

REVIEW

Basic mechanisms of itch

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Summary

Chronic itch represents a burdensome clinical problem that can originate from a variety of aetiologies. Pruriceptive itch originates following the activation of peripheral sensory nerve endings following damage or exposure to inflammatory mediators and ascends to the brain through the spinal thalamic tract. Much insight has been gained into the understanding of the mechanisms underlying pruriceptive itch through studies using humans and experimental animals. More than one sensory nerve subtype is thought to subserve pruriceptive itch which includes both unmyelinated C-fibres and thinly myelinated A δ nerve fibres. There are a myriad of mediators capable of stimulating these afferent nerves leading to itch, including biogenic amines, proteases, cytokines, and peptides. Some of these mediators can also evoke sensations of pain and the sensory processing underlying both sensations overlaps in complex ways. Studies have demonstrated that both peripheral and central sensitization to pruritogenic stimuli occur during chronic itch.

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Introduction

Itch, or pruritus, can be defined as an unpleasant sensation that evokes the desire to scratch. Although itch may be experienced acutely, chronic itch originates from many different aetiologies and is a burdensome condition with serious unmet clinical need. Broadly, subtypes of chronic itch have been delineated and termed pruriceptive, neuropathic, neurogenic, and psychogenic itch [1–3]. Each of these terms provides some information on the causative nature of itch. Pruriceptive itch is a sensation of itch that originates following activation of primary afferent nerve terminals. This type of itch is associated with insect bites or intradermal injection of pruritic substances, and is a very common symptom of inflammatory disorders of the skin [1]. Neuropathic itch is a chronic itch condition that results from nerve injury. Examples of neuropathic itch include itch following varicella zoster infection or nerve trauma [4]. Neurogenic itch refers to itch resulting from central nervous system activation without necessary activation of sensory nerve fibres and is thought to occur in a variety of visceral disease states such as renal disease and kidney failure [5]. Although neurogenic itch is associated with visceral diseases, the mechanisms underlying the pruritus are often complex and can also involve pruriceptive itch. For example, uraemic patients often experience changes in skin physiology such as xerosis (dry skin),

which would undoubtedly involve activation of sensory nerve fibres [6]. Psychogenic itch results from underlying mental illness and it often occurs with somatization and delirium [7]. The underlying factors mediating these subtypes of chronic itch are complex and can also involve components among the subtypes. There are virtually no drugs that can claim impressive efficacy at combating chronic or acute itch irrespective of their various aetiologies. As with our ancestors, the most effective treatment seems to be found in the nail of one's fingers. As biomedical scientists learn more about the mechanisms underlying itch, the paucity of effective therapies for itch will likely improve. The overall focus of this article is to review the neurophysiology of pruriceptive itch by emphasizing current understandings from studies of both humans and experimental animals. Where applicable, studies pertaining to pruritic skin diseases such as atopic dermatitis and prurigo nodularis will be emphasized.

Neural mechanisms of itch

Primary afferent nerves

Two broad theories have been postulated to explain pruriceptive sensory processing in the nervous system and they are named the *specificity* and *pattern* theories

[8–11]. The specificity theory of itch states that there are specific subtypes of sensory nerve fibres and spinal cord neurons that are responsible for transmitting itch-specific sensory information to the central nervous system. The pattern theory states that the sensation of itch is encoded across the activation of many sensory receptors and spinal cord neurons and the collective pattern of neuronal activity determines the ultimate sensation experienced. Thus, the pattern of activation determines whether itch is experienced rather than pain or non-noxious touch. Evidence has been slowly accumulating and currently favours the specificity theory over the pattern theory, though there remains much to be learned.

Pruriceptive itch originates when specific sensory nerve terminals, generally located in the skin, are activated. Pruriceptive itch can also originate from certain mucosal surfaces; however, a majority of research on itch has focused on sensory nerve fibres from skin [12]. The excitation of sensory nerve fibres in the skin leading to pruriceptive itch occurs upon exposure of certain sensory nerve terminals to a pruritic substance and frequently follows skin damage or inflammation. Sensory nerve fibres in the skin originate from the distal processes of primary afferent dorsal root ganglion (DRG) neurons. Sensory nerve fibres in the skin are broadly classified according to their condition of velocity and the sensory modalities that excite them. Fast conducting myelinated nerve fibres (A β) respond to non-noxious mechanical stimulation of the skin, while slow conducting myelinated (A δ) and unmyelinated (C) nerve fibres respond to noxious stimulation and temperature changes of the skin [13]. Although there are many subtypes of primary afferent nerves innervating the skin, it is intriguing to consider that a single nerve subtype or 'itch-receptor' is responsible for transducing pruriceptive stimuli. As is often the case with simple scenarios, this idea has not withstood critical scientific inquiry.

Studies in humans and non-human primates have demonstrated that slowly conducting unmyelinated nerve fibres (C-fibres) detect and signal pruriceptive information to the central nervous system. C-fibres are classified according to the stimuli that excite them. The most commonly used pruritogen, or itch producing stimulus, used in experimental studies of itch is histamine. Micro-neurography experiments in humans have been conducted to delineate the type of sensory nerve that responds immediately to a local application of histamine. Schmelz et al. [14, 15] noted that histamine activated mainly C-fibres. Moreover, the time-course of histamine-evoked responses (evoked action potentials) nicely matched the duration of the itch sensation. These histamine-sensitive C-fibres had conduction velocities slower than most C-fibres and were not stimulated by heat or mechanical forces and were classified as a subset of heat- and mechano-insensitive C-fibres (Fig. 1) [14, 15].

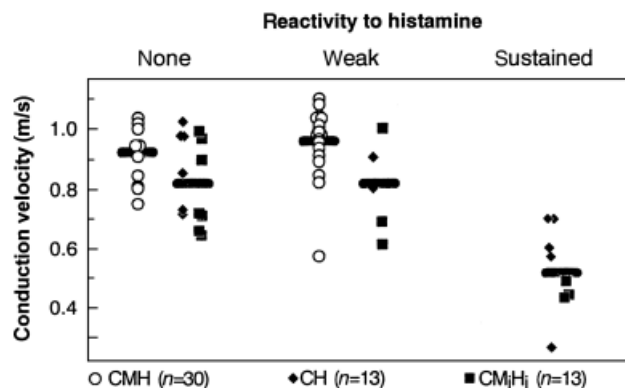


Fig. 1. Characterization of a subset of human cutaneous C-fibres that respond to histamine. Note that the fibres, which respond strongest to histamine are a subset of very slow conducting C-fibres (~0.6 m/s) that are relatively insensitive to heat or mechanical stimulation. Figure used with permission from [14]. CH, C-fibres that are heat responsive; CMH, C-fibres that are mechanically and heat responsive; CMiHi, C-fibres that are relatively unresponsive to mechanical forces or heat.

Further evidence demonstrating that C-fibres are involved in itch transduction has been obtained by using capsaicin, the pungent ingredient in chili peppers that evokes burning pain. A subpopulation of C-fibres express the capsaicin-receptor transient receptor potential, vanilloid 1 (TRPV1) and capsaicin not only activates these nerves, but upon repeated application can desensitize these fibres. Capsaicin-evoked desensitization not only affects the burning pain evoked by capsaicin, it also prevents the itch sensation evoked by histamine administration [16]. Clinically, capsaicin-induced desensitization has also been found to improve pathological itch associated with pruritic psoriasis [17] and itch associated with haemodialysis [18].

Another pruritogen used to study sensory nerve fibres in the skin are the spicules from the peapod of *Mucuna pruriens* or cowhage. In contrast to histamine-induced itch, the itch evoked following application of cowhage to human skin is not inhibited by a histamine H1 receptor antagonist [19]. As with histamine, cowhage was found to effectively stimulate cutaneous C-fibres. Unlike histamine, however, the subtype of C-fibre from human skin that was excited by cowhage was a mechanically-sensitive subtype of C-fibre [20]. Similarly, electrophysiological recordings made from non-human primate cutaneous nerve fibres also demonstrated preferential excitation of mechanically-sensitive C-fibres by cowhage [21]. In fact, mechano-insensitive C-fibres (that are often histamine sensitive) were not excited by cowhage [21]. Both histamine and cowhage evoke sensations of itch, but they stimulate different subtypes of C-fibres indicating more than one C-fibre subtype mediates the sensation of itch.

Histamine injection into the skin of mice evokes scratching behaviour, which is quantified and used as an

experimental measure of itch. Investigators have used histamine along with another pruritogen, chloroquine, in an attempt to define the afferent nerve phenotype responsible for itch transduction in mice. Chloroquine is an antimalarial drug known to evoke pruritus as one potential side-effect in humans [22]. Administration of chloroquine to mice also evokes scratching behaviour [23]. The mechanism by which chloroquine evokes itch in mice was found to be secondary to an interaction with a G-protein coupled receptor (GPCR) termed MrgA3 [24]. An evaluation of the sensory nerves that expressed MrgA3 revealed that they were also likely C-fibres. MrgA3-expressing DRG neurons also expressed receptors for histamine (H1) and capsaicin (TRPV1), but they only accounted for a very small subset of TRPV1-expressing DRG neurons. Studies have also been carried out implicating gastrin-releasing peptide (GRP) as an important neurotransmitter in the transduction of itch found in DRG neurons [25]. Consistent with this is the observation, a small subset of chloroquine sensitive (MrgA3-expressing) C-fibre neurons also express GRP [24]. Thus, a small subset of TRPV1-, MrgA3-expressing DRG neurons appear to mediate the transduction of itch in mice. Whether cowhage stimulates a subset of C-fibres in the mouse distinct from the histamine or chloroquine sensitive nerves is not known. Currently, it is also not known if other DRG neuron subtypes mediate itch in mice.

The role of myelinated (A-fibre) nerve fibres in mediating pruriceptive itch has been less extensively explored. Electrophysiological recordings made from non-human primate cutaneous nerve fibres found that some slowly conducting mechanically-insensitive A fibres (A δ fibres) were excited by histamine, but not cowhage [26]. By contrast, mechanically-sensitive A δ fibres were the predominant fibre type excited by cowhage [26]. This activation profile is similar to the aforementioned findings with C-fibres.

The complexity regarding the specific subtypes of sensory nerves transducing pruriceptive itch exists because more than one nerve subtype is activated by substances that cause itch. Based on their activation profile, both histamine and cowhage-sensitive fibres can also be classified as nociceptors (i.e. they respond to noxious or potentially damaging stimuli). Specifically, cutaneous C-fibres that respond to histamine also respond to capsaicin [20, 27]. Cowhage-responsive cutaneous C-fibres also respond to noxious mechanical and thermal stimuli [20]. Similarly, histamine- and chloroquine-sensitive nerve fibres in the mouse express receptors for capsaicin (TRPV1). Sensory spinal cord neurons that respond to histamine or cowhage also respond to noxious stimuli including noxious thermal and mechanical stimuli as well as capsaicin administration [28, 29].

Because sensory nerve fibres and spinal cord neurons that respond to itch-producing stimuli also respond to

noxious or potentially damaging stimuli, how the nervous system processing itch vs. noxious or pain-producing stimuli remains puzzling. Theories on how the nervous system processes itch and pain have been discussed by several excellent reviews [8, 30, 31]. A recent study in mice demonstrated that genetic ablation of a glutamate transporter (VGLUT2) in nociceptors resulted in a decreased response to thermal pain, but a substantial increase in scratching behaviour to certain pruritic substances like histamine [32]. Whether the interplay between itch and pain occurs at the level of the spinal cord, (i.e. glutamatergic transmission in the spinal cord regulate inhibitory input to secondary 'itch neurons'), or at higher levels of processing (or both) has not been clearly worked out.

Spinal cord

Following activation of peripheral sensory nerve endings of DRG neurons by itch producing stimuli, the central nerve terminals of these neurons form synapses on second-order neurons located in the dorsal horn of the spinal cord. Lesions in the anteriolateral quadrant of the spinal cord of humans ablate itch sensation contralaterally below the level of lesion [33–35]. The loss of itch sensation indicates that, like pain and temperature sensation, pruriception ascends to the brain via the anteriolateral quadrant of the spinal cord in the spinal thalamic tract (STT). A diagram of the STT can be seen in Fig. 2. Electrophysiological studies of non-human primates have also demonstrated that itch information ascends via the STT [28, 29, 36]. Electrophysiological studies of STT neurons from non-human primates reveal that they are activated in response to both histamine and cowhage applied to the skin. In other words, although histamine and cowhage stimulate nerve endings of distinct subtypes of primary afferent nerves, both subtypes form synapses on STT neurons. Interesting, histamine- and cowhage-sensitive afferent nerves do not seem to converge on the same STT neurons, because a given STT neuron responds to either histamine or cowhage, but not both [29].

Brain

Pruriceptive STT neurons were found to project to the posterior and ventral posterior regions of the thalamus in non-human primates and cats [29, 36]. Prior studies in humans have suggested that itch producing stimuli is processed in somatosensory areas of the brain. Imaging studies in humans have demonstrated activation of primary sensory cortex, primary motor cortex, supplementary motor area, and premotor cortex following administration of histamine into the skin [37–39].

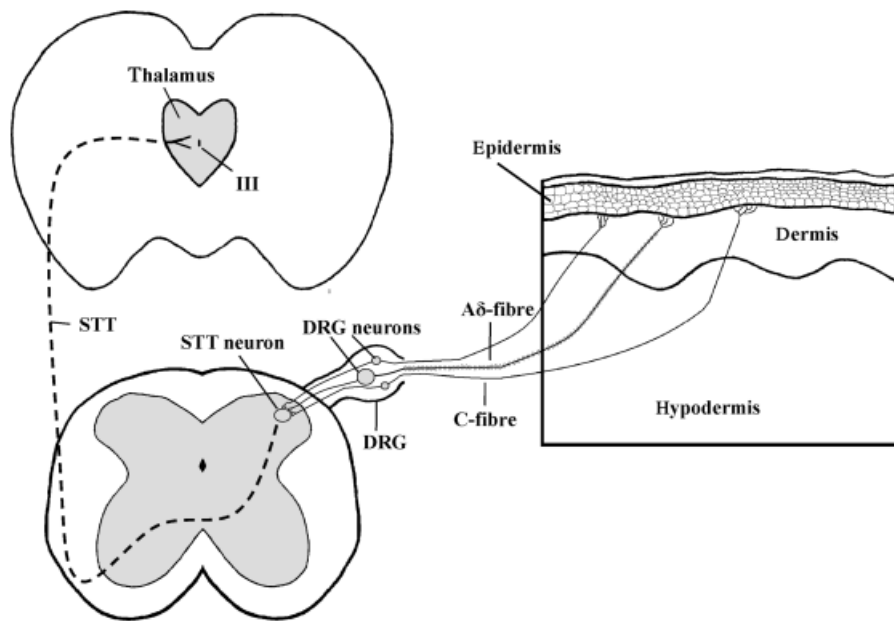


Fig. 2. Diagram of input to the spinal thalamic tract (STT) from cutaneous sensory nerve fibres. Cross-sections of the brain, spinal cord, and skin indicate the origin of sensory input from the skin to the spinal cord. The STT is indicated by the hatched line projecting from the dorsal horn of the spinal cord to the thalamus in the brain. Studies in both humans and experimental animals have demonstrated that both C- and A δ -fibres transduce itch sensation. DRG, dorsal root ganglion; III, third ventricle.

Biological mediators of itch

Many substances and inflammatory mediators have been demonstrated to evoke itch experimentally in humans and animals such as mice-rats, and non-human primates. Itch sensation in humans is quantified during psychophysical experiments where participants rate the intensity of itch sensations. In animals, scratching behaviour is quantified and used as an experimental measure of itch. Based on data obtained from these methods in a large number of studies, it is clear that the basic neurophysiology of itch is remarkably conserved among species. Rarely does a substance that evokes itch in humans, not evoke scratching behaviour in experimental animals. The use of experimental animals in the study of itch has allowed for in depth analysis of the molecular mechanisms underlying the sensation of itch. The mechanisms underlying how pruritogenic mediators affect sensory neuron excitability will be highlighted below in reference to both humans and experimental animals.

Histamine

Histamine is the most commonly studied pruritogen. Intradermal injection of histamine causes intense itching sensations, but not painful sensations. Histamine in the skin originates largely from dermal mast cells and is intimately linked to symptoms of allergy. It follows that the itch associated with acute allergen exposure is a

consequence largely due to histamine release from mast cells, presumably interacting with pruriceptive C-fibres.

Histamine binds to GPCRs and to date four histamine receptors have been isolated and cloned which are named as H1–4 [40]. Prior studies have localized H1, H3, and H4 receptors on DRG neurons [41–43]. The primary histamine receptor subtype responsible for evoking itch in response to histamine has been shown to be H1 receptors, while H2 receptors contribute a minor role in humans [44]. H1 receptor antagonists also inhibit itch associated with conjunctiva and nasal allergen exposure [45]. Studies have implicated H1 and H4 receptors in mediating experimental pruritus in mice [46–49]. H3 receptor activation in mice is associated with decreases in pruritogen-evoked scratching behaviour [50], while antagonism of H3 receptors evoked scratching behaviour [51, 52].

H1 receptor activation on DRG neurons leads to activation of G α_q G-proteins and increases in intracellular calcium through phospholipase C [53–55]. GPCR activation must couple in some fashion with a membrane ion channel to depolarize a nerve terminal. The inward (depolarizing) ionic current in DRG neurons secondary to H1 receptor activation (and other G α coupled receptors) has been shown to involve opening TRPV1 receptors [56]. Consistent with this observation, histamine-evoked scratching behaviour is attenuated by pretreatment with TRPV1 receptor antagonists and in TRPV1 receptor knock-out mice implicating TRPV1 receptors in histamine-evoked scratching behaviour [55]. These observations that TRPV1 receptors are involved in histamine

receptor signalling supports findings in humans and non-human primates demonstrating that mechanically-insensitive C-fibres that preferentially respond to histamine are also responsive to capsaicin [15].

Although histamine-induced itch depended on TRPV1 activation, the sensations induced by histamine (itch) are not mimicked by TRPV1 agonists. TRPV1 receptors are activated by a variety of nociceptive stimuli including capsaicin, noxious heat, and acid [57, 58]. Generally, substances that activate TRPV1 evoke sensations of burning pain rather than itch and one possible reason for this is that H1 receptors are only expressed on a subset of TRPV1 expressing DRG neurons. As alluded to earlier, it is possible that although capsaicin stimulates pruriceptive fibres, it also strongly activates pain fibres, and the pain sensation could potentially mask the sensation of itch. Studies in humans have found capsaicin injection into human skin inhibited the itch sensation evoked by histamine [16].

5-hydroxytryptamine (serotonin)

5-hydroxytryptamine (HT) is another endogenous biogenic amine that has been shown to evoke itch in humans. Consistent with this, 5-HT stimulates action potential discharge in a subset of human cutaneous C-fibres [15]. The itch sensation evoked by 5-HT was weaker than the sensation of itch evoked by histamine [15, 59, 60]. Although serotonin is a relatively weak pruritogen in normal non-lesioned skin, it should be kept in mind that it is a much stronger pruritogen when administered to lesioned skin from patients with atopic dermatitis [60]. 5-HT also evokes scratching behaviour in experimental animals. In rats, the scratching behaviour to 5-HT is associated with activation of C-fibres, but not faster conducting myelinated nerve fibres [61]. Based on studies with selective 5-HT receptor subtype agonists and antagonists it appears that in mice the 5-HT₂ receptor subtype is responsible for 5-HT-induced itch [62]. The receptor subtype involved with 5-HT-induced itch in humans has not been worked out. Nevertheless, it is noteworthy that the itch associated with polycythemia vera may involve 5-HT [63] and may be inhibited with pizotigen, an antagonist of 5-HT₂ receptors [64].

Acetylcholine

Intradermal injection of acetylcholine evokes itch in humans, but like 5-HT, it is less pruritogenic than histamine [15]. Acetylcholine can also evoke pain sensations [15]. The relative sensation of pain and itch may be influenced by underlying pathology. For example, in normal healthy participants, injection of acetylcholine consistently evoked sensations of pain, while acetylcholine injection into the skin of subjects with atopic dermatitis resulted in

the sensation of itch [65]. Acetylcholine evokes scratching behaviour in mice, which is blocked by muscarinic M3 receptor antagonists [66]. Interestingly, patients with atopic dermatitis have a greater than 10-fold concentration of acetylcholine in their skin compared with normal healthy controls [66]. The source of the acetylcholine is not clear, but choline acetyltransferase (the requisite enzyme for acetylcholine synthesis) can be found in keratinocytes and endothelial cells within skin [67].

Substance P

Substance P (SP) is a neuropeptide found largely in cutaneous nociceptive nerve terminals, which can be released and influence vascular and immune responses leading to processes that are collectively termed neurogenic inflammation [68]. Particularly relevant to allergic inflammation, SP is also found outside the nervous system and has been localized to human skin mast cells [69]. Cutaneous administration of SP in humans evoked itch in normal healthy individuals and in patients with atopic dermatitis [60, 70, 71]. Moreover, patients with prurigo nodularis and atopic dermatitis have an increased density of SP-positive cutaneous nerve fibres [72–74].

The mechanism underlying SP-evoked itch in humans is thought to involve, in large part, the release of histamine from mast cells [60, 63, 70]. SP stimulates histamine release from human skin mast cells and the pruritus evoked by administration SP into healthy and lesioned skin is inhibited by histamine H1 receptor antagonists [60, 70, 75]. SP preferentially activates neurokinin one (NK1) receptors with nanomolar potency. The SP-induced histamine from human skin mast cells, however, generally requires concentrations in excess of 1 µM. SP-induced histamine release from human skin has been partially antagonized by the NK1 receptor L732, 138; however, the concentration required was far in excess of the drug's affinity constant for the receptor [76]. It seems likely that NK1 receptor mechanisms may be involved in SP-induced mast cell degranulation, but other mechanisms may be involved. It has recently been found that SP stimulates MrgX2 receptors with an EC₅₀ of about 10 µM. MrgX2 receptors are GPCRs that are expressed in human mast cells and several other MrgX2 agonists (including SP) evoke histamine release [77]. SP also evokes scratching behaviour in mice, but in contrast to humans, the itch is not inhibited by histamine receptor antagonists [78, 79]. Pharmacological studies demonstrated that SP-evoked scratching behaviour was secondary to leukotriene B₄ (LTB₄) production [80].

Aprepitant, a selective NK1 receptor antagonist, given orally for 1 week, attenuated itch severity in patients with chronic pruritus [81]. Aprepitant was also found to be effective in treating malignancy-related pruritus [82, 83]. Consistent with these observations with aprepitant in

humans, studies using NG/Nga mice, a murine model of a atopic dermatitis, demonstrated that administration of NK1 receptor antagonist reduced scratching behaviour [84]. The mechanisms underlying the antipruritic effect of NK1 receptor antagonism is not currently known.

Leukotrienes

Intradermal injection of cysteinyl-leukotrienes and LTB₄ cause local inflammatory reactions in human skin, which was accompanied by itch in a subset of individuals [85]. Intradermal injections of LTB₄ evoked scratching behaviour in mice, which was inhibited by LTB₄ receptor antagonists [79]. SP-evoked scratching behaviour in mice is inhibited by 5-lipoxygenase inhibitors and LTB₄ receptor antagonism [80]. It is not known if LTB₄-induced itch or scratching behaviour is due to a direct effect on sensory nerve endings or secondary to other inflammatory effects, but LTB₄ has been found to directly sensitize rat cutaneous C-fibres [86].

Bradykinin

In humans, the subjective response to intradermal injection of bradykinin is an intense burning pain [87, 88]. Bradykinin injection also led to mild to moderate itch in about 60% of participants, but the itching was only noticed after cessation of the burning pain [87]. The preference for pain over itch may change in atopic dermatitis where administration of bradykinin in lesional skin causes less pain and a robust itch sensation [60].

Proteases

Endogenous serine proteases such as trypsin and tryptase evoke itch in humans [89–92]. Similarly, injection of trypsin, tryptase, and other proteases in experimental animals evokes scratching behaviour [93–95]. Endogenous proteases are upregulated in human skin with atopic dermatitis [91, 96]. Although proteases can produce a variety of biological effects, one mechanism through which they can evoke itch is through the activation of protease-activated receptors (PARs).

A total of four PARs have been isolated, named PAR1–PAR4. All are GPCRs and the ligands for PARs are found on the extracellular N-terminus, which is liberated following cleavage by a protease [97]. Although studies have demonstrated that scratching-evoked behaviour can be elicited by activation of PAR1 and PAR4, a majority of studies have focused on the role of PAR2 in pruritus [95]. Administration of selective PAR2 agonists in humans and experimental animals evokes itch and scratching behaviour, respectively [92, 98]. Many endogenous and exogenous proteases have been shown to act as PAR2 agonists and evoke itch. Although trypsin can activate all four

PARs, tryptase has been shown to selectively activate PAR2 [99]. This is relevant to allergic itch in that mast cells are a rich source of tryptase [100]. Indeed, pruritus is a common clinical manifestation of mastocytosis, a condition marked by elevations in serum tryptase [101]. Cathepsin S is another endogenous protease that can evoke itch in humans and activate PAR2 and PAR4 [102]. Kallikrein-related peptidases (KLKs), specifically KLK5 and KLK14, can also activate PAR2 and are upregulated in lesional skin of humans with atopic dermatitis [103].

Exogenous proteases have also been shown to evoke itch and activate PARs. As discussed above, cowhage evokes itch and is commonly used in pruritus research. The pruritogenic component of cowhage is mucunain which is a protease capable stimulating PAR2 and PAR4 receptors [104]. Administration of the plant-derived cysteine protease papain into skin evokes itch and activates PAR2 and PAR4 receptors [105]. Many allergens contain proteases capable of stimulating PARs such as proteases from dust mites and cockroaches, which have been found to activate PAR2 [106, 107].

PARs are expressed by numerous cell types, but relevant to the present discussion, they are richly expressed by DRG neurons [108, 109]. PAR2 receptors have been localized on cutaneous sensory nerve in humans and experimental animals [92, 110–112]. PAR2 expression is increased on cutaneous sensory nerves in humans with atopic dermatitis [92, 103]. DRG neurons that express PAR2 also express neuropeptides consistent with a C-fibre nociceptive phenotype. Activation of PAR2 on DRG neurons results in intracellular calcium responses, neuropeptide release, and sensitization of TRPV1 receptor responses [113, 114]. As with other GPCRs, PAR activation may lead to nerve stimulation by gating the TRPV1 ion channel. Trypsin-evoked scratching behaviour in mice is inhibited by TRPV1 receptor antagonists or by genetic knockdown of the TRPV1 gene expression [94]. Scratching behaviour to PAR2 agonists is also inhibited by bradykinin 2 antagonism implicating a potential indirect mechanism as well [115]. The expression of PAR2 receptors on non-neuronal cells suggests that the itch evoked may also involve indirect actions following PAR2 activation. In mice, PAR2 activation on keratinocytes can also modulate scratching evoked behaviour through the production of LTB₄ [79, 116].

Interleukin-31

Another potential mediator involved in pruriceptive itch is the Th2 cytokine IL-31. Prior studies in humans found that IL-31 is highly upregulated in the skin of patients with pruritic skin diseases such as atopic dermatitis and prurigo nodularis [117, 118]. IL-31 belongs to the IL-6 family of cytokines and it binds to heterodimeric receptors consisting of IL-31 receptor- α and oncostatin M receptor

β [119]. In NC/Nga mice, a murine model of atopic dermatitis, IL-31 is increased in skin and transgenic over-expression of IL-31 in mice results in persistent scratching behaviour [120]. Interestingly, blocking IL-31 via anti-IL-31 antibodies decreased scratching behaviour in NC/Nga mice [121]. Receptors for IL-31 have been localized on murine DRG ganglion neurons with nociceptive phenotypes [122, 123]. Although IL-31 can act on many different cell types in the skin, the studies detailed above indicate that IL-31 could potentially act directly on sensory nerve terminals to evoke itch.

Lysophosphatidic acid and autotaxin

Lysophosphatidic acid (LPA) is phospholipid-derived mediator that evokes scratching behaviour in mice [124, 125]. Although the mechanism underlying LPA-evoked scratching behaviour is not currently known, H1 receptor antagonism and capsaicin pretreatment attenuated the scratching behaviour [125]. Serum LPA and autotaxin, the enzyme that converts lysophosphatidylcholine into LPA, are strongly unregulated in patients with cholestatic pruritus [124]. Collectively, these studies implicate the role of LPA and autotaxin in cholestatic pruritus.

Toll-like receptor 7

Recently toll-like receptor 7 (TLR7) has been implicated in mediating scratching-evoked behaviour in mice [126]. Although TLR7 is used by the innate immune system to recognize single-stranded RNA from viruses, genetic knock out of TLR7 attenuated scratching behaviour to variety of pruritogens. Scratching behaviour evoked by chloroquine, PAR2 agonists, and serotonin were all attenuated in TLR7 knockout mice. Application of TLR7 agonists directly excited DRG neurons and TLR7 receptors were localized on DRG neurons and cutaneous sensory nerves with nociceptive phenotypes indicating they are likely present on C-fibres. Collectively, these results indicated that TLR7 is involved in the signalling of a variety of unrelated pruritogens, but the mechanism underlying this effect is unknown but may involve some general sensitizing mechanism.

Sensitization of itch

In addition to changes in the inflammatory environment of the skin, the mechanisms underlying chronic pruriceptive itch may also involve altered processing of itch by the nervous system. Studies have demonstrated that nociceptive stimuli that normally evoke pain in healthy individuals evoke itch when applied to lesional skin of patients with atopic dermatitis [127, 128]. The nociceptive stimuli that evoked itch included mechanical stimulation, heating, and acidic buffer administration into the skin [127,

128]. Bradykinin, a known algescic substance, also evoked itch when administered into lesional skin of patients with atopic dermatitis, whereas the major sensation in normal skin was pain [60].

Sensitization of itch can occur peripherally or centrally. Peripheral sensitization is most often studied in nociceptors and is characterized by a decreased threshold for activation, increased responsiveness, and the presence of ongoing activity [129–131]. Following exposure to inflammatory mediators or injury, the excitability of sensory nerve endings in the skin or other tissue that detect pruritic substances could be enhanced. Electrophysiological recordings made from a patient suffering from chronic pruriceptive itch due to prurigo nodularis demonstrated that mechano-insensitive C-fibres exhibited aberrant firing behaviour indicative of sensitization [27]. Results from psychophysical experiments have demonstrated enhanced responses to pruritogenic stimuli also possibly indicating peripheral sensitization. Itch sensation can be evoked in humans by transcutaneous electrical stimulation and the threshold for electrically evoked itch was lower in skin from patients with atopic dermatitis compared with normal healthy control skin [127, 132, 133]. The dose of histamine required to evoke itch in lesional skin of patients with atopic dermatitis was lower than in normal healthy skin [134]. Overall, it is likely that sensory nerve fibres mediating itch become sensitized during chronic itch.

Sensitization can also occur following changes in the activity CNS neurons, which is termed central sensitization. During central sensitization, the excitability of CNS neurons is altered in such a way where non-pruritic stimuli become coupled to pruritic sensations [8, 127, 135]. Although the mechanisms underlying central sensitization to itch are largely unknown, neuroimaging studies have demonstrated altered activity in certain brain regions in patients with atopic dermatitis compared with normal healthy controls [136–138].

Increased nerve fibre density could also potentially mediate chronic pruriceptive itch. Prior studies have implicated neurotrophins in the pathophysiological mechanisms of atopic dermatitis and prurigo nodularis. Neurotrophins are a class of growth factors including nerve growth factor (NGF), brain-derived neurotrophic factor, neurotrophin 3 (NT-3), and neurotrophin 4/5 (NT-4/5). Neurotrophins bind to three different subtypes of tropomyosin-receptor-kinase (Trk) receptors named TrkA, TrkB, and TrkC. All of the neurotrophins and their receptors have been localized in skin among various cell types [139, 140]. Studies in human subjects have demonstrated that skin from patients with atopic dermatitis have increased levels of NGF in keratinocytes and infiltrating leucocytes which are related to disease severity [141]. Skin from patients with prurigo nodularis also have increased presence of NGF, which is primarily found in infiltrating

leucocytes [142]. Patients with atopic dermatitis also have increased NT-4 expression in the skin, which is primarily localized to keratinocytes [143]. Although neurotrophins are not pruritogens, they can potentially enhance sensory nerve growth and sprouting. Prior studies have demonstrated that cutaneous nerve fibre density is increased in patients with prurigo nodularis and atopic dermatitis [72, 73, 144, 145].

Conclusion

Effective treatment for chronic itch is a serious unmet medical need. Although certain drugs can offer some relief, there is no evidence to support any given treatment is generally efficacious in the treatment of chronic pruritus. There are numerous inflammatory mediators asso-

ciated with pathological itch, which can also evoke itch when injected into the skin. Beyond histamine, there have been relatively few rigorous clinical studies that have evaluated the effect of antagonizing a given mediator in pathologic itch.

Logically, it may be more fruitful to target pruriceptive itch through mechanisms where several mediators converge. For example, many irritants as well as GPCR agonists stimulate C-fibres by interacting with TRPV1. Even TRPV1 antagonists, however, will only target itch evoked by a subset of mediators. A more general target may be the so-called 'itch receptors' themselves. As more knowledge is gained about these sensory A δ and C-fibres and the mediators that excite them, future strategies may avail themselves that will selectively inhibit their excitability and sensitization.

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