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REVIEW ARTICLE

Human Surrogate Models of Histaminergic and Non-histaminergic Itch

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Within the last decade understanding of the mechanistic basis of itch has improved significantly, resulting in the development of several human surrogate models of itch and related dysesthetic states. Well-characterized somatosensory models are useful in basic studies in healthy volunteers, in clinical studies for diagnostic and segmentation purposes, and in pharmacological studies to evaluate the antipruritic efficacy of existing and novel compounds. This review outlines recently introduced histamine-independent human models of itch, their mechanisms, their ability to induce clinically relevant phenomena, such as alloknesis, and the results obtained through their use. The article also introduces recent advances in the understanding of itch and provides an overview of the methods to assess experimentally-induced itch and associated manifestations. Major improvements are warranted in the treatment of chronic pruritus, and reliable human surrogate models are a valuable tool in achieving them, both for basic researchers and for clinicians. ***Key words: itch; pruritus; histamine; histamine-independent; surrogate model; cowhage.***

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Itch, also known as pruritus, is an unpleasant sensation that may prompt the sufferer to scratch the affected area. Itch may occasionally be dismissed as a minor nuisance, perhaps because everyone has experienced innocuous episodic itch (1–3). However, chronic pruritus (> 6 weeks (1)) profoundly impacts quality of life for the affected patients through disturbances relating to sleep, attention and sexual function (1, 4). Itch is associated with wide range of medical conditions, such as urticaria, atopic dermatitis (AD), psoriasis, primary biliary cirrhosis, and chronic renal failure, as well as several neurological, infectious, neoplastic, haematological, autoimmune, genetic and drug-induced conditions (5, 6). Moreover, due to a prevalence of approximately 10% and a largely suboptimal treatment regimen, chronic itch represents a significant socioeconomic burden (1).

Within the last decade understanding of the neural and molecular structures facilitating the sensation of itch during normal and pathophysiological conditions has been greatly enhanced (7). Perhaps most prominently, a long-suspected (8, 9), histamine-independent itch pathway has been uncovered, accompanied by a range of new peripheral receptors (i.e. new in a pruritogenic context) (7, 10–12). Moreover, histamine has been refuted as key mediator of itch in most of the clinical conditions presenting with chronic pruritus, which is in agreement with the fact that these conditions are frequently refractory to treatment with antihistamines (1, 13–15). Hence, the present treatment options for itch, beyond targeting the underlying disorder, are suboptimal, and the area is characterized by evidence originating from case-series or small-scale trials. This means that, as opposed to pain management, knowledge of treatment responsiveness in different itch patient subgroups is scarce (1). The progress made in elucidating the possibly distinct, histamine-independent itch modalities has sparked a new demand; the need for reliable human surrogate models. Such models facilitate the mimicking of activity in specific itch pathways in healthy human volunteers and patients with itch, whereby an improved understanding of itch can be achieved and novel diagnostic tools, targets and strategies for new pharmacological interventions can be revealed in a timely manner. This mechanistic approach, of using translatable symptom-specific surrogate models, has been highly advantageous within the field of pain research in terms of bridging the bench-to-bedside gap and of spurring the idea of mechanisms-based treatment (16, 17).

The present review provides an overview of the methods used to assess experimentally induced itch and analytically outlines the recently introduced histamine-independent human models of itch that have been reported in the research literature. This review does not intend to encompass a comprehensive summary of the mechanistic or therapeutic aspects of itch, but instead refers to recent reviews pertaining specifically to these subjects (1, 7, 18, 19).

ASSESSING EXPERIMENTALLY INDUCED ITCH

Experimentally induced itch provides the opportunity to study a particular itch pathway in a chosen anatomical location, while accurately assessing the quality, inten-

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sity, latency and duration of the acute itch, related nociceptive and dysesthetic states and the potential associated vasomotor aberrations. Perhaps most importantly, induction of itch in healthy volunteers improves the study of itch mechanisms, e.g. by functional magnetic resonance imaging (fMRI) or microneurography, and provides a shortcut for evaluating modulating factors or potential interventions (12, 20–22). Many of these modulatory factors, such as heat stimulation, cold stimulation, transient receptor potential (TRP)-modulation and scratch stimulation, have been characterized in histaminergic models, but remain uninvestigated or sparsely evaluated in non-histaminergic models.

Assessment of itch intensity and quality

With the exception of mechanically and electrically evoked itch, most human surrogate models produce itch lasting 5–15 min with a peak intensity rating elicited between 1 and 3 min after induction. In the case of clinical, as well as experimentally, induced itch, the sensation frequently presents with one or more associated sensations, such as pricking or burning. The most common approach is to instruct the participating subject to separately rate the sensory qualities of itch, pricking and burning on a generalized labelled magnitude scale (gLMS), a visual analogue scale (VAS) or a numerical rating scale (NRS), frequently (every 10–30 s) upon itch induction (10, 12, 14, 23). This allows for a temporal overview of the itch and other sensory qualities and reporting of itch latency, peak, area under the curve, etc.

Since most models of itch include some co-activation of nociceptors, sensations of pain and nociceptive dysesthesias should be assessed. For example, in a study addressing gender differences in surrogate models of itch, Hartmann et al. (24) reported itch intensity as "% itch of burning pain", i.e. as a ratio between the itch intensity score and the burning pain intensity score (both recorded by VAS). Interestingly, the study revealed that women reported a disproportionally higher intensity of burning pain than their male counterparts, particularly after histamine-induced itch (24).

Itch-related dysesthesias

Alloknesis. This is the itch analogue to the pain term "allodynia", in which a normally non-painful stimulus is perceived as painful (25). As such, alloknesis describes the dysesthetic state in which otherwise non-pruritic stimuli, such as brush strokes or light touch applied by von Frey hair, provoke a sensation of itch (26, 27). Alloknesis is a feature occurring not only in experimental models of itch, but also in many of the clinical conditions involving chronic itch, such as AD (28).

Hyperknesis. This is the itch-related analogue to the nociceptive state "hyperalgesia", in which a normally painful stimulus is associated with an increased pain response. In hyperknesis an increased itch response (in terms of magnitude or duration) is elicited upon a normally pruritogenic or prickling stimulus, e.g. by means of von Frey hair or weighted pinprick stimulators (29, 30). An area of hyperalgesia, including secondary hyperalgesia, to pinprick stimuli also occurs in response to cowhage- or histamine-evoked itch, although this is modest compared with that of epidermal or topical capsaicin. In experimentally induced itch, alloknesis and hyperknesis can be assessed both within the immediate area of pruritogen application and in the surrounding area, denoted "primary" and "secondary", respectively (28). Typically, the secondary area of alloknesis and hyperknesis is mapped by slowly approaching the application area by marked stimulus points and a pre-determined individualized stimulus. Hyperknesis is most commonly assessed by the use of von Frey or pinprick stimulators and, as such, represents "punctuate" or "dynamic" hyperknesis, but principally the parameter could also be assessed with a pruriceptive substance injected in the vicinity of the itchy area.

Upon itch induction alloknesis and hyperknesis spreads rapidly beyond the area of pruritogenic application (see Fig. 1) (23, 31). Mechanistically, alloknesis and hyperknesis are suggested to be a consequence of sensitization of the spinothalamic tract neurones conveying pruritogenic input, by a period of increased firing from itch-sensing primary afferents. Subsequently, these spinal neurones will become responsive to convergent input from Aβ primary afferents mediating touch (resulting in alloknesis) and additional itch-sensing primary afferents innervating the area surrounding the application site (resulting in hyperknesis) (28, 32–34). Induction of itch can also result in a moderate area of hyperalgesia, normally associated with induction of pain or inflammation, highlighting the overlap between nociception and pruritoception (31). In addition to the central mechanisms, injection of nerve growth factor has been shown to potentiate non-histaminergic itch and related mechanical hyperalgesia, suggesting peripheral sensitization of the primary afferents (34). In histaminergic and cowhage-induced itch, alloknesis spreads far beyond the immediate area of application within minutes and can extend to an area upwards of 20–30 cm dependent on the methodological approach. Typically, the area of alloknesis is reported to be slightly larger after cowhage-induced itch than after histamine-induced itch (23, 31). While the spatial profile of alloknesis is frequently mapped in experimental studies of itch, the temporal profile is very sparsely investigated.

Wheal and flare response

Flare is an increase in superficial perfusion normally assessed manually by recording the size of the affected area or by laser Doppler flowmetry, speckle contrast imaging/full-field laser perfusion imaging (FLPI) (Fig. 1), spectrophotometry or by infrared thermography,

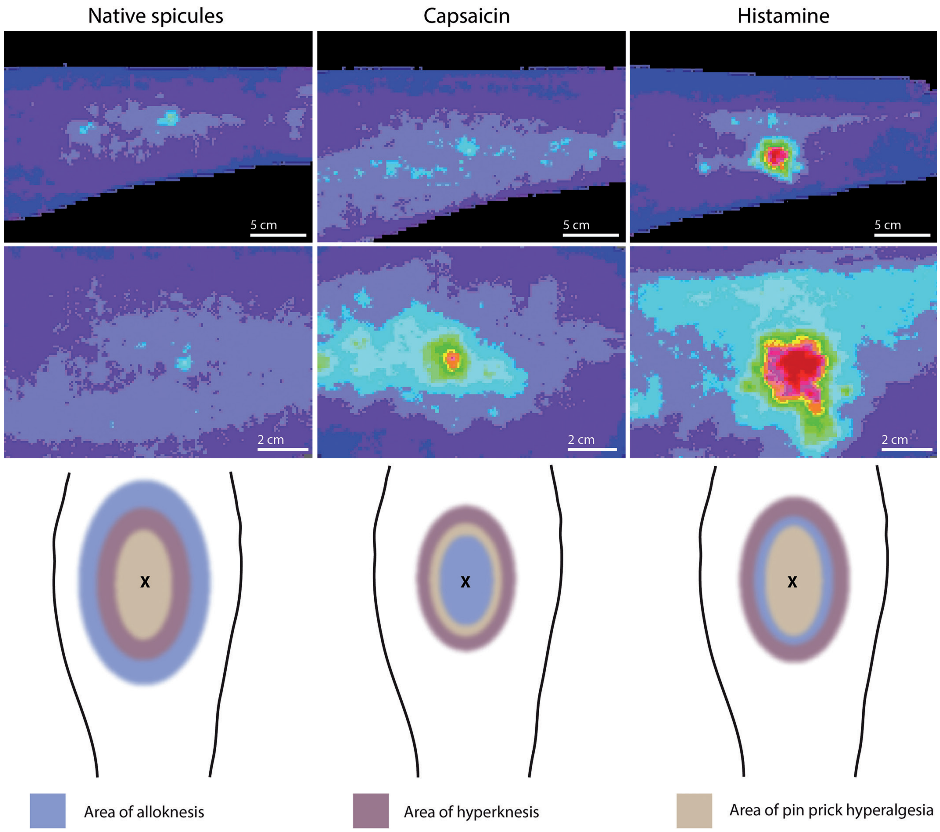

Fig. 1. A series of typical vasomotor and dysesthetic responses to epidermal insertion of 3–4 cowhage spicules; "native", capsaicin- or histamine-pretreated, in an area ≈1 mm^2 on the volar forearm. The 2 upper rows are obtained from a Moor Full-field Laser perfusion imager (FLPI-2, Moor Instruments, Axminster, UK) 10 min after spicule insertion. Note that the lowest row of arm charts represent the typical reported mean dispersion of alloknesis, pin hyperknesis and pinprick hyperalgesia in response to insertion of spicules with the denoted substances. The individual differences within these response parameters are considerable.

recording both flare size and intensity. Mechanistically, flare is a consequence of antidromic activation of terminal branches of CMi-fibres leading to the release of the vasoactive substance calcitonin-gene related peptide and substance P, which are important in the initiation of mast-cell activity (11, 35). Flare is dependent on the extent to which CMi-fibres, characterized by large receptive fields, are stimulated and is a typical feature of histamine-dependent itch, while histamine-independent itch, e.g. induced by cowhage spicules, appears to provoke no or very subtle vasomotor aberrations (35).

A wheal is a vascular leakage response to histamine, observed as a raised, often pale and circumscribed dermal oedema, caused by acute protein extravasation in the vascularized dermis. It is a cardinal response to application of histamine or introduction of any mast cell degranulation-provoking substance, such as allergens (23, 36). Wheals with a diameter of ~0.5 to 2.5 cm are common upon punctate or intradermal delivery of histamine (2–4, 23, 37, 38). Although cowhage spicules produce minimal vasomotor responses they have occasionally been shown to cause micro-skin reactions of slight oedema or flare no larger than 1 mm^2 (23).

DEFINING HISTAMINE-INDEPENDENT ITCH

Since the terms "histamine-independent" and "non-histaminergic" are essentially negative definitions it is necessary to recapitulate on histamine as an itch inducer. Moreover, histamine is by far the most-studied pruritogen, having been widely used as the prototypical experimental proxy of itch and, hence, despite the focus of this review being histamine-independent itch modalities, histamine-induced itch deserves a brief mentioning. Mechanistically, histamine activates the H1-receptor (H1-R) present on CMi-fibres and co-localized with the heat thermo-receptor, transient receptor potential vanillin 1 (TRPV1) (39, 40). Upon binding of histamine to the H1-R, TRPV1 is activated by downstream signalling, leading to an influx of Ca^{2+}, whereby the primary afferent initiates a pruritic signal.

To induce itch, histamine can be applied epicutaneously in combination with iontophoresis, by epidermal penetration with a lancet or functionally inert cowhage spicules coated with histamine or as an intradermal injection (3, 9, 14, 27, 41–44). All routes of administration are shown to produce a moderate to strong sensation of spontaneous itch, with slight differences in the reported presence of nociceptive sensations, alloknesis, and hyperknesis (27, 37). In particular, when injecting histamine the induced response ratio between nociception and itch appears to shift away from itch towards a more nociceptive sensation characterized by burning and pricking (15). Lastly, the use of histamine is accompanied by a significant wheal and flare reaction regardless of the route of administration (26, 35, 37, 42). Since histamine-dependent itch relies on the TRPV1-channel, a handful of studies have investigated the ability of capsaicin to induce itch, bypassing the H1-R, using epidermal, punctate and intradermal delivery (23, 24, 37). In general, capsaicin evokes burning pain and widespread flare when injected intradermally, while it produces significant itch and weaker burning pain when applied via inert cowhage spicules (23, 24, 37). This relative unspecificity of itch processing supports the notion of itch being conveyed in accordance with the selectivity theory, by a subpopulation of superficially residing itch-labelled afferents responding to pruritogens and algogens, such as capsaicin, while allowing the activation of the much larger population of TRPV1$^+$ nociceptors by capsaicin, to override the itch signal (24, 45–47). Since the flare response following punctate histamine is significantly more pronounced than that of capsaicin, it is probable that TPRV1$^+$ nociceptors, and not weakly capsaicin-sensitive histamine responsive itch fibres, are the primary facilitators (5). For practical purposes, in the experimental setting, a distinction between histamine-dependent and histamine-independent itch can be determined by showing that pre-administration of topical antihistamine, such as doxepine, reduces the itch intensity (11, 15).

Unlike histamine-dependent itch, histamine-independent itch is thought to rely mainly on a subpopulation of mechano-heat-sensitive/polymodal c-fibres (CMH) incapable of producing the extensive flare that is characteristic for histamine-induced itch (11, 27). In the non-histaminergic pathways the key second messenger role is played by transient receptor potential cation channel, subfamily A, member 1 (TRPA1), a downstream target of proteinase-associated receptor 2 (PAR) and Mas-related G-protein coupled receptor member G-signalling (Mrgpr) (48–50). TRPA1 appears to be crucial, not only in conveying chronic itch sensation, but also in processes such as neurogenic inflammation, epidermal hyperplasia and altered gene expression in sensory nerves, which frequently accompany chronic itch conditions (48, 51). It was recently shown that itch and neurogenic inflammation can be induced in human skin by direct TRPA1-stimulation using the natural agonist trans-cinnamaldehyde (52). Lastly, a notable difference is present between histaminergic and non-histaminergic itch in terms of higher processing. While both itch qualities activate brain structures such as thalamus, primary and secondary somatosensory cortices and cingulate cortices, histamine-independent itch was additionally associated with activation of areas such as the insular cortex, claustrum and basal ganglia (21). In a recent study it was shown that activation of nucleus accumbens and the septal nuclei mediated through the mixed action κ- and μ-opioid, butorphanol, completely abolished histaminergic itch, while only modestly reducing non-histaminergic itch, demonstrating that both peripheral and central processing differ between these itch pathways (20).

HUMAN SURROGATE MODELS OF ITCH

Non-chemical surrogate models of itch

Electrically-evoked itch. A few studies have explored the opportunity of using transcutaneous electrical stimulation to induce itch, with varying success (10, 53, 54). Ikoma et al. (10) explored numerous electrical stimuli paradigms designed to produce itch, and found that a 2 ms, 50 Hz, 0.05 mA stimulation with a 0.1×7 mm electrode, induced a highly selective sensation of moderate itch rated ≈ 3 on a NRS (VAS 0–10), while increasing the current intensity to 0.12 mA produced the most intense itch sensation, 4.5 (VAS 0–10). At this higher intensity level, itch occurred alongside a modest level of pain at 2.2 (VAS 0–10). Electrically induced itch was accompanied by very little axon reflex flare, in comparison than 1% histamine-iontophoresis, suggesting that it is not mediated by histamine-sensitive CMi-fibres (10). Electrically evoked itch was also associated with a significantly larger area of alloknesis, than histamine, thus displaying a pattern of effects similar to cowhage-induced histamine-independent itch. Interestingly, the extent of the area of alloknesis exhibited a significant negative correlation with the pain intensity (10).

A study designed to explore so-called "heterotopic pruritic conditioning" to itch as an analogue to "diffuse noxious inhibitory control" (DNIC) in pain used a stimulus paradigm of 0.3 ms, 100 Hz with a 3.5 cm diameter electrode, and determined the test stimulus intensity as 300% of the individually perceived unpleasantness threshold. For the conditioning stimulus the study applied 0.5% histamine delivered by iontophoresis, which produced a mean itch intensity of 2.5 ± 2.0 in the healthy control group and 2.9 ± 2.5 in patients with psoriasis. Of more concern is the fact that the electrical test stimuli evoked surprisingly low and variable itch levels at ~1.5 and ~0.55 (VAS 0–10) in healthy controls and psoriasis patients, respectively (54). This conditioning stimulus intensity is very low in comparison with the conditioning stimuli applied in various pain studies, with otherwise equivalent stimulus-test paradigms, to achieve a significant conditioned pain modulation-effect (55).

Mechanically-evoked itch. Apart from the above-mentioned electrical approach, itch can also be induced non-chemically with the use of mechanical stimulation. In a recent study, micro-vibration of the facial vellus hairs in a stimulus paradigm of 0–1 mm probe amplitude, at 1–50 Hz for 90 s resulted in a mean peak itch intensity at 5 (VAS 0–10). The chin was by far the most sensitive location, while the cheek and the forehead were considerably less responsive (both ~ 2.5, VAS 0–10), and stimulation on the forearm did not produce any itch. The mechanically evoked itch was unresponsive to antihistamine and did not entail flare or nociceptive sensations at any stimuli intensity, making the itch model unique. As opposed to mechanically evoked itch, histamine-induced itch was significantly more pronounced on the forearm compared with any facial areas, suggesting that the neural facilitation of itch may exhibit significant pathway heterogeneity depending on anatomical location (56).

Proteinase-activated receptor 2/4 (PAR) mediated itch

Cowhage spicules. The spicules found on the pod of the leguminous plant cowhage (*Mucuna pruriens*) and, more importantly, the sensory effects that these induce when inserted into the epidermis, were described in 1953 by Broadbent, who wrongfully concluded their itch inducing properties to be a consequence of an unknown substance causing histamine release (57). A few years later, Shelley & Arthur isolated mucunain, identified it as a proteinase, suggested it to be the principal itch-inducing compound in cowhage, and reported that the itch sensation it induced was "very unlike that of histamine" (8, 58). Fast-forward 50 years, the histamine-independent, PAR2/4 pathway of itch is uncovered, mucunain is revealed as a ligand of PAR2 and PAR4 (59), and the interest in using cowhage as a human experimental model of itch rapidly re-emerges (11, 14, 22–24, 31, 34, 37, 58). The quality of the somatosensory effects associated with PAR2-activation, e.g. through insertion of cowhage spicules, have been described as very similar to those reported in patients with AD (14, 58). Moreover, the level of the endogenous PAR2 agonist, tryptase, exhibits a 4-fold increase in serum from patients with AD, and expression of PAR2 on the primary afferent nerve fibres is markedly increased in skin biopsies from patients with AD, indicating that the receptor is probably involved in the somatosensory aberrations of AD (60).

Cowhage spicules are 1–3 mm in length, with a diameter of 1–3 μm at their tip. Inserted into the epidermis the spicules evoke a moderate to intense sensation of itch and, to a lesser extent, sensations of burning and stinging pain (11, 23, 61, 62). It has been reported that, in a majority of cases, a single spicule, estimated to occupy a skin area of $\approx 0.00003\ mm^3$, is sufficient to induce pruritic, nociceptive and dysesthetic sensations lasting 4–10 min (see Table SI[1]). The insertion of cowhage spicule(s) rapidly and consistently produces alloknesis, hyperknesis and hyperalgesia far beyond the immediate area of application, but no or very little flare, presumably due to the lack of CMi-fibre activation (12, 22).

Conveniently, cowhage spicules can be rendered functionally inert by heating and subsequently coated with another active compound, such as histamine or capsaicin. Hence, cowhage can serve as a convenient vehicle allowing delivery of any substance of interest to a very limited population of the most superficially residing nerve endings (15, 23, 31). However, the cowhage model has several drawbacks, e.g. the pods or spicules are often obtained from completely uncontrolled, unstandardized sources and numerous papers in the field completely fail to mention how or from where the pods were obtained. The content of the active itch-inducing cysteine protease mucunain could potentially differ widely between habitats and time of harvest. These problems collectively hamper the comparability of studies utilizing the model and the reproducibility of the results achieved. A potential solution to this problem could be to extract mucunain and use it in known concentrations via injections or on reconstituted inert spicules, as done by Reddy et al. (59), although good manufacturing practice requirements related to human use could make this approach laborious.

Other proteinases. The use of various proteinases, such as papain and tryptase, has been attempted to mimic non-histaminergic itch (58, 60, 62). The results are relatively sparse and variable. Arthur & Shelley conducted a comprehensive study, administering numerous proteases by means of inert cowhage spicules and intradermal injections. Here, papain was found to be the most effective itch inducer of 48 tested enzymatic substances (63). More recently, papain administered by intra-cutaneous injection was shown to produce highly variable responses, i.e. of 33 experiments in 8 subjects, 15 responded with itch and pain, 9 responded with itch, 2 responded with pain only, and 7 reported no evoked sensations (see Table SI[1]). Moreover, 13 of the 33 experiments resulted in significant flare. Itch-related dysesthesias were not assessed (27, 58).

Based on sparse evidence, papain primarily produces reliable itch upon intra-epidermal application. In the light of the variability and scarceness pertaining to the results on papain and importantly, recent studies on cowhage as a model of PAR2-dependent non-histaminergic itch, proteases such as papain pose a somewhat redundant opportunity as a model of itch.

Mas-related G-protein coupled receptor-mediated itch (Mrgprs)

Mrgprs are a family of approximately 50 receptors, of which several are exclusively expressed on small diameter dorsal root ganglia neurones. In humans these include MrgprX1, a receptor for chloroquine and bovine adrenal medulla 8–22 peptide (BAM8-22), and MrgprD, which is restricted to axons innervating the epidermis and is responsive to the itch-inducing amino acid; β-alanine (64–66).

Cellular and behavioural experiments have confirmed BAM8-22, a derivative of proenkephalin A, as an agonist of MrgprC11 (and hMrgprX1) (15, 66, 67). In healthy human volunteers, BAM8-22 induced an itch intensity profile peaking at "moderate" on a gLMS accompanied by almost equally intensely rated pricking/

[1]http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-2146

stinging sensation and weak burning sensations, not unlike that evoked by active cowhage (see Table SI[1]). Insertion of BAM8-22-soaked spicules evoked very similarly sized areas of alloknesis, hyperknesis and hyperalgesia, of approximately 10 cm^2, on average, and no wheal or flare. In accordance with the latter observation, the pruritic effect of BAM8-22 was shown to be completely histamine-independent, since it was not affected by antihistamine pretreatment. It remains to be elucidated whether the pruritic effect of BAM8-22 shifts towards algogenic if injected into the dermis, analogous to that of capsaicin, which is also pruritogenic when administered solely to the superficial pruritoceptive nerve terminals by spicules (15).

Similarly, β-alanine has been used as a model of itch by intradermal injection of 10 µl vehicle with 22.5–180 µg of dissolved β-alanine. This produced a weak to moderate sensation of itch, accompanied by slight pricking/stinging and weak burning sensations. All reported sensations were present when injecting the similar, but inactive, amino acid L-alanine, albeit at a much lower intensity and only in a subgroup of volunteers (68). No wheal or flare was present indicating histamine-independency. Potential dysesthesias were not recorded. Both itch induced through MrgprX1 and MrgprD with BAM8-22 and β-alanine, respectively, are histamine-independent and appear to evoke a similar, but perhaps slightly weaker, pattern of sensory qualities than those elicited by cowhage (15, 31, 68).

Itch induced by algogens: serotonin, bradykinin and substance P

Although being an established endogenous algogen the neurotransmitter serotonin has been shown to be a pruritogen in both healthy individuals and in patients with AD, and is suspected to play a role in the chronic pruritus often associated with cholestasis (69) and polycythemia vera (70, 71). However, the mechanistic basis of serotonin-induced itch remains unclear (72). In a study by Hosogi et al. (73) serotonin at a concentration of 17 mg/ml was delivered by iontophoresis and caused intense histamine-independent itch in lesional skin of patients with AD and in skin of healthy controls. In addition, serotonin elicited a significant axon-reflex-flare, but no wheal response in both lesional and non-lesional skin, indicating that serotonin induces itch distinct from that evoked by activation of histamine, PAR2 and Mrgpr receptors. In another study, iontophoretic delivery of serotonin (1%), induced moderate itch, a very large area of alloknesis, a large area of flare and, similar to other reports, no wheal. Thomsen et al. (74) found that intradermal injections of serotonin (0.25 mg/ml) elicited moderate to strong itch only in normal skin, but not in experimentally induced eczematous skin in healthy volunteers. Rausl et al. (72) found that healthy controls and patients with AD differed only in their vasomotor response to serotonin injections, in contrast to the results of Hosogi et al. (73).

The classic algogen substance P has been used to induce itch in healthy controls and patients with itch conditions, such as AD (73–75). As for serotonin, results exhibited some inconsistency. For instance, intradermal substance P has been found to induce a stronger itch than histamine in both normal and eczematous skin, while substance P delivered iontophoretically induced very little itch in normal skin, but significantly more intense itch in lesional skin of patients with AD. In addition, the vasomotor and somatosensory effects of substance P application can be abolished by anti-histamine pretreatment, suggesting the mechanism of substance P-induced itch to be histamine-dependent (73, 76). Dysesthetic responses, such as alloknesis and hyperknesis, have not been assessed in response to administration of substance P.

Lastly, the vasodilatory peptide bradykinin has been sparsely assessed for pruritogenic properties (73, 77). Interestingly, iontophoretic application of the peptide appears to produce little or no itch in healthy skin and in non-lesional skin of patients with AD, but induced considerable itch in lesional skin of patients with AD without evoking vasomotor responses (73).

CONCLUSION AND FUTURE PERSPECTIVES

Itch is a multifaceted sensation and although the general discourse mainly deals with histaminergic and non-histaminergic itch (11, 12, 78), more sub-classifications could be beneficial. Nakagawa & Hiura (18) suggested 4: (*i*) a $TRPV1^+$ histaminergic pathway; (*ii*) a TRPV1-independent histaminergic pathway (since histamine-induced itch is not completely abolished in TRPV1 knock-out mice); (*iii*) a PAR2/4- and Mrgpr-mediated non-histaminergic pathway; and (*iv*) a serotonin-mediated non-histaminergic pathway. However, it is currently indiscernible whether the multiple receptors mediating non-histaminergic itch are in fact associated with the same subpopulation of mechano-sensitive afferents or whether distinct pathways exist. Moreover, it is also unclear to what extent itch processing could differ between various anatomical locations; a notion that was recently rekindled (44, 55, 79). Beyond the complexity posed by the multiple neural pathways and peripheral receptors mediating itch, the sensation is also strongly modulated by other somatosensory submodalities, such as innocuous warmth, which facilitates itch, and pain and cold, which inhibit itch. These modulatory factors including their available chemical proxies, e.g. TRPM8-stimulation by l-menthol, have been investigated in relation to histamine-dependent itch, but not in a non-histaminergic context.

The fact that multiple parallel pathways can convey the sensation of itch in healthy individuals and in patients with chronic pruritus constitutes a challenge as well as an opportunity. On the one hand it complicates surrogate modelling and pharmacological development directed at itch, but on the other hand it could allow for increased diagnostic segmentation of itch-associated conditions and targeted therapy. This requires that the candidate mechanisms underlying itch must be validated in various different clinical itch disorders by psychophysical studies, assessment of biopsies, targeted interventional studies, microneurograhic studies and the use of validated surrogate models in patients. Currently, sparse and ambiguous evidence exists in relation to whether patients with chronic itch or subgroups within this population experience peripheral or central sensitization to itch, comparable with what has been shown repeatedly in patients with chronic pain (14, 43, 53, 72).

The development of diagnostic tools to aid clinicians in the sub-categorization of pruritus, i.e. by assessment of alloknesis, hyperknesis and response to pathway-specific itch-induction, is warranted. Progress in these areas would prompt the mapping of chronic pruritus by pathological mechanism, which, in conjunction with well-planned clinical trials, could pave the way for improved treatment.

The authors declare no conflicts of interest.

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