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].

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; moreover, they have a specific central connection which

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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 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]. In-

terestingly, 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 neurotrophins

[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 an 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 chemotactic protein-1). Intriguingly, this action is 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 ($\leq 43°C$), 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 activity 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 to 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 Freud'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 stimu-

lated 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 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 process 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 [309]. 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 [310].

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 [311, 312]. 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 [313-316]. Like TRPV1, TRPA1 is also considered as a key player of transduction of painful stimuli and a potential target of analgesic therapies [317-320].

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 MrgprC11, 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 [321].

Apparently, TRPA1 plays a crucial role in the downstream signaling of both MrgprA3 and C11 [322, 323]. 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 MrgprA3 was coupled to TRPA1 via the βγ subunit of G proteins whereas MrgprC11 most probably signals via a PLC dependent pathway [322].

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 [324]. In addition, PAR2 activation was also reported to sensitize TRPA1 agonists-evoked currents in DRG neurons, likely via PLC mediated hydrolysis of PIP2 [325]. 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 [326].

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 [327] 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 [328] (commented in [329]).

TRPA1, expressed on the mouse epidermal keratinocytes, was recently reported to contribute to skin permeabil-

ity 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 pretreatment 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 [330]. 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 [331-334].

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 [335, 336] 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 [337]. 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 [338]. Later, menthol was found to be effective in the treatment of mustard gas-induced pruritus in chemical warfare-injured veterans [339].

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 by capsaicin, mustard oil, menthol, acidic pH, ATP as well as by histamine and chloriquine [340]. 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 [341]. 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 [342].

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 [343]. 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 [344]. 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 [345].

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 [337]. 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 [346]. 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 [347].

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, 348]. For example, carvacrol, thymol and eugenol were reported to activate TRPV3 [349] 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, 350] which may contribute to their irritant actions by stimulating mast cell growth [351].

Despite of the difficulties in understanding their actions, TRP targeting compounds are very promising agents of antipruritic 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 [352] 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 [353]. 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 TRPV3, TRPM8, and TRPA1); produced a long-lasting inhibition of TRPV1, TRPV2 and TRPV3, and TRPM8; and transiently blocked TRPV4 and TRPA1 [354]. 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 [355, 356]. These features may be related to its differential actions on multiple TRP channels; namely, it activates TRPV1 and TRPA1 but inhibits TRPM8 *in vitro* [357].

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 [358-361] and, as we suggested above, altered differentiation of epidermal keratinocytes may play a role in pruritic diseases such as e.g. AD and psoriasis [362]. TRPC channels are important regulators of intracellular calcium homeostasis in several cells types [47, 154, 363, 364]. Epidermal or mucosal keratinocytes also express various TRPC channels (TRPC1, TRPC4, TRPC5, TRPC6, TRPC7), and their expression levels fluctuate in a differentiation-dependent manner [365-367].

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 [368, 369]. 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 [369]. 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 [370]. Furthermore, the activation of TRPC6 expressed on both HaCaT and NHEKs induced differentiation and inhibited proliferation via increasing Ca^{2+} influx to the cytoplasm [371-373]. 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 [374], 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 [375]. Likewise, pruritus of uremic patients completely disappeared after normalizing the concentration of magnesium Mg^{2+} in the dialysate [376]. Interestingly, EGF, a central growth factor in epidermal differentiation, has a crucial role in controlling the proper function of TRPM6 [377], 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 [378]. 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 [379-381].

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 [382].

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.

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REFERENCES

[1] Rothmann S. Physiology of itching. Physiol Rev 1941; 21: 357-81.
[2] Yosipovitch G, Greaves MW, Schmelz M. Itch. Lancet 2003; 361: 690-4.
[3] Stander S, Schmelz M. Chronic itch and pain--similarities and differences. Eur J Pain 2006; 10: 473-8.
[4] Biro T, Toth BI, Marincsak R, *et al.* TRP channels as novel players in the pathogenesis and therapy of itch. Biochim Biophys Acta 2007; 1772: 1004-21.
[5] Biro T, Ko MC, Bromm B, *et al.* How best to fight that nasty itch - from new insights into the neuroimmunological, neuroendocrine, and neurophysiological bases of pruritus to novel therapeutic approaches. Exp Dermatol 2005; 14: 225-40.
[6] von Frey M. Zur Physiologie der Juckempfindung. Arch Neerl Physiol 1922; 7: 142-5.
[7] Schmelz M, Schmidt R, Bickel A, *et al.* Specific C-receptors for itch in human skin. J Neurosci 1997; 17: 8003-8.
[8] Steinhoff M, Bienenstock J, Schmelz M, *et al.* Neurophysiological, neuroimmunological, and neuroendocrine basis of pruritus. J Invest Dermatol 2006; 126: 1705-18.
[9] Schmelz M. A neural pathway for itch. Nat Neurosci 2001; 4: 9-10.
[10] Davidson S, Giesler GJ. The multiple pathways for itch and their interactions with pain. Trends Neurosci 2010; 33: 550-8.
[11] Ross SE. Pain and itch: insights into the neural circuits of aversive somatosensation in health and disease. Curr Opin Neurobiol 2011; 21: 880-7.
[12] McMahon SB, Koltzenburg M. Itching for an explanation. Trends Neurosci 1992; 15: 497-501.
[13] Handwerker HO. Microneurography of pruritus. Neurosci Lett 2010; 470: 193-6.
[14] Schmidt R, Schmelz M, Forster C, *et al.* Novel classes of responsive and unresponsive C nociceptors in human skin. J Neurosci 1995; 15: 333-41.
[15] Schmidt R, Schmelz M, Ringkamp M, *et al.* Innervation territories of mechanically activated C nociceptor units in human skin. J Neurophysiol 1997; 78: 2641-8.
[16] Wahlgren CF, Ekblom A. Two-point discrimination of itch in patients with atopic dermatitis and healthy subjects. Acta Derm Venereol 1996; 76: 48-51.
[17] Schmelz M, Michael K, Weidner C, *et al.* Which nerve fibers mediate the axon reflex flare in human skin? Neuroreport 2000; 11: 645-8.
[18] Schmelz M, Schmidt R. Microneurographic single-unit recordings to assess receptive properties of afferent human C-fibers. Neurosci Lett 2010; 470: 158-61.
[19] Schmidt R, Schmelz M, Weidner C, *et al.* Innervation territories of mechano-insensitive C nociceptors in human skin. J Neurophysiol 2002; 88: 1859-66.
[20] Schmelz M, Schmidt R, Weidner C, *et al.* Chemical response pattern of different classes of C-nociceptors to pruritogens and algogens. J Neurophysiol 2003; 89: 2441-8.
[21] Namer B, Carr R, Johanek LM, *et al.* Separate peripheral pathways for pruritus in man. J Neurophysiol 2008; 100: 2062-9.
[22] Ikoma A, Handwerker H, Miyachi Y, *et al.* Electrically evoked itch in humans. Pain 2005; 113: 148-54.
[23] Ma C, Nie H, Gu Q, *et al. In vivo* responses of cutaneous C-mechanosensitive neurons in mouse to punctate chemical stimuli that elicit itch and nociceptive sensations in humans. J Neurophysiol 2012; 107: 357-63.
[24] Ringkamp M, Schepers RJ, Shimada SG, *et al.* A role for nociceptive, myelinated nerve fibers in itch sensation. J Neurosci 2011; 31: 14841-9.
[25] Andrew D, Craig AD. Spinothalamic lamina I neurons selectively sensitive to histamine: a central neural pathway for itch. Nat Neurosci 2001; 4: 72-7.
[26] Simone DA, Zhang X, Li J, *et al.* Comparison of responses of primate spinothalamic tract neurons to pruritic and algogenic stimuli. J Neurophysiol 2004; 91: 213-22.
[27] Davidson S, Zhang X, Yoon CH, *et al.* The itch-producing agents histamine and cowhage activate separate populations of primate spinothalamic tract neurons. J Neurosci 2007; 27: 10007-14.
[28] Davidson S, Zhang X, Khasabov SG, *et al.* Relief of itch by scratching: state-dependent inhibition of primate spinothalamic tract neurons. Nat Neurosci 2009; 12: 544-6.
[29] Sun YG, Zhao ZQ, Meng XL, *et al.* Cellular basis of itch sensation. Science 2009; 325: 1531-4.
[30] Sun YG, Chen ZF. A gastrin-releasing peptide receptor mediates the itch sensation in the spinal cord. Nature 2007; 448: 700-3.
[31] Fleming MS, Ramos D, Han SB, *et al.* The majority of dorsal spinal cord gastrin releasing peptide is synthesized locally whereas neuromedin B is highly expressed in pain- and itch-sensing somatosensory neurons. Mol Pain 2012; 8: 52.
[32] Gotoh Y, Andoh T, Kuraishi Y. Noradrenergic regulation of itch transmission in the spinal cord mediated by alpha-adrenoceptors. Neuropharmacology 2011; 61: 825-31.
[33] Gotoh Y, Omori Y, Andoh T, *et al.* Tonic inhibition of allergic itch signaling by the descending noradrenergic system in mice. J Pharmacol Sci 2011; 115: 417-20.
[34] Craig AD. How do you feel? Interoception: the sense of the physiological condition of the body. Nat Rev Neurosci 2002; 3: 655-66.
[35] Hsieh JC, Hagermark O, Stahle-Backdahl M, *et al.* Urge to scratch represented in the human cerebral cortex during itch. J Neurophysiol 1994; 72: 3004-8.
[36] Drzezga A, Darsow U, Treede RD, *et al.* Central activation by histamine-induced itch: analogies to pain processing: a correlational analysis of O-15 H2O positron emission tomography studies. Pain 2001; 92: 295-305.
[37] Mochizuki H, Tashiro M, Kano M, *et al.* Imaging of central itch modulation in the human brain using positron emission tomography. Pain 2003; 105: 339-46.
[38] Vogt BA, Vogt L, Laureys S. Cytology and functionally correlated circuits of human posterior cingulate areas. Neuroimage 2006; 29: 452-66.
[39] Mochizuki H, Inui K, Tanabe HC, *et al.* Time course of activity in itch-related brain regions: a combined MEG-fMRI study. J Neurophysiol 2009; 102: 2657-66.

[40] Kleyn CE, McKie S, Ross A, *et al.* A temporal analysis of the central neural processing of itch. Br J Dermatol 2012; 166: 994-1001.

[41] Paus R, Schmelz M, Biro T, *et al.* Frontiers in pruritus research: scratching the brain for more effective itch therapy. J Clin Invest 2006; 116: 1174-86.

[42] Ikoma A, Cevikbas F, Kempkes C, *et al.* Anatomy and neurophysiology of pruritus. Semin Cutan Med Surg 2011; 30: 64-70.

[43] Bergeret L, Black D, Theunis J, *et al.* Validation of a model of itch induction for brain positron emission tomography studies using histamine iontophoresis. Acta Derm Venereol 2011; 91: 504-10.

[44] Papoiu AD, Coghill RC, Kraft RA, *et al.* A tale of two itches. Common features and notable differences in brain activation evoked by cowhage and histamine induced itch. Neuroimage 2012; 59: 3611-23.

[45] Twycross R, Greaves MW, Handwerker H, *et al.* Itch: scratching more than the surface. QJM 2003; 96: 7-26.

[46] Ikoma A, Steinhoff M, Stander S, *et al.* The neurobiology of itch. Nat Rev Neurosci 2006; 7: 535-47.

[47] Clapham DE. TRP channels as cellular sensors. Nature 2003; 426: 517-24.

[48] Undem BJ, Riccio MM, Weinreich D, *et al.* Neurophysiology of mast cell-nerve interactions in the airways. Int Arch Allergy Immunol 1995; 107: 199-201.

[49] Ansel JC, Armstrong CA, Song I, *et al.* Interactions of the skin and nervous system. J Investig Dermatol Sympos Proc 1997; 2(1): 23-6.

[50] Botchkarev VA, Eichmuller S, Peters EM, *et al.* A simple immunofluorescence technique for simultaneous visualization of mast cells and nerve fibers reveals selectivity and hair cycle--dependent changes in mast cell--nerve fiber contacts in murine skin. Arch Dermatol Res 1997; 289: 292-302.

[51] Paus R, Theoharides TC, Arck PC. Neuroimmunoendocrine circuitry of the 'brain-skin connection'. Trends Immunol 2006; 27: 32-9.

[52] Luger TA. Neuromediators--a crucial component of the skin immune system. J Dermatol Sci 2002; 30: 87-93.

[53] Steinhoff M, Stander S, Seeliger S, *et al.* Modern aspects of cutaneous neurogenic inflammation. Arch Dermatol 2003; 139: 1479-88.

[54] Jeffry J, Kim S, Chen ZF. Itch signaling in the nervous system. Physiology (Bethesda) 2011; 26: 286-92.

[55] Metz M, Grundmann S, Stander S. Pruritus: an overview of current concepts. Vet Dermatol 2011; 22: 121-31.

[56] Metz M, Siebenhaar F, Maurer M. Mast cell functions in the innate skin immune system. Immunobiology 2008; 213: 251-60.

[57] Metz M, Maurer M. Innate immunity and allergy in the skin. Curr Opin Immunol 2009; 21: 687-93.

[58] Zuberbier T, Maurer M. Urticaria: current opinions about etiology, diagnosis and therapy. Acta Derm Venereol 2007; 87: 196-205.

[59] Ortonne JP. Urticaria and its subtypes: the role of second-generation antihistamines. Eur J Intern Med 2012; 23: 26-30.

[60] Klein PA, Clark RA. An evidence-based review of the efficacy of antihistamines in relieving pruritus in atopic dermatitis. Arch Dermatol 1999; 135: 1522-5.

[61] Akiyama T, Carstens MI, Carstens E. Enhanced scratching evoked by PAR-2 agonist and 5-HT but not histamine in a mouse model of chronic dry skin itch. Pain 2010; 151: 378-83.

[62] Bell JK, McQueen DS, Rees JL. Involvement of histamine H4 and H1 receptors in scratching induced by histamine receptor agonists in Balb C mice. Br J Pharmacol 2004; 142: 374-80.

[63] Rossbach K, Nassenstein C, Gschwandtner M, *et al.* Histamine H(1), H(3) and H(4) receptors are involved in pruritus. Neuroscience 2011; 190: 89-102.

[64] Steinhoff M, Buddenkotte J, Shpacovitch V, *et al.* Proteinase-activated receptors: transducers of proteinase-mediated signaling in inflammation and immune response. Endocr Rev 2005; 26: 1-43.

[65] Vu TK, Hung DT, Wheaton VI, *et al.* Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. Cell 1991; 64: 1057-68.

[66] Steinhoff M, Neisius U, Ikoma A, *et al.* Proteinase-activated receptor-2 mediates itch: a novel pathway for pruritus in human skin. J Neurosci 2003; 23: 6176-80.

[67] Vergnolle N, Ferazzini M, D'Andrea MR, *et al.* Proteinase-activated receptors: novel signals for peripheral nerves. Trends Neurosci 2003; 26: 496-500.

[68] Steinhoff M, Vergnolle N, Young SH, *et al.* Agonists of proteinase-activated receptor 2 induce inflammation by a neurogenic mechanism. Nat Med 2000; 6: 151-8.

[69] Reddy VB, Iuga AO, Shimada SG, *et al.* Cowhage-evoked itch is mediated by a novel cysteine protease: a ligand of protease-activated receptors. J Neurosci 2008; 28: 4331-5.

[70] Papoiu AD, Tey HL, Coghill RC, *et al.* Cowhage-induced itch as an experimental model for pruritus. A comparative study with histamine-induced itch. PLoS One 2011; 6: e17786.

[71] Stefansson K, Brattsand M, Roosterman D, *et al.* Activation of proteinase-activated receptor-2 by human kallikrein-related peptidases. J Invest Dermatol 2008; 128: 18-25.

[72] Buddenkotte J, Stroh C, Engels IH, *et al.* Agonists of proteinase-activated receptor-2 stimulate upregulation of intercellular cell adhesion molecule-1 in primary human keratinocytes via activation of NF-kappa B. J Invest Dermatol 2005; 124: 38-45.

[73] Reis ML, Maeda S, Rosatelli JB, *et al.* Kininogens and kallikrein in pruritic papular eruption. Immunopharmacology 1999; 45: 115-20.

[74] Briot A, Deraison C, Lacroix M, *et al.* Kallikrein 5 induces atopic dermatitis-like lesions through PAR2-mediated thymic stromal lymphopoietin expression in Netherton syndrome. J Exp Med 2009; 206: 1135-47.

[75] Church MK, Okayama Y, el-Lati S. Mediator secretion from human skin mast cells provoked by immunological and non-immunological stimulation. Skin Pharmacol 1991; 4 (Suppl 1): 15-24.

[76] Thomsen JS, Sonne M, Benfeldt E, *et al.* Experimental itch in sodium lauryl sulphate-inflamed and normal skin in humans: a randomized, double-blind, placebo-controlled study of histamine and other inducers of itch. Br J Dermatol 2002; 146: 792-800.

[77] van der Kleij HP, Ma D, Redegeld FA, *et al.* Functional expression of neurokinin 1 receptors on mast cells induced by IL-4 and stem cell factor. J Immunol 2003; 171: 2074-9.

[78] Kulka M, Sheen CH, Tancowny BP, *et al.* Neuropeptides activate human mast cell degranulation and chemokine production. Immunology 2008; 123: 398-410.

[79] Salomon J, Baran E. The role of selected neuropeptides in pathogenesis of atopic dermatitis. J Eur Acad Dermatol Venereol 2008; 22: 223-8.

[80] Abadia Molina F, Burrows NP, Jones RR, *et al.* Increased sensory neuropeptides in nodular prurigo: a quantitative immunohis-tochemical analysis. Br J Dermatol 1992; 127: 344-51.

[81] Haas S, Capellino S, Phan NQ, *et al.* Low density of sympathetic nerve fibers relative to substance P-positive nerve fibers in lesional skin of chronic pruritus and prurigo nodularis. J Dermatol Sci 2010; 58: 193-7.

[82] Trivedi M, Bergasa NV. Serum concentrations of substance P in cholestasis. Ann Hepatol 2010; 9: 177-80.

[83] Ekblom A, Lundeberg T, Wahlgren CF. Influence of calcitonin gene-related peptide on histamine- and substance P-induced itch, flare and weal in humans. Skin Pharmacol 1993; 6: 215-22.

[84] Jarvikallio A, Harvima IT, Naukkarinen A. Mast cells, nerves and neuropeptides in atopic dermatitis and nummular eczema. Arch Dermatol Res 2003; 295: 2-7

[85] Liang Y, Jacobi HH, Reimert CM, *et al.* CGRP-immunoreactive nerves in prurigo nodularis--an exploration of neurogenic inflammation. J Cutan Pathol 2000; 27: 359-66.

[86] Yamaoka J, Di ZH, Sun W, *et al.* Erratum to "changes in cutaneous sensory nerve fibers induced by skin-scratching in mice". J Dermatol Sci 2007; 47: 172-82.

[87] Botchkarev VA, Yaar M, Peters EM, *et al.* Neurotrophins in skin biology and pathology. J Invest Dermatol 2006; 126: 1719-27.

[88] Groneberg DA, Serowka F, Peckenschneider N, *et al.* Gene expression and regulation of nerve growth factor in atopic dermatitis mast cells and the human mast cell line-1. J Neuroimmunol 2005; 161: 87-92.

[89] Urashima R, Mihara M. Cutaneous nerves in atopic dermatitis. A histological, immunohistochemical and electron microscopic study. Virchows Arch 1998; 432: 363-70.

[90] Hefti FF, Rosenthal A, Walicke PA, *et al.* Novel class of pain drugs based on antagonism of NGF. Trends Pharmacol Sci 2006; 27: 85-91.

[91] Lazar J, Szabo T, Marincsak R, *et al.* Sensitization of recombinant vanilloid receptor-1 by various neurotrophic factors. Life Sci 2004; 75: 153-63.

[92] Toyoda M, Nakamura M, Makino T, *et al.* Nerve growth factor and substance P are useful plasma markers of disease activity in atopic dermatitis. Br J Dermatol 2002; 147: 71-9.

[93] Johansson O, Liang Y, Emtestam L. Increased nerve growth factor- and tyrosine kinase A-like immunoreactivities in prurigo nodularis skin -- an exploration of the cause of neurohyperplasia. Arch Dermatol Res 2002; 293: 614-9.

[94] Nakamura M, Toyoda M, Morohashi M. Pruritogenic mediators in psoriasis vulgaris: comparative evaluation of itch-associated cutaneous factors. Br J Dermatol 2003; 149: 718-30.

[95] Grewe M, Vogelsang K, Ruzicka T, *et al.* Neurotrophin-4 production by human epidermal keratinocytes: increased expression in atopic dermatitis. J Invest Dermatol 2000; 114: 1108-12.

[96] Yamaguchi J, Aihara M, Kobayashi Y, *et al.* Quantitative analysis of nerve growth factor (NGF) in the atopic dermatitis and psoriasis horny layer and effect of treatment on NGF in atopic dermatitis. J Dermatol Sci 2009; 53: 48-54.

[97] Roggenkamp D, Falkner S, Stab F, *et al.* Atopic keratinocytes induce increased neurite outgrowth in a coculture model of porcine dorsal root Ganglia neurons and human skin cells. J Invest Dermatol 2012; 132: 1892-900.

[98] Verge VM, Richardson PM, Wiesenfeld-Hallin Z, *et al.* Differential influence of nerve growth factor on neuropeptide expression *in vivo*: a novel role in peptide suppression in adult sensory neurons. J Neurosci 1995; 15: 2081-96.

[99] Raychaudhuri SP, Raychaudhuri SK. Role of NGF and neurogenic inflammation in the pathogenesis of psoriasis. Prog Brain Res 2004; 146: 433-7.

[100] Chang SE, Han SS, Jung HJ, *et al.* Neuropeptides and their receptors in psoriatic skin in relation to pruritus. Br J Dermatol 2007; 156: 1272-7.

[101] Ji RR, Samad TA, Jin SX, *et al.* p38 MAPK activation by NGF in primary sensory neurons after inflammation increases TRPV1 levels and maintains heat hyperalgesia. Neuron 2002; 36: 57-68.

[102] Zhang X, Huang J, McNaughton PA. NGF rapidly increases membrane expression of TRPV1 heat-gated ion channels. EMBO J 2005; 24: 4211-23.

[103] Amaya F, Shimosato G, Nagano M, *et al.* NGF and GDNF differentially regulate TRPV1 expression that contributes to development of inflammatory thermal hyperalgesia. Eur J Neurosci 2004; 20: 2303-10.

[104] Tal M, Liberman R. Local injection of nerve growth factor (NGF) triggers degranulation of mast cells in rat paw. Neurosci Lett 1997; 221: 129-32.

[105] Marshall JS, Gomi K, Blennerhassett MG, *et al.* Nerve growth factor modifies the expression of inflammatory cytokines by mast cells via a prostanoid-dependent mechanism. J Immunol 1999; 162: 4271-6.

[106] Metz M, Botchkarev VA, Botchkareva NV, *et al.* Neurotrophin-3 regulates mast cell functions in neonatal mouse skin. Exp Dermatol 2004; 13: 273-81.

[107] Stempelj M, Ferjan I. Signaling pathway in nerve growth factor induced histamine release from rat mast cells. Inflamm Res 2005; 54: 344-9.

[108] Reeh PW, Kress M. Effects of classical algogens. Seminars in the Neurosciences 1995; 7: 221-6.

[109] Bickford RG. Experiments relating to the itch sensation, its peripheral mechanism and central pathway. Clin Sci 1938; 3: 377-86.

[110] Stander S, Steinhoff M, Schmelz M, *et al.* Neurophysiology of pruritus: cutaneous elicitation of itch. Arch Dermatol 2003; 139: 1463-70.

[111] Toth BI, Olah A, Szollosi AG, *et al.* "Sebocytes' makeup": novel mechanisms and concepts in the physiology of the human sebaceous glands. Pflugers Arch 2011; 461: 593-606.

[112] Hagermark O, Strandberg K. Pruritogenic activity of prostaglandin E2. Acta Derm Venereol 1977; 57: 37-43.

[113] Boss M, Burton JL. Lack of effect of the antihistamine drug clemastine on the potentiation of itch by prostaglandin E1. Arch Dermatol 1981; 117: 208-9.

[114] Lovell CR, Burton PA, Duncan EH, *et al.* Prostaglandins and pruritus. Br J Dermatol 1976; 94: 273-5.

[115] Neisius U, Olsson R, Rukwied R, *et al.* Prostaglandin E2 induces vasodilation and pruritus, but no protein extravasation in atopic dermatitis and controls. J Am Acad Dermatol 2002; 47: 28-32.

[116] Andoh T, Kuraishi Y. Intradermal leukotriene B4, but not prostaglandin E2, induces itch-associated responses in mice. Eur J Pharmacol 1998; 353: 93-6.

[117] Andoh T, Nishikawa Y, Yamaguchi-Miyamoto T, *et al.* Thromboxane A2 induces itch-associated responses through TP receptors in the skin in mice. J Invest Dermatol 2007; 127: 2042-7.

[118] Zhu Y, Wang XR, Peng C, *et al.* Induction of leukotriene B(4) and prostaglandin E(2) release from keratinocytes by protease-activated receptor-2-activating peptide in ICR mice. Int Immunopharmacol 2009; 9: 1332-6.

[119] Andoh T, Katsube N, Maruyama M, *et al.* Involvement of leukotriene B(4) in substance P-induced itch-associated response in mice. J Invest Dermatol 2001; 117: 1621-6.

[120] Sugimoto M, Arai I, Futaki N, *et al.* Putative mechanism of the itch-scratch circle: repeated scratching decreases the cutaneous level of prostaglandin D2, a mediator that inhibits itching. Prostaglandins Leukot Essent Fatty Acids 2007; 76: 93-101.

[121] Kaur S, Sur R, Liebel FT, *et al.* Induction of prostaglandin D2 through the p38 MAPK pathway is responsible for the antipruritic activity of sertaconazole nitrate. J Invest Dermatol 2010; 130: 2448-56.

[122] Hashimoto Y, Arai I, Tanaka M, *et al.* Prostaglandin D2 inhibits IgE-mediated scratching by suppressing histamine release from mast cells. J Pharmacol Sci 2005; 98: 90-3.

[123] Sugimoto M, Arai I, Futaki N, *et al.* COX-1 inhibition enhances scratching behaviour in NC/Nga mice with atopic dermatitis. Exp Dermatol 2006; 15: 582-8.

[124] Inoue T, Sugimoto M, Sakurai T, *et al.* Modulation of scratching behavior by silencing an endogenous cyclooxygenase-1 gene in the skin through the administration of siRNA. J Gene Med 2007; 9: 994-1001.

[125] Cormia FE, Dougherty JW. Proteolytic activity in development of pain and itching. Cutaneous reactions to bradykinin and kallikrein. J Invest Dermatol 1960; 35: 21-6.

[126] Hagermark O. Studies on experimental itch induced by kallikrein and bradykinin. Acta Derm Venereol 1974; 54: 397-400.

[127] Walker K, Perkins M, Dray A. Kinins and kinin receptors in the nervous system. Neurochem Int 1995; 26: 1-16; discussion 7-26.

[128] Lang E, Novak A, Reeh PW, *et al.* Chemosensitivity of fine afferents from rat skin *in vitro*. J Neurophysiol 1990; 63: 887-901.

[129] Koppert W, Martus P, Reeh PW. Interactions of histamine and bradykinin on polymodal C-fibres in isolated rat skin. Eur J Pain 2001; 5: 97-106.

[130] Averbeck B, Reeh PW. Interactions of inflammatory mediators stimulating release of calcitonin gene-related peptide, substance P and prostaglandin E(2) from isolated rat skin. Neuropharmacology 2001; 40: 416-23.

[131] Hosogi M, Schmelz M, Miyachi Y, *et al.* Bradykinin is a potent pruritogen in atopic dermatitis: a switch from pain to itch. Pain 2006; 126: 16-23.

[132] Costa R, Manjavachi MN, Motta EM, *et al.* The role of kinin B1 and B2 receptors in the scratching behaviour induced by proteinase-activated receptor-2 agonists in mice. Br J Pharmacol 2010; 159: 888-97.

[133] Costa R, Marotta DM, Manjavachi MN, *et al.* Evidence for the role of neurogenic inflammation components in trypsin-elicited scratching behaviour in mice. Br J Pharmacol 2008; 154: 1094-103.

[134] Liang J, He Y, Ji W. Bradykinin-evoked scratching responses in complete Freund's adjuvant-inflamed skin through activation of B1 receptor. Exp Biol Med (Maywood) 2012; 237: 318-26.

[135] Gaspari AA, Lotze MT, Rosenberg SA, *et al.* Dermatologic changes associated with interleukin 2 administration. JAMA 1987; 258: 1624-9.

[136] Wahlgren CF, Tengvall Linder M, Hagermark O, *et al.* Itch and inflammation induced by intradermally injected interleukin-2 in atopic dermatitis patients and healthy subjects. Arch Dermatol Res 1995; 287: 572-80.

[137] Darsow U, Scharein E, Bromm B, *et al.* Skin testing of the pruritogenic activity of histamine and cytokines (interleukin-2 and tumour necrosis factor-alpha) at the dermal-epidermal junction. Br J Dermatol 1997; 137: 415-7.

[138]   Martin HA, Murphy PR. Interleukin-2 activates a sub-population of cutaneous C-fibre polymodal nociceptors in the rat hairy skin. Arch Physiol Biochem 1995; 103: 136-48.

[139]   Martin HA. Bradykinin potentiates the chemoresponsiveness of rat cutaneous C-fibre polymodal nociceptors to interleukin-2. Arch Physiol Biochem 1996; 104: 229-38.

[140]   Fallahzadeh MK, Roozbeh J, Geramizadeh B, *et al.* Interleukin-2 serum levels are elevated in patients with uremic pruritus: a novel finding with practical implications. Nephrol Dial Transplant 2011; 26: 3338-44.

[141]   Chan LS, Robinson N, Xu L. Expression of interleukin-4 in the epidermis of transgenic mice results in a pruritic inflammatory skin disease: an experimental animal model to study atopic dermatitis. J Invest Dermatol 2001; 117: 977-83.

[142]   Nordlind K, Chin LB, Ahmed AA, *et al.* Immunohistochemical localization of interleukin-6-like immunoreactivity to peripheral nerve-like structures in normal and inflamed human skin. Arch Dermatol Res 1996; 288: 431-5.

[143]   Zheng T, Oh MH, Oh SY, *et al.* Transgenic expression of interleukin-13 in the skin induces a pruritic dermatitis and skin remodeling. J Invest Dermatol 2009; 129: 742-51.

[144]   Dillon SR, Sprecher C, Hammond A, *et al.* Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. Nat Immunol 2004; 5: 752-60.

[145]   Takaoka A, Arai I, Sugimoto M, *et al.* Expression of IL-31 gene transcripts in NC/Nga mice with atopic dermatitis. Eur J Pharmacol 2005; 516: 180-1.

[146]   Bilsborough J, Leung DY, Maurer M, *et al.* IL-31 is associated with cutaneous lymphocyte antigen-positive skin homing T cells in patients with atopic dermatitis. J Allergy Clin Immunol 2006; 117: 418-25.

[147]   Nobbe S, Dziunycz P, Muhleisen B, *et al.* IL-31 expression by inflammatory cells is preferentially elevated in atopic dermatitis. Acta Derm Venereol 2012; 92: 24-8.

[148]   Sonkoly E, Muller A, Lauerma AI, *et al.* IL-31: a new link between T cells and pruritus in atopic skin inflammation. J Allergy Clin Immunol 2006; 117: 411-7.

[149]   Kim S, Kim HJ, Yang HS, *et al.* IL-31 Serum Protein and Tissue mRNA Levels in Patients with Atopic Dermatitis. Ann Dermatol 2011; 23: 468-73.

[150]   Ezzat MH, Hasan ZE, Shaheen KY. Serum measurement of interleukin-31 (IL-31) in paediatric atopic dermatitis: elevated levels correlate with severity scoring. J Eur Acad Dermatol Venereol 2011; 25: 334-9.

[151]   Bando T, Morikawa Y, Komori T, *et al.* Complete overlap of interleukin-31 receptor A and oncostatin M receptor beta in the adult dorsal root ganglia with distinct developmental expression patterns. Neuroscience 2006; 142: 1263-71.

[152]   Kasraie S, Niebuhr M, Baumert K, *et al.* Functional effects of interleukin 31 in human primary keratinocytes. Allergy 2011; 66: 845-52.

[153]   Ishii T, Wang J, Zhang W, *et al.* Pivotal role of mast cells in pruritogenesis in patients with myeloproliferative disorders. Blood 2009; 113: 5942-50.

[154]   Nilius B, Voets T. TRP channels: a TR(I)P through a world of multifunctional cation channels. Pflugers Arch 2005; 451: 1-10.

[155]   Biro T, Acs G, Acs P, *et al.* Recent advances in understanding of vanilloid receptors: a therapeutic target for treatment of pain and inflammation in skin. J Investig Dermatol Symp Proc 1997; 2: 56-60.

[156]   Spassova MA, Soboloff J, He LP, *et al.* Calcium entry mediated by SOCs and TRP channels: variations and enigma. Biochim Biophys Acta 2004; 1742: 9-20.

[157]   Schlingmann KP, Waldegger S, Konrad M, *et al.* TRPM6 and TRPM7--Gatekeepers of human magnesium metabolism. Biochim Biophys Acta 2007; 1772: 813-21.

[158]   Nilius B, Owsianik G, Voets T, *et al.* Transient receptor potential cation channels in disease. Physiol Rev 2007; 87: 165-217.

[159]   Dong XP, Wang X, Xu H. TRP channels of intracellular membranes. J Neurochem 2010; 113: 313-28.

[160]   Colsoul B, Vennekens R, Nilius B. Transient receptor potential cation channels in pancreatic beta cells. Rev Physiol Biochem Pharmacol 2011; 161: 87-110.

[161]   Talavera K, Nilius B, Voets T. Neuronal TRP channels: thermometers, pathfinders and life-savers. Trends Neurosci 2008; 31: 287-95.

[162]   Shapovalov G, Lehen'kyi V, Skryma R, *et al.* TRP channels in cell survival and cell death in normal and transformed cells. Cell Calcium 2011; 50: 295-302.

[163]   Denda M, Tsutsumi M. Roles of transient receptor potential proteins (TRPs) in epidermal keratinocytes. Adv Exp Med Biol 2011; 704: 847-60.

[164]   Cortright DN, Szallasi A. TRP channels and pain. Curr Pharm Des 2009; 15: 1736-49.

[165]   Chung MK, Jung SJ, Oh SB. Role of TRP channels in pain sensation. Adv Exp Med Biol 2011; 704: 615-36.

[166]   Cosens DJ, Manning A. Abnormal electroretinogram from a Drosophila mutant. Nature 1969; 224: 285-7.

[167]   Montell C, Rubin GM. Molecular characterization of the Drosophila trp locus: a putative integral membrane protein required for phototransduction. Neuron 1989; 2: 1313-23.

[168]   Moran MM, Xu H, Clapham DE. TRP ion channels in the nervous system. Curr Opin Neurobiol 2004; 14: 362-9.

[169]   Pedersen SF, Owsianik G, Nilius B. TRP channels: an overview. Cell Calcium 2005; 38: 233-52.

[170]   Nilius B, Owsianik G. The transient receptor potential family of ion channels. Genome Biol 2011; 12: 218.

[171]   Nilius B. TRP channels in disease. Biochim Biophys Acta 2007; 1772: 805-12.

[172]   Nilius B, Owsianik G. Transient receptor potential channelopathies. Pflugers Arch 2010; 460: 437-50.

[173]   Caterina MJ, Schumacher MA, Tominaga M, *et al.* The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 1997; 389: 816-24.

[174]   Szallasi A, Blumberg PM. Vanilloid (Capsaicin) receptors and mechanisms. Pharmacol Rev 1999; 51: 159-212.

[175]   Kedei N, Szabo T, Lile JD, *et al.* Analysis of the native quaternary structure of vanilloid receptor 1. J Biol Chem 2001; 276: 28613-9.

[176]   Vriens J, Nilius B, Vennekens R. Herbal compounds and toxins modulating TRP channels. Curr Neuropharmacol 2008; 6: 79-96.

[177]   Szallasi A, Blumberg PM. Resiniferatoxin, a phorbol-related diterpene, acts as an ultrapotent analog of capsaicin, the irritant constituent in red pepper. Neuroscience 1989; 30: 515-20.

[178]   Winter J, Dray A, Wood JN, *et al.* Cellular mechanism of action of resiniferatoxin: a potent sensory neuron excitotoxin. Brain Res 1990; 520: 131-40.

[179]   Zygmunt PM, Petersson J, Andersson DA, *et al.* Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 1999; 400: 452-7.

[180]   Dinis P, Charrua A, Avelino A, *et al.* Anandamide-evoked activation of vanilloid receptor 1 contributes to the development of bladder hyperreflexia and nociceptive transmission to spinal dorsal horn neurons in cystitis. J Neurosci 2004; 24: 11253-63.

[181]   Huang SM, Bisogno T, Trevisani M, *et al.* An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. Proc Natl Acad Sci U S A 2002; 99: 8400-5.

[182]   Hwang SW, Cho H, Kwak J, *et al.* Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. Proc Natl Acad Sci U S A 2000; 97: 6155-60.

[183]   Shin J, Cho H, Hwang SW, *et al.* Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia. Proc Natl Acad Sci U S A 2002; 99: 10150-5.

[184]   Chuang HH, Prescott ED, Kong H, *et al.* Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature 2001; 411: 957-62.

[185]   Tominaga M, Wada M, Masu M. Potentiation of capsaicin receptor activity by metabotropic ATP receptors as a possible mechanism for ATP-evoked pain and hyperalgesia. Proc Natl Acad Sci U S A 2001; 98: 6951-6.

[186]   Lopshire JC, Nicol GD. The cAMP transduction cascade mediates the prostaglandin E2 enhancement of the capsaicin-elicited current in rat sensory neurons: whole-cell and single-channel studies. J Neurosci 1998; 18: 6081-92.

[187]   Mohapatra DP, Nau C. Desensitization of capsaicin-activated currents in the vanilloid receptor TRPV1 is decreased by the cyclic AMP-dependent protein kinase pathway. J Biol Chem 2003; 278: 50080-90.

[188]   Kim BM, Lee SH, Shim WS, *et al.* Histamine-induced Ca(2+) influx via the PLA(2)/lipoxygenase/TRPV1 pathway in rat sensory neurons. Neurosci Lett 2004; 361: 159-62.

[189] Nicol GD, Lopshire JC, Pafford CM. Tumor necrosis factor enhances the capsaicin sensitivity of rat sensory neurons. J Neurosci 1997; 17: 975-82.

[190] Khan AA, Diogenes A, Jeske NA, *et al.* Tumor necrosis factor alpha enhances the sensitivity of rat trigeminal neurons to capsaicin. Neuroscience 2008; 155: 503-9.

[191] Zhang N, Inan S, Cowan A, *et al.* A proinflammatory chemokine, CCL3, sensitizes the heat- and capsaicin-gated ion channel TRPV1. Proc Natl Acad Sci U S A 2005; 102: 4536-41.

[192] Amadesi S, Nie J, Vergnolle N, *et al.* Protease-activated receptor 2 sensitizes the capsaicin receptor transient receptor potential vanilloid receptor 1 to induce hyperalgesia. J Neurosci 2004; 24: 4300-12.

[193] Dai Y, Moriyama T, Higashi T, *et al.* Proteinase-activated receptor 2-mediated potentiation of transient receptor potential vanilloid subfamily 1 activity reveals a mechanism for proteinase-induced inflammatory pain. J Neurosci 2004; 24: 4293-9.

[194] Premkumar LS, Ahern GP. Induction of vanilloid receptor channel activity by protein kinase C. Nature 2000; 408: 985-90.

[195] Vellani V, Mapplebeck S, Moriondo A, *et al.* Protein kinase C activation potentiates gating of the vanilloid receptor VR1 by capsaicin, protons, heat and anandamide. J Physiol 2001; 534: 813-25.

[196] Tang HB, Inoue A, Oshita K, *et al.* Sensitization of vanilloid receptor 1 induced by bradykinin via the activation of second messenger signaling cascades in rat primary afferent neurons. Eur J Pharmacol 2004; 498: 37-43.

[197] Lukacs V, Thyagarajan B, Varnai P, *et al.* Dual regulation of TRPV1 by phosphoinositides. J Neurosci 2007; 27: 7070-80.

[198] Rohacs T, Thyagarajan B, Lukacs V. Phospholipase C mediated modulation of TRPV1 channels. Mol Neurobiol 2008; 37: 153-63.

[199] Nilius B, Owsianik G, Voets T. Transient receptor potential channels meet phosphoinositides. EMBO J 2008; 27: 2809-16.

[200] Voets T, Nilius B. Modulation of TRPs by PIPs. J Physiol 2007; 582: 939-44.

[201] Lishko PV, Procko E, Jin X, *et al.* The ankyrin repeats of TRPV1 bind multiple ligands and modulate channel sensitivity. Neuron 2007; 54: 905-18.

[202] Cesare P, Moriondo A, Vellani V, *et al.* Ion channels gated by heat. Proc Natl Acad Sci U S A 1999; 96: 7658-63.

[203] Voets T, Droogmans G, Wissenbach U, *et al.* The principle of temperature-dependent gating in cold- and heat-sensitive TRP channels. Nature 2004; 430: 748-54.

[204] Nilius B, Talavera K, Owsianik G, *et al.* Gating of TRP channels: a voltage connection? J Physiol 2005; 567: 35-44.

[205] Caterina MJ, Julius D. The vanilloid receptor: a molecular gateway to the pain pathway. Annu Rev Neurosci 2001; 24: 487-517.

[206] Tominaga M, Caterina MJ, Malmberg AB, *et al.* The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 1998; 21: 531-43.

[207] Caterina MJ, Leffler A, Malmberg AB, *et al.* Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 2000; 288: 306-13.

[208] Davis JB, Gray J, Gunthorpe MJ, *et al.* Vanilloid receptor-1 is essential for inflammatory thermal hyperalgesia. Nature 2000; 405: 183-7.

[209] Holzer P. Capsaicin: cellular targets, mechanisms of action, and selectivity for thin sensory neurons. Pharmacol Rev 1991; 43: 143-201.

[210] Alenmyr L, Hogestatt ED, Zygmunt PM, *et al.* TRPV1-mediated itch in seasonal allergic rhinitis. Allergy 2009; 64: 807-10.

[211] Naono-Nakayama R, Sunakawa N, Ikeda T, *et al.* Differential effects of substance P or hemokinin-1 on transient receptor potential channels, TRPV1, TRPA1 and TRPM8, in the rat. Neuropeptides 2010; 44: 57-61.

[212] Liang J, Xiao G, Ji W. Capsaicin induces reflex scratching in inflamed skin. Pharmacology 2011; 88: 82-7.

[213] Shim WS, Tak MH, Lee MH, *et al.* TRPV1 mediates histamine-induced itching via the activation of phospholipase A2 and 12-lipoxygenase. J Neurosci 2007; 27: 2331-7.

[214] Imamachi N, Park GH, Lee H, *et al.* TRPV1-expressing primary afferents generate behavioral responses to pruritogens via multiple mechanisms. Proc Natl Acad Sci U S A 2009; 106: 11330-5.

[215] Kim AY, Tang Z, Liu Q, *et al.* Pirt, a phosphoinositide-binding protein, functions as a regulatory subunit of TRPV1. Cell 2008; 133: 475-85.

[216] Patel KN, Liu Q, Meeker S, *et al.* Pirt, a TRPV1 modulator, is required for histamine-dependent and -independent itch. PLoS One 2011; 6: e20559.

[217] Liu T, Gao YJ, Ji RR. Emerging role of Toll-like receptors in the control of pain and itch. Neurosci Bull 2012; 28: 131-44.

[218] Liu T, Xu ZZ, Park CK, *et al.* Toll-like receptor 7 mediates pruritus. Nat Neurosci 2010; 13: 1460-2.

[219] Kim SJ, Park GH, Kim D, *et al.* Analysis of cellular and behavioral responses to imiquimod reveals a unique itch pathway in transient receptor potential vanilloid 1 (TRPV1)-expressing neurons. Proc Natl Acad Sci U S A 2011; 108: 3371-6.

[220] Liu T, Berta T, Xu ZZ, *et al.* TLR3 deficiency impairs spinal cord synaptic transmission, central sensitization, and pruritus in mice. J Clin Invest 2012; 122: 2195-207.

[221] Liu Y, Abdel Samad O, Zhang L, *et al.* VGLUT2-dependent glutamate release from nociceptors is required to sense pain and suppress itch. Neuron 2010; 68: 543-56.

[222] Lagerstrom MC, Rogoz K, Abrahamsen B, *et al.* VGLUT2-dependent sensory neurons in the TRPV1 population regulate pain and itch. Neuron 2010; 68: 529-42.

[223] Bodo E, Biro T, Telek A, *et al.* A hot new twist to hair biology: involvement of vanilloid receptor-1 (VR1/TRPV1) signaling in human hair growth control. Am J Pathol 2005; 166: 985-98.

[224] Bodo E, Kovacs I, Telek A, *et al.* Vanilloid receptor-1 (VR1) is widely expressed on various epithelial and mesenchymal cell types of human skin. J Invest Dermatol 2004; 123: 410-3.

[225] Toth BI, Benko S, Szollosi AG, *et al.* Transient receptor potential vanilloid-1 signaling inhibits differentiation and activation of human dendritic cells. FEBS Lett 2009; 583: 1619-24.

[226] Toth BI, Dobrosi N, Dajnoki A, *et al.* Endocannabinoids modulate human epidermal keratinocyte proliferation and survival via the sequential engagement of cannabinoid receptor-1 and transient receptor potential vanilloid-1. J Invest Dermatol 2011; 131: 1095-104.

[227] Toth BI, Geczy T, Griger Z, *et al.* Transient receptor potential vanilloid-1 signaling as a regulator of human sebocyte biology. J Invest Dermatol 2009; 129: 329-39.

[228] Amantini C, Mosca M, Lucciarini R, *et al.* Distinct thymocyte subsets express the vanilloid receptor VR1 that mediates capsaicin-induced apoptotic cell death. Cell Death Differ 2004; 11: 1342-56.

[229] Birder LA, Kanai AJ, de Groat WC, *et al.* Vanilloid receptor expression suggests a sensory role for urinary bladder epithelial cells. Proc Natl Acad Sci U S A 2001; 98: 13396-401.

[230] Biro T, Brodie C, Modarres S, *et al.* Specific vanilloid responses in C6 rat glioma cells. Brain Res Mol Brain Res 1998; 56: 89-98.

[231] Biro T, Maurer M, Modarres S, *et al.* Characterization of functional vanilloid receptors expressed by mast cells. Blood 1998; 91: 1332-40.

[232] Denda M, Fuziwara S, Inoue K, *et al.* Immunoreactivity of VR1 on epidermal keratinocyte of human skin. Biochem Biophys Res Commun 2001; 285: 1250-2.

[233] Inoue K, Koizumi S, Fuziwara S, *et al.* Functional vanilloid receptors in cultured normal human epidermal keratinocytes. Biochem Biophys Res Commun 2002; 291: 124-9.

[234] Basu S, Srivastava P. Immunological role of neuronal receptor vanilloid receptor 1 expressed on dendritic cells. Proc Natl Acad Sci U S A 2005; 102: 5120-5.

[235] Stander S, Moormann C, Schumacher M, *et al.* Expression of vanilloid receptor subtype 1 in cutaneous sensory nerve fibers, mast cells, and epithelial cells of appendage structures. Exp Dermatol 2004; 13: 129-39.

[236] Southall MD, Li T, Gharibova LS, *et al.* Activation of epidermal vanilloid receptor-1 induces release of proinflammatory mediators in human keratinocytes. J Pharmacol Exp Ther 2003; 304: 217-22.

[237] Lee YM, Kim YK, Kim KH, *et al.* A novel role for the TRPV1 channel in UV-induced matrix metalloproteinase (MMP)-1 expression in HaCaT cells. J Cell Physiol 2009; 219: 766-75.

[238] Lee YM, Li WH, Kim YK, *et al.* Heat-induced MMP-1 expression is mediated by TRPV1 through PKCalpha signaling in HaCaT cells. Exp Dermatol 2008; 17: 864-70.

[239] Li WH, Lee YM, Kim JY, *et al.* Transient receptor potential vanilloid-1 mediates heat-shock-induced matrix metalloproteinase-1 expression in human epidermal keratinocytes. J Invest Dermatol 2007; 127: 2328-35.

[240] Lee YM, Kang SM, Chung JH. The role of TRPV1 channel in aged human skin. J Dermatol Sci 2012; 65: 81-5.

[241] Denda M, Sokabe T, Fukumi-Tominaga T, *et al.* Effects of skin surface temperature on epidermal permeability barrier homeostasis. J Invest Dermatol 2007; 127: 654-9.

[242] Yun JW, Seo JA, Jang WH, *et al.* Antipruritic effects of TRPV1 antagonist in murine atopic dermatitis and itching models. J Invest Dermatol 2011; 131: 1576-9.

[243] Szallasi A, Cruz F, Geppetti P. TRPV1: a therapeutic target for novel analgesic drugs? Trends Mol Med 2006; 12: 545-54.

[244] Stander S, Luger T, Metze D. Treatment of prurigo nodularis with topical capsaicin. J Am Acad Dermatol 2001; 44: 471-8.

[245] Gooding SM, Canter PH, Coelho HF, *et al.* Systematic review of topical capsaicin in the treatment of pruritus. Int J Dermatol 2010; 49: 858-65.

[246] Blom HM, Van Rijswijk JB, Garrelds IM, *et al.* Intranasal capsaicin is efficacious in non-allergic, non-infectious perennial rhinitis. A placebo-controlled study. Clin Exp Allergy 1997; 27: 796-801.

[247] Ellis CN, Berberian B, Sulica VI, *et al.* A double-blind evaluation of topical capsaicin in pruritic psoriasis. J Am Acad Dermatol 1993; 29: 438-42.

[248] Lacroix JS, Buvelot JM, Polla BS, *et al.* Improvement of symptoms of non-allergic chronic rhinitis by local treatment with capsaicin. Clin Exp Allergy 1991; 21: 595-600.

[249] Lotti T, Teofoli P, Tsampau D. Treatment of aquagenic pruritus with topical capsaicin cream. J Am Acad Dermatol 1994; 30: 232-5.

[250] Lysy J, Sistiery-Ittah M, Israelit Y, *et al.* Topical capsaicin--a novel and effective treatment for idiopathic intractable pruritus ani: a randomised, placebo controlled, crossover study. Gut 2003; 52: 1323-6.

[251] Makhlough A, Ala S, Haj-Heydari Z, *et al.* Topical capsaicin therapy for uremic pruritus in patients on hemodialysis. Iran J Kidney Dis 2010; 4: 137-40.

[252] Stjarne P, Rinder J, Heden-Blomquist E, *et al.* Capsaicin desensitization of the nasal mucosa reduces symptoms upon allergen challenge in patients with allergic rhinitis. Acta Otolaryngol 1998; 118: 235-9.

[253] Tarng DC, Cho YL, Liu HN, *et al.* Hemodialysis-related pruritus: a double-blind, placebo-controlled, crossover study of capsaicin 0.025% cream. Nephron 1996; 72: 617-22.

[254] Wallengren J, Klinker M. Successful treatment of notalgia paresthetica with topical capsaicin: vehicle-controlled, double-blind, crossover study. J Am Acad Dermatol 1995; 32: 287-9.

[255] Weisshaar E, Dunker N, Gollnick H. Topical capsaicin therapy in humans with hemodialysis-related pruritus. Neurosci Lett 2003; 345: 192-4.

[256] Kim DK, Kim HJ, Kim H, *et al.* Involvement of serotonin receptors 5-HT1 and 5-HT2 in 12(S)-HPETE-induced scratching in mice. Eur J Pharmacol 2008; 579: 390-4.

[257] Sekine R, Satoh T, Takaoka A, *et al.* Anti pruritic effects of topical crotamiton, capsaicin, and a corticosteroid on pruritogen-induced scratching behavior. Exp Dermatol 2012; 21: 201-4.

[258] FolsterHolst R, Brasch J. Effect of topically applied capsaicin on pruritus in patients with atopic dermatitis. J Dermatol Treatment 1996; 7: 13-5.

[259] Li H, Wang S, Chuang AY, *et al.* Activity-dependent targeting of TRPV1 with a pore-permeating capsaicin analog. Proc Natl Acad Sci U S A 2011; 108: 8497-502.

[260] Moran MM, McAlexander MA, Biro T, *et al.* Transient receptor potential channels as therapeutic targets. Nat Rev Drug Discov 2011; 10: 601-20.

[261] Lim KM, Park YH. Development of PAC-14028, a novel transient receptor potential vanilloid type 1 (TRPV1) channel antagonist as a new drug for refractory skin diseases. Arch Pharm Res 2012; 35: 393-6.

[262] Yun JW, Seo JA, Jeong YS, *et al.* TRPV1 antagonist can suppress the atopic dermatitis-like symptoms by accelerating skin barrier recovery. J Dermatol Sci 2011; 62: 8-15.

[263] Pereira U, Boulais N, Lebonvallet N, *et al.* Mechanisms of the sensory effects of tacrolimus on the skin. Br J Dermatol 2010; 163: 70-7.

[264] Caterina MJ, Rosen TA, Tominaga M, *et al.* A capsaicin-receptor homologue with a high threshold for noxious heat. Nature 1999; 398: 436-41.

[265] Axelsson HE, Minde JK, Sonesson A, *et al.* Transient receptor potential vanilloid 1, vanilloid 2 and melastatin 8 immunoreactive nerve fibers in human skin from individuals with and without Norrbottnian congenital insensitivity to pain. Neuroscience 2009; 162: 1322-32.

[266] Rutter AR, Ma QP, Leveridge M, *et al.* Heteromerization and colocalization of TrpV1 and TrpV2 in mammalian cell lines and rat dorsal root ganglia. Neuroreport 2005; 16: 1735-9.

[267] Radtke C, Sinis N, Sauter M, *et al.* TRPV channel expression in human skin and possible role in thermally induced cell death. J Burn Care Res 2011; 32: 150-9.

[268] Zhang D, Spielmann A, Wang L, *et al.* Mast-cell degranulation induced by physical stimuli involves the activation of transient-receptor-potential channel TRPV2. Physiol Res 2012; 61: 113-24.

[269] Park U, Vastani N, Guan Y, *et al.* TRP vanilloid 2 knock-out mice are susceptible to perinatal lethality but display normal thermal and mechanical nociception. J Neurosci 2011; 31: 11425-36.

[270] Neeper MP, Liu Y, Hutchinson TL, *et al.* Activation properties of heterologously expressed mammalian TRPV2: evidence for species dependence. J Biol Chem 2007; 282: 15894-902.

[271] Qin N, Neeper MP, Liu Y, *et al.* TRPV2 is activated by cannabidiol and mediates CGRP release in cultured rat dorsal root ganglion neurons. J Neurosci 2008; 28: 6231-8.

[272] Stokes AJ, Shimoda LM, Koblan-Huberson M, *et al.* A TRPV2-PKA signaling module for transduction of physical stimuli in mast cells. J Exp Med 2004; 200: 137-47.

[273] Eid SR, Cortright DN. Transient receptor potential channels on sensory nerves. Handb Exp Pharmacol 2009; 261-81.

[274] Peier AM, Reeve AJ, Andersson DA, *et al.* A heat-sensitive TRP channel expressed in keratinocytes. Science 2002; 296: 2046-9.

[275] Xu H, Ramsey IS, Kotecha SA, *et al.* TRPV3 is a calcium-permeable temperature-sensitive cation channel. Nature 2002; 418: 181-6.

[276] Smith GD, Gunthorpe MJ, Kelsell RE, *et al.* TRPV3 is a temperature-sensitive vanilloid receptor-like protein. Nature 2002; 418: 186-90.

[277] Delany NS, Hurle M, Facer P, *et al.* Identification and characterization of a novel human vanilloid receptor-like protein, VRL-2. Physiol Genomics 2001; 4: 165-74.

[278] Liedtke W, Choe Y, Marti-Renom MA, *et al.* Vanilloid receptor-related osmotically activated channel (VR-OAC), a candidate vertebrate osmoreceptor. Cell 2000; 103: 525-35.

[279] Strotmann R, Harteneck C, Nunnenmacher K, *et al.* OTRPC4, a nonselective cation channel that confers sensitivity to extracellular osmolarity. Nat Cell Biol 2000; 2: 695-702.

[280] Wissenbach U, Bodding M, Freichel M, *et al.* Trp12, a novel Trp related protein from kidney. FEBS Lett 2000; 485: 127-34.

[281] Suzuki M, Watanabe Y, Oyama Y, *et al.* Localization of mechanosensitive channel TRPV4 in mouse skin. Neurosci Lett 2003; 353: 189-92.

[282] Benham CD, Gunthorpe MJ, Davis JB. TRPV channels as temperature sensors. Cell Calcium 2003; 33: 479-87.

[283] Guler AD, Lee H, Iida T, *et al.* Heat-evoked activation of the ion channel, TRPV4. J Neurosci 2002; 22: 6408-14.

[284] Watanabe H, Vriens J, Suh SH, *et al.* Heat-evoked activation of TRPV4 channels in a HEK293 cell expression system and in native mouse aorta endothelial cells. J Biol Chem 2002; 277: 47044-51.

[285] Todaka H, Taniguchi J, Satoh J, *et al.* Warm temperature-sensitive transient receptor potential vanilloid 4 (TRPV4) plays an essential role in thermal hyperalgesia. J Biol Chem 2004; 279: 35133-8.

[286] Lee H, Iida T, Mizuno A, *et al.* Altered thermal selection behavior in mice lacking transient receptor potential vanilloid 4. J Neurosci 2005; 25: 1304-10.

[287] Moqrich A, Hwang SW, Earley TJ, *et al.* Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. Science 2005; 307: 1468-72.

[288] Huang SM, Li X, Yu Y, *et al.* TRPV3 and TRPV4 ion channels are not major contributors to mouse heat sensation. Mol Pain 2011; 7: 37.

[289] Chung MK, Lee H, Caterina MJ. Warm temperatures activate TRPV4 in mouse 308 keratinocytes. J Biol Chem 2003; 278: 32037-46.

[290] Chung MK, Lee H, Mizuno A, *et al.* TRPV3 and TRPV4 mediate warmth-evoked currents in primary mouse keratinocytes. J Biol Chem 2004; 279: 21569-75.

[291] Lee H, Caterina MJ. TRPV channels as thermosensory receptors in epithelial cells. Pflugers Arch 2005; 451: 160-7.

[292] Mandadi S, Sokabe T, Shibasaki K, *et al.* TRPV3 in keratinocytes transmits temperature information to sensory neurons via ATP. Pflugers Arch 2009; 458: 1093-102.

[293] Huang SM, Lee H, Chung MK, *et al.* Overexpressed transient receptor potential vanilloid 3 ion channels in skin keratinocytes modulate pain sensitivity via prostaglandin E2. J Neurosci 2008; 28: 13727-37.

[294] Miyamoto T, Petrus MJ, Dubin AE, *et al.* TRPV3 regulates nitric oxide synthase-independent nitric oxide synthesis in the skin. Nat Commun 2011; 2: 369.

[295] Steinhoff M, Biro T. A TR(I)P to pruritus research: role of TRPV3 in inflammation and itch. J Invest Dermatol 2009; 129: 531-5.

[296] Yamamoto-Kasai E, Imura K, Yasui K, *et al.* TRPV3 as a Therapeutic Target for Itch. J Invest Dermatol 2012; 132: 2109-12.

[297] Asakawa M, Yoshioka T, Matsutani T, *et al.* Association of a mutation in TRPV3 with defective hair growth in rodents. J Invest Dermatol 2006; 126: 2664-72.

[298] Xiao R, Tian J, Tang J, *et al.* The TRPV3 mutation associated with the hairless phenotype in rodents is constitutively active. Cell Calcium 2008; 43: 334-43.

[299] Imura K, Yoshioka T, Hikita I, *et al.* Influence of TRPV3 mutation on hair growth cycle in mice. Biochem Biophys Res Commun 2007; 363: 479-83.

[300] Borbiro I, Lisztes E, Toth BI, *et al.* Activation of transient receptor potential vanilloid-3 inhibits human hair growth. J Invest Dermatol 2011; 131: 1605-14.

[301] Yoshioka T, Imura K, Asakawa M, *et al.* Impact of the Gly573Ser substitution in TRPV3 on the development of allergic and pruritic dermatitis in mice. J Invest Dermatol 2009; 129: 714-22.

[302] Lin Z, Chen Q, Lee M, *et al.* Exome sequencing reveals mutations in TRPV3 as a cause of Olmsted syndrome. Am J Hum Genet 2012; 90: 558-64.

[303] Cheng X, Jin J, Hu L, *et al.* TRP channel regulates EGFR signaling in hair morphogenesis and skin barrier formation. Cell 2010; 141: 331-43.

[304] Sokabe T, Tominaga M. The TRPV4 cation channel: A molecule linking skin temperature and barrier function. Commun Integr Biol 2010; 3: 619-21.

[305] Sokabe T, Fukumi-Tominaga T, Yonemura S, *et al.* The TRPV4 channel contributes to intercellular junction formation in keratinocytes. J Biol Chem 2010; 285: 18749-58.

[306] Kida N, Sokabe T, Kashio M, *et al.* Importance of transient receptor potential vanilloid 4 (TRPV4) in epidermal barrier function in human skin keratinocytes. Pflugers Arch 2011; 463: 715-25.

[307] Grant AD, Cottrell GS, Amadesi S, *et al.* Protease-activated receptor 2 sensitizes the transient receptor potential vanilloid 4 ion channel to cause mechanical hyperalgesia in mice. J Physiol 2007; 578: 715-33.

[308] Gopinath P, Wan E, Holdcroft A, *et al.* Increased capsaicin receptor TRPV1 in skin nerve fibres and related vanilloid receptors TRPV3 and TRPV4 in keratinocytes in human breast pain. BMC Womens Health 2005; 5: 2.

[309] Yang WZ, Chen JY, Yu JT et al. Effects of low power laser irradiation on intracellular calcium and histamine release in RBL-2H3 mast cells. Photochem Photobiol 2007; 83: 979-84.

[310] Kim KS, Shin DH, Nam JH, *et al.* Functional Expression of TRPV4 Cation Channels in Human Mast Cell Line (HMC-1). Korean J Physiol Pharmacol 2010; 14: 419-25.

[311] Story GM, Peier AM, Reeve AJ, *et al.* ANKTM1, a TRP-like channel expressed in nociceptive neurons, is activated by cold temperatures. Cell 2003; 112: 819-29.

[312] Kobayashi K, Fukuoka T, Obata K, *et al.* Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with adelta/c-fibers and colocalization with trk receptors. J Comp Neurol 2005; 493: 596-606.

[313] Bandell M, Story GM, Hwang SW, *et al.* Noxious cold ion channel TRPA1 is activated by pungent compounds and bradykinin. Neuron 2004; 41: 849-57.

[314] Jordt SE, Bautista DM, Chuang HH, *et al.* Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. Nature 2004; 427: 260-5.

[315] McNamara CR, Mandel-Brehm J, Bautista DM, *et al.* TRPA1 mediates formalin-induced pain. Proc Natl Acad Sci U S A 2007; 104: 13525-30.

[316] Talavera K, Gees M, Karashima Y, *et al.* Nicotine activates the chemosensory cation channel TRPA1. Nat Neurosci 2009; 12: 1293-9.

[317] Story GM. The emerging role of TRP channels in mechanisms of temperature and pain sensation. Curr Neuropharmacol 2006; 4: 183-96.

[318] McMahon SB, Wood JN. Increasingly irritable and close to tears: TRPA1 in inflammatory pain. Cell 2006; 124: 1123-5.

[319] Andrade EL, Meotti FC, Calixto JB. TRPA1 antagonists as potential analgesic drugs. Pharmacol Ther 2012; 133: 189-204.

[320] Baraldi PG, Preti D, Materazzi S, *et al.* Transient receptor potential ankyrin 1 (TRPA1) channel as emerging target for novel analgesics and anti-inflammatory agents. J Med Chem 2010; 53: 5085-107.

[321] Liu Q, Tang Z, Surdenikova L, *et al.* Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. Cell 2009; 139: 1353-65.

[322] Wilson SR, Gerhold KA, Bifolck-Fisher A, *et al.* TRPA1 is required for histamine-independent, Mas-related G protein-coupled receptor-mediated itch. Nat Neurosci 2011; 14: 595-602.

[323] Xiao B, Patapoutian A. Scratching the surface: a role of pain-sensing TRPA1 in itch. Nat Neurosci 2011; 14: 540-2.

[324] Liu T, Ji RR. Oxidative stress induces itch via activation of transient receptor potential subtype ankyrin 1 in mice. Neurosci Bull 2012; 28: 145-54.

[325] Dai Y, Wang S, Tominaga M, *et al.* Sensitization of TRPA1 by PAR2 contributes to the sensation of inflammatory pain. J Clin Invest 2007; 117: 1979-87.

[326] Chen Y, Yang C, Wang ZJ. Proteinase-activated receptor 2 sensitizes transient receptor potential vanilloid 1, transient receptor potential vanilloid 4, and transient receptor potential ankyrin 1 in paclitaxel-induced neuropathic pain. Neuroscience 2011; 193: 440-51.

[327] Liang J, Ji Q, Ji W. Role of transient receptor potential ankyrin subfamily member 1 in pruritus induced by endothelin-1. Neurosci Lett 2011; 492: 175-8.

[328] Atoyan R, Shander D, Botchkareva NV. Non-neuronal expression of transient receptor potential type A1 (TRPA1) in human skin. J Invest Dermatol 2009; 129: 2312-5.

[329] Biro T, Kovacs L. An "ice-cold" TR(i)P to skin biology: the role of TRPA1 in human epidermal keratinocytes. J Invest Dermatol 2009; 129: 2096-9.

[330] Denda M, Tsutsumi M, Goto M, *et al.* Topical application of TRPA1 agonists and brief cold exposure accelerate skin permeability barrier recovery. J Invest Dermatol 2010; 130: 1942-5.

[331] Bautista DM, Siemens J, Glazer JM, *et al.* The menthol receptor TRPM8 is the principal detector of environmental cold. Nature 2007; 448: 204-8.

[332] Colburn RW, Lubin ML, Stone DJ, Jr., *et al.* Attenuated cold sensitivity in TRPM8 null mice. Neuron 2007; 54: 379-86.

[333] McKemy DD, Neuhausser WM, Julius D. Identification of a cold receptor reveals a general role for TRP channels in thermosensation. Nature 2002; 416: 52-8.

[334] Peier AM, Moqrich A, Hergarden AC, *et al.* A TRP channel that senses cold stimuli and menthol. Cell 2002; 108: 705-15.

[335] Frolich M, Enk A, Diepgen TL, *et al.* Successful treatment of therapy-resistant pruritus in lichen amyloidosis with menthol. Acta Derm Venereol 2009; 89: 524-6.

[336] Haught JM, Jukic DM, English JC, 3rd. Hydroxyethyl starch-induced pruritus relieved by a combination of menthol and camphor. J Am Acad Dermatol 2008; 59: 151-3.

[337] Bromm B, Scharein E, Darsow U, *et al.* Effects of menthol and cold on histamine-induced itch and skin reactions in man. Neurosci Lett 1995; 187: 157-60.

[338] Yosipovitch G, Szolar C, Hui XY, *et al.* Effect of topically applied menthol on thermal, pain and itch sensations and biophysical properties of the skin. Arch Dermatol Res 1996; 288: 245-8.

[339] Panahi Y, Davoodi SM, Khalili H, *et al.* Phenol and menthol in the treatment of chronic skin lesions following mustard gas exposure. Singapore Med J 2007; 48: 392-5.

[340] McCoy ES, Taylor-Blake B, Zylka MJ. CGRPalpha-expressing sensory neurons respond to stimuli that evoke sensations of pain and itch. PLoS One 2012; 7: e36355.

[341] Linte RM, Ciobanu C, Reid G, *et al.* Desensitization of cold- and menthol-sensitive rat dorsal root ganglion neurones by inflammatory mediators. Exp Brain Res 2007; 178: 89-98.

[342]   Zhang X, Mak S, Li L, *et al.* Direct inhibition of the cold-activated TRPM8 ion channel by Galpha(q). Nat Cell Biol 2012; 14: 851-8.

[343]   Cho Y, Jang Y, Yang YD, *et al.* TRPM8 mediates cold and menthol allergies associated with mast cell activation. Cell Calcium 2010; 48: 202-8.

[344]   Medic N, Desai A, Komarow H, *et al.* Examination of the role of TRPM8 in human mast cell activation and its relevance to the etiology of cold-induced urticaria. Cell Calcium 2011; 50: 473-80.

[345]   Denda M, Tsutsumi M, Denda S. Topical application of TRPM8 agonists accelerates skin permeability barrier recovery and reduces epidermal proliferation induced by barrier insult: role of cold-sensitive TRP receptors in epidermal permeability barrier homoeostasis. Exp Dermatol 2010; 19: 791-5.

[346]   Yosipovitch G, Fast K, Bernhard JD. Noxious heat and scratching decrease histamine-induced itch and skin blood flow. J Invest Dermatol 2005; 125: 1268-72.

[347]   Wang S, Lee J, Ro JY, *et al.* Warmth suppresses and desensitizes damage-sensing ion channel TRPA1. Mol Pain 2012; 8: 22.

[348]   Vriens J, Appendino G, Nilius B. Pharmacology of vanilloid transient receptor potential cation channels. Mol Pharmacol 2009; 75: 1262-79.

[349]   Xu H, Delling M, Jun JC, *et al.* Oregano, thyme and clove-derived flavors and skin sensitizers activate specific TRP channels. Nat Neurosci 2006; 9: 628-35.

[350]   Corsini E, Primavera A, Marinovich M, *et al.* Selective induction of cell-associated interleukin-1alpha in murine keratinocytes by chemical allergens. Toxicology 1998; 129: 193-200.

[351]   Kameyoshi Y, Morita E, Tanaka T, *et al.* Interleukin-1 alpha enhances mast cell growth by a fibroblast-dependent mechanism. Arch Dermatol Res 2000; 292: 240-7.

[352]   Macpherson LJ, Hwang SW, Miyamoto T, *et al.* More than cool: promiscuous relationships of menthol and other sensory compounds. Mol Cell Neurosci 2006; 32: 335-43.

[353]   Karashima Y, Damann N, Prenen J, *et al.* Bimodal action of menthol on the transient receptor potential channel TRPA1. J Neurosci 2007; 27: 9874-84.

[354]   Stotz SC, Vriens J, Martyn D, *et al.* Citral sensing by Transient [corrected] receptor potential channels in dorsal root ganglion neurons. PLoS One 2008; 3: e2082.

[355]   del Palacio A, Ortiz FJ, Perez A, *et al.* A double-blind randomized comparative trial: eberconazole 1% cream versus clotrimazole 1% cream twice daily in Candida and dermatophyte skin infections. Mycoses 2001; 44: 173-80.

[356]   Schopf R, Hettler O, Brautigam M, *et al.* Efficacy and tolerability of terbinafine 1% topical solution used for 1 week compared with 4 weeks clotrimazole 1% topical solution in the treatment of interdigital tinea pedis: a randomized, double-blind, multi-centre, 8-week clinical trial. Mycoses 1999; 42: 415-20.

[357]   Meseguer V, Karashima Y, Talavera K, *et al.* Transient receptor potential channels in sensory neurons are targets of the antimycotic agent clotrimazole. J Neurosci 2008; 28: 576-86.

[358]   Micallef L, Belaubre F, Pinon A, *et al.* Effects of extracellular calcium on the growth-differentiation switch in immortalized keratinocyte HaCaT cells compared with normal human keratinocytes. Exp Dermatol 2009; 18: 143-51.

[359]   Bikle DD, Ng D, Tu CL, *et al.* Calcium- and vitamin D-regulated keratinocyte differentiation. Mol Cell Endocrinol 2001; 177: 161-71.

[360]   Tu CL, Oda Y, Komuves L, *et al.* The role of the calcium-sensing receptor in epidermal differentiation. Cell Calcium 2004; 35: 265-73.

[361]   Yuspa SH, Hennings H, Tucker RW, *et al.* Signal transduction for proliferation and differentiation in keratinocytes. Ann N Y Acad Sci 1988; 548: 191-6.

[362]   Hanifin JM. Evolving concepts of pathogenesis in atopic dermatitis and other eczemas. J Invest Dermatol 2009; 129: 320-2.

[363]   Nilius B, Mahieu F. A road map for TR(I)Ps. Mol Cell 2006; 22: 297-307.

[364]   Ramsey IS, Delling M, Clapham DE. An introduction to TRP channels. Annu Rev Physiol 2006; 68: 619-47.

[365]   Cai S, Fatherazi S, Presland RB, *et al.* TRPC channel expression during calcium-induced differentiation of human gingival keratinocytes. J Dermatol Sci 2005; 40: 21-8.

[366]   Cai S, Fatherazi S, Presland RB, *et al.* Evidence that TRPC1 contributes to calcium-induced differentiation of human keratinocytes. Pflugers Arch 2006; 452: 43-52.

[367]   Fatherazi S, Presland RB, Belton CM, *et al.* Evidence that TRPC4 supports the calcium selective I(CRAC)-like current in human gingival keratinocytes. Pflugers Arch 2007; 453: 879-89.

[368]   Barfield RL, Barrett KR, Moon CM, *et al.* Pruritic linear papules on a 75-year-old woman: a case of localized Darier-White disease. Cutis 2002; 70: 225-8.

[369]   Pani B, Cornatzer E, Cornatzer W, *et al.* Up-regulation of transient receptor potential canonical 1 (TRPC1) following sarco(endo)plasmic reticulum Ca2+ ATPase 2 gene silencing promotes cell survival: a potential role for TRPC1 in Darier's disease. Mol Biol Cell 2006; 17: 4446-58.

[370]   Beck B, Lehen'kyi V, Roudbaraki M, *et al.* TRPC channels determine human keratinocyte differentiation: new insight into basal cell carcinoma. Cell Calcium 2008; 43: 492-505.

[371]   Leuner K, Kraus M, Woelfle U, *et al.* Reduced TRPC channel expression in psoriatic keratinocytes is associated with impaired differentiation and enhanced proliferation. PLoS One 2011; 6: e14716.

[372]   Muller M, Essin K, Hill K, *et al.* Specific TRPC6 channel activation, a novel approach to stimulate keratinocyte differentiation. J Biol Chem 2008; 283: 33942-54.

[373]   Woelfle U, Laszczyk MN, Kraus M, *et al.* Triterpenes promote keratinocyte differentiation *in vitro*, ex vivo and *in vivo*: a role for the transient receptor potential canonical (subtype) 6. J Invest Dermatol 2010; 130: 113-23.

[374]   Sun XD, You Y, Zhang L, *et al.* The possible role of TRPC6 in atopic dermatitis. Med Hypotheses 2012; 78: 42-4.

[375]   Thomsen JS, Nielsen PL, Serup J. The hypomagnesic rat model: dermatitis-prone hairless rats with mild magnesium depletion fed a diet low in lipids did not develop pruritic dermatitis. Skin Res Technol 2005; 11: 42-6.

[376]   Graf H, Kovarik J, Stummvoll HK, *et al.* Disappearance of uraemic pruritus after lowering dialysate magnesium concentration. Br Med J 1979; 2: 1478-9.

[377]   Muallem S, Moe OW. When EGF is offside, magnesium is wasted. J Clin Invest 2007; 117: 2086-9.

[378]   Groenestege WM, Thebault S, van der Wijst J, *et al.* Impaired basolateral sorting of pro-EGF causes isolated recessive renal hypomagnesemia. J Clin Invest 2007; 117: 2260-7.

[379]   Agero AL, Dusza SW, Benvenuto-Andrade C, *et al.* Dermatologic side effects associated with the epidermal growth factor receptor inhibitors. J Am Acad Dermatol 2006; 55: 657-70.

[380]   Porzio G, Aielli F, Verna L, *et al.* Efficacy of pregabalin in the management of cetuximab-related itch. J Pain Symptom Manage 2006; 32: 397-8.

[381]   Wu PA, Balagula Y, Lacouture ME, *et al.* Prophylaxis and treatment of dermatologic adverse events from epidermal growth factor receptor inhibitors. Curr Opin Oncol 2011; 23: 343-51.

[382]   Luo J, Stewart R, Berdeaux R, *et al.* Tonic Inhibition of TRPV3 by Mg(2+) in Mouse Epidermal Keratinocytes. J Invest Dermatol 2012; 132: 2158-65.