demonstrated that direct application of TSLP to cultured DRG neurons elicits a marked increase of intracellular Ca^{2+} concentration in a subset of cells. TSLP-induced scratching is absent in TRPA1 knockouts, suggesting that TRPA1 is required for TSLP-mediated itch sensation [25] (Figure 2). However, how activation of the heterodimeric TSLP receptor activates TRPA1 remains unknown.

3.2. Endothelin 1 and endothelin receptors

ET-1 is highly expressed by epidermal keratinocytes and is released after cutaneous injury. Emerging evidence demonstrates that ET-1 plays significant roles in both itch and pain sensations. Application of ET-1 to human forearm by iontophoresis elicits itching in human subjects. Furthermore, the expression of ET-1 is increased in the epidermis of patients with chronic pruritic diseases [94]. In mice, intradermal (i.d.) injection of ET-1 produces a robust scratching response in numerous studies [2, 94–97]. ET-1-induced itching is mediated mainly by the endothelin receptor type A (ETA) as the ETA antagonist BQ123, but not the ETB antagonist BQ788, significantly reduces the scratching evoked by ET-1 [95]. The activation of ETA increases extracellular-signal-regulated kinases 1/2 (ERK1/2) phosphorylation in DRG neurons, and inhibition of ERK1/2 activation abolishes ET-1-induced pruritus in mice, suggesting that ERK1/2 is the downstream target of ETA [94]. Treatment of DRG neurons with an endothelin-converting enzyme-1 (ECE1) inhibitor SM-19712 decreases ET-1 degradation in the endosome and prevents the recycling of internalized ETA back to the plasma membrane, leading to ERK1/2 activation and facilitation of ET-1-evoked scratching behavior in mice [94]. Further studies demonstrated that both pharmacological blockade and genetic ablation of TRPA1 function reduce ET-1-evoked scratching, suggesting that TRPA1 is required for ET-1-induced itch although the relationship between TRPA1 and ETA -ERK1/2 signaling remains unclear [94, 98] (Figure 2). On the other hand, TRPV1, TRPM8 and H1R are not associated with the itch sensation evoked by ET-1 [94, 99].

In addition to producing itch sensation, ET-1 also elicits nociceptive behaviors and hyperalgesia when injected into the hind paws of mice and rats [100, 101]. In humans, administration of ET-1 to the non-dominant brachial artery induces deep muscular pain and allodynia in the injected limb [102]. ET-1 evokes not only scratching (itch sensation) but also wiping (pain sensation) towards the injection site in the mouse cheek injection model. The ETA antagonist BQ123 but not the ETB antagonist BQ788 suppresses both ET-1-evoked scratching and wiping, suggesting that ETA also mediates ET-1-induced pain

responses [97]. On the other hand, BQ788 enhances ET-1-induced pain-like behavior and local injection of a selective ETB agonist IRL-1620 inhibits the ET-1-induced pain responses in rats, suggesting that activation of ETB produces analgesic effects [103]. Further studies demonstrated that ET-1 activates keratinocytes through the ETB leading to the release of β-endorphin which in turn activates μ- and κ-opioid receptors in nociceptors, which activates G protein-coupled inwardly rectifying potassium (GIRK) channels, resulting in hyperpolarization of nociceptors and analgesic effects [104]. Furthermore, genetic studies demonstrated that selective deletion of ET-1 in neurons increases the sensitivity to acute nociceptive stimuli and exacerbates both inflammatory and neuropathic pain [105]. Thus, the distinct roles of ETA and ETB in nociception may explain the pain phenotype in these neuron-specific ET-1 knockout mice.

3.3. Histamine and histamine receptors

Histamine is a classic monoamine and one of the best known endogenous inflammatory mediators for induction of pain and itch by exciting a subset of unmyelinated C-fibers of primary sensory neurons through four G protein-coupled histamine receptors, H1-4R [106, 107]. Most histamine is generated and stored in granules of mast cells and released through degranulation when allergens interact with IgE molecules bound to Fc receptors on the surface of mast cells. Histamine increases the permeability of capillaries, allowing white blood cells to migrate out and engage pathogens in allergic conditions [106].

Allergen-mediated release of histamine is the primary cause of allergic itch [106]. Histamine-elicited itch can be effectively suppressed by H1R antagonists [108]. The H3R antagonist thioperamide increases scratching in both ICR (CD-1) and mast cell-deficient W/Wv mice, which is suppressed by a H3R agonist (R)-alpha-methylhistamine. Therefore, activation of H3R likely inhibits itch. However, thioperamide also exhibits affinities to the H4R [109]. The H4R plays an important role in dermatitis-related pruritus, and the H4R antagonist JNJ7777120 effectively reduces itching in chronic allergic dermatitis [110]. Both the H1R and H4R agonists elicit itch when injected into the skin of mice, and blocking these histamine receptors inhibits histamine- and allergen-induced itch [108, 110]. H1R-mediated scratching requires activation of both PLCβ3 and TRPV1 in C-fiber nociceptive neurons [99] (Figure 2). Moreover, TRPV1 is a downstream effector of the histamine-activated phospholipase A2 (PLA2)/lipoxygenase pathway, and is directly activated by lipoxygenase metabolites such as 12-Hydroxyeicosatetraenoic acid (12-HETE) [111, 112]. Consistent with these findings, histamine-induced scratching is severely attenuated in TRPV1-deficient mice [99, 112]. Therefore, multiple intracellular signaling pathways may converge on TRPV1 channels to mediate histamine responses in allergic itch.

Besides its predominant role in mediating itch sensation, H1R is also associated with thermally-, mechanically- and chemically-induced pain sensations, suggesting that it has a general role in detecting painful and itching stimuli [113]. Interestingly, although H2R is not a major itch receptor, it displays similar pain-sensing properties as the H1R, and genetic ablation of the H2R severely attenuates pain responses to thermal, mechanical and chemical stimuli [114].

3.4. Serotonin and serotonin receptors

Serotonin (5-HT) plays a pivotal role in regulating pain and itch signaling through both peripheral and central mechanisms. Most of the 7 families of 5-HT receptors are GPCRs with the exception of 5-HT3R which is a ligand-gated ion channel. Topically applied 5-HT evokes a robust scratching in rats [115]. Direct i.d. injection of 5-HT to the cheek or rostral back also produces a scratching response in rats and mice [2, 116]. In humans, application of 5-HT to the skin elicits a weak itch sensation [117]. Patients associated with several chronic itch conditions, including allergic contact dermatitis and AD, exhibit increased levels of 5-HT in the skin lesions and plasma [118]. 5-HT has also been proposed to be involved in the pathogenesis of pruritus in polycythaemia vera, a myeloproliferative neoplasm (MPN) associated with intense itching [119]. Indeed, selective serotonin reuptake inhibitors (SSRI) have displayed beneficial effects in palliative care patients with pruritus of different natures [120].

5-HT appears to initiate itch sensation by directly activating DRG neurons, since topically applied and intradermally injected 5-HT evoke action potential firing in C-type DRG neurons and spinal dorsal horn neurons, which is also evident by elevated expression of the c-fos protein in response to 5-HT application [115, 121]. Alpha-methylserotonin, a selective 5-HT2 receptor agonist, induces similar scratching behaviors in mice and the scratching induced by both 5-HT and α-methylserotonin is inhibited by a 5-HT2 receptor antagonist, ketanserin [122] which also attenuates experimental dry skin-induced chronic itch [123]. Genetic ablation of PLCβ3 but not TRPV1 substantially attenuates 5-HT- and α-methylserotonin-induced scratching, suggesting that PLCβ3 signaling but not the TRPV1 channel is required for 5-HT-induced itch [99] (Figure 2).

Although ketanserin effectively suppresses the scratching response elicited by acute application of 5-HT, it has no effect on the scratching in a rat model of contact dermatitis generated by repeated application of dinitrofluorobenzene (DNFB) [124], suggesting that endogenous 5-HT is unlikely involved in the pathogenesis of itch response in allergic dermatitis. On the other hand, the scratching responses elicited by PAR2 agonists and 5-HT but not histamine are enhanced in a mouse model of chronic dry skin itch [123]. Therefore, plasticity induced by peripheral 5-HT signaling contributes to the sensitization of itch-signaling pathways in some but not all types of chronic itch.

Besides involvement in itch sensation, 5-HT also plays a complex modulatory role in pain signaling by acting on distinct 5-HT receptors in the periphery and CNS. The superficial dorsal horn neurons displaying single unit firing in response to i.d. injection of 5-HT also respond to capsaicin and mustard oil, suggesting that these neurons might also signal pain in response to noxious stimuli [121]. In fact, intraplantar administration of 5-HT produces both local inflammation and hyperalgesia [125]. Pain responses to the algogen formalin are also reinforced by 5-HT and blocked by 5-HTR antagonists [126]. Many 5-HT receptors including 5-HT2A, 5-HT2B, 5-HT3, 5-HT4, 5-HT6, and 5-HT7 receptors, have been reported to mediate 5-HT-induced pain responses in the periphery [126, 127]. There is also accumulating evidence that peripheral inflammation up-regulates the expression of several 5-HT receptors in primary nociceptors [128]. Pharmacological inhibition of these receptors using selective antagonists has been effective in reducing inflammatory pain [129].

Furthermore, CCI-induced mechanical hyperalgesia is inhibited by selective 5-HT2A and 5-HT3 receptor antagonists [130]. Therefore, distinct peripheral 5-HTRs are involved in the pathogenesis of inflammatory and/or neuropathic pain.

5-HT7R and 5-HT3R are the two major 5-HT receptors mediating complex modulatory effects of 5-HT in CNS descending pathways [131]. Central terminal TRPV1 sensitization is maintained by upregulated descending serotonergic (5-HT) input from the rostral ventromedial medulla (RVM) in the brainstem, as central blockade of 5-HT/5-HT3A signaling attenuates TRPV1-dependent central terminal sensitization, excitatory primary afferent inputs, and mechanical hyperalgesia in the territories of injured and uninjured nerves. This finding reveals a molecular mechanism in which descending 5-HT from the RVM maintains central terminal sensitization by activation of presynaptic 5-HT3A receptors and subsequent facilitation of TRPV1 activity [10]. Interestingly, 5-HT3R also mediates the descending serotonergic facilitation through activation of a reciprocal neuron-glial signaling cascade leading to enhanced activation of NMDA receptors in the spinal dorsal horn [132]. Therefore, complex mechanisms are involved in 5-HT3R-mediated descending facilitation of pain signaling. Paradoxically, activation of the 5-HT7 receptors produces both excitatory and inhibitory effects on pain signaling, leading to either hyperalgesia or antinociception. For instance, in healthy rats 5-HT7 receptor agonists elicit a pronociceptive response while in rats with peripheral neuropathy they suppress ongoing pain [129].

3.5. Toll-like receptors

Recognition of conserved pathogen-associated molecular patterns (PAMPs) activates the toll-like receptors (TLRs), which initiate both innate and adaptive immune responses. Different subtypes of TLRs are widely distributed in the central and peripheral nervous systems, and their activation is involved in both pain and itch processing under normal and pathological conditions.

Increasing evidence indicates that many TLRs are involved in sensory hypersensitivity in response to nerve injury and tissue inflammation. For example, genetic ablation of TLR2 attenuates spinal nerve injury-induced expression of chemokines and cytokines in DRG as well as spontaneous pain behaviors [133]. TLR3 is associated with the increased expression of IL-1β, TNF-α, IL-6 and MCP-1 in spinal astrocytes, which contributes to the visceral pain in chronic pancreatitis [134]. Interestingly, although intrathecal injections of TLR2, TLR3, or TLR4 ligands all elicit tactile allodynia, different downstream adaptor proteins are involved in TLR-mediated responses, i.e. toll-interleukin 1 receptor domain containing adaptor protein (TIRAP) is involved in the pain signaling by TLR2 and TLR4 but toll-like receptor adaptor molecule 1 (TICAM-1) contributes to the TLR3-mediated pain response. Furthermore, TLR2 and TLR4 responses are TNF-dependent but the TLR3 response is TNF-independent [135].

TLR4 is functionally expressed by many different cell types in both the periphery and CNS. Peripheral blood mononuclear cells (PBMCs) from chronic pain patients display increased responses to agonists of TLR2, 4, and 7, suggesting that TLR system in these blood born cells might be primed in response to inflammation and nerve injury [136]. TLR4 is also co-expressed with TRPV1 and CGRP in DRG and TG, and activation of TLR4 by LPS

increases TRPV1 activity and release of CGRP, leading to enhanced pain perception [137, 138]. Activation of TLR4 expressed on spinal microglia and astrocytes produces tactile allodynia through release of PGE2 and TNFα, which is attenuated by microglial inhibitors minocycline and pentoxifylline [139]. Activation of microglial TLR4 also contributes to the initiation of CNS neuroimmune activation after L5 nerve transection, which causes painful neuropathy [140]. Interestingly, it has also been demonstrated that opioids are TLR4 agonists, and microglial TLR4 mediates opioid-induced proinflammatory and hyperalgesic actions [141].

Activation of TLR3, TLR7 and TLR9 in DRG neurons by their respective ligands also induces expression and production of proinflammatory chemokines and cytokines CCL5, CXCL10, IL-1β, and PGE2, which increases pain sensitivity [142]. Pharmacological blockade and genetic ablation of TLR9 can reduce pain-like hypersensitivity [143, 144]. TLR3 is primarily expressed by the TRPV1-positive nociceptive neurons and activation of TLR3 elicits an inward current and causes action potential firing in DRG neurons. Interestingly, it seems that TLR3 is also required for excitatory spinal neurotransmission and central sensitization as central sensitization-driven pain but not acute pain is impaired in the TLR3 knockout mice [145]. Pain-like behavior elicited by the microRNA lethal-7 is mediated by activation of colocalized TLR7/TRPA1 in nociceptors, which is abolished in mice lacking TLR7 or TRPA1 [146]. On the other hand, TLR7 null mice are reported to exhibit normal heat, mechanical sensitivity, and acute nociceptive responses to capsaicin, mustard oil, and formalin, and normal carrageenan-induced inflammatory pain [147].

In addition to pain, activation of TLR3 or TLR7 also generates itch sensation. Indeed, both TLR3 and TLR7 are present in the itch-sensing small diameter TRPV1-positive DRG neurons co-expressing the itch mediator gastrin-releasing peptide (GRP). Moreover, both TLR3 agonist, poly (I:C) (a double-stranded RNA mimetic), and TLR7 agonist imiquimod produce an excitation of DRG neurons to elicit scratching [145, 147], although a distinct TLR7-independent, inositol 1,4,5-trisphosphate (IP3)-mediated pathway is also reported to mediate imiquimod-induced itch [148]. Genetic studies also showed that global deletion of TLR3 or TLR7 abolishes scratching behaviors induced by their respective agonists and the pruritogen chloroquine (CQ). Of interest, TLR7 null mice exhibit normal scratching responses elicited by histamine, HTMT, or compound 48/80, suggesting the involvement of TLR7 in histamine-independent itch [147]. In contrast, TLR3 is required for both histamine-dependent and histamine-independent itch [149]. These results suggest that both TLR3 and TLR7 are important itch receptors that regulate the excitability of pruriceptors (Figure 2). Therefore, TLRs may serve as new therapeutic targets for developing anti-itch treatment.

3.6. Sensory neuron-derived neuropeptides and their receptors

DRG neuron-derived neuropeptides are essential to pain perception in mammals. Among these neuropeptides, tachykinins are a large family of structurally-related peptides acting on three tachykinin receptors (NK1R-NK3R). Disruption of the gene for Tac1 (also known as preprotachykinin A, which encodes the tachykinin peptide hormone family) significantly reduces behavioral responses to moderate and intense noxious stimuli and eliminates neurogenic inflammation, suggesting that sensory neuron-derived tachykinins are critical to

neurogenic inflammation and pain [150]. Interestingly, African naked mole rats (Heterocephalus glaber) express functional TRPV1 but not SP in the small-diameter DRG neurons. Even though capsaicin strongly activates TRPV1 channels, it fails to induce SP release from DRG central terminals in the spinal dorsal horn to elicit nocifensive responses in these animals. Remarkably, when Tac1 is re-introduced into DRG neurons of African naked mole rats by a neurotropic herpes virus expressing the Tac1 gene, the nocifensive responses to capsaicin can be “rescued”, which further highlights the importance of sensory neuron-derived SP in pain physiology [151].

The neurokinin 1 receptor (NK1R) binds high affinity ligands, including SP, and the SP-like hemokinin and endokinin peptides [152]. NK1R is a GPCR which couples to Gq/11, activation of which leads to activation of PLCβ and results in a transient increase in intracellular IP3 and cytosolic free Ca^{2+} concentration [153] (Figure 2). NK1R is expressed by nociceptors and activation of NK1R sensitizes TRPV1 channel function, presumably through PKCε-mediated phosphorylation [154]. NK1R is also expressed by interneurons located in the superficial layer of the spinal dorsal horn. NK1R knockout mice display altered nociceptive responses during tissue damage [155] and ablation of lamina I spinal cord neurons expressing the NK1R using a conjugate of SP and saporin (SP-saporin) markedly attenuates responses to highly noxious stimuli and mechanical and thermal hyperalgesia [156]. This toxin-based ablation of NK1R-positive spinal neurons also prevents the development of mechanical hyperalgesia in response to a painful mechanical joint injury in rats [157]. Therefore, NK1R-expressing spinal neurons are key integrators of both thermal and mechanical hyperalgesia.

Interestingly, NK1R also couples to Gi/o proteins to induce release of reactive oxygen species (ROS) which serve as second messengers to promote oxidative modification and enhanced function of M-type K^+ channels in primary sensory neurons, leading to attenuation of pain [158]. Peripheral NK1R also mediates SP-induced anti-nociceptive effects on acid-induced chronic muscle pain by enhancing activity of the M-type K^+ channels [159]. These studies suggest that NK1R is a versatile pain modulator that can either enhance or attenuate pain response through activation of distinct downstream signaling pathways. However, NK1R antagonists have not exhibited efficacy in clinical trials [160], which might partly result from functional redundancy of other pain-related neuropeptides, such as CGRP.

The CGRP-expressing DRG neurons in mice also express TRPV1 but not TRPM8 [161]. Accordingly, capsaicin treatment produces neurotoxicity and severely reduces the number of CGRP-expressing neurons in DRG explants [162] and CGRP expression in cultured DRG neurons [163]. Genetic depletion of CGRP-expressing neurons has a profound effect on thermally- and chemically-induced nociception and pruritus without affecting mechanosensation [161]. Surprisingly, genetic ablation of CGRP-expressing neurons also promotes cold hypersensitivity, suggesting that these neurons tonically inhibit cold signaling in the spinal cord [161]. CGRP released into the spinal dorsal horn produces nocifensive responses, and peripherally released CGRP causes neurogenic vasodilatation [164]. In fact, CGRP is a well-established neuromediator of migraine, and selective CGRP receptor antagonists as well as anti-CGRP antibodies have shown promising efficacy in the treatment of migraine [165].

Administration of SP elicits scratching in both mice and humans through activation of the NK1R [166, 167]. Intrathecal injection of SP also rescues the loss of histamine-induced scratching in African naked mole rats [168]. In addition to SP-induced itch, NK1R mediates itch responses to other pruritogens. For example, blockade of NK1R partially suppresses the non-histaminergic itch responses induced by i.d. injection of CQ and the PAR2 agonist SLIGRL [169]. Ablation of NK1R-positive spinal dorsal horn neurons in rats using SP-saporin also results in a loss of serotonin-evoked itch [170]. In fact, a Food and Drug Administration (FDA)-approved NK1 antagonist, aprepitant, has been used in clinical trials and showed promising effectiveness in treating patients with refractory pruritus [171]. The other piece of evidence supporting a role of SP and NK1R in pruritus is that the numbers of both SP-positive nerves and NK1R-positive mast cells are increased in patients with chronic plaque psoriasis associated with pruritus [172]. These studies suggest that both SP and NK1R are involved in the pathogenesis of chronic itch associated with skin disorders.

3.7. Sensory neuron-derived glutamate

Glutamate is the principal fast excitatory neurotransmitter in the vertebrate nervous system. Conditional knockout of the VGLUT2 transporter, which packages glutamate into synaptic vesicles and controls excitatory neurotransmission, from Nav1.8-positive sensory neurons [4, 5] not only reduces thermally-, mechanically-, and chemically-induced pain but also dramatically increases scratching elicited by pruritogens as well as promoting spontaneous scratching and skin lesions [4, 5]. Therefore, glutamate likely mediates the crosstalk between pain and itch signaling in the spinal cord. Interestingly, glutamate also mediates histamine-dependent itch as the scratching responses elicited by histamine are abolished by the AMPA/Kainate antagonist CNQX. On the other hand, a combination of CNQX, GRPR, and NK1R antagonists is required to eliminate CQ-evoked firing in spinal dorsal horn neurons as well as CQ-elicited scratching responses [173], suggesting that both glutamate and neuropeptides are required to generate non-histaminergic pruritus.

3.8. Mas-related G-protein-coupled Receptors (Mrgprs)

Mrgprs, also known as Mrg/SNSR, are a family of orphan GPCRs consisting of more than 50 members in the mouse genome and are grouped into several subfamilies: MrgprA1-22, MrgprB1-13, MrgprC1-14, and MrgprD-G. Mouse MrgprA3 (corresponding to human MrgprX1) was initially found to mediate the scratching response evoked by CQ, and the Mrgpr family has since emerged as an important class of histamine-independent itch receptors [174]. Further studies have identified TRPA1 as a critical downstream target of both MrgprA3 and MrgprC11 in primary sensory neurons and genetic ablation of TRPA1 eliminates the scratching response to both CQ and BAM8-22 (a mast cell amide that activates MrgprC11) [175] (Figure 2).

A recent study using conditional knockout mice demonstrated that ablation of the MrgprA3-positive DRG neurons causes a substantial reduction in scratching evoked by multiple pruritogens and spontaneous scratching under chronic itch conditions. Importantly, pain sensitivity remains intact in these mice. Moreover, in mouse skin, MrgprA3-positive fibers exclusively innervate the epidermis and are activated by multiple pruritogens, suggesting

that they are peripheral itch-specific fibers [87]. However, the endogenous agonist (s) of MrgprA3 has yet to be identified.

MrgprD is a receptor for β-alanine which induces itch and tingling sensations after ingestion [176]. In primates, β-alanine can also activate mechanically sensitive rapidly responding polymodal sensory fibers that might correspond to the MrgprD-expressing, non-peptidergic nociceptive afferents in mice [177]. MrgprD can detect noxious mechanical stimulation such as pinching while MrgprB4 is tuned to pleasant, gentle touch such as massage-like stroking of hairy skin in mice [178].

Consistent with their roles in detecting noxious pain and itch stimuli, the functions of both MrgprA3- and MrgprD-positive neurons are enhanced by chemically-induced allergic contact dermatitis in mice as reflected by enhanced membrane excitability and action potential firing in response to mechanical and thermal stimuli [179]. These neurons also display more depolarized resting membrane potential, more action potentials, and a significant increase in the peak amplitude of both tetrodotoxin-sensitive and resistant Na^+ currents. Ablation of the MrgprA3-positive neurons also significantly reduces the incidence of scratching during chemical-induced allergic contact dermatitis. These findings provide mechanistic insights into the critical role of Mrgprs in the pathogenesis of allergic itch [179].

3.9. Proteinase-activated receptor 2 (PAR2)

PARs are a unique family of protease-activating GPCRs. Proteases cleave the N-terminus of the receptors and liberate a tethered ligand that activates G protein- and/or β-arrestin-dependent signaling. Thrombin activates PAR1, PAR3, and PAR4, and trypsin selectively activates PAR2 and PAR4 [180]. PARs are expressed throughout both the peripheral and central nervous systems and play important roles in the pathogenesis of pain and itch. Among the 4 PARs, PAR2 is a major contributor to the initiation of both pain and itch [181]. PAR2 is involved in generating several types of inflammatory, neuropathic and visceral pain [41, 182], and activation of PAR2 sensitizes nociceptors, which is involved in the development of bone cancer pain [183] and hyperalgesia after DRG compression [184]. The molecular mechanisms underlying PAR2-induced sensitization involve cAMP-dependent alterations [184] and facilitation of pain-initiating TRPV1, TRPA1, and TRPV4 channels, which are key players in initiating thermal and mechanical hyperalgesia under inflammatory and neuropathic conditions [41, 185, 186].

Consistent with PAR2 being a receptor mediating both itch and pain, activation of PAR2 by a cysteine protease from cowhage elicits both itch and nociceptive sensations [187]. I.d. injection of a selective PAR2 peptide agonist SLIGRL can elicit scratching in mice (Figure 2) as well as enhanced c-fos expression in mouse spinal dorsal horn neurons, and enhanced spontaneous scratching in a mouse model of chronic dry skin itch [188]. Interestingly, Liu et al. demonstrated that SLIGRL elicits scratching via MrgprC11 rather than PAR2. Furthermore, a shorter peptide, SLIGR, which specifically activates PAR2 but not MrgprC11, induces thermal pain hypersensitivity but not scratching in mice, suggesting that PAR2 activation is likely to generate pain rather than itch sensation [189].

3.10. Transient receptor potential (TRP) channels

3.10.1 TRPV1—TRPV1 is the first cloned TRP member, and is gated by capsaicin, heat, low pH, and voltage [190]. In addition, TRPV1 is also activated or sensitized by numerous molecules associated with inflammation and tissue damage, including bradykinin, prokineticin, prostaglandins, anandamide, retinoids, N-arachidonoyldopamine, ROS, NO, oxidized linoleic acid metabolites, amines, ATP, thrombin and trypsin proteases, and CCL2 [191–203]. TRPV1 sensitization by inflammatory mediators generally involves phosphorylation by protein kinases C and A (PKC, PKA) [204] as well as complex regulation by PIP2 [205]. Moreover, activation of PLCγ and Src signaling pathways increases trafficking of TRPV1 to the plasma membrane [206, 207]. TRPV1 sensitization by signaling of in ammatory mediators reduces the threshold for detecting painful stimuli during in ammation, leading to inflammatory hyperalgesia [208]. TRPV1 sensitization by inflammatory mediators may also contribute to nociceptor hyperexcitability, spontaneous activity, and neuropathic pain after spinal cord injury (SCI) [209].

Although numerous studies have demonstrated that the expression of TRPV1 in DRG neurons is increased in inflammatory pain models [210–212], TRPV1 immunoreactivity is reduced in the DRG in a variety of neuropathic pain models generated by spinal nerve ligation (SNL) [213], CCI [214] and partial nerve section [215]. Decreased TRPV1 expression has also been reported in diabetic neuropathy [216] and in sural nerve and skin nerve fibers of patients with painful neuropathy [217]. In marked contrast to the damaged neurons, undamaged DRG neurons in neuropathic pain models show increased expression of TRPV1 [209, 215], which may account for the enhanced heat responsiveness of DRG neurons dissociated from the SNL mice [218]. Although selective TRPV1 antagonists show efficacy in suppressing both inflammatory and neuropathic pain in rodents, they produced undesirable hyperthermia and blunted noxious heat perception in clinical trials, which presents a major roadblock for the employment of these drugs [219]. On the other hand, agonist-induced TRPV1 desensitization, especially through intrathecal administration of intrathecal resiniferatoxin, has displayed promising pain relief in patients with intractable cancer pain [220].

Unlike the well-recognized role of TRPV1 in pain, the function of TRPV1 in itch sensation has just recently been established. TRPV1 null mice display significantly attenuated itch behavior in response to i.d. injection of histamine [99, 112]. Consistent with TRPV1 as a downstream target of histamine signaling, TRPV1 mediates histamine-induced excitation of DRG neurons, and histamine can activate TRPV1 channels heterologously expressed in HEK293 cells [112]. Further studies demonstrated that histamine-evoked scratching requires PLC signaling since itch sensation is attenuated in mice deficient in PLCβ3, a PLC isoform downstream of the G_q-coupled H1 receptor [99] (Figure 2). Although the mechanism underlying the opening of TRPV1 by PLCβ3 remains unknown, it seems that distinct functions of TRPV1 in pain and itch are determined by pattern of activation: direct activation of TRPV1 induces pain whereas GPCR-mediated indirect activation of TRPV1 produces itch, probably in different subsets of sensory neurons. It should also be noted that signaling by inflammatory mediators is generally associated with many downstream effectors besides TRPV1. For instance, downstream effectors of the bradykinin-initiated

signaling cascade include TRPA1, M-type K^+ channels, and Ca^{2+}-activated Cl^- channels, all of which are implicated in the pathogenesis of inflammatory pain. Therefore, multiple ion channels are downstream targets of inflammatory mediators and thereby contribute to the initiation and/or maintenance of pain and itch sensations.

3.10.2. TRPA1—TRPA1 is selectively expressed by a subpopulation of small-diameter neurons in dorsal root, trigeminal, and nodose ganglia [221, 222], as well as in epidermal keratinocytes [223]. Interestingly, TRPA1 expression in human sensory neurons appears to be up-regulated by nerve injury [224]. Like TRPV1, TRPA1 can be activated by an enormous range of molecules, including exogenous pungent agents from plants (such as wasabi, allium and ginger) [221, 222, 225], endogenous products of oxidative or nitrative stress (such as 4-hydroxynonenal) [226, 227], and by in ammatory mediators that cause pain (such as bradykinin or ATP) [222, 225]. TRPA1 can also be activated by a metal ion, Zn^{2+}, leading to an acute pain sensation [228].

A gain-of-function mutation (N855S) in the S4 transmembrane segment of TRPA1 causes familial episodic pain syndrome, which is the first example of a human pain-associated TRP channelopathy [229]. Pharmacological studies have shown that TRPA1-expressing nociceptors mediate both inflammatory and neuropathic pain. The TRPA1 antagonists AP18 and HC-030031 reduce nocifensive behaviors induced by paw injection of formalin and suppress mechanical hyperalgesia in complete Freund's adjuvant (CFA)-induced inflammatory pain as well as SNL-induced neuropathic pain [230, 231]. Intrathecal administration of TRPA1 antisense oligodeoxynucleotides suppress SNL-induced cold allodynia [232]. TRPA1 is also involved in painful diabetic neuropathy and TRPA1 expression is significantly increased in the DRG of rats with peripheral nerve injury [233] and SCI [234]. ROS, produced during inflammation and tissue injury in the diabetic tissues, activate TRPA1 and leads to nociceptor sensitization [235], which is consistent with the findings that acute treatment with TRPA1 antagonists decreases mechanical hypersensitivity in rodents with CFA-induced inflammation [236] or diabetic peripheral neuropathy [237]. Umbellulone, a major active constituent of the "headache tree" *umbellularia californica*, activates TRPA1 and produces a nocifensive response by evoking CGRP release from trigeminal ganglion neurons. Furthermore, intranasal application of acrolein or umbellulone elicits TRPA1-dependent meningeal vasodilatation, suggesting that TRPA1 might be involved in the initiation of migraine [238]. Thus, TRPA1 serves as an important polymodal molecular detector sensing environmental irritants and endogenous proalgesic agents, leading to excitation of nociceptors and pain sensation [239, 240].

TRPA1 is also a key mediator of histamine-independent itch sensation downstream of MrgprA3 and MrgprC11 signaling in sensory neurons, which can be activated by the pruritogens CQ and BAM8-22, respectively. MrgprC11 and MrgprA3 use two distinct signaling pathways involving PLC or $G\beta\gamma$ to mediate the activation of TRPA1 [175, 241]. Complementary studies have shown that a specific subpopulation of TRPA1-positive sensory neurons mediates histamine-independent itch. These used entry of the charged sodium channel blocker QX-314 into primary sensory neurons through activated TRPA1 channels to block activity selectively in TRPA1-positive axons [242]. TRPA1 is a promiscuous ion channel activated and/or modulated by many signaling pathways, thus it

serves as a general downstream mediator of itch signaling elicited by many other itch-producing molecules including ET-1 [98], ROS [243], TSLP [25], IL-31 [78], IL-13 [244], LTB4 [245], bile acids [246] (Figure 2).

In addition to acute itch evoked by exogenously applied pruritogens, TRPA1 plays critical roles in chronic itch. Wilson et al. have shown that in a dry skin-induced chronic itch model the scratching response and epidermal hyperplasia are markedly attenuated by genetic ablation or pharmacological blockade of TRPA1 function. TRPA1 is also responsible for the changes in gene expression in both sensory neurons and skin cells [247]. TRPA1 knockout mice display reduced scratching behaviors compared with wild-type mice in a chronic allergic contact dermatitis model [248]. Furthermore, Oh and colleagues reported that TRPA1 expression is increased in dermal afferent nerves and mast cells in skin biopsy from patients with AD, and TRPA1 mediates spontaneous itch in an IL-13-transgenic mouse model of AD [244].

3.10.3. TRPM8—TRPM8 channels are widely expressed in small-diameter DRG and TG neurons, and are activated by cold to cool temperatures (8–28°C) as well as compounds such as menthol and icilin that evoke cooling sensations [249, 250]. A subset of TRPM8-expressing neurons also express TRPV1 [251, 252]. Liposome reconstitution studies have shown that both TRPV1 and TRPM8 are intrinsically temperature sensitive, i.e. their activation by thermal stimuli does not require other cellular proteins or second messengers [253, 254]. Like TRPV1 and TRPA1, TRPM8 function is also strongly regulated by inflammatory mediators through PLC and PIP2 signaling [205]. Because PIP2 hydrolysis has opposite effects on TRPV1 and TRPM8, an in ammatory agent that activates a PLC-coupled receptor could potentially produce hyperalgesia by sensitizing heat-sensitive bers while simultaneously suppressing any counteracting analgesic action from the TRPM8-expressing cold-sensitive sensory fibers.

In a rat CCI model, the number of TRPM8-immunoreactive neurons and the percentage of sensory neurons activated by both menthol and capsaicin increased in the injured rats [255]. Additionally, the amplitude of cold-evoked currents was significantly enhanced in the same population of neurons cultured from rats with CCI. However, a decrease of TRPM8 mRNA has also been reported in rat sensory ganglia following SNI [256]. Therefore, whether TRPM8 mediates cold allodynia in neuropathic conditions is still an open question. Interestingly, Genome-wide association analysis has also identified TRPM8 as one of the susceptibility loci for common migraine in the general population [257].

Unlike TRPV1 and TRPA1, which promote itch signaling, TRPM8 seems to inhibit itch signaling because cooling is known to soothe and relieve itching. Even though direct evidence supporting TRPM8 in itch inhibition is lacking, cooling and the TRPM8 agonists, menthol and icillin, have shown effectiveness in inhibiting histamine and lichenification-associated itch in humans [258]. Likewise, cooling the skin significantly attenuates the activation of spinal neurons induced by cutaneous application of histamine [259]. Interestingly, a recent study showed that activation of Gαq, a downstream signal of MrgprA3 activation by CQ, inhibits the function of TRPM8, suggesting that the inhibition of TRPM8 might be an additional mechanism underlying CQ-induced itch [260] (Figure 2).

3.10.4. Other TRP channels—Extensive efforts have been made to define the roles of several other temperature- sensitive TRP channels, including TRPV3, TRPV4, and TRPM3, in the initiation of pain and itch. Being most abundantly expressed in keratinocytes, TRPV3 and TRPV4 are potential epidermal contributors to pain and itch. Activation of TRPV3 [261] and TRPV4 [262, 263] has been shown to produce nociceptive behaviors, although their presence and function in the primary afferents remain controversial [264]. TRPM3 is expressed in a subset of DRG and TG neurons [265, 266] and is activated by heat and neurosteroid pregnenolone sulfate to elicit pain responses [267]. TRPM3-mediated pain is enhanced when it is activated concomitantly through an alternative pathway evoked by clotrimazole-related drugs [268]. Consistent with their roles in pain perception, blockade of TRPV3 [269, 270] or TRPM3 [271] produces analgesic effects, while spinal administration of oligodeoxynucleotides antisense to TRPV4 abolishes paclitaxel-induced painful peripheral neuropathy [272]. In addition, TRPV3- and TRPM3-deficient mice exhibit deficits in response to noxious heat [273, 274], whereas keratinocyte-specific TRPV3 knock-in mice display increased avoidance of noxious heat, in the absence of functional TRPV1 channels [24]. Genetic ablation of TRPV4 function also attenuates the sensitization of nociceptors by inflammatory mediators PGE2 and serotonin [275]. Combined, these studies suggest that TRPV3, TRPV4, and TRPM3 may play significant roles in pain transduction.

A contribution of TRPV3 to pruritus is evidenced by severe scratching in DS non-hair (DS-Nh) mice associated with spontaneously developed AD-like lesions. The DS-Nh mice carry a gain-of-function mutation of Gly573 to serine in TRPV3, which renders the channel constitutively active [276, 277]. Similar gain-of-function TRPV3 mutations have also been found in humans associated with Olmsted syndrome, which is characterized by bilateral mutilating palmoplantar keratoderma (PPK) and periorificial keratotic plaques with severe pruritus [278]. The role of TRPV3 in chronic itch has been further confirmed by the lack of spontaneous scratching in TRPV3-deficient mice subject to a chronic dry skin itch model by treating mice with acetone-ether-water (AEW) which causes severe spontaneous scratching in wild-type mice [279]. These findings provide strong evidence that TRPV3 plays a major role in the development of chronic itch in both humans and mice.

3.11. Opioids and opioid receptors

Opioid receptors are divided into three classical subtypes, mu, kappa, and delta opioid receptors (MOR, KOR and DOR) [280]. Opioid receptors also have multiple functional splice variants, heteromers and nociceptin/orphanin FQ peptide (NOP) receptors [281]. Once opioids bind to their receptors, Gi-proteins dissociate into Gα and Gβγ subunits, which inhibit adenylyl cyclase and cAMP production and/or modulate the function of ion channels, thereby decreasing neuronal excitability and ultimately inhibiting the transmission of the nociceptive information. For instance, activation of presynaptic opioid receptors inhibits N-type voltage-gated Ca^{2+} channels, leading to a decreased release of neurotransmitters [282]. This is consistent with the finding that DOR deficient mice exhibit enhanced mechanical allodynia and thermal hyperalgesia [283].

The significant attenuation of analgesia mediated by MOR or DOR in GIRK knockout mice suggests that Gβγ activation of GIRK channels is a major downstream effect of opioid stimulation, hyperpolarizing and reducing the excitability of nociceptors [284]. Although most clinical drugs act primarily on MOR and DOR, the activation of KOR also produces a strong analgesic effect via distinct cellular mechanisms, e.g., MOR- and DOR-induced analgesia depend on spinal 5-HT-expressing neurons while KOR-induced analgesia requires supraspinal 5-HT-expressing neurons [285].

Despite beneficial analgesic effects, administration of opioids often results in serious side effects, including pruritus. Severe itching is evident in postoperative patients with epidural infusion of morphine [286] and experimental animals with i.d. injection of morphine [287]. Administration of MOR but not KOR or DOR agonists also elicits scratching in monkeys [288], and pretreatment with the MOR antagonists, naloxone and naltrexone, inhibits scratching elicited by morphine and various pruritogens [287]. On the other hand, dynorphin, released from inhibitory B5-I interneurons in the dorsal horn, is a key neuromodulator of pruritus through activation of KOR. Decreased KOR signaling, due to loss of dynorphin-expressing spinal interneurons, contributes to the abnormally increased itch in *Bhlhb5*$^{-/-}$ mice while locally applied KOR agonists in the spinal cord selectively reduce histamine-dependent and histamine-independent itch (but not pain) [289].

Interestingly, although MOR agonists induce itch, itch does not always accompany analgesia [290]. Neither the shift in the dose-response curve nor the time course of morphine-induced scratching are proportional to that of morphine-induced analgesia [290]. Furthermore, morphine-induced scratching in mice with morphine tolerance is not attenuated when the analgesic effect is reduced. In fact, knockdown of one MOR isoform, MOR1D, in the spinal cord significantly attenuates morphine-induced scratching while knockdown of MOR1 reduces morphine-induced analgesia, suggesting that MOR1D and MOR1 play central roles in morphine-induced itch and pain inhibition, respectively [290].

3.12. Gastrin-releasing peptide receptor (GRPR) and natriuretic polypeptide receptor subtype A (NPRA)

Both gastrin-releasing peptide (GRP) and B-type natriuretic peptide (BNP) are expressed by subsets of DRG neurons and their respective receptors GRPR and NPRA are expressed in neurons located in the superficial lamina of the spinal cord dorsal horn [3, 291]. Genetic ablation of GRPR has no effect on thermal, mechanical, inflammatory, and neuropathic pain measures compared with wild-type mice. In marked contrast, the scratching responses elicited by a variety of pruritogens are significantly reduced in GRPR mutant mice [291]. In addition, selective ablation of lamina I dorsal horn interneurons that express GRPR using bombesin-saporin also causes profound scratching deficits to many pruritogens without affecting pain behaviors [2]. Moreover, intrathecal injection of a GRPR antagonist also significantly inhibits scratching in three independent itch models, while intrathecal injection of $GRP_{18–27}$, a selective GRPR agonist, elicits robust scratching [291].

Interestingly, although bombesin-related peptides including GRP, neuromedin B and bombesin, elicit scratching when injected intrathecally, distinct mechanisms seem to be involved because pharmacological blockade of GRPR only effectively suppresses the

scratching induced by a GRPR agonist but not neuromedin B or bombesin [292, 293]. Furthermore, neuromedin B produces local swelling and nocifensive responses in mice and ablation of neuromedin B receptor-expressing neurons severely reduces thermal pain behavior without affecting responses to mechanical and pruritic stimuli [294].

The expression of GRPR in spinal neurons is under the control of the homeobox gene Tlx3, and conditional knockout of Tlx3 from the spinal dI5 and dILB cells causes developmental defects of GRPR-expressing neurons in laminae I and II leading to marked deficits of itch responses elicited by a variety of pruritogens [295]. A recent study also showed that GRPR forms a heterodimer with MOR1D and the unidirectional cross-activation of GRPR signaling by MOR1D heterodimerization is critically involved in the opioid-induced itch [290] (Figure 2). Combined, these studies support the hypothesis that GRPR is a specific spinal itch receptor that transmits itch signals upon activation by GRP released from primary sensory neurons.

Likewise, toxin-mediated ablation of natriuretic polypeptide receptor subtype A (NPRA)-expressing interneurons eliminates both BNP- and histamine-evoked itch behaviors without affecting GRP-elicited scratching [3]. Furthermore, BNP null mice lack almost all behavioral responses to itch-inducing agents without affecting responses evoked by noxious thermal or mechanical stimuli [3]. These studies demonstrate a key role for NPRA in the spinal cord dorsal horn in mediating itch sensation and suggest that NPRA is the immediate spinal pruriceptor that is activated by BNP released from the central terminals of primary sensory neurons to relay the itch information to downstream GRPR-expressing spinal interneurons [3].

On the other hand, Liu et al. have shown that the scratching induced by intrathecal injection of BNP is not affected by pharmacological inhibition or genetic ablation of GRP-GRPR signaling, suggesting that GRPR is not downstream of the BNP-NPRA signaling cascade [296]. Likewise, the expression and quantity of GRP in DRG are also questioned [297–299]. However, these experiments cannot exclude the possibility that GRP may act in parallel with BNP to mediate itch signaling. Therefore, although it is clear that both GRPR and NPRA are important components for itch signaling in the spinal cord, the site of BNP action in itch and its relationship to GRPR signaling remain to be clarified.

4. Conclusion and perspectives

Persistent pain and itch are major clinical issues associated with many disease conditions and represent a significant unmet medical need for patients around the globe. Both chronic pain and itch significantly degrade the quality of life and may lead to depression or even suicide of the affected individuals [300]. Current treatments for chronic pruritus are limited to antihistamines, topical and systemic corticosteroids, or certain antidepressants, while opioids and non-steroidal anti-inflammatory drugs (NSAIDs) are still primary options for the treatment of chronic pain [301, 302]. However, the efficacy of these drugs is limited and systemic application of opioids, NSAIDS, corticosteroids, and antidepressants is associated with many severe side effects [303]. Therefore, more effective and safer treatments for chronic pain and itch are urgently needed. Recent exciting findings have identified many

cells, molecules, and circuits that are critically involved in the generation of chronic pain and itch at multiple levels from the skin to the spinal cord. Although pain and itch use similar neural pathways, distinct cellular and molecular mechanisms are involved in the pathogenesis of itch and pain. Thus, accumulating knowledge about the unique and shared mechanisms driving pain and itch should enable the development of more effective treatments for these major clinical problems.

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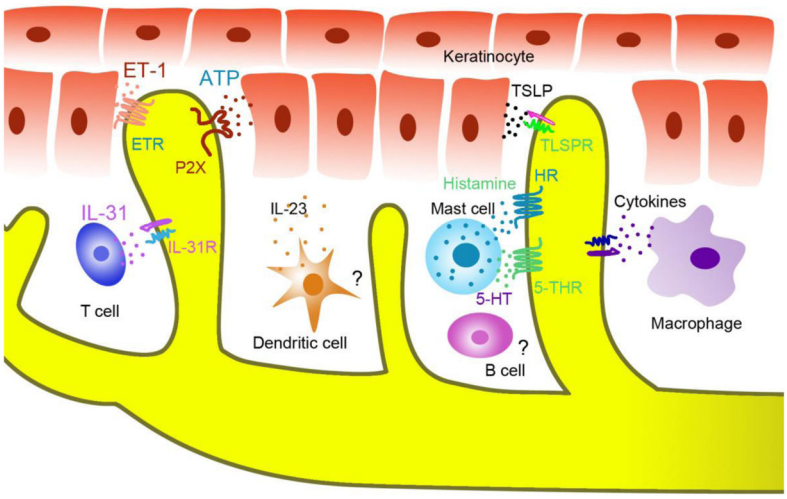

Figure 1. Cells and molecules that are involved in pain and itch signaling in the skin

Multiple cell types in the skin interact with sensory terminals by releasing bioactive molecules to initiate pain and itch signaling. Keratinocytes release ATP, ET-1, and TSLP that bind to P2XR, ETR, and TSLPR expressed by sensory nerve endings leading to excitation of primary afferent neurons in pain and itch pathways. Immune cells also play important roles in the development of pain and itch. Upon activation, cutaneous mast cells, macrophages, and T cells release histamine, 5-HT, IL-31, and many inflammatory cytokines that bind to receptors on sensory neuron terminals to induce hypersensitivity. Nociceptors also interact with dermal dendritic cells to initiate IL-23 release to participate in skin inflammation.

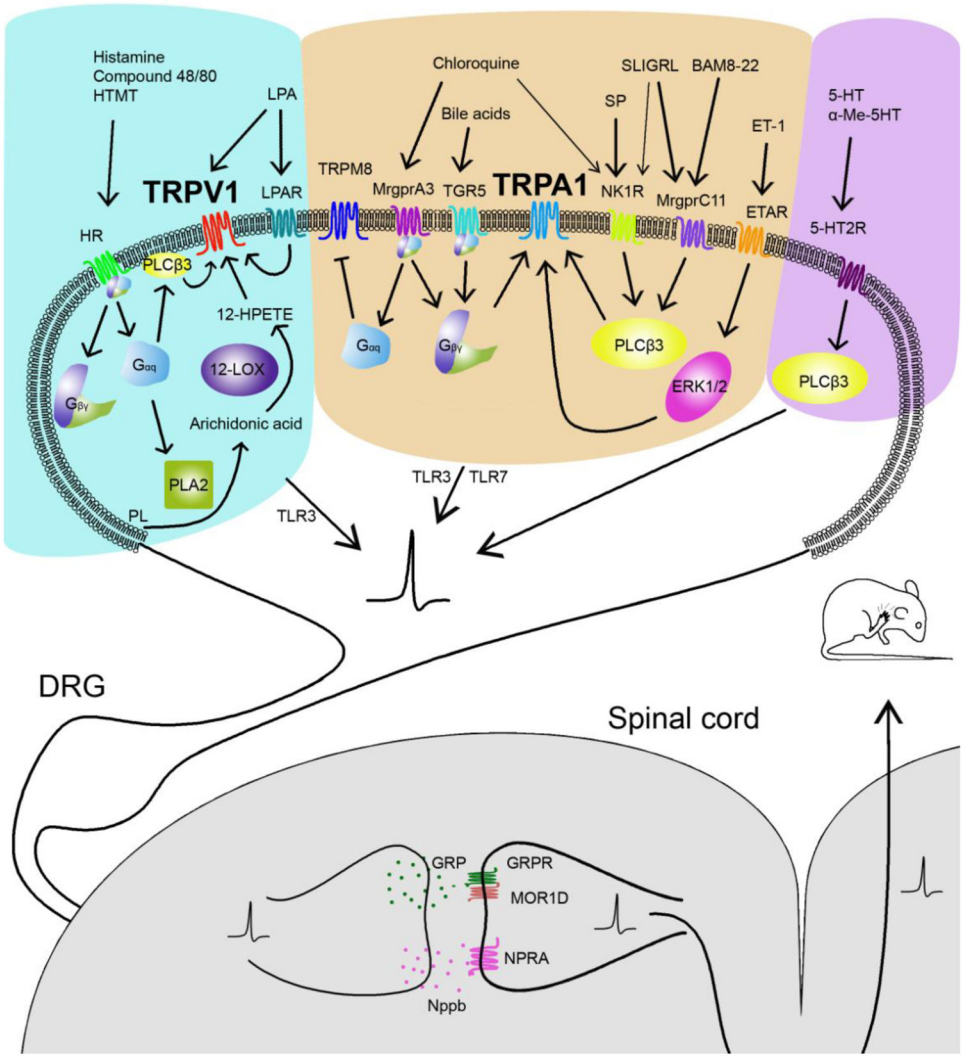

Figure 2. Molecular basis of itch signaling

There are three classes of pruritogen-elicited itch: TRPV1-dependent, TRPA1-dependent, and non-TRPV1-TRPA1-dependent. Histamine, compound 48/80, and HTMT activate HRs, which activate TRPV1 through both PLCβ3 and PLA2/12-LOX signaling pathways. TRPV1 could also mediate LPA-induced itch, either through direct activation or indirect sensitization of TRPV1 through G protein-coupled LPAR as described for LPA-induced pain responses. TRPA1 is required for itch signlaing induced by CQ, SLIGRL, BAM8–22, ET-1, SP, and bile acids, which bind to their respective receptors MrgprA3, MrgprC11, ETAR, NK1R, and TGR5. $G_{\beta\gamma}$ subunit, PLCβ3, and ERK1/2 participate in signaling downstream from these GPCRs and contribute to the activation of TRPA1. TLR3 is involved in both TRPV1- and TRPA1-dependent itch sensations, whereas TRL7 is specificaly involved in TRPA1-dependent itch. The itch sensation induced by serotonin is unique since neither TRPA1 nor TRPV1 is involved, although PLCβ3 plays important roles in both 5-HTR- and TRPA1-dependent itch. In the spinal cord, presynaptically released GRP and Nppb relay itch signals to the second-order spinal neurons by binding to

corresponding heteromeric GRPR/MOR1D and NPRA receptors, respectively, which in turn transmit itch signals to the brain, leading to scratching.