Review Article

Four Possible Itching Pathways Related to the TRPV1 Channel, Histamine, PAR-2 and Serotonin

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Abstract

The following four possible pathways for itching sensation have been suggested by recent reports. 1) Histaminergic TRPV1-positive pathway: Although histamine-positive nerve fibers cannot strictly be classified as "itch specific" due to their excitation also by pure algogens (making them itch-selective), the existence of a subpopulation of nociceptors responsible for itching is strongly suggested. Moreover, the TRPV1-expressing neurons have been suggested to be the main sensors and mediators of itching. 2) Histaminergic TRPV1-negative pathway: The scratching behavior caused by itching was not different between capsaicin-pre-treated and vehicle-treated (control) mast cell-rich NC mice. This result suggests the existence of a capsaicin-insensitive (TRPV1-negative) histaminergic pathway. 3) Non-histaminergic PAR-2 pathway: Protease-activated receptor 2 (PAR-2) has been shown to play a role in the itching of atopic dermatitis (AD). The itch evoked by cowhage (a non-histaminergic pruritogen that activates PAR-2) is very similar in characteristics to the itch evoked by conditions such as AD. 4) Non-histaminergic serotonin (5-HT) pathway: 5-HT alone applied to the human skin evokes an itching sensation and has been suggested to be involved in the itching associated with pruritic diseases, such as polycythemia vera and cholestasis.

Keywords: *itch, TRPV1, histamine, PAR-2, serotonin*

Introduction

Why do we have an itching sensation? What is the purpose of the itching sensation in the body? It is not like pain but represents a signal that something is wrong and/or injured. It seems to us to be nothing more than a sensation of displeasure. If we would not perceive the itching sensation, would we be any worse off? Itch is sensitive so that undangerous factors frequently trigger it; scratching is an "unintended" side effect by which itch can be evaluated. There has been an explosion of research on itching in several medical fields. We attempted to understand itching at a fundamental level, that is, the participation of neuronal cells and pruritic agents. Investigations on the molecular mechanism underlying the itching sensation have begun. Itching is a multifaceted pathophysiological phenomenon involving multiple mediators at the cellular and molecular levels. Different etiologies may underlie the complaint of itching. Recent reports have suggested four putative itching pathways. This review also deals briefly with whether or not capsaicin-sensitive nerve fibers might be involved in itching.

Theoretical Basis of the Itching Sensation

Chronic pruritus (atopic dermatitis, or AD) severely disturbs quality of life for patients, frequently causing insomnia and severe scratching of the skin as a consequenceof severe itching. Thus, alleviation of the itching sensation is as serious an issue in patients as pain. Despite growing experimental evidence of the existence of a close relationship between histamine and pruritus, the precise mechanism underlying the development of the itching sensation is still unclear. There are four theories to explain itching (1); 1. The theory of the existence of specific afferents only responsive to itching (specificity theory); 2. The theory that intense activation of nociceptive neurons evokes pain, whereas a weak activation induces itching (the intensity

theory); 3. The theory of the presence of a subset of nociceptive afferents that could activate central neurons responsible for itching (the selectivity theory); 4. The theory that temporal or spatial discharge patterns in cutaneous afferents that can transmit other senses determine the itching sensation (the pattern theory). The question of whether "itch specific" neurons exist has not yet been resolved (2), because they could not be excluded as other sensory modalities (activation of the neurons by sensory experience other than itching).

The skin response to allergen exposure involves an itching sensation followed by a flare (vasodilatation) and wheal (plasma protein extravasation). In one reported study, capsaicin pre-treatment of human skin prevented the flare and decreased the itching sensation upon cutaneous allergy challenge, while the wheal response remained unchanged (3). It was therefore argued that the activation of capsaicin-sensitive neurons by allergens played a major role in the flare and itching reactions but not in the wheal response (3). The wheal response was considered to be a direct effect of histamine released from the mast cells and independent of mediator release from the capsaicin-sensitive nerve endings (3).

Based on the report of Andrew and Craig (4), who demonstrated a specific group of secondary neurons (spinothalamic tract, or STT) in the lamina I of the cat spinal dorsal horn clearly transmitting the itch signal to the ventral posterior or lateral nuclei of the thalamus, Schmelz (2) claimed support for the specific theory to explain itching.

Molecular Mechanisms Underlying itching

Histaminergic Itch Signaling

Histamine receptors (HR) are members of the G-protein coupled receptor (GPCR) family, and four receptors (H1R-H4R) have been recognized (5,6). H1R is a major receptor concerned with the itching sensation (7,8). H1R exerts its influence through activation of Transient Receptor Potential Vanilloid-1 (TRPV1) (7,8). The relationship between histamine and TRPV1 is also supported by the finding in rat dorsal root ganglion (DRG) neurons that histamine-induced calcium increase was attenuated by TRPV1 antagonists (8). Mice lacking TRPV1 were demonstrated to show impaired scratching behavior in response to histamine injection (9). Importantly, it was shown in human embryonic kidney 293 (HEK293) cells that histamine evoked inward currents only when TRPV1 and H1R were co-expressed on the cells, and not when either receptor was expressed alone (9). Together, these studies indicate the existence of a molecular link between H1R and TRPV1.

Several recent studies have shown the involvement of H4R in chronic itching, particularly in allergic and inflammatory conditions (6,10-12). On the other hand, there are only limited reports on the involvement of H2R and H3R in itching (13). The H2R agonist dimaprit failed to cause scratching, and the H2R antagonist cimetidine could not prevent histamine-induced itching in mice (6). No expression of H3R neurons has been reported in mouse sensory neurons (14). Thus, it is reasonable to consider that H2R and H3R have a minimal role in the itching sensation.

Cowhage and Protease Activated Receptor (PAR) Signaling

Cowhage plant (*Mucuna pruriens*) is a classic non-histaminergic pruritogen that causes intense itching when injected into the skin (15). Cowhage stimulates mechanically sensitive C fibers, whereas histamine activates mechanically insensitive C fibers (16–19). Interestingly, unlike histamine-induced itch, itching caused by cowhage is not associated with the classical wheal and flare response (17). The itch-inducing component of cowhage is a cysteine protease called mucunain, which is a ligand for protease activated receptor 2 (PAR-2) (20). Clinically, PAR-2 expression has been reported to be increased in the skin of AD patients with chronic itching (21) and also in a mouse model of dry skin (22). About 40–60% of small DRG neurons with unmyelinated fibers co-express substance P (SP), calcitonin gene-related peptide (CGRP), and PAR-2 (23).

Several TRP channels have functional correlations with PAR-2. TRPV4 and PAR-2 are co-expressed on peptidergic DRG neurons, and the PAR-2 agonist Ser-Leu-Ile-Gly-Arg-Leu-NH2 (SLIGRL-NH2) has been shown to evoke TRPV4-mediated calcium ($Ca2^{+}$) increase (24). Moreover, PAR-2 expression has also been shown to correlate with transient receptor potential ankyrin 1 (TRPA1) expression (25). Eighty percent of TRPA1-positive cells also express PAR-2, while 56% of PAR-2-positive cells express TRPA1 (25). TRPV1 is co-expressed with PAR-2 in DRG neurons (26). SLIGRL-NH2 also amplified afferent signaling by sensitizing TRPV1.

In addition, to peripherally mediated primary hyperalgesia, increase in the afferent signals may also subserve secondary hyperalgesia by central sensitization (26–28). In contrast to TRPA1, inhibitors of both phospholipase C (PLC)

and protein kinase C (PKC) suppressed PAR-2-induced central sensitization of TRPV1, as measured by calcium ($Ca2^{+}$) increase and TRPV1-amplified currents (26). Thus, both PLC and PKC seem to have roles in the intracellular signaling pathway in the sensitization of TRPV1 via PAR-2.

These results suggest the presence of multiple PAR-2-mediated intracellular signalling pathways for pruriceptive processing. It is likely that some TRP channels (TRPV1, TRPV4, and TRPA1) are regulated downstream of PAR-2.

Four Pathways for the Itching Sensation

Differences in the experimental animals, concentrations of pruritogens used, nature of the study (in vivo or in vitro) and complicated intracellular pathways may underlie the conflicting reports (for instance, with respect to TRPV1 participation in itching) among the reported studies. In short, four possible pathways for the itching sensation have been proposed (Figure 1), including the histaminergic (TRPV1-expressing/non-TRPV1-expressing) and non-histaminergic (PAR-2 or serotonin) pathways (29).

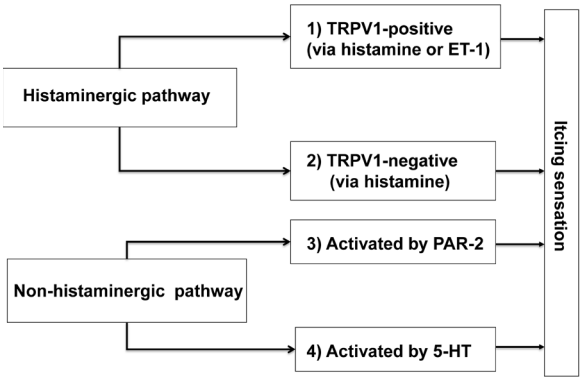

Figure 1: A schematic diagram showing the four putative itch signaling pathways. 1) TRPV1-expressing small sensory neurons that mediate itching *via* histamine or ET-1, as well as inflammatory pain. 2) TRPV1-negative (capsaicin-insensitive) pathway activated by histamine; this pathway likely transmits acute noxious heat as well. Non-histaminergic pathway activated by 3) PAR-2 or 4) 5-HT, which terminate in the lateral part of lamina I as separate from the histaminergic pathways.

TRPV1-positive histaminergic pathway

Recently, it was shown that histamine, a potent itch-inducing agent, excited the TRPV1 expressed on sensory neurons both in vivo and in vitro downstream of phospholipase A2 and lipoxygenases (30). Therefore, rather than the type of channels, it was suggested that specific types of primary afferent neurons might initiate itching (30). The TRPV1-expressing neurons have been suggested as the main sensors and mediators of itching (30). Phospholipase C (PLC) β3, a critical intracellular mediator in C-fiber nociceptors link the histamine and 5-hydroxytryptamine/serotonin (5-HT), with no relation to noxious pain sensation (31). Endothelin (ET)-1-evoked itching requires TRPV1, but not PLCβ3, suggesting a different intracellular downstream pathway for ET-1 and histamine (31). Curiously, the absence of any reduction of the scratching behavior caused by 5-HT or ET-1 in TRPV1-deficient mice contradicts the marked decrease of the response in mice with loss of the TRPV1-expressing neurons induced by intrathecal injection of capsaicin (31). On the other hand, H1R was also suggested to be involved in not just itching but histamine-induced TRPV1 sensitization (inflammatory pain) through the PLC/PKC pathway, because histamine-sensitive mouse DRG neurons are mostly also capsaicin-sensitive neurons (32).

Although there is increasing evidence to suggest that capsaicin-sensitive or TRPV1-expressing primary afferents participate in itching signaling (3,30–32), Nicolson et al., emphasized the existence of a specific subset of non-polymodal nociceptive primary afferent nerve fibers that mediate itching (1). In short, no specific nerve fibers participating only in the development of the itching sensation have been identified.

TRPV1-negative histaminergic pathway

Itching sensations could be mediated mostly by specific histamine-sensitive nerve fibers (specific theory) (1). Ninety per cent of histamine-sensitive neurons from the adult rat DRG were exclusively of small diameter (mean: 17.8 μm), and the majority were distinct from capsaicin-sensitive nerve fibers (1). In experiments of intradermal microdialysis application of vasoactive substances (prostagladin E, 5-HT, acetylcholine, bradykinin, and capsaicin) into the skin of healthy human volunteers, histamine had the highest pruritic potency, and capsaicin did not induce an itching sensation but intense pain (33). Although histamine-positive nerve fibers cannot exactly be classified as "itch specific" due to their excitation by pure algogens, they are "itch-

selective", and the existence of a subpopulation of nociceptors responsible for itching is strongly suggested (33).

Histamine release into the dermis by mast cell degranulation seems to be the first event in the sensation of itching via excitation of cutaneous unmyelinated C fibers (1). Whether capsaicin-sensitive C fibers or mast cells are mainly involved in plasma extravasation and itching was studied using mast cell-deficient W/Wv and mast cell-rich NC mice (34). No plasma exudation occurred in adult NC mice neonatally injected with subcutaneous (s.c.) capsaicin, even when stimulated by compound (Co) 48/80, a potent mast cell-degranulating agent (34). The scratching behavior caused by itching was not different between capsaicin-pretreated and vehicle-treated control NC mice. Nor was there any difference in the scratching behaviors elicited by Co 48/80 between rats pre-treated with s.c. capsaicin within two days of birth and the vehicle-treated control animals (35). Thus, itch-specific C fibers were speculated to be capsaicin-insensitive.

The opioidergic pathway enhancing itchiness was thought to be activated by capsaicin treatment (35), because scratching was shown to be inhibited by a morphine antagonist, naloxone, in rats treated with capsaicin in the neonatal stage (36). This could be explained by the pain pathway not being functional due to the marked decrease of nociceptive C fibers by neonatal capsaicin treatment, and the remaining nociceptive Aδ (pain)-fibers being affected by treatment with an opioid or opioid antagonist, resulting in a reduction or enhancement, respectively, of the itching sensation. On the assumption that neonatal capsaicin treatment results in a reduction of the unmyelinated C fibers in the primary afferents by 70% (37), the remaining capsaicin-insensitive C fibers (about 30%), could be involved in the itching sensation induced by histamine release from the mast cells (34); furthermore, these fibers could sense acute noxious heat as well (37–40). These assumptions may be supported by the finding that most histamine-sensitive small neurons in cultured rat DRG cells are capsaicin-insensitive (1).

Non-histaminergic PAR-2 pathway

The existence of a non-histaminergic pathway mediating itching as distinct from the histaminergic pathway was indicated by intradermal injection of a PAR-2 agonist with pruritic or algesic agents (5-HT, capsaicin, or mustard oil) commonly activating the superficial dorsal horn neurons (41).

From neurophysiological studies, a non-histaminergic pathway mediated by PAR-2 has been discovered by Steinhoff (21). Upon skin contact, cowhage spicules release mucunain, a cysteine protease that serves as a ligand for PAR-2 (20) and elicits a strong sensation of "itch without flare" that lasts for several minutes (42,43). It has been reported recently that a model of PAR-2-mediated itching may be a more appropriate model for pathological pruritus than models based on histamine, since antihistamines are not effective in clinical settings as anti-pruritic agents (21). PAR-2 has been shown to play a crucial role in the itching associated with atopic eczema (21). The symptoms of itching evoked by this plant are very similar to the characteristics of itching in certain diseases like AD (20). The serum level of the endogenous PAR-2 agonist tryptase was increased by up to fourfold in cases of AD (21). Increased expression of PAR-2 on the primary afferent nerve fibers has been shown in skin biopsies of AD patients (21), and cowhage has been shown to accurately reproduce the itch. Intracutaneous injection of the endogenous PAR-2 agonist enhanced and prolonged the itching sensation. Suppression of mast cell degranulation by antihistamine agents led to the assumption that mast cell mediators other than histamine could act as key mediators of itching in AD (21). Besides neuronal cells, PAR-2 signals are also increased in keratinocytes, endothelial cells, smooth muscle cells, and inflammatory cells, all of which are involved in the pathophysiology of various chronic inflammatory diseases (21). PAR-2 activation has been shown to increase the release of IL-6 and granulocyte-macrophage colony-stimulating factor from keratinocytes in AD patients (21). Since PAR-2 is expressed in various inflammatory cells, including mast cells and T cells, it is considered to be involved in both neurogenic and non-neurogenic inflammation in human skin (21). Existence of a histamine-independent, protease-dependent and PAR-2-mediated itch pathway may open up the possibility for development of beneficial therapies for pruritus and cutaneous inflammation.

Currently, research on itching has shifted from histamine-based models to cowhage-based models (44). Accordingly, researchers worldwide are trying to develop PAR-2 antagonists that would bind to the PAR-2 receptor to block the development of the particular itching sensation. The simplest way then to alleviate itching would be to develop peripherally acting agents such as topical creams or systemic agents that would

block the PAR-2 pathway (45).

Non-histaminergic 5-HT pathway

5-HT elicits an itching sensation when applied to human skin (46,47) and has been thought to be involved in the itching associated with pruritic diseases, such as polycythemia vera (48) and cholestasis (49). 5-HT is a more potent inducer of scratching in mice as compared to histamine (50,51). Algesic chemicals such as capsaicin elicited little scratching in mice (50), suggesting that histamine-induced scratching behavior is different from the behavior elicited by algesic chemicals. To investigate the pruritogenic activity of 5-HT in animals, Yamaguchi et al. examined whether an intradermal (ID) injection of 5-HT might elicit itch-associated behaviors in mice and point to the 5-HT receptor subtypes involved in this response (51). The scratching induced by ID injection of 5-HT is likely to be an itch-associated response (51). This action of 5-HT may be mediated partly by cutaneous 5-HT_2 receptors (51).

Jink and Carstens (52), investigated whether intracutaneous 5-HT might excite the superficial dorsal horn neurons, and pruritic (5-HT, histamine) or algesic chemicals (capsaicin, formalin) might elicit scratching behavior in the rat. 5-HT appears to be more pruritogenic than histamine, as assessed by the scratching and shaking behaviors elicited by it. Superficial dorsal horn neurons were excited over a behaviorally relevant time course (52). However, they are not itch-specific, because most neurons responded to pain-producing stimuli as well (52). Rodent mast cells contain 5-HT and unmyelinated nerve endings express 5-HT_2 receptors at the dermal-epidermal junction where itch is evoked in humans (52). Intraplantar administration of 5-HT has been reported to produce inflammation and hyperalgesia. Behavioral responses to the algogen formalin are reinforced by 5-HT and blocked by 5-HT antagonists. In mice, 5-HT elicited scratching behavior via the 5-HT_2 receptor (52). The relationship between the scratching response and the dose of 5-HT showed a bell-shaped curve, therefore, it was speculated that 5-HT is pruritic at lower doses, but elicits pain at higher doses, along with a decrease of the scratching behavior (52).

The superficial dorsal horn neurons might nonetheless contribute to neural pathways that distinguish between pain and itching by some neural mechanism, such as by changing the frequency coding.

Conclusion

Four possible pathways mediating the development of the itching sensation have been suggested: histaminergic 1) TRPV1-expressing or 2) non-TRPV1-expressing pathways, and non-histaminergic 3) protease-activating receptor (PAR-2) or 4) serotonin (5-HT) pathways. However, no itch-specific unmyelinated C-fiber pathway has been identified as yet.

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Conflict of Interest

None.

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Conception and design: HN, AH
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Critical revision of the article for the important intellectual content and final approval of the article: AH

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