

Peripheral mechanisms of itch

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Abstract: Detection of environmental stimuli that provoke an aversive response has been shown to involve many receptors in the periphery. Probably the least-studied of these stimuli are those that induce the perception of itch (pruritus), an often-experienced unpleasant stimulus. This review covers the ligands and their receptors which are known to cause primary sensory neuron activation and initiate itch sensation. Also covered are several itch-inducing substances which may act indirectly by activating other cell types in the periphery which then signal to primary neurons. Finally, progress in identifying candidate neurotransmitters that sensory neurons use to propagate the itch signal is discussed.

Keywords: itch; primary sensory neurons; receptors

1 Introduction

Detection of environmental stimuli that provoke an aversive response, such as heat, cold, and noxious chemicals, involves many receptors in the periphery. Probably the least-studied of these stimuli are those inducing the perception of itch (pruritus), an often-experienced unpleasant stimulus. Until recently only one receptor family, the histamine receptors, was linked to pruritus; this is clearly inadequate since histamine can also evoke pain. Histamine is not a component of most external pruritogens, and antihistamines are ineffective in treating itch in many disorders. In fact, whether itch-specific receptors even exist has been unclear, due in part to the lack of consensus on how itch is perceived. Is itch uniquely encoded, with specific receptors for different pruritogens and a dedicated neuronal circuit in the periphery and spinal cord? Or, does

the nervous system “decide” that certain combinations of signals that on their own communicate other sensations, should together trigger itch?

Numerous studies have been designed to test these possibilities, but the most prominent are a pair of breakthrough publications in 2007 and 2009 that reported direct evidence for itch as a distinctly encoded sensation^[1,2]. In these experiments, it was shown that a subset of neurons in the spinal cord is required for scratching induced by several chemicals, but dispensable for pain behavior^[2]. These neurons are characterized by the expression of receptors for a small peptide called bombesin, though it is unclear whether all signaling onto these neurons is carried out through these receptors^[1,2]. The identification of an itch-specific circuit in the spinal cord raised the exciting possibility that primary neurons in the periphery that feed into this circuit may also be specific for itch. Indeed, several studies^[3-5] had already shown that specific subsets of neurons respond to pruritic stimuli, though they also respond to other types of stimuli, and the cellular and molecular characterization of these neurons was lacking due to technical constraints.

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The notion of itch-specific neurons was given a boost by another study published in 2009, in which receptors for histamine-independent pruritic stimuli were reported^[6]. MrgprA3 and MrgprC11, two members of the Mas-related G protein-coupled receptor (Mrgpr) family of G protein-coupled receptors (GPCRs) in mice, were shown to be activated by the pruritic chemicals chloroquine and BAM8-22, respectively^[6]. Even more interesting was that their expression patterns mostly overlap, with both expressed on a very small subset of primary sensory neurons^[6]. These neurons

also respond to histamine, indicating that they may be specialized for the detection of pruritogens^[6]. This review covers these ligands and their receptors, which directly cause neuron activation, as well as plant cysteine proteases, which may also act directly on neurons (Fig. 1). Also covered are several pruritogens which may act indirectly by activating other cell types in the periphery that then signal to primary neurons (Fig. 1). Finally, progress in identifying candidate neurotransmitters that sensory neurons use to propagate the itch signal are discussed. Pruritogens

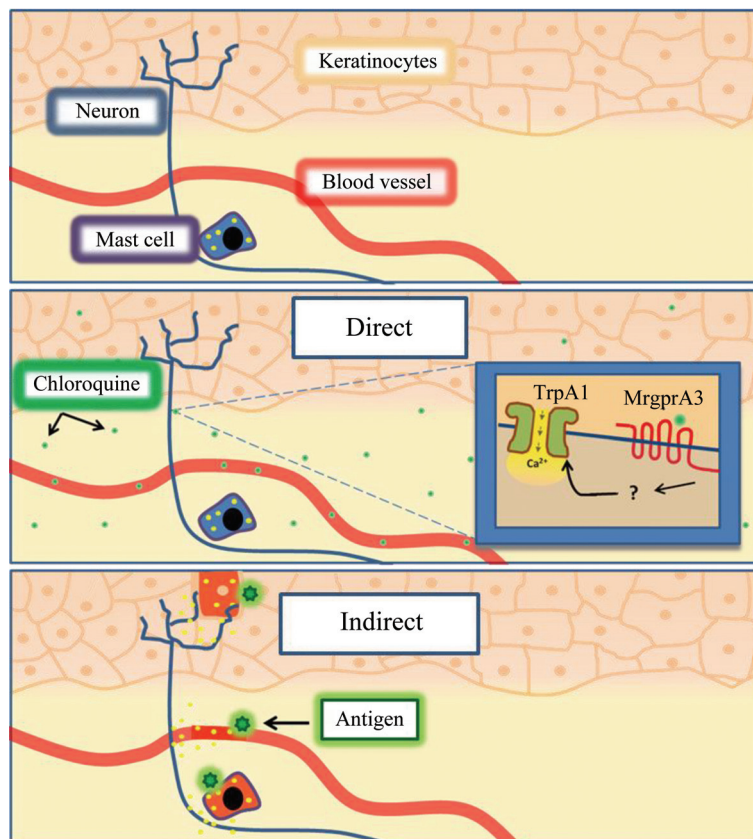


Fig. 1. Mechanisms of pruritus in the periphery. Top: Cartoon of the epidermis and dermis, and cell types implicated in pruritus. Sensing of pruritogens in the periphery depends on the activation of peripheral neurons that communicate the signal to the central nervous system. In some instances pruritogens induce neuronal excitation directly, via binding to receptors on neurons. In many cases, however, pruritogens likely indirectly activate neurons by inducing other cell types to signal onto neurons. Middle: Example of a direct interaction between a pruritogen and peripheral neurons. The antimalarial drug chloroquine induces scratching by binding to a receptor in mice called MrgprA3 that is expressed only in a subset of peripheral neurons, including a fraction of those in the dorsal root, nodose, and trigeminal ganglia. Receptor activation leads to opening of TrpA1, a calcium-permeable channel on the plasma membrane. Calcium influx leads to excitation of the neuron. Bottom: Some cell types proposed to mediate the indirect activation of neurons. Keratinocytes, endothelial cells in blood vessels, and mast cells all are capable of secreting substances that excite peripheral neurons. For example, histamine, presumably originating from mast cells, is a critical mediator of itch in response to several allergens. In most cases, how pruritogens activate these cell types is unknown; however, several of the transmitters secreted by these cells are known and are covered in this review.

that can bypass the sensory neurons and act directly on the itch circuitry in the central nervous system are not addressed. The most prominent of these is the opioid receptor agonist morphine, which can cause histamine release but appears to induce pruritus mostly through acting on a heterodimer of a splice variant of the μ -opioid receptor and the gastrin-releasing peptide receptor (GRPR)^[7].

2 Receptors in sensory neurons mediating external pruritogens

2.1 The Mrgpr family of GPCRs The Mrgprs comprise a family of receptors that are similar to each other, while their closest relative outside the family is the oncogene Mas^[8]. Unlike most families of GPCRs, they do not have a common ligand. In fact, for most members, it is not clear what the primary ligand is, or whether they have more than one. Humans have 10 members, while rodents have undergone a significant gene expansion and have many members with no clear human ortholog^[8,9]. Among the human Mrgprs, most of the focus in the sensory field has been on MrgprX1, as it and its mouse ortholog MrgprC11 are expressed in a small subset of neurons in the dorsal root ganglia (DRGs), which convey sensory information from most parts of the body apart from the head^[8–11]. MrgprX1 can be activated by several ligands of the endogenous opioid family of peptides with relatively low affinity compared to their canonical receptors, and it is not clear how relevant these associations are^[8,10]. However, among these ligands, BAM8-22 is very potent and appears to be specific for MrgprX1, showing no activation of opioid receptors, making it a good tool to examine MrgprX1 function^[10]. Strikingly, subcutaneous injection of BAM8-22 in mouse hairy skin induces scratching behavior^[6]. Human volunteers also report that intradermal BAM8-22 evokes an itch sensation^[12]. It appears, then, that BAM8-22 is an endogenous promoter of itch. Its origin in the body is not entirely known but it is derived from proenkephalin A, which is abundant in the periphery^[10].

MrgprX1 also responds to the antimalarial drug chloroquine, which often induces itch in African populations^[6]. Interestingly, the mouse receptor MrgprA3, not MrgprC11,

responds to chloroquine and is required for chloroquine-induced itch in mice, as well as the activation of DRG neurons by chloroquine^[6]. Thus, it appears that MrgprX1 has two orthologs in mice. Notably, MrgprC11 and MrgprA3 largely overlap in DRG neurons, and these neurons also are histamine-sensitive, giving rise to the hypothesis that this is a selective subset of neurons that is specialized to detect pruritic stimuli and transmit the itch signal^[6,9].

The intracellular pathways utilized by the Mrgpr family are not completely known, and may be diverse given the number of family members. MrgprA3, C11 and human MrgprX1 induce rises in intracellular calcium^[6] and MrgprA3 and C11 require TrpA1 for neuronal activation^[13], although how the receptors and the Trp channel are linked has not been determined. One interesting report showed that activation of heterologously expressed MrgprX1 in cultured hippocampal neurons actually decreases a high voltage-activated calcium current, which could suppress neurotransmission^[14]. The relevance to sensory neurons is unclear but the finding is intriguing, as it hints at complicated roles for the Mrgpr family in sensation, and perhaps differences between short- and long-term stimulation or differences in signaling between cell types. Indeed, MrgprX1 agonists appear to be able to inhibit some types of pain^[15].

A recent study hints at a more expanded role for the Mrgprs in itch than what is currently known^[16]. This study found that mouse MrgprC11 responds to an agonist for protease-activated receptor 2 (PAR2), a receptor implicated in itch (covered in the next section)^[16]. This highly unexpected finding raises the possibility that other pruritogens may signal itch not through their presumed receptors but through Mrgprs. This may carry over to the human Mrgprs, as human X2, which has also been detected in the DRG, responds to the human PAR2 agonist SLIGKV^[16].

2.2 PAR2/PAR4 The PAR family of GPCRs is the second major family associated with direct activation of neurons by exogenous pruritogens. PARs are very different from most GPCRs in their mechanism of activation: they are activated by cysteine proteases, which cleave part of the extracellular N-terminus from these receptors, allowing the N-terminus to become a ligand for its own receptor and

activate it^[17,18]. Of these, PAR2 is the best studied in nociception. It couples to phospholipase C β (PLC β) and activates protein kinase C (PKC). It is apparently capable of raising cAMP levels as well^[19]. Downstream effectors are not fully characterized, but it has been shown that PAR2 and other members of the family can sensitize TrpV1 when activated, which would make cells more susceptible to activation by other stimuli^[19-22]. All members of the family have been detected in primary sensory neurons, and DRG neurons have been shown to respond to PAR family agonists, supporting the hypothesis that at least part of the effects of their agonists are due to direct targeting of the peripheral nervous system^[22-24]. Interestingly, the PAR family's earliest link to nociception was to inflammation and pain^[17], while their role in itch was inferred from studies showing that endogenous proteases like trypsin and mast cell tryptase that activate the PAR family cause itch or scratching behavior when injected into the skin^[25-27]. This was bolstered by studies showing that a PAR2 ligand, SLIGRL, which is derived from the N-terminus of PAR2, is pruritogenic^[26,28]. Later, pruritic plant cysteine proteases like papain and mucunain, the principal active substance in cowhage (*Mucuna pruriens*, a tropical vine), were shown to activate PAR2 and PAR4, suggesting a possible target for these external pruritogens^[29,30].

However, the family's role in itch has recently been called into question despite these strong correlations. In a 2011 study, trypsin and the PAR2 ligand SLIGRL were shown to induce scratching to an equal or greater extent in PAR2 knockouts compared with in wild-type animals^[16]. Surprisingly, the scratch-inducing activity of SLIGRL, as mentioned in the previous section, was actually dependent on MrgprC11 and was found to activate the receptor directly^[16]. The ability of SLIGRL to evoke pain-like behavior was still dependent on PAR2, and abolished in the PAR2 knockout, showing that the two defined roles for SLIGRL rely on two different receptors^[16]. In light of these results, further testing of plant cysteine proteases in PAR2 and PAR4 knockout mice would be worthwhile to determine whether these, too, utilize other receptors to evoke itch.

3 Indirect activation of neurons by pruritogens

It is highly likely that not all environmental pruritogens directly activate neurons. In fact, chloroquine, opioids, and perhaps the plant cysteine proteases are the only well-defined pruritogens not native to the body with known receptors on sensory neurons. Of these, only chloroquine may act primarily through sensory neuron activation, as opioids likely act mostly through central neurons^[7], and even if PARs are required for the effects of plant proteases, these receptors are found on several other cell types and part of their effect may be mediated indirectly. Most pruritogens with known or suspected receptors are endogenous to the body, meaning that they are intermediate signaling molecules in the sensation of itch. Other cell types in the periphery most likely are activated by external pruritogens via unknown mechanisms, and secrete these intermediate molecules either onto neurons or onto other cell types which then signal to neurons. By far the best-studied of these is histamine, a transmitter secreted by mast cells that has multiple peripheral effects and is responsible for pruritus evoked by several stimuli, including several allergens^[31,32]. In the mouse DRG, most chloroquine-sensitive neurons are also sensitive to histamine, suggesting that the two pruritogens target neurons directly^[6]. Other endogenous pruritogens include 5-hydroxytryptamine (5-HT, also known as serotonin), the small peptide endothelin (ET), and interleukins IL-13 and IL-31. 5-HT and ET may also induce the sensation of pain (see below for more details), but they are covered here because their ability to induce itch is relatively well-studied. Several other compounds, like prostaglandin E2 and substance P, which also have been shown to induce scratching behavior, are not covered because results have been inconsistent and/or there are reports that pain is the dominant sensation evoked after application.

3.1 Histamine Histamine is the best-studied transmitter in the field of itch. Intradermal or subcutaneous injection of histamine in mice and humans induces scratching behavior and itch, respectively, though rats appear to be much less sensitive^[33]. Histamine receptors are the targets of many drugs that alleviate the symptoms of allergic disorders

involving itch, implicating it as a common effector in these disorders^[32]. The sources of histamine in the periphery include mast cells and basophils, with mast cells thought to be the most important^[32]. Mast cells are components of the innate immune system and can be activated by many substances, as well as by IgE antibodies^[34]. Histamine is capable of activating sensory neurons directly (about 15% of DRG neurons), and while the number of neurons responding to histamine is larger, most or all DRG neurons responsive to chloroquine (i.e. those expressing MrgprA3) also respond to histamine^[6]. This supports the general notion of itch-specific neurons, and sensory neurons as a target for histamine in particular.

Four histamine receptors have been identified in mammals; of these, the H1 receptor (H1R) is the best-studied and the target of most therapeutic drugs, while recent evidence supports a role for the H4R, as well, in evoking itch. Both H1R and H4R have been detected on DRG neurons^[35-37], and agonists for both can induce scratching in mice^[35,38-40]. H1R couples to G_q/G_{11} and ligand binding induces PLC β activation, leading to rises in intracellular calcium and PKC activation^[32]. Mice lacking PLC β show deficiencies in scratching in response to histamine^[35]. H4R is coupled to $G_{i/o}$, which lowers cAMP levels but may be excitatory through other pathways^[32]. Evidence for a downstream role for TrpV1 in histamine-mediated pruritus has been shown, in line with experiments showing that several pruritogens require TrpV1 or TrpV1-expressing neurons to induce itch^[41,42]. A combination of H1R and H4R antagonists is more effective than either alone^[43], indicating that they have non-redundant roles in itch. However, more work must be done to determine which of the receptors has a greater role in activating sensory neurons, and which one has a more dominant role in evoking itch. This is of particular interest since H1R antagonists are ineffective in treating itch in many diseases, and a general role for histamine in pruritus is currently in doubt^[44].

3.2 5-HT 5-HT appears to have different peripheral effects in different species. In rats, intradermal application induces scratching with almost no pain behavior^[33], while in mice^[45] and humans^[4] it produces a more mixed itch/

pain response. Some of the differences may be due to the different sites of application, though species differences are not unheard of (also see section on histamine). 5-HT, like histamine, is found in large amounts in mast cells^[46], and this likely is a primary source of 5-HT in the periphery. Pharmacological experiments indicate that 5-HT1 and 5-HT2 receptors are important for itch perception^[42,47,48]. 5-HT1 receptors are coupled to G_i pathways, which lower cAMP levels and, while in some cases can excite cells, are generally thought to be inhibitory. 5-HT2 receptors, on the other hand, signal through $G_{q/11}$, which activates PLC and leads to mitogen-activated protein kinase (MAPK) and PKC activation^[49]. 5-HT fails to induce scratching in PLC β 3 knockout mice^[42], supporting a more dominant or essential role for the 5-HT2 receptor family.

5-HT has been shown to activate sensory neurons directly^[33,45]. The population of sensory neurons responsive to 5-HT only partially overlaps with histamine-sensitive and chloroquine-sensitive neurons, indicating either that several populations of neurons contribute to itch perception, or that some 5-HT-sensitive neurons are unrelated to itch^[33,45]. Knockouts of many 5-HT receptors are available but since the 5-HT1 and 5-HT2 receptor families comprise at least 3 members each, elucidation of exactly which receptors are most important would be an arduous task using this approach.

3.3 ET ET-1 is a small (21 amino acids) peptide found, among other places, in human and mouse skin and endothelial cells^[50]. Intradermal application causes scratching behavior and also induces pain, more so than the compounds previously mentioned^[51-56]. It signals through two subtypes of GPCRs, ET_A and ET_B, though it appears that ET_A is more important in the induction of itch and pain^[56,57]. ET_A is thought to act via G_s to raise cAMP levels and activate PLC, while ET_B has been shown to reduce cAMP levels via G_i signaling, though, as with many other GPCRs, the receptors can be promiscuous and the pathways may differ in different cell types and conditions^[58].

ET receptors have been detected in DRG neurons^[59], and ET-1 has direct effects on a subset of these neurons, though the effect is subtle, mostly sensitizing the neurons

to other stimuli and inducing little calcium influx on its own^[60-63]. In stark contrast, ET-1 strongly activates mast cells, glia and keratinocytes, the primary cell type in the epithelium^[63,64]. ET-1 is also present in keratinocytes and can be secreted in what may be an autocrine or paracrine signaling pathway^[65]. It therefore seems more likely that the effects of ET-1 are indirect, perhaps being secreted after an insult by non-neuronal cells that then act on other cells before neurons are activated.

Why ET-1 can cause scratching and itch in addition to pain is unclear. Perhaps the nervous system processes the same stimulus differently, depending on which part of the body is presented with the stimulus. Alternatively, different dosages could determine which – pain or itch – the organism predominantly experiences.

3.4 IL-13 and IL-31 Pruritus is a common symptom of disease, and perhaps no disease is more closely linked to itch than atopic dermatitis. The levels of cytokines IL-13 and IL-31 are higher in patients with this disorder, attracting attention to a possible role for these transmitters in its symptoms^[66]. These cytokines are secreted from T cells, and receptors in the periphery have been detected in keratinocytes^[66]. IL-13 and IL-31 receptors are very different from the GPCRs mentioned so far in this review. These receptors, along with most interleukin receptors, are each dimers of single transmembrane proteins. The IL-13 receptor is a dimer of IL-13R α 1 and IL-4R α , which, as the name suggests, also is a component of the IL-4 receptor; “sharing” of receptor subunits among several cytokine receptors is fairly common^[67]. Another receptor exists, IL-13R α 2, which does not have an intracellular signaling domain and may act as a decoy to regulate local signaling^[66]. The IL-31 receptor is a dimer of IL-31 receptor alpha (IL-31RA) and the oncostatin M receptor (OSMR)^[68]. Both receptor complexes directly activate members of the JAK family of tyrosine kinases, which leads to the activation of the transcription factor STAT, as well as induction of the PI3K and MAPK signaling cascades^[67,68].

Mice overexpressing IL-13 in keratinocytes^[69] or IL-31 in lymphocytes or ubiquitously^[70] develop a phenotype similar to severe atopic dermatitis, including strong

scratching behavior. It is unclear, however, whether these cytokines acutely promote itch or instead lead to the development of other problems, like defective wound repair or inflammation, that cause itch. Direct stimulation of sensory neurons has not been reported, though IL-31 receptors have been detected on a fraction of DRG neurons^[70,71]. Further studies should elaborate on the short-term *versus* long-term effects of these cytokines in pruritus, as well as their site(s) of action.

4 Do itch-specific neurons exist?

It has been hypothesized that the neurons activated directly or indirectly by pruritogens are specialized to transmit the itch signal, implying that they are dedicated to itch and no other sensations. Support for this most recently was provided by a study showing that receptors for histamine, BAM8-22, and chloroquine all were localized on the same neurons^[6], and *in vivo* studies showing that DRG neurons responding to cowhage and histamine largely, though not completely, overlapped^[72-74]. More than one population may exist, given the lack of complete overlap between these receptors and responding neurons, as well as the finding that cowhage and histamine activate at least partly different populations of monkey spinothalamic neurons, indicating that the signals diverge at some point^[75]. These studies support the hypothesis that the mechanisms used to detect pruritogens directly are concentrated on a small group of neurons.

These findings are not easily compatible, however, with reports that neurons sensitive to pruritogens also respond to non-pruritic stimuli like heat and mechanical stimulation, as well as capsaicin^[4-6,42,72,74]. A detailed discussion of central sensory processing is outside the scope of this review, but brief points concerning this topic are addressed. First, it is possible that these neurons do contribute to the detection of other sensations, perhaps on the grade of the sensation, and only when activated selectively would they transmit itch. Second, they might selectively signal itch but be drowned out when too many other neurons are also activated. Third, activation by other stimuli might serve primarily to induce changes in these

neurons that influence their ability to detect pruritogens, for instance, to sensitize or desensitize them.

The confusion over the exact type of information that these neurons transmit would be greatly reduced if they could be selectively activated. Intriguingly, a pair of recent studies demonstrated that capsaicin, normally a painful stimulus, induces itch in human subjects when applied in the very shallow epidermis^[76,77]. It would be of great interest to see exactly which neurons are activated by this type of application, since it has been shown that the shallowest layers of the epidermis are innervated by a subset of putative nociceptive neurons^[78]. This may be a way to activate primarily the putative itch neurons and leave other capsaicin-sensitive neurons relatively unstimulated. Other options for selective activation might include a technique like optogenetics. More detailed studies are needed to understand how exactly itch is transmitted by peripheral neurons.

5 Transmitters

Considering the multiple receptors and cell types implicated in the peripheral signaling of itch, searching for a single target to treat all causes may appear to be impractical. However, one could be identified if itch is transmitted through a single circuit, or multiple circuits that use the same transmitter to signal to the spinal cord. Some work has addressed this issue, but the results do not yet lead to a coherent model. The most striking evidence implicates gastrin-releasing peptide (GRP), a member of a family of 3 mammalian peptides related to bombesin, a peptide found in several species of frogs, as a common transmitter for itch neurons^[1,2]. First, knockout of its primary receptor, GRPR, impairs scratching induced by several chemicals^[1], and ablation of neurons expressing GRPR blocks scratching induced by all examined substances^[2]. GRPR-expressing neurons are found in the superficial dorsal horn, consistent with a role in receiving sensory input^[1]. However, studies have not been performed in knockouts for GRP, its ligand, and given that its close relative neuromedin B (NMB) also can activate GRPR, the identity of the endogenous spinal GRPR ligand is still very much in question. Both peptides have been detected in DRG neurons^[1,79], though no evidence

exists to show that either is released in response to pruritic stimuli. Another issue is that GRPR deletion does not eliminate the effects of all of the pruritogens examined. It is unclear whether this is due to compensation by NMBRs, the presence of additional transmitters, or combinations of these and unknown factors. A third issue is that the neuronal ablation was achieved by a toxin conjugated to bombesin, which binds to not only GRPR but its related family members NMBR and BRS-3^[80]. Finally, it is not clear whether GRPR-positive neurons actually receive input from primary neurons, or if they are instead part of the spinal processing circuitry downstream of sensory input.

A role for glutamate has also been examined. Vglut2, the primary transporter for glutamate from the cytoplasm into secretory vesicles in the DRG, was deleted in various subsets of DRG neurons^[81,82]. In every case, the animals exhibited increased spontaneous scratching^[81,82]. In one study, this could be blocked by antihistamines, indicating that basal histamine release can trigger the activation of itch circuits in the absence of other nociceptive stimuli^[81]. Many models could explain these studies, especially since the central circuits for itch are almost completely unknown; however, if glutamate secretion was abolished in itch neurons – and it was claimed in one study that MrgprA3-positive neurons were indeed targeted^[82] – the studies indicate that another transmitter is used by primary sensory neurons. However, these studies raise more questions than they answer. One issue is that Vglut1 or Vglut3 could compensate for the absence of Vglut2. Another, and more important, is that it must be ascertained with absolute certainty that all neurons that respond to pruritic stimuli are targeted and do not secrete glutamate in the knockouts. It is also possible that Vglut2 deletion removes a constitutive inhibitory circuit driven by pain or other neurons, and that in wild type mice, Vglut2 is indeed required to overcome this inhibition.

A recent paper challenged both sets of studies, claiming instead that glutamate is the neurotransmitter used by primary itch neurons and that GRP is not required for signaling to the dorsal horn^[83]. In this study, dorsal horn neurons that received direct input from C-fibers were tested, and over half were sensitive to GRP^[83]. However, the C-fiber

input could be blocked by glutamate receptor antagonists, suggesting that, while the postsynaptic neurons can be activated by GRP, the sensory neurons do not utilize this peptide^[83]. The authors' final evidence came from a GFP-fos mouse in which neurons express GFP after strong activation. The study reported that after histamine application to skin, several neurons in the dorsal horn expressed GFP at high levels, and the authors assumed that these were the neurons receiving primary input^[83]. Only 1 of 4 of these neurons tested was responsive to GRP, while input to all of them could be blocked by glutamate receptor antagonists^[83]. The *n* values are far too small to make any definite conclusion, and whether the GFP-positive neurons represent all secondary neurons receiving input from histamine-sensitive primary neurons is unclear, but it raises enough doubt about other studies to prevent any clear conclusion to be made about the nature of the transmitter(s) used. Selective deletion of putative transmitters in MrgprA3-positive neurons is required to answer this question, at least in this putative itch population.

6 Conclusion

In the past few years, significant advances have been made in understanding the peripheral mechanisms of itch. Receptors that detect pruritogenic compounds were found, which are expressed solely on sensory neurons, raising the exciting possibility that they can be used to label itch-specific neurons, allowing circuits to be defined and additional receptors to be found. However, despite the discovery of the receptor for chloroquine, an external pruritogen, most of what is known concerns substances endogenous to the body. In one sense this is the primary goal of the field, as endogenous signaling likely underlies the uncontrollable itching in many disease states. On the other hand, these are intermediate compounds and there is scant knowledge of what events, resulting either from disease or exposure to external stimuli, lead to the release of these compounds. For example, it is unknown just how common it is for primary neurons to sense pruritogens directly. In instances where other cells detect the pruritogens, little information is available on how they are activated and whether certain

transmitters are more common than others in signaling to sensory neurons.

Another issue concerns the polymodal nature of many primary neurons sensitive to pruritogens. These neurons may have electrophysiological profiles unlike most other neurons, but they can respond to heat, cold, and pressure and are not solely activated by pruritogens^[3-5, 42]. The central processing of itch is still being worked out, and the various models for processing are outside the scope of this review. However, it is important to note that it is not clear how stimuli such as heat and cold affect neurons sensitive to itch; for instance, detection of these other stimuli might not serve to transmit those stimuli, but instead sensitize or desensitize these neurons to enhance or suppress the itch sensation. In other words, processing might begin with the primary sensory neurons. Another possibility is that itch is integrated with other sensations to clarify the nature or severity of those sensations.

A third issue concerning peripheral itch mechanisms is species difference. As described in this review, responses to chemicals including histamine and 5-HT are not uniform between humans, rats, and mice. It is possible, even likely, that not only the placement and sensitivity of various receptors, but the circuitry of itch differs according to the type of animal examined. This should be taken into account when attempting to generalize findings from any one species. A combined, multi-organism (including human subjects) approach is needed before a hypothesis about itch can be accepted.

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