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TRP Channels and Pruritus

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Abstract: Itch (pruritus) is one of the most often seen sensory phenomena in clinical practice. Recent neurophysiological findings proposed the existence of a novel pruriceptive system which includes a multitude of pruritogenic (itch-inducing) peripheral mediators, itch-selective pruriceptors, sensory afferent networks, spinal cord neurons, and certain central nervous system regions. In this review, we first introduce major features of the pruriceptive system. We then focus on defining the roles of transient receptor potential (TRP) ion channels in skin-coupled itch and provide compelling evidence that certain thermosensitive TRP channels (especially TRPV1, TRPV3, TRPV4, and TRPA1) are indeed key players in pruritus pathogenesis. Finally, we propose TRP-centered future experimental directions towards the therapeutic targeting of TRP channels in the clinical management of itch.

Keywords: TRP channels, pruritus, pruritogenic, thermosensitive

1. INTRODUCTION – THEORIES OF ITCH

Classically, itch (pruritus) is defined as an "unpleasant sensation that elicits the desire or reflex to scratch" [1]. Although this definition has been standing the test of time, intense research efforts of the last decades have significantly broadened our knowledge and reformed some of the basic concepts of pruritus.

Although both itch and pain are unpleasant sensations associated with protective motor responses, the phenomenon of itch clearly differs from that of pain in the subjective sensation as well as the resulting motor activity. In spite of the resulted sensational differences, the itch processing "pruriceptive" system (similar to the nociceptive system) can be characterized as an evolutionarily ancient neuronal network for the avoidance of potentially harmful agents (e.g. mechanical objects, insects, skin irritants, allergens, toxic plants) endangering the integrity of the body. With respect to its aim, the itch-induced scratching (defined as a goal-directed movement against noxious agents that have successfully passed the skin barrier and have already invaded the organism) can be compared to the withdrawal reflex evoked by painful stimuli. However, the appearances of these two motor responses are quite distinct and they represent different strategies of protection [2-4]. Due to the intimate relationship of itch and pain sensations (see below), the distinction between the pruri- end nociceptive systems is not a trivial task, and there are different approaches existing even till today [5].

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Older studies defined itch as a "sub-threshold pain". Indeed, it was thought that itch sensation is evoked by weak activation of the nociceptors and the resulted neural activity is transmitted and processed by the same pathways and higher centers, which, in case of a more intense activation, are responsible for the processing of pain sensation [6]. Theories arguing for this hypothesis are summarized as the *intensity theory of itch*; based on this theory, itch and pain sensations are differentiated only by the intensity of the given stimulus activating the same branch of sensory system; i.e. pruriceptive system = nociceptive system [2, 4].

This concept was strongly challenged by a multitude of neurobiological findings which collectively support the existence of an autonomous *pruriceptive system* organized (more or less) independently from the pain sensation. Indeed, a growing number of evidence supports the presence of itch-specific sensory fibers which are different from the pain sensing ones [7, 8]. Furthermore, it is well known that pain inhibits itch [3, 5, 8-11]; actually, the itch-induced scratching response itself is a motor activity to induce pain and hence to alleviate itch. The logical consequence of these data and other observations together led to establish the so called *specificity theory* which proposes the existence of specialized receptor structures, neural pathways as well as higher centers processing exclusively itch, but not pain. Although recent data (see below) strongly support the specificity theory, it is also clear that the pain and itch sensing systems are intimately related to each other [3, 10, 11]. Below, we introduce several overlaps of the pruriceptive and nociceptive systems, especially regarding receptors and mediators shared by the two systems. Based on these data, the *selectivity* (rather than the specificity) *theory* gives a better explanation about their relationship. The selectivity theory states that a subpopulation of nociceptors is sensitive for pruritogenic stimuli;

1876-3863/12 2012 Bentham Open

moreover, they have a specific central connection which differs from other nociceptors. The selective activation of these pathways (i.e. by pruritogenic stimuli) results in itch sensation whereas painful stimuli, activating both the itch sensitive and insensitive nociceptors mask/inhibit the itch sensation and result in a (pure) pain sensation [12, 13].

2. ORGANIZATION OF THE PRURICEPTIVE SYSTEM

2.1. Pruriceptive Sensory Fibers

A dense network of unmyelinated sensory C-afferent axons innervates the skin and hence ensures the appropriate detection of potentially harmful stimuli. Approximately 80% of these slow conducting fibers are mechano-sensitive polymodal nociceptors, responding to both chemical and thermal stimuli; the remaining ~ 20% are mechano-insensitive ones activated by chemical signals [14, 15]. In the mechano-insensitive group, an "itch-sensitive" subset of neurons shows strong and sustained activation by histamine, the prototypical itch mediator (see below). These unmyelinated, histamine sensitive sensory neurons have large receptive fields and poor discrimination threshold for histamine induced itch. Furthermore, they are characterized by a particularly low conduction velocity and high transcutaneous electrical thresholds [7, 16-20]. These C-fibers are also suggested to be involved in the generation of the axon reflex erythema [17].

Importantly, the existence of other, histamine-insensitive (but most probably mechano- and heat-sensitive) pruriceptors was also proposed [8]. Intradermal insertion of spicules from the pods of the cowhage plant (*Mucuna pruriens*), in contrast to histamine, activated only mechano-sensitive (but not mechano-insensitive) C-fibers indicating the existence of distinct, histamine-insensitive pruriceptive pathways [21]. In addition, Ikoma *et al* [22] demonstrated that low intensity and high frequency focal electrical stimulation was also able to evoke itch sensation, but without erythema. The lack of the axon reflex also indicates that itch sensation could be transmitted by a subset of sensory neurons clearly different from the above mentioned histamine sensitive ones. It was also shown that some of the mechano- and heat sensitive nociceptive C-fibers (but not the heat-insensitive ones) can be activated by histamine or other pruritogenic substances [23]. Furthermore, a recent study demonstrated that nociceptive, myelinated A-fibers may also contribute to the itch sensation evoked by cowhage [24]. This intriguing diversity of afferent pruriceptive fibers suggests that the various "submodalities" of itch experienced by humans could be due to the selective "encoding" from the very first level of the sensory system.

2.2. Spinal Itch Processing

Certain studies also suggest that encoding of itch sensation is separated from the nociceptive system also at the spinal cord level. Indeed, in cats, histamine-responsive mechano-insensitive neurons were described in the dorsal horn (lamina I) of the spinal cord [25]. However, histamine-responsive spinothalamic tract neurons were also identified among the mechano-, heat- and capsaicin-sensitive ones in primates [26]. It is therefore suggested that spinal projection neurons may receive innervation from both itch sensitive and nociceptive primary fibers [13] which supports the selectivity over the specificity hypothesis regarding the relation of itch and pain transmitting pathways. However, in spite of the overlap with the pain sensitive pathways, distinct, non-overlapping populations of spinothalamic tract neurons, transmitting histaminergic and non-histaminergic (cowhage evoked) itch sensations, were described [27]. Indeed, the two sensations can be selectively inhibited, even if they are transmitted by the same spinothalamic tract neurons; it was demonstrated that only the histamine (but not capsaicin) evoked activity of the same spinal projecting neurons can be inhibited by eliciting scratching within the respective cutaneous receptive field [28].

Of further importance, another subset of itch-specific spinal cord neurons was identified recently. In mice, ablation of gastrin-releasing peptide receptor (GRPR) expressing neurons in the lamina I of the spinal cord (without affecting pain sensation and motor activity) abolished the scratching response to all pruritogenic stimuli, including histamine-sensitive and insensitive ones. It is important to note that the practical disappearance of the scratching behavior was related to the loss of the neurons and not to the loss of the receptors [29], although the mutation of the GRPR was also reported to slightly decrease the scratching. Consequently, GRPR agonists were able to induce itch [30]. A most recent study suggests that neuromedin B, a GRP and bombesin analogue peptide, is highly and selectively expressed in on itch and pain sensitive neurons in dorsal root ganglia (DRG). Moreover, neuromedin B was suggested to play a role in neurotransmission between DRG sensory neurons and spinal cord interneurons whereas GRP is mostly expressed on spinal cord neurons [31]. These results indicate the urgent need to re-evaluate the concept about the role of GRP in itch transmission since it may also act at another level of itch processing pathway than earlier studies suggested.

Interestingly, in contrast to nociceptive second-order spinothalamic neurons, the itch-specific projection neurons do not exhibit spontaneous activity. It was proposed that the lack of spontaneous activity may be generated by an active (tonic) inhibition exerted by pain-processing neurons [9-13]. Recently, a subpopulation of inhibitory interneurons expressing the transcription factor Bhlhb5 during the development was identified as the mediator of the pain evoked itch inhibition [11]. Furthermore, descending adrenergic pathways were also suggested to control the inhibitory interneurons of the spinal cord [32, 33].

2.3. Higher Itch Centers

The itch-sensitive spinal neurons project from lamina I of the spinal cord to the ventrocaudal part of medial dorsal nucleus of the thalamus which has connections to the anterior cingulate and dorsal insular cortex [25, 34]. Beyond these regions, alterations in neuronal activities in several brain areas were reported in relation to itch processing above the thalamus; these include the primary and secondary somatosensory cortex, the premotor and supplementary motor cortex, the inferior parietal lobe, the cerebellum as well as certain temporal regions [34-40]. Although there are slight variations in the itch-related regions reported among studies, which could be due to different experimental designs [40], there is a consensus in the literature about the

following: (i) the sensory-discriminative component of itch is likely processed in the primary somatosensory cortex; (ii) the activities of the anterior cingulate and insular cortex may be related to the motivational and affective components; (iii) the motor component of the goal-directed scratching is attributed to the premotor and supplementary motor areas; (iv) these brain regions are also involved in the central pain processing; therefore, distinction of the two sensations is mainly based on the differential activation patterns of mostly identical centers [4, 10, 35-37, 41, 42].

Most recent studies aimed to identify specific central nervous system activity patterns related to quantitative and qualitative characteristic of itch sensation. In a PET study, a positive correlation was found between the subjective intensity of histamine evoked itch sensation and the activity of the insula and the anterior cingulate cortex [43]. Moreover, Papoiu and colleagues [44] identified different cerebral activity patterns in itch evoked by histamine or cowhage.

2.4. Types of Itch

Although different itch classifications have been promoted, we (as well as others) suggest distinction between the below types of itch. These widely accepted categories extensively reflect the above introduced hierarchical organization of the pruriceptive system; moreover, they are based on operational, clinically relevant definitions [2, 4, 42, 45, 46].

- ✓ **Pruriceptive itch**: The most "trivial" category of itch which involves all peripherally induced pruritus arising from skin injuries (e.g. insect bite, botanical irritation) or diseases such as dry skin, atopic dermatitis (AD), psoriasis, infestations (e.g. scabies, pediculosis), urticaria.
- ✓ **Neurogenic itch**: Centrally induced pruritus caused by systemic disorders such as chronic liver disease (cholestasis), chronic renal failure, and thyroid dysfunction which directly trigger higher levels of the pruriceptive system. In this case, the cause of itch is not a primary failure of the itch processing nervous system, although the pathological activation of the nervous system is a key element in the development of neurogenic itch.
- ✓ **Neuropathic itch**: In this case, pruritus develops due to a primary neurological disorder of the central or peripheral nervous systems. For example, neuropathic itch can be induced by certain brain tumors, multiple sclerosis, peripheral neuropathy (e.g. postherpetic pruritus), nerve compression or irritation (e.g. notalgia paresthetica, brachoradial pruritus).
- ✓ **Psychogenic itch**: Pruritus is related to psychological or psychiatric disorders such as parasitophobia, obsessive-compulsive disorder or different psychoses leading to "neurotic or psychotic excoriations".

With respect to the versatile role of TRP channels in different cellular sensory functions [47], below we focus on the first itch category (pruriceptive itch), i.e. the characteristics of pruritus originating from the skin as a result of a peripherally applied stimulus-induced sensation.

2.5. Pruritogens

Pruriceptive itch is induced by various agents, collectively referred to as pruritogens, stimulating the itch-selective afferent fibers. These molecules are mostly released from various cell types of the skin which are in close proximity to or even in direct physical contact with sensory nerve endings [48-51]. These mediators stimulate and/or sensitize the itch-selective sensory afferent fibers hence evoking action potential firing. The release of these mediators can be provoked by external effects (e.g. insect bite), by various skin diseases or by pathological (e.g. inflammatory) conditions. However, the cutaneous cell – sensory neuron connection is not a "one-way-street": as neurons can be stimulated by various mediators liberated from the non-neural cells of dermis and epidermis, sensory neurons can also release certain neuropeptides which may act not only on other neurons but also on various non-neuronal cutaneous cell types [49]. Therefore, via these interactions, alterations in the general condition and homeostatic balance of the skin (e.g. barrier function, inflammatory responses) may significantly affect the release of pruritogens and hence may induce itch [8, 41, 51-53]. Since TRP channels play pivotal roles in these epithelial-neuronal interactions (see below), first we shortly summarize the roles of the most important pruritogen mediators and their related cellular mechanisms involved in itch sensation.

2.5.1. Histamine

Histamine is probably the most-known pruritogen [54, 55]. It is mostly released from activated mast cells and basophil granulocytes, central players in local cutaneous inflammatory as well as systemic allergic responses [56, 57]. Although histamine was shown to directly activate sensory neurons and evoke itch both in humans and rodents, its exclusive etiological role in pruritic skin diseases is quite rarely documented. Histamine is the key pruritogen in various forms of urticarial diseases in which antihistamines are mostly effective [58, 59]. However, several other skin diseases (e.g. AD) with chronic pruritus are very often resistant to antihistamine therapies [54, 55, 60]. In a mouse dry skin model, histamine (in contrast of other itch inductors) did not stimulate scratching behavior [61], further supporting the hypothesis that histamine is not a central mediator of chronic pruritus.

So far, four histamine receptor subtypes (H_1-H_4) are identified; all of them are G–protein coupled receptors [54]. Among them, H_1 and H_4 are widely accepted as receptors responsible for itch sensation; however, quite interestingly, an H_3 receptor inverse agonist was also shown to induce scratching behavior in mice [54, 62, 63].

2.5.2. Proteases and their Receptors

Serine proteases (trypsin, chymotrypsin, chymase) are capable of activating metabotropic, G-protein coupled proteinase-activated receptors (PARs) [64, 65]. Among them, PAR2, expressed by sensory neurons, is considered as a key molecule in itch sensation [66-68]. In experimental models, by using cowhage spicules (which contain the protease mucunain) to activate PAR2, a histamine-independent parallel itch pathway was identified and found to significantly contribute to chronic itch sensation [10, 27, 44,

69, 70]. Interestingly, the activation of PAR2 by kallikrein (a tryptic enzyme) related proteases was also shown in epidermal keratinocytes [71] which further argue for the impact of the pathway in pruriceptive itch.

Of great clinical importance, in AD patients the dysregulation of PAR2 and the activating proteases were observed both in sensory fibers and in epidermal keratinocytes [72]. In addition, kallikrein activity was also increased in pruritic papular eruptions [73]. Furthermore, mutations in the serine protease inhibitor Kazal-type 5 in Netherton syndrome lead to increased PAR2 stimulation due to epidermal protease hyperactivity; this alteration may have a causative role in the development of the AD-like symptoms in this disease [74].

2.5.3. Role of Neuropeptides and Neurotrophins

Various neuropeptides, which are locally released from afferent nerve endings of activated sensory neurons, via the aforementioned intercellular communication circuits, can evoke the release of pruritogenes from non-neuronal cell types of the skin. For example, substance P (SP) is not only a well-known mediator of pain but it is also able to induce the degranulation of mast cells and hence the release of histamine [75-78]. Beyond histamine, mast cells may also release several other (mostly inflammatory) mediators which process can be also triggered by SP or vasointestinal polypeptide [78]. These mast cell-derived mediators may, in turn, further activate sensory neurons resulting in further SP liberation [41, 49, 51-53]. Participating in this "positive feedback," SP can be considered as an important etiological factor of itch in several diseases. Indeed, in AD patients, significantly elevated plasma SP levels were reported [79]. Likewise, increased density of SP+ nerve fibers was found in prurigo nodularis lesions and chronic pruritus-affected skin regions compared to non-affected skin areas of the same patients [80, 81]. Furthermore, in cholestasis associated with itch, increased plasma SP concentrations were found when compared to cholestasis without itch or to healthy controls [82].

The pruriceptive role of another neuropeptide, calcitonin gene related peptide (CGRP) is less documented and rather controversial. CGRP was reported to prolong the latency of SP induced itch [83]. Interestingly, lower CGRP plasma levels were found in AD patients during the exacerbation period, although significantly higher values were measured in patients with severe pruritus than in patients with mild pruritus [79]. In other studies, increased levels of CGRP were found in several patients with AD and with other pruritic dermatoses (e.g. nummular eczema, prurigo nodularis) [84, 85]. In anesthetized mice, the scratching itself increased the outgrowth of CGRP+ nerve fibers [86]. Based on these results, it is tempting to hypothesize that in some pruritic disorders the decreased CGRP level might be a primary itch-inducing factor whereas the elevation of CGRP might be only a delayed consequence of scratching.

Beyond sensory neuron-derived neuropeptides, neurotrophins (such as nerve growth factor [NGF], neurotrophin-3 and -4, and glial cell-line-derived neurotrophic factor [GDNF]), well-known regulators of cutaneous nerve development and regeneration [87], may also play an etiological role in the development of itch sensation and pruritic diseases. Several cell-types of the skin (e.g. keratinocytes, mast cells, and fibroblasts) produce and release neurothrophins [88]. Neurotrophins significantly influence itch sensation in various pathophysiological conditions via modulating innervation density of the skin as well as expressions and/or sensitivity of receptors expressed by sensory neurons. For example, in inflammation and injuries of the skin, expression of NGF is highly increased which initiates acute sensitization and sprouting (leading to chronic sensitization) of C-type afferent fibers via activating specific TrkA receptors [87, 89-91]. Increased expression and plasma level of NGF were also reported in pruritic skin diseases such as in AD [92], prurigo nodularis [93] and psoriasis vulgaris [94]. Likewise, increased expressions of neurotrophin-4 were found in lesional skin of AD patients [95]. Of further importance, NGF levels were well-correlated with symptom severity in AD and decreased by effective therapies [96], further supporting the etiological role of this neurotrophin. In good accord with these findings, atopic human keratinocytes, co-cultured with porcine sensory neurons, were shown to induce an increased neurite outgrowth which effect was mediated by NGF and GDNF [97]. It was also shown that NGF not only stimulates the sprouting of itch sensitive C-fibers but also upregulates the expression of certain neuropeptides (SP, CGRP) [97-100] and receptors (e.g. TRPV1, see below) [101-103] involved in itch. Finally, it should be noted that neurotrophins may exert their pruritogenic effect also via activating non-neuronal cell types of the skin. Indeed, NGF and neurotrophin-3 were reported to induce degranulation and histamine release of mast cells [104-107].

2.5.4. Inflammatory Mediators as Peripheral Itch Sensitizers

Nociceptive sensory neurons can be sensitized by a multitude of inflammatory mediators produced in acute or chronic inflammation of the skin; the sensitization is referred to as inflammatory hyperalgesia [108]. The plethora of mediators are released from various cell types of the skin such as mast cells (histamine, tryptase, serotonin, tumor necrosis factor-α (TNF-α), leukotriens, prostaglandins, etc.), keratinocytes (prostaglandins, interleukins, neurotrophins, etc.), sebocytes (prostaglandins, leukotriens, interleukines, etc.), endothelial cells (kinins, endothelins, etc.) or immune cells (interleukins, chemokines, etc.). Importantly, the majority of these mediators possess marked pruritogenic potentials [5, 8, 41, 46, 109-111].

Prostaglandins PGE1 and E2 were shown to initiate or potentiate itch responses, which effect (at least in some cases) may be independent of histamine liberation from mast cells [112-115]. In addition, intradermal injection of other eicosanoids, such as leukotriene B4 (LTB4) or a non-hydrolysable thromboxan A2 analog evoked itch-associated responses in mice [116, 117]. Furthermore, PAR2 activation on keratinocytes resulted in LTB4 (and PGE2) release *in vitro* and evoked scratching behavior *in vivo* which effects were reduced by 5-lipoxygenase inhibition arguing for that LTB4 may mediate the effect of PAR2 activation [118]. Interestingly, LTB4 is also suggested to play a role in mediating SP-evoked itch [119].

On the other hand, PGD2 exerts an anti-pruritic effect [120, 121] and was shown to be able to suppress IgE induced histamine release from the RBL-2H3 mast cell line [122].

Further supporting its anti-pruritic actions, both pharmacological inhibition and *in vivo* silencing of cyclooxygenase COX1 resulted in (among else) decreased PGD2 levels and enhanced the scratching behavior in NC/Nga mice, a murine model of AD [123, 124].

The vasodilative, pro-inflammatory, and pain-inducing mediator, bradykinin also induces itch [20, 125, 126]. Its mechanism of action involves increased histamine release from mast cells [127], augmentation of histamine evoked responses [128, 129] as well as sensitization of sensory afferent fibers and their receptors (e.g. TRPV1) [128]. Bradykinin applied to the skin elevates the release of neuropeptides and prostaglandins [130] and was also reported to evoke itch on lesional atopic skin via a histamine independent manner [131]. Of further importance, both B1 and B2 bradykinin receptors were shown to mediate protease and PAR2 activation-evoked scratching in mice [132, 133] and bradykinin was found to evoke scratching responses in complete Freund's adjuvant-inflamed skin [134].

Immune-competent cells of the skin can produce several cytokines and interleukins (ILs) which also contribute to the pathogenesis of pruritus and pruritic disorders. For example, IL-2 was found to induce itch [135, 136] and to activate a histamine and bradykinin sensitive subpopulation of sensory C-fibers [137, 138]. Interestingly, bradykinin potentiated the chemo-responsiveness of polymodal nociceptors to IL-2 [139] resulting in a bi-directional augmentation. Clinical relevance of IL-2 in pruritic disorders was also implicated since elevated IL-2 serum levels were reported in hemodialyzed patients with uremic pruritus compared to non-pruritic subjects [140]. Moreover, cutaneous overexpression of IL-4 in mice resulted in a AD-like pruritic inflammatory skin disease [141] and increased IL-6 like immunreactivity was reported in cutaneous nerve fibers of prurigo nodularis patients [142].

Recently, IL-13 and IL-31 were closely linked to AD. Skin specific overexpression of IL-13 resulted in a chronic inflammatory phenotype associated with pruritus, infiltration of various immune cells, and up-regulation of chemokine and cytokine genes [143]. As in the case of IL-13, transgenic overexpression of IL-31 also induced severe pruritus and dermatitis in mice [144]. Further supporting its role, increased expression of IL-31 was found both in an atopic mouse model [145] and in AD patients [146, 147] in good correlation with the scratching behavior in mice [148] and the symptom severity in humans [149, 150]. The exact pruritogenic mechanism of action of IL-31 is still unclear although IL-31 receptors were detected on a fraction of small diameter sensory neurons [151] as well as on normal human epidermal keratinocytes (NHEKs) [152]. On NHEKs, stimulation of toll-like receptor TLR-2 resulted in the elevation of IL-31 receptor expression levels, followed by an enhanced secretion of the pro-inflammatory chemokine ligand CCL2 (also known as monocyte chemotractic protein-1). Intriguingly, this action in missing from epidermal keratinocytes of AD patients where TLR-2 expression is impaired [152]. Finally, it should be mentioned that in pruritic myeloproliferative disorders, mast cells release higher amount of IL-31 and other pruritogenic mediators [153].

3. ROLES OF TRP CHANNELS IN PRURITUS

As shown above, pruritus is a complex sensory modality processed by mostly selective neural pathways and influenced by a plethora of mediators released from both sensory neurons and cutaneous non-neuronal cell types. So, the key question is: why do we think that the transient receptor potential TRP channels are of great interest in further understanding the (patho)physiological mechanisms underlying pruriceptive itch?

Here are a few "convincing" examples:

- ✓ TRP channels function as broadly expressed polymodal "cellular sensors" sensitive for alterations and agents of the physico-chemical environment (e.g. temperature, pH, osmolarity, ionic concentrations, endogenous mediators, external chemical irritants, etc.) [47, 154]. Therefore, they are "prone" to be sensitive for pruritogenic effects, substances and mediators as well.
- ✓ TRP channels not only act as "sensors" but also as key "effectors" of various (patho)physiological processes (e.g. cellular homeostasis of different ions, secretory mechanisms, sensory functions of the nervous system, inflammation, etc.) [155-160]. In addition, they exert fundamental influence on cell-fate as they regulate differentiation and proliferation of different cell types [161-163]. Hence, it is conceivable that they also influence a multitude of processes involved in pruritus.
- ✓ Due to their expression patterns, they are especially promising candidates to "molecularly control" the itch-coupled cellular mechanisms. Indeed, TRP channels are widely expressed on both sensory neurons and on several cutaneous non-neuronal cell types. Therefore, they hold perfect "molecular positions" in the pruritogenic crosstalk of cutaneous cells and sensory neurons [8, 41].
- ✓ Some members of the TRP channel family were reported to play crucial roles in pain sensation [164, 165] which, as was shown above, is closely related to the overlapping itch sensation [4, 5, 8, 41].

3.1. The family of TRP ion Channels

The *Drosophila* TRP channel [166, 167] has become the founding member of the TRP ion channel superfamily which recently counts 28 mammalian members divided into 6 subfamilies based on structural homology. These are the canonical (or classical, TRPC), the vanilloid (TRPV), the melastatin (TRPM), the mucolipin (TRPML), the polycystin (TRPP) and the ankyrin (TRPA) subfamilies. The TRP proteins are non-specific cationic channels (mostly permeable for Ca^{2+} ions) containing 6 trans-membrane domains [154, 168-170]. Besides functioning as sensors of external stimuli in physiological circumstances, they are also involved in the development of several pathological conditions and diseases [158, 171, 172].

With respect to pruritus, certain thermosensitive TRPs (especially TRPV1, TRPV3, and TRPA1) have the greatest significance. However, some other member of TRP family may also contribute to development of itch and related diseases [4].

3.2. TRPV1

3.2.1. Activation and Sensitization of TRPV1

The most extensively studied TRPV1 is the firstly described member of the TRPV subfamily. It was identified in rat DRGs as the receptor for capsaicin, the pungent ingredient of red hot chili peppers (*Capsaicum sp.*) [173, 174]. The receptor itself is a highly Ca^{2+} permeable non-specific cation channel, which, like TRP channels in general, is formed in a tetrameric structure [173-175]. Not only capsaicin and other capsaicinoids, but a plethora of other botanical substances can activate TRPV1. The list of these herbal compounds is quite long and (among others) involves piperine, eugenol, zingerone, ginger-derived substances and, maybe most importantly, resiniferatoxin (RTX), an ultrapotent capsaicin analog isolated from *Euphorbia resinifera* [176-178].

Although these compounds serve as excellent experimental tools to study TRPV1 properties and, at the same time, are potential candidates for therapeutic applications, at least from physiological point-of-view the multitude of the below endogenous TRPV1 activating agents and mechanisms are possibly of greater importance. These involve heat (≤43°C), acidosis (pH<5.9) [173] and mostly arachidonic acid derived lipid mediators such as the endocannabinoid anandamide [179, 180], the "endovanilloid" N-arachidonoyl-dopamine [181], and lypoxigenase products [182, 183]. Besides direct activation mechanisms, several molecules are able to act on TRPV1 via first activating their specific receptors and then initiating downstream signaling pathways leading to the sensitization of TRPV1. Indeed, bradykinin [183, 184], ATP [185], lipoxygenase products [182, 183], prostaglandins [186, 187], histamine [188], various neurotrophins (NGF, neurotrophin-3 and -4) [91, 102, 184], TNF-α [189, 190], pro-inflammatory chemokines [191], and PAR2 [192, 193] were all reported to sensitize TRPV1 via various signal transduction pathways. TRPV1 was shown to be modulated by the protein kinase C (PKC) [183, 194-196], phospholipase C (PLC), and phosphatidylinositol 4,5-bisphosphate (PIP_2) [184, 197-200] systems as well as by intracellular ATP [201]. The sensitization process eventually results in the shift of the activation temperature and voltage threshold towards more physiological ranges [195, 202-204].

3.2.2. Function of TRPV1 Expressed on Sensory Afferents

TRPV1, expressed by the polymodal C-type nociceptive sensory neurons, was shown to play a key role in peripheral pain sensation. Acute activation of TRPV1 first excites the polymodal nociceptive neurons by initiating depolarization and concomitant action potential firing resulting in the induction of pain [174, 205]. Via sensitization by the above endogenous mechanisms, TRPV1 can integrate the effect of various noxious, inflammation-related stimuli resulting in the activation of TRPV1 even at physiological temperature leading in thermal hyperalgesia [205, 206] which phenomenon was not found in TRPV1 KO mice [205, 207, 208]. In addition to the "classical" nociceptive afferent functions, these neurons may also release their neuropeptide content upon TRPV1 activation (efferent functions of the sensory afferents). The neuropeptides (e.g. SP and/or CGRP), by acting on the neighboring cell populations in the skin, may then initiate the onset of neurogenic inflammation [53, 64, 209]. Importantly, prolonged stimulation of TRPV1 may induce desensitization and emptying of the neuropeptide stores resulting in the suspension of the interplay between sensory neurons and cutaneous non-neuronal cells and hence inhibit the further exacerbation of the inflammatory processes [155, 205].

3.2.3. Contribution of Neural TRPV1 to Itch Sensation Induced by Various Pruritogens

Increasing amount of evidence strongly argue that the activation of TRPV1 can evoke not only pain but also itch. Indeed, as detailed above, most of the endogenous agents and mechanisms that activate and/or sensitize TRPV1 are also considered as potent pruritogens. Moreover, it is apparent that the quality of the evoked itch sensation depends on the presence of these pro-inflammatory mediators and neuropeptides. For example, during seasonal allergen exposure in allergic rhinitis, nasal application of TRPV1-activators induce itch [210]. In addition, intrathecal application of RTX also evoked scratching responses in animal experiments, which effect was potentiated in the presence of SP [211]. Interestingly, intradermally injected capsaicin evoked itch responses (scratching) if skin inflammation was previously induced by complete Freund's adjuvant; however, in control animals, capsaicin induced only pain responses (licking) [212].

These results also indicate that the neuronal expression of the TRPV1 is not restricted to the nociceptive system; instead, it is also expressed (and hence plays a functional role) in the pruriceptive system. The prototypic TRPV1-selective activator capsaicin reportedly activates both histamine-reactive and non-reactive mechano-insensitive C-fibers as well as mechano-sensitive ones [20]. With respect to the mechanisms how TRPV1 can contribute to itch sensation, it is noteworthy that histamine was found to be able to activate TRPV1 via the phospholipase A2 (PLA2) – lipoxygenase pathway. Moreover, histamine-evoked itch is reduced in TRPV1 KO mice as well as by inhibiting H1 histamine receptor, PLA2, lipoxygenase or TRPV1 [188, 213]. The pharmacological blockade or genetic deletion of TRPV1 also inhibited trypsin-evoked itch in mice [133], which implicates the role of TRPV1 in PAR2-coupled pruriceptive signaling. Furthermore, Imamachi *et al* [214] found that TRPV1 is required for the transmission of histamine induced scratching behavior, but not for serotonin or endothelin-1 (ET-1) induced itch. Interestingly, however, ablation of the TRPV1+ sensory neurons led to deficit in behavioral responses to all above itch inductors suggesting that although TRPV1 is apparently not involved in mediating the actions of all pruritogens, the functional existence of TRPV1-expressing afferents is a pre-requisite for their actions.

Recently, a membrane associated phosphoinositide-binding protein, Pirt was identified as an important positive regulator of TRPV1 [215]. Pirt KO mice showed not only impaired responsiveness to noxious heat and capsaicin [215] but also decreased scratching responses to both histaminergic and non-histaminergic itch evoked by chloroquine, alpha-methyl-serotonin, ET-1 or PAR2 activation [216]. Although the authors concluded that the itch mediating effect of Pirt may be partly independent of TRPV1, it did not affect formalin induced TRPA1 activation and the concomitant scratching response [216].

Recently, TLRs were also identified as new players in itch sensation [217]. TLR7 was co-localized with TRPV1 on

sensory neurons of the mouse and mediated the imiquimod (TLR7 agonist) evoked scratch responses. In TLR7 KO mice, the non-histaminergic itch response (evoked by serotonin, ET-1, PAR2 activation or chloroquine) was impaired. The ablation of TRPV1+ neurons by RTX pretreatment terminated the imiquimod response but the deletion of TRPV1 channel had no effect, again arguing for the crucial role of TRPV1+ sensory afferents, but not the TRPV1 channel itself, in mediating TLR7-induced itch [218]. Controversially, another study [219] concluded that the pruritic effect of imiquimod was not related to TLR7; yet, the authors also confirmed the role of TRPV1 expressing neurons in the process. In animal experiments using respective KO mice, TLR3 (expressed in the subset of sensory neurons positive for TRPV1 and GRP) was also reported to mediate both histamine dependent and independent itch. Importantly, genetic deletion of TLR3 attenuated itch responses and central sensitization-driven pain but it did not affect acute pain sensation. In these animals, the responsiveness of DRG neuronal cell bodies was normal both for algogenic and pruritogenic agents; in contrast, the excitatory synaptic transmission in the spinal cord was impaired [220].

Recent data also suggested that the contribution of TRPV1 both to itch and pain sensation may be related to distinct populations of sensory neurons. The conditioned genetic deletion from DRG neurons of vesicular glutamate transporter type 2 (VGLUT2) (leading to a marked removal of glutamatergic transmission) resulted in a dramatic increase in itch-related behavior and, at the same time, a significant deficiency in thermal pain sensation. The deletion of the VGLUT2 affected the majority of TRPV1 expressing neurons which effect was thought to be responsible for the pain transmission. However, another, partly overlapping subpopulation of TRPV1+ neurons, also expressing GRP, is suggested to be responsible for itch sensation. It is proposed that due to impairment of the nociceptive system by VGLUT2 deletion, the itch inhibitory effect of the pain processing pathway was also ablated and this, in turn, resulted in a dramatic increase in spontaneous as well as histamine-dependent and independent scratching behavior. Furthermore, in animals with VGLUT2 deleted from nociceptors, capsaicin evoked mostly itch but it caused clearly pain in their littermate controls [221, 222].

These data clearly argue for the existence of functionally different populations of TRPV1 expressing sensory neurons in pain and itch transmission and introduce VGLUT2 as a functionally important marker of the processes. Evidently, further investigations are needed to clarify the molecular characteristic of the itch processing neurons and to explore the exact contribution of other neurotransmitters (e.g. GRP) to the diversity of itch evoking effects and related pathways.

3.2.4. The Role of TRPV1 Expressed By Non-neuronal Cells

Numerous reports indicate that TRPV1 is widely expressed, beyond sensory afferents, on several non-neuronal cell-types of the skin, including epidermal and hair follicle keratinocytes, mast cells, dendritic (Langerhans) cells, sebocytes and endothelial cells [223-236]. Activation of non-neuronal TRPV1 channels significantly influences key functions of these cutaneous cells which may lead to the development of pruritus and pruritic disorders. Indeed, TRPV1 activity influences the local immunological processes in the skin. The activation of TRPV1 was reported to induce the expression of COX-2 and the release of PGE2 and IL-8 from cultured epidermal keratinocytes [236] as well as it stimulated IL-4 release from mast cells [231]. Likewise, TRPV1 influenced (i) synthesis of numerous pro-inflammatory mediators produced by human hair follicles [223]; (ii) cytokine and lipid production of human sebaceous gland-derived sebocytes [227]; and (iii) maturation of dendritic cells [225, 234]. Furthermore, TRPV1 was found to decrease proliferation, increase apoptosis [226], and induce matrix metalloproteinase production [237-239] of epidermal keratinocytes; these effects may all contribute to skin aging and altered barrier functions and hence to the development of related pruritus [237, 240]. In good agreement with these data, activation of TRPV1 delayed the barrier recovery after mechanical injury (tape stripping) which effect was blocked by capsazepine, a TRPV1 antagonist [241]. Likewise, the novel TRPV1 antagonist PAC-14028 also accelerated barrier recovery and alleviated the AD like symptoms in a hairless mouse model [242]. In addition, increased expression of TRPV1 was reported on epidermal keratinocytes of prurigo nodularis patients which further support the role of non-neuronal TRPV1 in pruritic diseases [235].

3.2.5. Potential Itch Therapies Targeting TRPV1

As was presented above, TRPV1 has a central role in the development of pruriceptive itch on both sensory neurons and non-neuronal cutaneous structures; therefore, targeting the TRPV1 to alleviate its activity seems to be a promising therapeutic strategy to treat itch. Interestingly, the most well-known compound to decrease the TRPV1 activity is its agonist capsaicin itself [155, 174, 205, 243]. As we have presented above, prolonged application of capsaicin (and other exogenous vanilloid substances) results in depletion of neuropeptides in the C-type neurons causing desensitization of TRPV1-coupled responses which can lead to disruption of the pruritogenic crosstalk of sensory neurons and other skin cells [2, 5, 8, 41, 45, 46, 244]. Although very few studies, investigating the therapeutic effect of topical capsaicin, fulfill the criteria of a well-designed, correctly controlled human clinical trial [245], numerous publications report on the beneficial effects of its topical application in different pruritic syndroms such as notalgia paresthetica, psoriasis, prurigo nodularis, aquagenic pruritus, uremic pruritus, cholestasis, pruritus ani or allergic rhinitis [244, 246-255]. Likewise, the anti-pruritic effect of capsaicin was also reported in animal experiments [256, 257].

The major problem of topical capsaicin application is the undesired algogenic side effect; namely, via activating TRPV1 on nociceptors, it evokes an acute burning pain sensation which greatly reduces patient compliance [258]. Therefore, the use of such agonists which cause only minor excitation but hold strong desensitization power is highly desirable [4]. RTX is one of the most promising candidates as it has a threefold higher potency to induce desensitization than activation of the channel [174]. Another novel strategy could be the activity dependent targeting of TRPV1; this may be reached by using pore permeable capsaicin analogs (e.g. permanently charged capsaicinoids) which target and desensitize only (hyper)active channels [259, 260].

Another straightforward approach to decrease TRPV1 activity is the application of TRPV1 antagonists. As mentioned before, pilot studies using topical capsazepine or the novel antagonist PAC-14028 reported beneficial effects to alleviate itch in different AD models [241, 242, 261, 262].

Intriguingly, TRPV1 can be involved in mediating the effects of therapeutic tools designed to target other molecules. Tacrolimus, a potent calcineurin (protein phosphatase 2B) inhibitor is a widely used drug in the treatment of AD. Although further studies are needed to clarify its exact mechanism of anti-pruritic action, it was revealed that tacrolimus can influence the phosphorylation state of TRPV1 and hence inhibit (pruritogenic) Ca^{2+} currents in porcine DRG neurons [263]

3.3. Role of Other Thermosensitive TRP Channels in Pruritus

3.3.1. TRPV2

TRPV2 was originally described on a subset of medium- to large-diameter sensory neurons as a detector of noxious hot temperature (> 52 °C) ranges [264]. Yet, later on, it was also identified on C-type sensory fibers of human skin [265] and co-localization with its closest relative, TRPV1, was also reported [266]. In addition, TRPV2 was found not only on sensory nerve fibers but also on human epidermal keratinocytes and on human mast cell line HMC1 [267, 268].

Although this expression pattern can "nominate" TRPV2 as an important player in the development of itch, role of the TRPV2 channel even in the noxious temperature sensation is controversial. Indeed, TRPV2 KO mice display normal thermal and mechanical nociception [269]. Moreover, although the rodent channel was activated by high temperature, the human TRPV2 seems to be insensitive for noxious heat [270]. However, the non-psychotropic phytocannabinoid cannabidiol was shown to activate TRPV2 expressed on sensory DRG neurons and resulted in a release of CGRP [271] which may influence pain and/or itch sensation. Furthermore, activation of TRPV2 by various physical stimuli caused the degranulation of human mast cell line HMC1 as well as induced a transmembrane current and increased the intracellular Ca^{2+} concentrations. Importantly, all of the above effects were inhibited by SKF96365, a blocker of TRPV2 [268]. It was also suggested that the (potentially pruritogenic) PKA dependent phosphorylation can modulate the activity of TRPV2 expressed on mast cells [272]. By activating mast cells, TRPV2 may therefore contribute to the development of pruritus.

3.3.2. TRPV3 and TRPV4

Due to their similar temperature sensitivities and expression patterns, which are associated with partly overlapping physiological functions, it is reasonable to discuss the role of TRPV3 and TRPV4 together. TRPV3 is most abundantly expressed on epidermal keratinocytes; yet, it was also described on sensory neurons in co-expression with TRPV1 [273-276]. TRPV4 was originally described as an osmoreceptor expressed in various tissues including sensory neurons [277-280] and keratinocytes [281].

Both TRPV3 and TRPV4 are activated by physiological, innocuous warm temperature ranges (>33°C for TRPV3 and ca. >30 °C for TRPV4) [273-276, 282-284] and their deletion results in altered sensation of thermal stimuli [285-287] – although recent results suggest that their contribution to heat sensation is not essential in mice [288]. Of further importance, the thermo-sensory functions of TRPV3 and TRPV4 are apparently linked to keratinocytes; namely, the moderate warm temperature elevation, that increases the activity of TRPV3 and/or TRPV4 expressed in keratinocytes, resulted in the release of ATP release which, in turn, can transmit the thermal information toward sensory neurons [289-292]. It was also shown that TRPV3, overexpressed in epidermal keratinocytes, might modulate sensory modalities by regulating PGE2 secretion in the skin cells [293]. Finally, a recent study has introduced nitric oxide as a key keratinocyte-derived mediator regulating thermosensory behavior (and other cutaneous functions) when released from epidermal keratinocytes upon stimulation of TRPV3 expressed by these cells [294].

Beyond thermosensation, TRPV3 and TRPV4 are also potential key players in pruritus [4, 8, 41, 265, 295]. In an experimentally induced dry skin pruritus model, the application of acetone-ether-water treatment resulted in less intense scratching behavior, associated with decreased sprouting of sensory fibers, in TRPV3 KO mice than in wild-type controls [296]. In good accordance with the itch-inhibitory effect of the deletion of TRPV3, a gain-of-function (Gly573Ser) mutation of the *trpv3* gene resulted in a spontaneously developing itchy dermatitis and a closely related hairless phenotype both on mice and rats [297, 298]. Keratinocytes from these animals showed a highly augmented Ca^{2+} elevation in response to thermal stimulation and histological analysis of the skin revealed the inhibition of hair follicle growth [299]. In line with these findings, activation of TRPV3 was also found to be a negative regulator of the human hair cycle [300]. Transgenic mice overexpressing the above gene-of-function mutation in the epidermal keratinocytes also exhibited symptoms of severe itch, AD-like dermatitis, and increased serum levels of IgE and various pro-inflammatory cytokines. Similar to AD, hyperkeratosis, increased infiltration of mast cells and CD4+ lymphocytes, increased tissue NGF levels and elevated NGF production for thermal stimuli, greater skin sensory fiber innervation densities as well as associated scratching behavior were observed in these mice [301]. Of greatest importance, most recently, partly identical gain-of-function mutations of TRPV3 were identified in Olmsted syndrome, a rare congenital disorder characterized by palmoplantar and periorificial keratoderma, alopecia, and severe itching. Mutant TRPV3 channels overexpressed in HEK cells exhibited an increased inward current and apoptotic cell death which was also detected in keratinocytes of affected patients [302]. Olmsted syndrome therefore can be regarded as the first "cutaneous channelopathy" related to thermosensitive TRP channels.

TRPV3 and TRPV4 also play crucial roles in regulation of skin barrier functions, impairment of which may result in pruritus (e.g. in AD). In keratinocytes, TRPV3 forms a functional complex with the receptor of epidermal growth factor (EGF) which is indispensable for the formation of the physiological skin barrier. Deletion of TRPV3 resulted in wavy hair phenotype and impaired epidermal barrier formation due to decreased transglutaminase activity, a key proc-

ess of keratinocyte differentiation [303]. Temperature ranges activating TRPV3 and TRPV4, similar to the effects of TRPV4 agonists (but, interestingly, not to those of TRPV3 activators), accelerated barrier recovery after tape striping. In contrast, challenging with higher temperatures and (as mentioned above) application of the TRPV1 activator capsaicin delayed barrier regeneration [241]. TRPV4 was also shown to play an important role in the formation of intercellular junction in keratinocytes, another component of the healthy barrier [304]. Namely, the channel was found to interact with β-catenin and was localized in cell-cell junctions. In TRPV4 KO mice, leaky cell-cell junctions and delayed actin rearrangement and stratification were observed which were associated with reduced intracellular Ca^{2+} levels and suppressed Rho activation [305]. Furthermore, using human keratinocytes and skin cultures, it was demonstrated that the activation of TRPV4, either by agonists or by temperature, promoted cell-cell junction formation and barrier recovery [306].

Finally, it should also be mentioned that the mechanisms of action of certain pruritogens may also involve TRPV3 and/or TRPV4. For example, PAR2 activation sensitizes TRPV4-coupled cellular responses in DRG neurons resulting in increased neuropeptide release and mechanical hyperalgesia [307]. Furthermore, on surgical samples from breast operations, increased *in situ* expressions of TRPV3 and TRPV4 were found in the basal layers of keratinocytes in the breast pain affected group. This expression pattern was associated with a higher NGF and TRPV1 expressions on sensory fibers [308] which correlation might also contribute not only pain but also to itch. Of further importance, mast cell functions also seem to be affected by TRPV3 and TRPV4. Namely, laser irradiation of mouse RBL-2H3 mast cells evoked intracellular Ca^{2+} increase and histamine release via TRPV4 (Yang *et al*, 2007). TRPV4 (like TRPV2) was also expressed by HMC1 human mast cells and the increase of temperature to 37-39 °C, as well as the TRPV4 agonist 4α-phorbol 12,13-didecanoate, induced a weakly outward rectifying current and increase of the intracellular Ca^{2+} concentration [309].

Taken together, these intriguing findings collectively suggest that both TRPV3 and TRPV4 are promising, novel targets in the future management of pruritic dermatoses.

3.3.3. TRPA1

TRPA1 was originally described as a noxious cold (<17 °C) activated channel expressed by sensory neurons [310, 311]. Besides cold, it is also activated by various, mostly pungent and/or irritant compounds such as botanical substances like mustard oil, delta-9-tetrahydrocannabinol, eugenol, gingerol, methyl salicylate, allyl isothiocyanate, cinnamaldehyde, formalin, and nicotine [312-315]. Like TRPV1, TRPA1 is also considered as a key player of transduction of painful stimuli and a potential target of analgesic therapies [316-319].

Recently, the intriguing role of TRPA1 was reported in histamine independent itch. Chloroquine, an anti-malaria drug, was shown to activate mouse and rat MrgprA3 and human MrgprX1, members of Mrgpr family (Mas-related G protein-coupled receptors, exclusively expressed in peripheral sensory neurons) and, by this, evoked itch – a mechanism which can be responsible for the undesired side effect of chloroqiune. In mouse, chloriquine responsive (MrgprA3 expressing) sensory neurons also responded for capsaicin, histamine, and BAM 8-22, a specific agonist of the MrgpcrC11, suggesting the co-expression of the Mrgprs with TRPV1 and histamine receptors. Furthermore, these Mrgprs were also co-expressed with GRP, another neurotransmitter of the "itch pathway" (see above). Importantly, the deletion of the *mrgpr* gene cluster resulted in an impaired chloriquine response but it did not affect the histamine evoked scratching behavior indicating the role of Mrgprs in non-histaminergic itch [320].

Apparently, TRPA1 plays a crucial role in the downstream signaling of both MrgprA3 and C11 [321, 322]. Indeed, in TRPA1 KO mice, but not in TRPV1 KO and wild-type animals, the activation of neither MrgprA3 (by chloroquine) nor MrgprC11 (by BAM 8-22) resulted in itch related scratching behavior. In addition, although both substances evoked an elevation of the intracellular Ca^{2+} concentration and firing of action potentials on a capsaicin and mustard oil sensitive subset of DRG neurons from wild-type animals, this effect was abolished in TRPA1 KO mice (but not in TRPV1 KO animals). Furthermore, in heterologous expression systems, activation of the Mrgprs was able to evoke Ca^{2+} responses only in those cells in which they were co-expressed with TRPA1. Finally, pharmacological experiments suggested that MrgpcA3 was coupled to TRPA1 via the βγ subunit of G proteins whereas MrgpcrC11 most probably signals via a PLC dependent pathway [321].

TRPA1 is also involved in other pruritogenic mechanisms. Hydrogen peroxide induced itch was mediated by TRPA1 (as it was inhibited by the pharmacological blockade of TRPA1) but not by TRPV1, although the ablation of TRPV1+ neurons also abolished the itch response. The hydrogen peroxide induced itch was not influenced by histamine receptor blockers, which, similar to the above findings, further argue for the role of a TRPA1+ subpopulation within the TRPV1+ sensory afferent in the mediation of histamine-independent itch [323]. In addition, PAR2 activation was also reported to sensitize TRPA1 agonists-evoked currents in DRG neurons, likely via PLC mediated hydrolysis of PIP [324]. Paclitaxel, a microtubule de-organizer used in cancer chemotherapies, was found to increase mast cell tryptase activity. In mice, this results in the onset of neuropathic pain (a known side effect of paclitaxel administration) via the activation of PAR2 and consequent sensitization of TRPA1, as well as TRPV1 and TRPV4 [325].

In contrast with the above findings, it was also reported that the pharmacological inhibition of TRPA1 enhanced ET-1 (but, notably, not histamine) induced itch responses [326] suggesting an inhibitory role of TRPA1 in (at least some forms of) histamine-independent itch. This controversy indicates the urgent need for further studies to clarify the contribution of TRPA1 to other forms of itch.

Recently, TRPA1 expression was also reported on keratinocytes and melanocytes in the human epidermis as well as on dermal fibroblast. The treatment of primary human epidermal keratinocytes with icilin (activator of both TRPA1 and TRPM8) caused marked alteration in the expressions of several genes encoding (among else) adhesion and extracellular matrix components, heat shock proteins, and molecules regulating cell cycle, apoptosis, differentiation

and proliferation. Furthermore, expressions of pro-inflammatory cytokines IL-1α and IL1-β were also increased [327] (commented in [328]).

TRPA1, expressed on the mouse epidermal keratinocytes, was recently reported to contribute to skin permeability barrier recovery. Following the disruption of the epidermal barrier of hairless mice by tape striping, topical application of allyl isothiocyanate or cinnemaldehyde accelerated the barrier recovery, which effect was prevented by pre-treatment with the TRPA1 specific antagonist HC030031. Local cooling of the skin (10-15 °C for 1 min) evoked a similar effect, most probably via accelerated secretion of (barrier-forming) lamellar bodies at the interface of stratum granulosum and corneum; this action was also inhibited by the TRPA1 antagonist [329]. Although the above exciting data clearly suggest a possible role of TRPA1 in the control of various biological processes of skin keratinocytes, the exact role of the channel expressed by non-neuronal cells and its contribution to itch sensation needs to be clarified in forthcoming studies.

3.3.4. TRPM8

TRPM8 is a cold sensitive (<25 °C) member of TRPM subfamily, expressed by a specific subset of sensory neurons which usually do not express TRPV1 or CGRP. The channel is considered as a major sensor of environmental cold stimuli and it is also sensitive for cooling agents such as menthol, eucalyptol or the synthetic icilin [330-333].

Menthol has a long tradition in topical anti-itch therapy although its success is rather controversial [41]. Case studies reported successful treatment using menthol in various forms of pruritus [334, 335] although the results obtained in controlled studies are not obvious. Moderate cooling of skin as well as topical application of menthol decreased the subjective intensity of histamine-induced itch [336]. However, another study reported that menthol was ineffective to suppress histamine-induced itch and, moreover, increased the transepithelial water loss, suggesting an irritant nature of the compound [337]. Later, menthol was found to be effective in the treatment of mustard gas-induced pruritus in chemical warfare-injured veterans [338].

According to the available data, it appears that the neuronal TRPM8 do not contribute to the development and/or exacerbation of itch sensation, although it may exert an inhibitory role. TRPV1+/CGRP+ DRG neurons very limitedly expressed TRPM8 and did not respond to icilin, although ca. 10-50% of them could be activated to capsaicin, mustard oil, menthol, acidic pH, ATP as well as by histamine and chloriquine [339]. Pruritogenic pro-inflammatory mediators such as e.g. bradykinin and PGE2, desensitized the cooling-evoked (and most probably TRPM8 mediated) currents and shifted the activation threshold towards more negative membrane potentials [340]. Most recently, it was also proven that this inhibitory effect of the pro-inflammatory mediators targets TRPM8 via a quite "unusual" signaling mechanism; i.e. α subunit of a G protein directly bound to TRPM8 [341].

Our knowledge about the role of non-neuronal TRPM8 in itch is very limited and often controversial. The expression of TRPM8 was described on RBL-2H3 basophilic leukemia mast cell line and its activation, both by menthol and cold stimuli, evoked an elevation in the intracellular Ca^{2+} concentration and induced the release of histamine. These effects were blocked by both pharmacological blockade and siRNA based silencing of TRPM8 [342]. In contrast, another study could not identify TRPM8 expression in human mast cells. Furthermore, the role of TRPM8 in mast cell degranulation was not identified either in human or in mouse mast cells [343]. Recently, the expression of TRPM8 (both at mRNA and protein levels) was suggested on epidermal keratinocytes isolated from hairless mice. In these animals, following the epidermal barrier disruption by tape stripping, topical application of menthol or the TRPM8 agonist WS12 potentiated the barrier recovery, which effect was blocked by the general TRP antagonist ruthenium red as well as by the TRPM8 specific antagonist BTCT [344]

3.3.5. Targeting Termosensitive TRP Channels in Pruritus – Multiple Targets, Multiple Effects

The most obvious "protocols" which may target thermosensitive TRP channels during itch therapies are applying various temperature ranges – in other words, warming and cooling the skin. It is traditionally known in dermatological practice that cooling alleviates whereas warming potentiates itch sensation [4]; however, the quite few studies investigating the effect of "thermal therapies" are not always consistent with this mostly "anecdotal evidence". Indeed, as we mentioned above, moderate cooling was found to increase histamine induced itch sensation [336]. However, in another study, noxious heat reduced histamine-induced skin blood flow and itch intensity whereas cooling suppressed only itch intensity (without affecting histamine induced blood flow); moderate warming did not exert any effect [345]. Although the exact mechanisms of these intriguing findings remain to be determined, one possibility could be the simultaneous activation of cold sensitive receptors and the desensitization of the heat responsive ones. Alternatively, interactions between the warm/heat and cold sensitive mechanisms are also possible. Indeed, warming was shown to inhibit mustard oil induced TRPA1 currents in both TRPA1 overexpressing HEK cells and sensory neurons, similar to as described in TRPM8 overexpressing HEK cells activated by menthol [346].

The application of different TRP channel targeting substances often results in rather controversial effects due to the general lack of highly selective TRP channel agonists and antagonists. Among the well-known botanical substances used to modulate TRP channels, capsaicin is possibly the only one which acts exclusively on one member of TRP family [176, 347]. For example, carvacrol, thymol and eugenol were reported to activate TRPV3 [348] which might be responsible for the warm (and sometimes irritant) sensations, evoked by these substances, when applied on the skin surface [4]. However, they are also able to activate other TRP channels such as TRPV1, TRPA1 and TRPM8 [176]. In addition, although the exact mechanism of action is not known, eugenol and capsaicin were shown to evoke IL-1α and PGE2 release, respectively, from mouse keratinocytes [236, 349] which may contribute to their irritant actions by stimulating mast cell growth [350].

Despite of the difficulties in understanding their actions, TRP targeting compounds are very promising agents of anti-pruritic therapies. Indeed, modulating multiple TRP channel activities (e.g. activating TRPM8 and inhibiting TRPVs in

parallel) might be especially effective. Camphor is one of these promiscuous substances with good anti-itch potential: it was described to activate TRPV3, to activate and then desensitize TRPV1, and also to inhibit TRPA1 [4]. The often used menthol also possesses multiple mechanisms of action: beyond its action on TRPM8, menthol was also described to inhibit TRPA1 [351] and to stimulate TRPV3 [176]. Interestingly, a concentration dependent effect was also reported; i.e. menthol, at low doses, was shown to activate whilst, at high doses, block TRPA1 [352]. These variable effects may explain why, in some cases, menthol was found to be pruritogenic although it is generally considered as an anti-pruritic agent. At last, another botanical substance, citral should be mentioned. On sensory neurons, it was found to activate multiple TRP channels (TRPV1 and V3, TRPM8, and TRPA1); produced a long-lasting inhibition of TRPV1, V2, V3, and TRPM8; and transiently blocked TRPV4 and TRPA1 [353]. This intriguing activation and desensitization profile makes citral a highly promising candidate of topical itch therapies.

Finally, it should also be noted that not only anti-pruritic but itch evoking pruritogenic compounds may also act (often simultaneously) on multiple TRP channels. For example, clotrimazole, an antifungal agent, often evokes burning or itching side effects [354, 355]. These features may be related to its differential actions on multiple TRP channels; namely, it activates TRPV1 and TRPA1 but inhibits TRPM8 *in vitro* [356].

3.4. Potential Roles of thermo-insensitive TRP Channels in Pruritus

3.4.1. TRPCs and Keratinocyte Differentiation

Calcium signaling is a key element of keratinocyte differentiation [357-360] and, as we suggested above, altered differentiation of epidermal keratinocytes may play a role in pruritic diseases such as e.g. AD and psoriasis [361]. TRPC channels are important regulators of intracellular calcium homeostasis in several cells types [47, 154, 362, 363]. Epidermal or mucosal keratinocytes also express various TRPC channels (TRPC1, TRPC4, TRPC5, TRPC6, TRPC7), and their expression levels fluctuate in a differentiation-dependent manner [364-366].

TRPC1 was overexpressed in epidermis of patients with Darier's disease (DD) (or keratosis follicularis), a genetic disorder with loss-of-function mutations in the *SERCA2b* gene encoding the Ca^{2+}-pump of the endoplasmic reticulum. This malformation causes a severe differentiation disorder of keratinocytes and is very often associated with intense pruritus [367, 368]. TRPC1-mediated Ca^{2+} influx was significantly higher in keratinocytes obtained from DD patients. Furthermore, DD keratinocytes show enhanced proliferation and apoptosis resistance, suggesting that TRPC1 is involved in the abnormal keratinization in DD epidermis. Importantly, experiments performed on SERCA2b KO mice as well as on human epidermal HaCaT keratinocytes, in which expression of SERCA2b was silenced by siRNA, concluded similar results [368]. Other studies also show that TRPC1/TRPC4 channels were important for keratinocyte differentiation as siRNA based silencing of these channels prevented the induction of Ca^{2+}-induced differentiation. On cells derived from basal cell carcinoma, the lack of TRPC1/TRPC4 was coupled to impaired differentiation and enhanced proliferation [369]. Furthermore, the activation of TRPC6 expressed on both HaCaT and NHEKs induced differentiation and inhibited proliferation via increasing Ca^{2+} influx to the cytoplasm [370-372]. In addition, decreased expression of TRPC1/3/4/5/6/7 was found in keratinocytes from psoriasis patients, another pruritic dermatosis with a disturbed proliferation-differentiation program of the cells. Since recent findings also raise the possible involvement of TRPC6 in AD [373], these data collectively argue for the role of certain TRPCs in itch development.

3.4.2. Mg^{2+} Homeostasis in Itch – Possible Roles of TRPM6 and TRPM7

Recent data attract the attention to certain TRP channels involved in Mg^{2+} homeostasis. In rats, insufficient dietary magnesium intake leads to low serum Mg^{2+} concentration which results in the development of dermatitis and intense scratching [374]. Likewise, pruritus of uremic patients completely disappeared after normalizing the concentration of magnesium Mg^{2+} in the dialysate [375]. Interestingly, EGF, a central growth factor in epidermal differentiation, has a crucial role in controlling the proper function of TRPM6 [376], besides that of TRPV3 (see above). Indeed, EGF stimulates Mg^{2+} reabsorption in the renal distal convoluted tubule. Moreover, impaired sorting of pro-EGF at the basolateral membrane of the tubular epithelial cells was shown to disturb this mechanism and resulted in renal magnesium loss [377]. Supporting the link between EGF–TRPM6–Mg^{2+}–itch, several studies reported itch as a common side effect of cetuximab, a chemotherapeutic monoclonal antibody which inhibits the EGF-receptor [378-380].

A direct link between Mg^{2+} ions and itch could be the fact that Mg^{2+} ions very often inhibit TRP channels. Of great importance, Mg^{2+} was shown to constitutively inhibit TRPV3 expressed by epidermal keratinocytes; therefore, in overall body Mg^{2+} deficiency, TRPV3 channel activity could be augmented which may lead to the development of pruritic skin diseases [381].

4. CONCLUDING REMARKS

In this paper, we have attempted to review features of the recently identified members of the pruriceptive system (i.e. pruritogens, selective sensory pathways, higher central nervous system centers) involved in the pathogenesis of pruritus and the generation of itch sensation. Moreover, we have detailed a plethora of compelling evidence that certain thermosensitive and -insensitive TRP channels indeed play key roles in the pathogenesis of skin-derived pruriceptive itch.

The major massages of this review can be summarized as follows:

- ✓ Pruriceptive pruritus is generated by a bi-directional interplay of sensory neurons and various non-neuronal cell types of the skin
- ✓ Pruriceptive itch is processed and transmitted by a highly selective pruriceptive system which shows significant overlapping with the cutaneous nociceptive system
- ✓ On sensory neurons, certain TRP channels (especially the thermosensitive TRPV1, TRPV3, TRPV4, and

TRPA1) are targets of pruritogenic mediators; therefore, these TRP channels may function as neuronal "transducers" of pruriceptive stimuli

- ✓ Most of these TRP channels are also expressed on various non-neuronal cutaneous cells (e.g. keratinocytes, mast cells) where their activation (via the release of numerous soluble factors) also contributes to the augmentation of the above neuron – non-neuronal cell communication network resulting in itch
- ✓ TRP channels also regulate homeostatic skin functions (e.g. proliferation, differentiation, barrier formation, immune competence and tolerance) whose pathological alterations may result in the development of pruritic skin disorders such as e.g. AD and psoriasis.

Evidently, more extensive *in vitro* and *in vivo* studies are needed to uncover the exact molecular roles of TRP channels in the neurophysiology of itch and in the pathophysiology of related dermatoses. However, we strongly hope that the presented intriguing findings will foster future, highly sophisticated clinical trials to explore the seemingly rich potential of TRP channel-targeted management of this very often devastating sensory condition which impairs quality of life of millions worldwide.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

ACKNOWLEDGEMENTS

This work was supported by Hungarian (LP2011-003/2011, TÁMOP 4.2.1./B-09/1/KONV-2010-0007, OTKA NK78398 and 101761) and EU (FP7-REGPOT-2008-1/229920) research grants. BIT is supported by the FWO Vlaanderen and the Bijzonder Onderzoeksfonds (BOF) KU Leuven (TRPLe Research Platform).

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Received: August 09, 2012 Revised: August 09, 2012 Accepted: August 16, 2012