ANNUAL REVIEWS

Annual Review of Neuroscience

Neural Mechanisms of Itch

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Annu. Rev. Neurosci. 2020. 43:187–205

First published as a Review in Advance on Feburary 19, 2020

The *Annual Review of Neuroscience* is online at neuro.annualreviews.org

https://doi.org/10.1146/annurev-neuro-083019-024537

Keywords

pruritus, itch, skin, DRG, spinal cord, brain

Abstract

Itch is a unique sensation that helps organisms scratch away external threats; scratching itself induces an immune response that can contribute to more itchiness. Itch is induced chemically in the peripheral nervous system via a wide array of receptors. Given the superficial localization of itch neuron terminals, cells that dwell close to the skin contribute significantly to itch. Certain mechanical stimuli mediated by recently discovered circuits also contribute to the itch sensation. Ultimately, in the spinal cord, and likely in the brain, circuits that mediate touch, pain, and itch engage in cross modulation. Much of itch perception is still a mystery, but we present in this review the known ligands and receptors associated with itch. We also describe experiments and findings from investigations into the spinal and supraspinal circuitry responsible for the sensation of itch.

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INTRODUCTION

Itch, also called pruritus, is an unpleasant sensation that precipitates an urge to scratch. Itch is evolutionarily conserved across species, including fish, birds, mice, and humans, suggesting a beneficial role of scratching in ridding an organism of potential external threats. Scratching can induce an inflammatory response toward the site of the scratches as an endogenous immunological defense for the host. In certain metabolic, dermatological, or neurological conditions, itch can be debilitating, dramatically reducing quality of life (Dong & Dong 2018). Beyond the basic understanding of this fundamental sensation, elucidation of the molecular components of pruritus as well as its underlying neuronal circuitry may aid in the development of alleviatory therapies.

Historically, somatosensation research has focused on understanding modalities such as pain and touch. Researchers have made many important advancements in these efforts, elucidating specific gene expression patterns and neuronal circuits responsible for these modalities. Recently, more scientists have begun to appreciate that many pain- and touch-sensing neurons and circuits are also responsible for itch sensation. Furthermore, there is now evidence that some neurons transmit itch messages independently of other modalities. Cross-disciplinary efforts as well as the implementation of modern tools have ultimately resulted in surprising new findings. Notably, more dermatologists, biomedical engineers, immunologists, and neuroscientists are contributing to the advancement of our understanding of itch sensation. There is still much to uncover, but substantial groundwork has been laid for these basic and clinical endeavors. In this review, we discuss what is currently known about the neural components of itch somatosensation.

PERIPHERAL MECHANISMS OF ITCH

Although itch may involve direct activation of spinal neurons or arise via idiopathic means, most known mechanisms of itch begin with the activation of peripheral neurons. Itch-detecting neurons in the periphery are also called pruriceptors. Pruriceptors belong to a class of neurons

called nociceptors that have specialized functionality in sensing pain. In this section, we briefly discuss ways researchers have classified pruriceptors. Following that, we present proposed itch receptors, categorized as G protein–coupled receptors (GPCRs), cytokine receptors, Toll-like receptors (TLRs), and ion channels. We conclude this section with a discussion of the proposed sources of itch-related ligands that bind to these receptors on pruriceptors.

Pruriceptor Classification

Not all itch-transmitting neurons are the same. Some of the earliest work on characterizing itch involved in vivo electrophysiological recording of nerve fibers in humans, monkeys, and mice. Those experiments showed that itch is transmitted via Aδ and C fibers (LaMotte et al. 2014). C fibers are unmyelinated and transmit action potentials more slowly than the myelinated Aδ fibers, and these fibers can further be characterized by their sensitivity to mechanical force. Mechanically insensitive C fibers can be activated by histamine, while mechanically and heat-sensitive C (CMH) fibers do not respond to histamine. These CMH fibers can instead be stimulated by nonhistaminergic pruritogens like cowhage, a plant that produces very itchy spicules.

More recently, genetic tools like RNA sequencing have helped to further clarify the identity of neurons responsible for itch signaling. One group in particular processed about 800 individual dorsal root ganglion (DRG) neurons and used principal component analysis to categorize them based on gene expression (Usoskin et al. 2015). The analyses placed unmyelinated nonpeptidergic (NP) C-type nociceptors into three categories: NP1, NP2, and NP3. The NP1 group of neurons seems to be tuned to cholestatic itch [categorized by Mas-related G protein–coupled receptor D (MRGPRD) expression]. While histamine receptors are assigned to both NP2 and NP3 groups, NP2 neurons likely specifically mediate chloroquine-associated itch (i.e., *MRGPRA3* expression), and NP3 neurons seem to be involved in chronic inflammatory itch (i.e., *Il31r* expression). These categorizations can be tremendously helpful in identifying previously unknown itch-related receptors, as they might be found on neurons expressing other known pruriceptors. Furthermore, these RNA sequencing efforts can corroborate conclusions about the functionality of itch neurons by providing more comprehensive profiles of gene expression. With these RNA sequencing results as a reference, we start the next section with a discussion of putative itch receptors. We also discuss the proposed functions and ligands associated with these receptors.

PERIPHERAL G PROTEIN–COUPLED RECEPTORS

Receptors for Biogenic Amines

The best-characterized component of itch involves histamine signaling. Histamine, a potent vasodilator, produces a triple response of local vasodilation, swelling, and flare that was found to be concurrent with the sensation of itchiness (Lewis 1942). This histaminergic sensation of itch was found to be mediated via C fibers that transmit the signal toward the spinal cord (Schmelz et al. 1997).

In mouse studies, it has been shown that histamine acts primarily via H1r and H4r to induce itch (Bell et al. 2004). Signaling of this type of itch has been shown to involve an itch-selective population of MRGPRA3-expressing neurons (Han et al. 2013) as well as Trpv1 and the lipoxygenase pathway (Imamachi et al. 2009). Past research has shown that blocking H1r attenuates the vast majority of histamine-associated itch conditions, as compared to the other histamine receptors (Coulie et al. 1991). Interestingly, even though H3r antagonists are ineffective in attenuating many forms of itch, they have been shown to have a role in mediating nasal itch (Stokes et al. 2012).

Histamine is the most well-known biogenic amine that induces itch. However, other biogenic amines like bradykinin and serotonin can also play a role in itch induction. Bradykinin has been shown in human subjects to produce mildly pruritic sensations, while administration into atopic dermatitis patients leads to intense pruritus (Hosogi et al. 2006). Serotonin has been shown to be a potent mediator of itch. Using selective antagonists for serotonin receptors [5-hydroxytryptamine receptors (5hts)], researchers have shown that 5ht2 but not 5ht1 or 5ht3 is involved in serotonin-induced itch (Yamaguchi et al. 1999). There is also evidence that 5ht7 activation along with downstream Trpa1 activity explains itch associated with serotonin administration (Morita et al. 2015). Downstream of serotonin receptors, there is evidence from the use of knockout (KO) mice that phospholipase beta 3, but not Trpv1, is involved in serotonin-induced itch (Imamachi et al. 2009).

Mas-Related G Protein–Coupled Receptors

Another class of GPCRs implicated in itch signaling is the family of receptors termed the MRGPRs. These receptors were found using a complementary DNA (cDNA) subtractive screening approach to isolate a cDNA clone expressing a GPCR specifically enriched in DRG neurons (Dong et al. 2001). Due to the large number of MRGPRs present in the mouse genome, we wanted to create a mouse line with multiple MRGPRs knocked out as a tool to further study the function of these receptors in vivo. We deleted an 845-kb stretch of DNA containing 12 full *Mrgpr* genes, generating an MRGPR cluster–KO line (Liu et al. 2009). Using this tool, we found that chloroquine, a widely used malaria drug with pruritic side effects, induced itch significantly more in wild type (WT) compared to the MRGPR cluster–KO mice. We then used in vitro DRG calcium and observed that a small subset (5% of the total population) of DRG neurons responded to chloroquine, whereas DRG from MRGPR cluster–KO mice exhibited no calcium transients (Liu et al. 2009). Importantly, we saw that transfection of MRGPR cluster–KO DRG with the human *MRGPRX1* rescued reactivity to chloroquine, suggesting that *MRGPRX1* serves as a human ortholog to *MRGPRA3* (Liu et al. 2009). We further characterized the expression pattern of MRGPRA3+ DRG neurons using a genetic labeling strategy (Han et al. 2013). MRGPRA3+ neurons were found to innervate superficial areas of the skin. They also innervated the regions in the eye between the conjunctiva and the cornea to mediate ocular itch (C.C. Huang et al. 2018). In vivo electrophysiological recording from MRGPRA3+ neurons showed that these neurons are typically polymodal, responding to noxious heat, noxious mechanical force, and capsaicin; they were also found to be activated by various pruritogens like histamine, Bam8-22, chloroquine, and cowhage (Han et al. 2013). We then used a genetic strategy of expressing Trpv1 only in MRGPRA3+ DRG neurons in a background of Trpv1 KO. Capsaicin, which usually induces pain-associated behaviors, instead only generated itch behaviors in this unique mouse line (Han et al. 2013). These data provide evidence for a population of itch-selective neurons in mice marked by MRGPRA3 (Han et al. 2013).

Bam8-22, a C-terminal fragment of proenkephalin, was found to potently activate the human MRGPRX1 as well as the mouse MRGPRC11 receptor (Han et al. 2002, Lembo et al. 2002). Indeed, in mouse as well as human behavioral experiments, our collaborators have shown that Bam8-22 can elicit itch independent of histamine-associated signaling (Sikand et al. 2011). There has been research suggesting that Bam8-22 activation of MRGPRC11+ neurons is mediated by Trpa1 (Wilson et al. 2013), though our recent findings have suggested that Bam8-22-related activation of MRGPRC11 and the human ortholog MRGPRX1 is instead mediated by the TTX-resistant voltage-gated sodium channel (Nav) Nav1.9 (Tseng et al. 2019). SLIGRL, a 6-amino-acid peptide cleaved from the Par2 receptor, had been accepted to be the ligand for Par2. We compared the itch phenotypes of SLIGRL administration in Par2 KO, MRGPR cluster–KO, and WT mice

(Q. Liu et al. 2011). Surprisingly, we observed that SLIGRL-induced itch was still present in Par2 KO compared to WT, yet itch was significantly reduced in MRGPR cluster–KO mice (Q. Liu et al. 2011). A truncated version of SLIGRL with the sequence SLIGR was instead found to activate Par2; thus, we concluded that SLIGRL activates MRGPRC11, while SLIGR activates Par2 (Q. Liu et al. 2011). In a detailed study into the functionality of MRGPRC11, our collaborators found that cathepsin S–induced itch is mediated by MRGPRC11 via cleavage of the receptor's N terminus. Unlike the Par2 receptor's functionality, where the cleaved peptide activates the receptor itself, MRGPRC11 undergoes a proposed conformational change to induce downstream signaling of itch (Reddy et al. 2015). MRGPRs seem to be involved in pruritus induced by protease exposure. Recent work has shown that mucunain, the pruritic component of cowhage, activates MRGPRX1, the human ortholog of MRGPRC11 (Reddy et al. 2018). As a candidate for mediating pruritus, MRGPRC11 has also been implicated in itch associated with cholestasis (Sanjel et al. 2019).

Previous work had been done to show that β-alanine can activate the human and mouse forms of *Mrgprd* (Shinohara et al. 2004). We administered β-alanine orally as well as into the cheek of MRGPRD-KO mice versus WT and found that both modes of administration led to dramatically more itch in the WT mice (Q. Liu et al. 2012). Human subjects injected with β-alanine also reported increased itch, accompanied by tingling and mild stinging (Q. Liu et al. 2012).

Our recent investigations into MRGPRA1 function using the MRGPR cluster–KO mouse line have shown that this receptor, along with its human ortholog MRGPRX4, may contribute to the pruritus elicited by bilirubin in jaundice and cholestasis (Meixiong et al. 2019b). We showed that in a mouse model of cholestasis, KO of a single gene, *Mrgpra1*, led to decreased pruritus as compared to WT controls. Interestingly, we found that plasma bilirubin levels did not correlate well with itchiness. Skin bilirubin content seemed to correlate better with itch severity.

We have also recently shown that certain bile acids can activate MRGPRX4 at pathophysiological concentrations (Meixiong et al. 2019c). Bile acids previously have been shown in mice to induce itch via the receptor Tgr5, which is discussed below. In humans, though, the mechanism for bile acid–induced pruritus is less clear. We addressed this issue by generating a humanized mouse with MRGPRX4 specifically expressed in an itch-selective DRG population. These humanized MRGPRX4+ mice exhibited greater itch severity when challenged with bile acids compared to WT control mice. Similar disparity was seen when comparing MRGPRX4+ mice and WT control mice in a cholestatic model of pruritus (Meixiong et al. 2019c). A parallel study also found that bile acids can induce itch via MRGPRX4 in human subjects (Yu et al. 2019). In contrast, Tgr5 agonists are unable to induce itch in human subjects. Clinically, bile acid and bilirubin levels correlate well with reported levels of pruritus in patients with cholestatic itch. Pruritus associated with hepatological disorders is among the oldest described maladies, and these recent studies provide an underappreciated connection involved in this form of pruritus.

Protease-Activated Receptors

Besides MRGPRs, another class of GPCRs termed protease-activated receptors (PARs) has been found to mediate itch. PARs are involved in nonhistaminergic itch. They are a unique class of receptors whereby exogenous or endogenous proteases cleave the N terminus of the receptors, converting the cleaved peptide into a ligand that can then activate the receptor itself. Certain endogenous serine proteases like kallikreins (KLK5 and KLK14) have been shown to activate Par2 in vitro (Stefansson et al. 2008). Cathepsin S, another endogenous protease, was shown to induce itch in humans as well as activate human forms of Par2 and Par4 in vitro (Reddy et al. 2010). A recent paper has also shown that cathepsin S–induced itch can be attenuated by inhibitors

of cathepsin S, antagonists of Par2, and KO of Trpv1 (Chung et al. 2019). Tryptase, a protease secreted by mast cells, especially in certain chronic itch conditions, was also shown to activate Par2 (Wilson et al. 2013). Recent research also corroborates the role of Par2 in chronic pruritus. PZ-235, an antagonist of Par2 signaling, is sufficient in attenuating itch induced by house dust mite as well as by mast cell degranulation (Barr et al. 2019).

Exogenous proteases can also activate this class of receptors to induce itch. Mucunain, a proteolytic enzyme found in cowhage, was shown to be pruritic, an effect involving the activation of Par2 and Par4 (Reddy et al. 2008). Other plant-derived proteases like bromelain, ficin, and papain also activate Par2 and Par4, implicating PARs in this form of pruritus (Reddy et al. 2010).

Receptors for Endogenous Peptides

Certain endogenous peptides are also implicated in itch induction. Intradermal administration of Endothelin-1 leads to increased spontaneous scratching, which can be attenuated with an antagonist of the Eta receptor (Trentin et al. 2006). Surprisingly, intradermal injection of Etb antagonist leads to increased scratching, while coinjection of Etb agonist decreases scratching (Trentin et al. 2006). Substance P is another endogenous peptide implicated in itch. Substance P has been known to activate mast cells, which are key mediators of itch. In an experiment comparing mast cell–deficient mice to WT, substance P was shown to induce itch even in mast cell–deficient mice (Andoh et al. 1998). Furthermore, antagonists for Nk1, a receptor for substance P, attenuates substance P–associated itch, while Nk1 agonists induce spontaneous scratching (Andoh et al. 1998). Data from our lab also show that substance P can induce itch in mice. However, our data suggest that substance P activates mast cells via the receptor MRGPRB2 and not Nk1 (Green et al. 2019).

Receptors for Products of Cellular Metabolism

Products of cellular metabolism have also been implicated in the induction of itch in vivo. Bile acids administered intradermally can induce itch, an effect attenuated in mice with the receptor Tgr5 knocked out (Alemi et al. 2013). In regard to signaling, bile acid–associated itch seems to involve Trpa1 activity (Lieu et al. 2014). Additionally, bile acid–induced itch can be attenuated with an intrathecal gastrin-releasing peptide receptor (GRPR) antagonist as well as with intravenous naloxone. Tgr5 expression is found on DRG neurons, and bile acids can induce hyperexcitability of certain DRG neurons. Recent work, however, suggests that Tgr5 is salient for bile acid pruritus only in mice and not in humans.

Another metabolic product, lysophosphatidic acid (LPA), is a phospholipid derivative that can also induce itch that is attenuated by H1r antagonists (Hashimoto et al. 2004). Recent findings show that sphingosine-1-phosphate (S1p), another endogenous lipid, acts on S1pr3 to induce itch; there is also evidence that Trpa1 is involved with S1p-induced itch (Hill et al. 2018).

PERIPHERAL CYTOKINE AND CHEMOKINE RECEPTORS

Besides GPCRs, another big class of itch-related receptors is the receptor group activated by cytokines and/or chemokines. Cytokines and chemokines, released by keratinocytes and immune cells near the epidermis, have been particularly implicated in pruritus that occurs during chronic itch conditions.

Interleukin (IL)-31 is the most characterized cytokine involved in pruritus. Hints of this molecule's pruritic capacity came as anti-IL-31 antibodies were shown to attenuate the

spontaneous scratching in an NC/Nga mouse model of atopic dermatitis (Grimstad et al. 2008). Further research showed that intradermal IL-31 administration in mice can induce scratching behavior that lasts over 2 h postinjection (Arai et al. 2013). The neuronal mechanism of IL-31-associated itch was shown to involve direct activation of IL-31 receptors on DRG neurons in vitro (Cevikbas et al. 2014). KO of Trpa1 and Trpv1 in mice attenuated itch induced by IL-31, while pretreatment with Erk1/2 inhibitors also attenuated IL-31-associated pruritus (Cevikbas et al. 2014).

IL-31 has been known to be secreted by activated T cells, and its overexpression in mice leads to dermatitis (Dillon et al. 2004). Pretreatment of mice with IL-31 antibody inhibits IL-31-associated itch and surprisingly reduces dermatitis and ear thickness in a mouse model of atopic dermatitis (Kasutani et al. 2014).

Other Th2-associated cytokines have also been implicated in itch. The keratinocyte-derived thymic stromal lymphopoietin (TSLP) is sufficient to induce itch when administered into the mouse cheek. TSLP was further characterized as able to directly activate a subset of Trpa1-expressing DRG neurons (Wilson et al. 2013). Similarly, the cytokine IL-33 was found to activate DRG neurons directly via the ST2 receptor. This is especially salient in explaining the itch associated with poison ivy–mediated contact dermatitis (Liu et al. 2016). Other important itch-associated Th2 cytokines include IL-4 and IL-13, where overexpression of either cytokine in mice leads to pruritic dermatitis (Chan et al. 2001, Zheng et al. 2009). In a comprehensive study to explore the roles of IL-4 and IL-13 in itch, both cytokines were found to directly activate neurons (Oetjen et al. 2017). Administration of either cytokine did not induce as pronounced an acute itch compared to IL-31. However, pretreatment with either IL-4 or IL-13 enhances the itch associated with subsequent histamine administration.

TLRs, which play a significant role in pathogen detection for the innate immune system, have also been found to be expressed on DRG neurons. Specifically, application of agonists for TLR3 as well as for TLR7 can activate certain subsets of DRG neurons. Furthermore, KO of TLR3 or TLR7 attenuates itch behavior when mice are challenged with nonhistaminergic pruritogens (T. Liu et al. 2010, 2012).

PERIPHERAL ION CHANNELS

Some ion channels have been shown to have important roles in itch signaling. Notably, it has been shown that KO of Nav1.9 leads to reduced histaminergic as well as nonhistaminergic itch. Nav1.9 was found to be coexpressed on most MRGPRA11- and MRGPRA3-expressing nonmyelinated small-diameter neurons (Salvatierra et al. 2018). Interestingly, a gain-of-function mutation of this channel leads to increased spontaneous scratching (Salvatierra et al. 2018). Electrophysiological data suggest that around 40% of itch fibers can be silenced with Nav1.7 blockers, while Nav1.8 blockers alone have no effect (Jurcakova et al. 2019).

Another ion channel that has been implicated in pruritus is Piezo2, which is responsible for mechanically activated currents. A recent study showed that aged mice exhibit fewer Merkel cells per touch dome; this decrease was found to be correlated with greater alloknesis scores (Feng et al. 2018). Chemogenetic activation of Merkel cells attenuated alloknesis, while mice with genetic deletion of Merkel cells displayed increased alloknesis (Feng et al. 2018). Ultimately, it was found that mice with genetically knocked out Piezo2 in keratinocytes as well as in Merkel cells displayed greater alloknesis scores compared to WT control mice (Feng et al. 2018).

Two notable ion channels implicated in nociception are Trpv1 and Trpa1. One group showed that the itchiness of SADBE-induced allergic contact dermatitis is attenuated in mice with Trpv1, Trpa1, or both receptors knocked out as compared to WT mice (Feng et al. 2017). Interestingly,

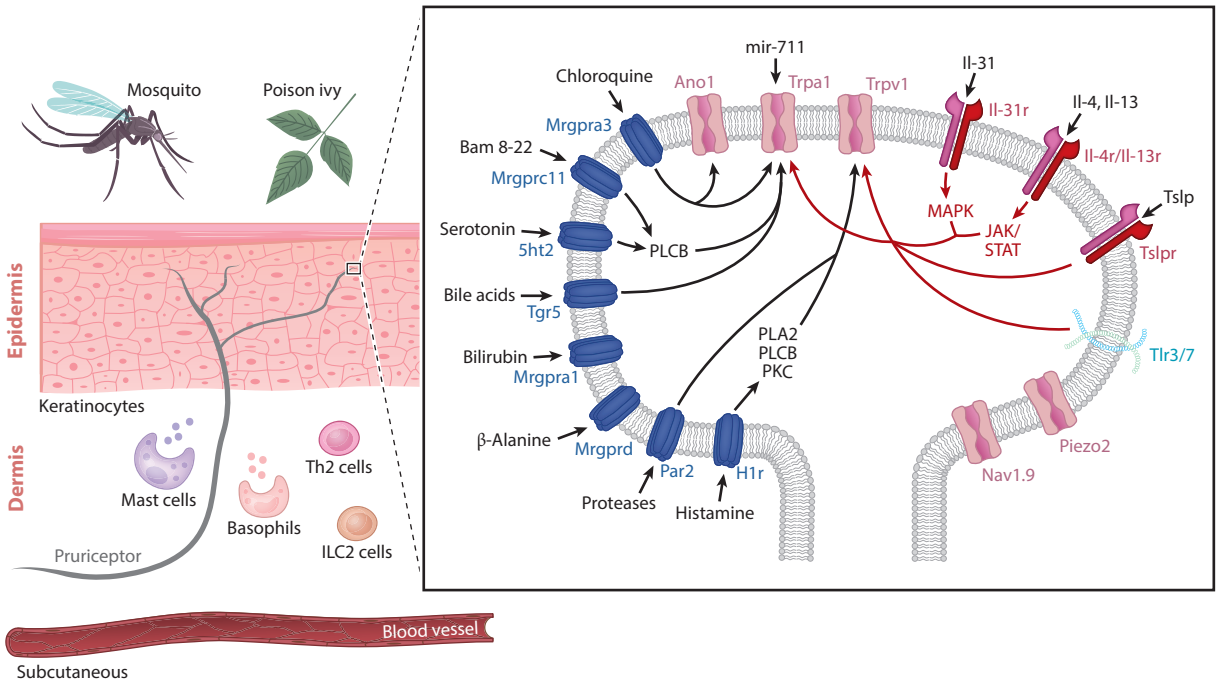

Figure 1

Peripheral sources of itch mediators and itch receptors. Pruriceptors terminate peripherally at superficial levels of the skin. External irritants or metabolic byproducts from internal organs can stimulate pruriceptors, keratinocytes, and/or nearby immune cells. Stimulated keratinocytes and/or dermal immune cells can release pruritogens that bind to a broad array of receptors, represented on the expanded nerve ending (representing receptors found in mice; *black box*). Itch-related G protein–coupled receptors, represented on the left with purple transmembrane domains, as well as cytokine/chemokine receptors (*pink and red receptors*, *top right*) require ion channels like TRPA1 and Trpv1 to excite pruriceptors. Abbreviations: 5HT, 5-hydroxytryptamine; Ano1, anoctamin 1; H1R, histamine 1 receptor; IL, interleukin; ILC2, type 2 innate lymphoid cell; MRGPR, Mas-related G protein–coupled receptor; PAR2, protease-activated receptor 2; Th2, T helper cell type 2; TLR3, Toll-like receptor 3; TRPA1, transient receptor potential ankyrin 1; Trpv1, transient receptor potential vanilloid 1; TSLPR, thymic stromal lymphopoietin receptor.

SADBE can directly activate both receptors at high concentrations (Feng et al. 2017). Other pruritogens like LPA and S1p also seem to induce itch via both Trpv1 and Trpa1 (Hill et al. 2018, Kittaka et al. 2017).

There is also evidence that microRNA like mir711 can activate Trpa1 directly and induce itch in mice in vivo (Han et al. 2018). Trpa1 also seems to be an important mediator of itch induced by other pruritogens like serotonin and bile acids (Lieu et al. 2014, Morita et al. 2015). Trpv1 seems to have its biggest role in mediating pruritus involving H1r signaling (Shim et al. 2007).

PERIPHERAL ITCH MEDIATORS AND SOURCES

Itch is a unique sensation that is distinct from other modalities like pain and touch. Unlike other modalities, itch is localized to the most superficial portion of the skin, the epidermis (**Figure 1**). Nerve fibers that transmit itch have terminals that reside beside keratinocytes in the epidermis. Given this localization, itch-transducing neurons can be activated by surface mechanical forces (e.g., insects) as well as by chemicals released by nearby cells (e.g., keratinocytes, mast cells). In this section, we highlight proposed sources of itch mediators.

Immune Cells

Mast cells are one of the main sources of histamine, which is a potent pruritogen (Metcalfe et al. 1997). Tryptase is another pruritogen that can be released by mast cells to induce itch. Mast cells have also been shown to release IL-4, which can potentiate certain forms of pruritus (Metcalfe et al. 1997). A recent paper has shown that mast cells can release LTC4, serotonin, and S1p, which were all shown to be pruritogens (Solinski et al. 2019). Furthermore, receptors for these pruritic compounds were found to be expressed on a class of itch-specific neurons labeled with Nppb (Solinski et al. 2019).

Our lab has previously shown that the gene *Mrgprb2* is specifically expressed on connective-tissue mast cells in mice (McNeil et al. 2015). In regard to pruritus, we showed that secretagogue-induced histamine release and airway contraction were attenuated in mice with *Mrgprb2* KO (McNeil et al. 2015). Recently, we have also shown in vivo that activation of this receptor leads to faster release timing compared to IgE-mediated mast cell degranulation, activating neurons at earlier time points on average (Meixiong et al. 2019a). Our in vivo experiments have also shown that, compared to IgE activation, MRGPRB2 activation leads to the release of larger amounts of tryptase, a known biomarker of certain pruritic mast cell diseases (Meixiong et al. 2019a). Clinically, we have found that MRGPRB2 plays a key role in the development of itch in a mouse model of allergic contact dermatitis. Investigations of human contact dermatitis patient skin samples corroborate data from mouse studies in finding higher levels of an endogenous ligand of MRGPRX2 (PAMP8-22) in patient samples versus healthy samples (Meixiong et al. 2019a).

In certain chronic itch conditions such as atopic dermatitis, certain immune cells like basophils, Th2, and ILC2 have been found to be elevated in the skin (Mack & Kim 2018). Basophils are especially pertinent because, like mast cells, they are granulocytes that can release itch-related mediators such as IL-4, histamine, and tryptase. Th2 and ILC2 cells are also known to be involved in mediating itch via the release of cytokines such as IL-31, IL-4, and IL-13 (Mack & Kim 2018).

Keratinocytes

Keratinocytes have been shown to play a critical role in itch signaling. In addition to secreting histamine under certain conditions (Schwendinger-Schreck et al. 2015), keratinocytes can also release the pruritogen endothelin-1 (Kido-Nakahara et al. 2014). The cytokine TSLP is another pruritogen released by keratinocytes, especially in mouse models of atopic dermatitis (Wilson et al. 2013). Recently, it has been shown that a transmembrane protein linked to atopic dermatitis, Tmem79, plays a role in limiting reactive species produced by keratinocytes (Emrick et al. 2018). The increase of reactive species in keratinocytes can lead to secretion of prostaglandin E2, which can recruit mast cells, known contributors to pruritus in atopic dermatitis (Emrick et al. 2018).

Indirectly, keratinocytes also play a big role in the induction of pruritus. Trpv4, an ion channel expressed on sensory neurons as well as on keratinocytes, was shown to be important for the induction of histaminergic itch. Interestingly, in mice with Trpv4 specifically knocked out from keratinocytes, histaminergic itch was reduced while chloroquine-induced itch was unaffected (Chen et al. 2016).

SPINAL MECHANISMS OF ITCH

Removing irritating insects from the skin can be crucial for an organism's survival, especially in preventing the transmission of dangerous disease vectors. Parasites on the skin can induce a mechanical force that is perceived as itchy to hosts, leading to stereotyped scratching to alleviate the

itch and remove parasites. Such mechanical forms of itch have been found to involve inhibitory spinal interneurons expressing neuropeptide Y (Npy) (Bourane et al. 2015). Ablating and silencing these Npy interneurons leads to alloknesis, without subsequent sensitization to chemical itch stimuli like chloroquine and histamine (Bourane et al. 2015). Recent research has extended the understanding of this neuronal circuitry, implicating Npy in the inhibition of another population of excitatory interneurons labeled by Ucn3. Investigators have found that mechanosensitive DRG neurons expressing TLR5 synapse onto Ucn3+ interneurons and drive itch signaling (Pan et al. 2019).

Consistently, administration of H1r antagonists or the chemical ablation of GRPR neurons in the spinal cord has no effect on the alloknesis developed when Npy neurons are silenced or ablated (Bourane et al. 2015), suggesting the existence of a neural pathway specific to mechanically induced itch. This pathway specificity was observed in humans, where the vibration of vellus hairs on human skin induced mechanical itch without any burning or stinging characteristic of histamine-induced itch. This mechanical itch was also found to be resistant to histamine antagonist administration (Fukuoka et al. 2013).

The spinal circuitry involved in chemically induced itch has been better characterized than that for mechanical itch (**Figure 2**). Notably, a GRPR expressed in the spinal cord was found to be critically important for itch induced by chemical stimuli like compound 48/80, SLIGRL, and chloroquine (Sun & Chen 2007). Its ligand, gastrin-releasing peptide (GRP), was found to be expressed in the DRG (Sun & Chen 2007), though this claim remains controversial (Fleming et al. 2012, Mishra & Hoon 2013, Solorzano et al. 2015). Nevertheless, the role of GRPR in the mediation of chemically induced itch in the spinal cord has been well validated (Fleming et al. 2012, Mishra & Hoon 2013, Sun et al. 2009). In a paper that elucidated a labeled line marker for chemically induced itch (Nppb), evidence was provided suggesting that GRP is released by a secondary pruriceptor neuron in the spinal cord to activate GRPR (Mishra & Hoon 2013). A recent paper has shown that single optogenetic activation of GRP neurons is not enough to induce excitatory postsynaptic currents in downstream GRPR+ neurons; only with burst activation of these GRP neurons can GRPR neurons be effectively stimulated (Pagani et al. 2019). These GRP+ spinal neurons were found to receive monosynaptic input from itch-selective neurons, and the specific activation of these GRP+ neurons in the spinal cord induced a dose-dependent itch phenotype (Sun et al. 2017). Another type of chemically induced itch involves activation of mu opioid receptors found in the spinal cord. This spinal cord–mediated, morphine-induced itch has been shown in monkeys to be dose dependent (Ko & Naughton 2000). In mice, GRPR neurons have also been shown to be important for morphine-induced pruritus (X. Liu et al. 2011). In the spinal cord, there is evidence that GRPR forms a heterodimer with Mor1D for transmission of mu opioid–induced itch (X. Liu et al. 2011).

One advance in the study of molecular characteristics of spinal cord itch-transducing neurons is the discovery that neurokinin B is a pruritic compound when introduced intrathecally and can be released by DRG neurons stimulated with IL-31 (Sakata et al. 2019). With ablation experiments, the researchers also showed that neurokinin B acts upstream of GRP signaling in the spinal cord. Other investigators used a conditional KO strategy to show that a subclass of excitatory spinal cord neurons expressing Tlx3 are critically important for the transduction of itch induced by compound 48/80, Par2 agonist, and chloroquine (Xu et al. 2013). Deletion of testicular orphan receptor 4 (Tr4) in spinal cord neurons also attenuates itch induced by histamine, serotonin, and chloroquine (Wang et al. 2013). The roles of certain spinal cord–associated receptors have also been elucidated. Ablation of Nk1r attenuated spontaneous itch, alloknesis, and hyperknesis in a model of atopic dermatitis, while ablation of GRPR neurons only attenuated the hyperknesis in this model (Akiyama et al. 2015).

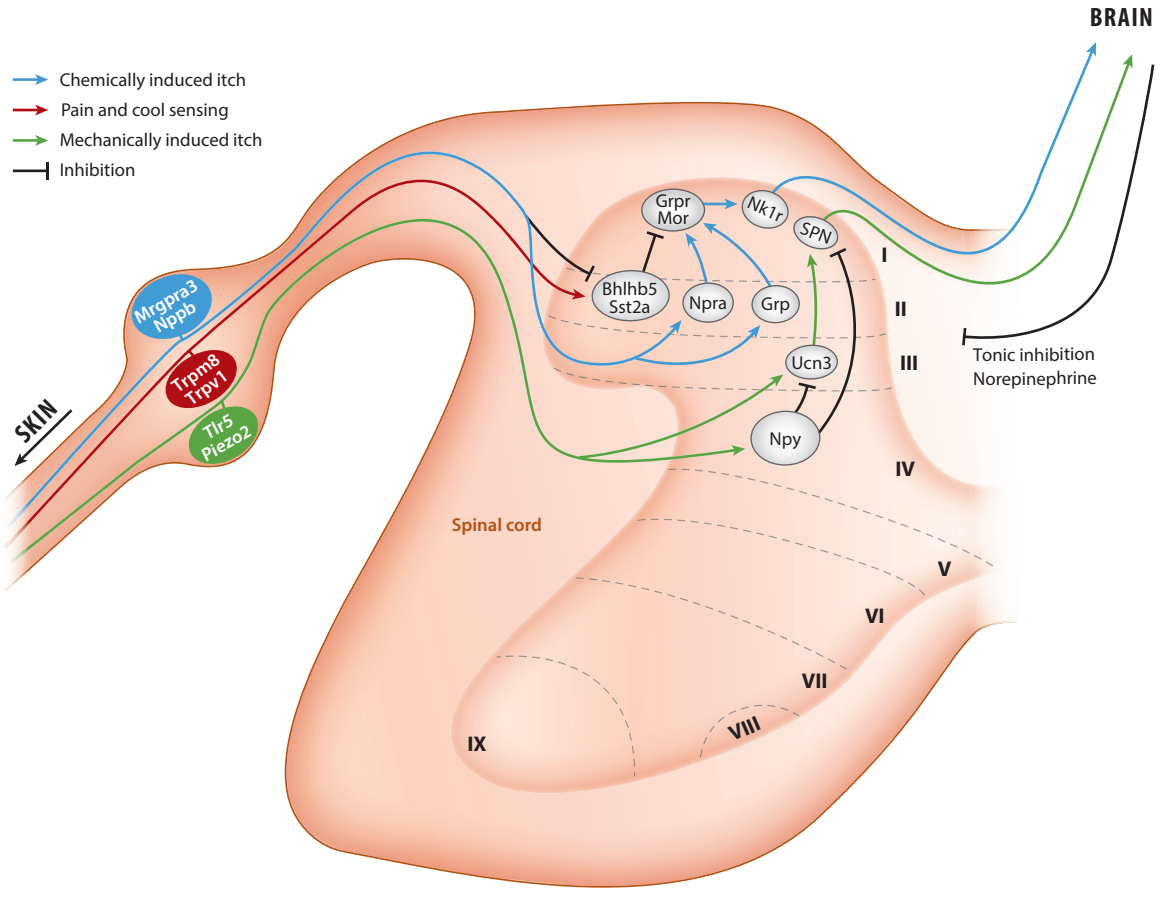

Figure 2

Spinal circuitry for itch transmission. In the pathway represented by blue lines, chemically induced itch is represented by itch-sensitive neurons expressing MRGPRA3 or Nppb, which form excitatory synapses with Npra+ or GRP+ neurons. Npra+ and GRP+ interneurons can form excitatory synapses with interneurons expressing GRPR (neurons that also coexpress mu opioid receptor). GRPR+ neurons finally form excitatory synapses with NK1R+ projection neurons that then send itch signals supraspinally. Some Mrpgra3+ or Nppb+ neurons can form inhibitory synapses with neurons expressing Bhlhb5 and the somatostatin receptor, sst2a. These Bhlhb5+ inhibitory interneurons can inhibit the activity of GRPR+ interneurons that mediate chemically induced itch. Pain- (Trpv1+) and cool-sensing (Trpm8+) neurons (*red pathway*) can stimulate Bhlhb5 neurons to ultimately inhibit itch signals. Mechanical itch (*green pathway*) is mediated by TLR5+ neurons that synapse onto Ucn3+ excitatory interneurons. DRG neurons responsible for touch (i.e., Piezo2+) can stimulate Npy+ inhibitory interneurons. The mechanical itch signals converge onto SPNs that send signals supraspinally. Tonic adrenergic inhibition of spinal itch signaling from the brain is also shown. Abbreviations: Bhlhb5, class B basic helix loop helix protein 5; GRP, gastrin-releasing peptide; GRPR, gastrin-releasing peptide receptor; Mor, mu opioid receptor; MRGPRA3, Mas-related G protein–coupled receptor A3; Nk1r, neurokinin 1 receptor; Nppb, natriuretic peptide B; Npy, neuropeptide Y; SPN, spinal projection neuron; Sst2a, somatostatin receptor 2a; TLR5, Toll-like receptor 5; Trpm8, transient receptor potential m8; Trpv1, transient receptor potential vanilloid 1; Ucn3, urocortin 3.

An important aspect of spinal cord circuitry of itch involves the attenuation of itch via counterstimuli. One of the oldest treatments of itch involves menthol application onto the skin, and recent research has confirmed that the amelioration of itch by cooling requires cold-sensing peripheral neurons that express Trpm8 (Palkar et al. 2018). In a human study of this counterstimuli phenomenon, heat and mechanical noxious stimuli were found to be sufficient

to inhibit histamine-induced itch as well as histamine-induced blood flow to the skin site of histamine administration (Yosipovitch et al. 2005). A landmark primate itch study also found that scratching the receptive field (on the skin) of specific pruritogen-sensitive spinothalamic tract neurons can attenuate the firing rates of those STT neurons (Davidson et al. 2009). In mouse studies, glutamate signaling from nociceptors was found to be important for the inhibition of itch transduction by noxious stimulation. Knocking out Vglut2+ nociceptor neurons leads to increased spontaneous scratching as well as enhanced itch responses (Lagerstrom et al. 2010, Y. Liu et al. 2010). It was further shown that a specific class of inhibitor interneurons expressing Bhlhb5 (B5) is responsible for inhibiting itch transduction (Ross et al. 2010). B5-KO mice exhibit excessive self-inflicted licking/scratching, inducing lesions on the skin. This effect was further narrowed down to the inhibitor interneuron population expressing Pax2 (Ross et al. 2010). A large population of B5 interneurons were found to also express Sst2a, where somatostatin application led to the hyperpolarization of these neurons (Kardon et al. 2014). Thus, intrathecal administration of the somatostatin analog octreotide was able to induce spontaneous itch dose dependently as well enhance chloroquine-induced itch (Kardon et al. 2014). These Sst2a interneurons also express dynorphin, an endogenous kappa opioid receptor (Kor) agonist, compelling researchers to suggest a hypothesis in which dynorphin released from these Sst2+ interneurons inhibited itch. A recent study suggested that the attenuation of nonhistaminergic itch via Kor agonism involves the phosphorylation of GRPR via PLC and PKCdelta (Munanairi et al. 2018).

Consistent with previous findings, optogenetic stimulation of spinal somatostatin neurons enhanced itch, while administration of somatostatin receptor antagonist reversed this itch (Christensen et al. 2016). Another study used a chemogenetic strategy to activate dynorphin-expressing spinal cord neurons to also show inhibition of evoked itch (J. Huang et al. 2018). Interestingly, somatostatin was also found to be expressed in the periphery, where optogenetic stimulation of Sst+ neurons in the trigeminal ganglia elicited itch. Ultimately, Sst and dynorphin signaling were found to converge upon GRPR neurons for itch transduction (J. Huang et al. 2018).

SUPRASPINAL AND CORTICAL MECHANISMS OF ITCH

After an itch stimulus is detected and transmitted to the spinal cord, processing as described above occurs and ultimately leads to a signal sent upstream toward the brain for itch perception (**Figure 3**). In this section, we discuss research on the supraspinal pathways involved in itch sensation as well as some descending regulatory pathways that can modulate those itch signals.

The spinothalamic tract is a major tract involved in central itch processing (Davidson et al. 2012). Histamine- and cowhage-sensitive spinothalamic tract neurons were found to innervate the ventral posterior lateral, ventral posterior inferior, and posterior nuclei of the thalamus. Cowhage-sensitive neurons were also found to terminate in the suprageniculate and medial geniculate nuclei (Davidson et al. 2012). After administration of histamine or chloroquine, cFos expression in the parabrachial nucleus (PBN) increased (Mu et al. 2017). Calcium imaging of the PBN has shown increased signaling during histamine/chloroquine administration (Mu et al. 2017). Furthermore, inhibitory chemogenetics as well as conditional KO of Vglut2 in the PBN can attenuate scratches induced by histamine and chloroquine, as well as spontaneous scratches in chronic itch models of atopic dermatitis and contact dermatitis (Mu et al. 2017). Another study found that Tac1+ spinal neurons project to the medial thalamus as well as to the superior lateral PBNs (T.L. Huang et al. 2018). When these Tac1- and Lbx1-coexpressing neurons are ablated, scratching induced by compound 48/80 and chloroquine is significantly reduced (T.L. Huang et al. 2018). Another population of Tac1-expressing neurons in the periaqueductal gray (PAG) was also found to be critical for itch sensation (Gao et al. 2019). While calcium imaging glutamatergic and GABAergic

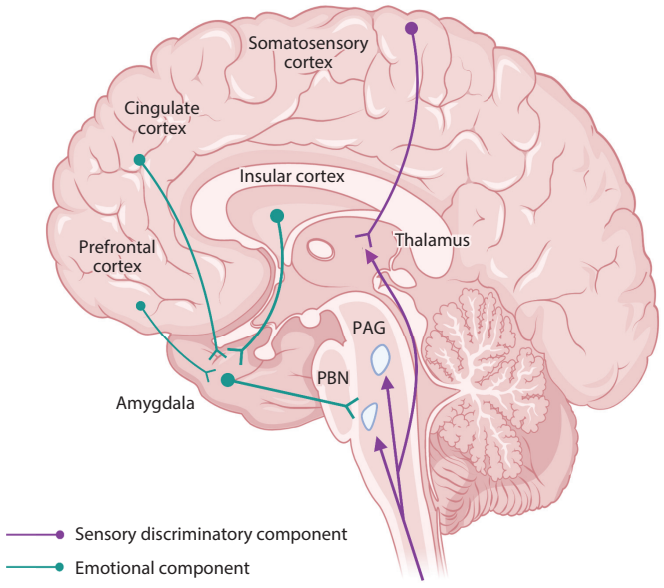

Figure 3

Supraspinal and cortical regions mediating itch. Regions that have been implicated in itch processing include the periaqueductal gray (PAG), parabrachial nucleus (PBN), amygdala, thalamus, insular cortex, cingulate cortex, prefrontal cortex, and primary/secondary somatosensory cortex.

neurons in lateral/ventral lateral PAG (l/vlPAG), researchers observed increased activity of both subtypes of neurons during scratching induced by histamine or chloroquine (Gao et al. 2019). A recent study has further shown that GABAergic but not glutamatergic PAG neurons encode the aversive components of itch (Samineni et al. 2018). In vivo calcium imaging research has also been done on subsets of neurons in the ventral tegmental area, where GABAergic neurons seem to fire before dopaminergic neurons in response to pruritogen challenge (Su et al. 2019).

Past the thalamus and midbrain, itch signals are transmitted to many areas of the cortex. Human functional MRI experiments have shown that the presentation of chemical itch stimuli leads to increased blood oxygen levels to prefrontal areas, supplementary motor areas, premotor cortex, anterior insula, anterior midcingulate cortex, primary/secondary somatosensory cortex, basal ganglia, and cerebellum (Herde et al. 2007).

There is also evidence of certain descending neural pathways that can modulate the itch signals being transmitted supraspinally. In an interesting experiment, mice were spinalized at the upper cervical spinal cord level, while lumbar spinal cord neurons were recorded (Akiyama et al. 2011). Spinalized mice were presented with an itch stimuli in the hindpaw, leading to increased firing of lumbar spinal cord neurons. Yet, scratching is unable to decrease the firing of those itch-responsive neurons, suggesting some form of top-down modulation of itch signaling in the spinal cord that is disrupted in spinalized mice (Akiyama et al. 2011). Other researchers have shown that the descending modulation may be due to adrenergic signaling. Intrathecal injection of an adrenergic antagonist leads to increased serotonin-associated itch, while alpha1/2 agonists decreased serotonin-induced itch, suggesting that tonic inhibition via the adrenergic system modulates itch transmission (Gotoh et al. 2011). Serotonin also has been implicated in the descending modulation of itch signaling. Genetic deletion or chemical degeneration of 5HT neurons in the central nervous system attenuates chloroquine-induced itch (Zhao et al. 2014). Further investigation of

the spinal localization of this signaling concluded that the 5HT1A receptor is coexpressed with GRPR. Functional studies show that 5HT1A activates GRPR excitability, while the blockade of 5HT1A attenuates scratches observed in a model of chronic itch (Zhao et al. 2014). Inhibitory chemogenetics suppressing the activity of l/vlPAG neurons that innervate the rostral ventromedial medulla (RVM) leads to attenuation of itch induced by histamine and chloroquine (Gao et al. 2019). Specifically, it was shown that Tac1+ and not Sst+ neurons in the l/vlPAG are responsible for this descending modulation of itch sensation. Excitatory chemogenetics as well as optogenetics were utilized to stimulate Tac1+ l/vlPAG neurons that innervate the RVM, leading to increased spontaneous itch (Gao et al. 2019). Investigations into RVM ON and OFF cells provide evidence that itch signals generally lead to the activation of RVM ON cells and inhibition of OFF cells (Follansbee et al. 2018).

CONCLUDING REMARKS

While past investigations into pruritus mainly interrogated the effects of histamine, modern genetic and imaging tools have dramatically expanded our knowledge of the nonhistaminergic components of itch. With the rapid expansion of pruritus research methods and lines of inquiry, a previously unappreciated complexity of mechanisms for itch beyond classic molecules like histamine is being uncovered. This is exemplified by many chronic itch conditions in which antihistamine treatments have been found to be mostly refractory. As the itch field expands, it is beginning to overlap with adjacent fields. In recent years, many receptors and ligands that were previously considered to be purely pain related have been found to also contribute to pruritus in specific circuits. Furthermore, many interactions among keratinocytes, immune cells, some internal organs, and itch-sensing neurons have been elucidated, possibly accelerating the development of more specific therapies for pruritus. On the basis of recent findings, indeed many new and existing companies have begun investing the development of drugs for chronic itch conditions.

Harnessing tools like in vivo calcium imaging, optogenetics, and chemogenetics has led to a more sophisticated understanding of the circuitry mediating itch in the spinal cord as well as in the brain. In the near future, scientists can hopefully begin to use viral delivery and genomic manipulation tools to study higher organisms, including nonhuman primates, with finer precision. Similarly, optimization and application of newer tools like histamine and voltage sensors may aid greatly in delineating the neuronal circuitry responsible for different components of itch. It is an exciting time for this burgeoning field, and we look forward to its continued development.

DISCLOSURE STATEMENT

X.D. is a cofounder of Escient Pharmaceuticals.

ACKNOWLEDGMENTS

We thank Mr. Pin-Tsung Lee for illustrating the figures. We also thank James Meixiong, Xintong Dong, and Elizabeth Sypek for discussions and critical reading of the manuscript. The work was supported by a grant from the National Institutes of Health (NS054791) to X.D.

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