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Biochimica et Biophysica Acta 1772 (2007) 1004–1021

Biochimica et Biophysica Acta BBA

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Review

# TRP channels as novel players in the pathogenesis and therapy of itch

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Received 4 December 2006; received in revised form 8 March 2007; accepted 8 March 2007
Available online 19 March 2007

**Abstract**

Itch (pruritus) is a sensory phenomenon characterized by a (usually) negative affective component and the initiation of a special behavioral act, i.e. scratching. Older studies predominantly have interpreted itch as a type of pain. Recent neurophysiological findings, however, have provided compelling evidence that itch (although it indeed has intimate connections to pain) rather needs to be understood as a separate sensory modality. Therefore, a novel pruriceptive system has been proposed, within which itch-inducing peripheral mediators (pruritogens), itch-selective receptors (pruriceptors), sensory afferents and spinal cord neurons, and defined, itch-processing central nervous system regions display complex, layered responses to itch. In this review, we begin with a current overview on the neurophysiology of pruritus, and distinguish it from that of pain. We then focus on the functional characteristics of the large family of transient receptor potential (TRP) channels in skin-coupled sensory mechanisms, including itch and pain. In particular, we argue that – due to their expression patterns, activation mechanisms, regulatory roles, and pharmacological sensitivities – certain thermosensitive TRP channels are key players in pruritus pathogenesis. We close by proposing a novel, TRP-centered concept of pruritus pathogenesis and sketch important future experimental directions towards the therapeutic targeting of TRP channels in the clinical management of itch.


*Keywords:* Itch; Pruriceptive pruritus; Pruritogens; TRP channels

## 1. Neurophysiology of itch

### 1.1. Basic phenomenon — Itch is "created" by our brain

From a phenomenological point of view, itch (pruritus) can be defined as an unpleasant cutaneous sensation associated with urge desire to scratch, a more or less voluntary yet often subconscious motor activity [1,2]. Hence, itch (as a "product" of the central nervous system, CNS) [3,4] is clearly distinct from pain with respect to not only the subjective sensation but also the resulting motoric activities and behavioral patterns (e.g. pain-related withdrawal reflexes) [5]. This suggests that the neuronal "mechanism" for itch has developed as an evolutionarily ancient system for the removal or avoidance of potentially harmful agents (e.g. skin irritants, allergens, toxic plants) endangering the integrity of the body. Itch-induced scratching can then be interpreted as a goal-directed movement against noxious agents that have successfully passed the epidermal barrier and have already invaded the skin (where, against which, withdrawal would make no sense).

### 1.2. Categories of itch

From a clinical point of view, pruritus is one of the most common symptoms not only in dermatology, but also in general, pediatric and geriatric medicine, and requires immediate and effective therapy [2–8]. The importance of itch in general medicine is evident e.g. in various malignancies (including lymphoma, leukemia, and metastatic cancer), metabolic and endocrine diseases (such as diabetes mellitus and thyroid dysfunction), uremia, iron deficiency, generalized infection

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doi:10.1016/j.bbadis.2007.03.002

and inflammation, application of certain drugs, chronic psycho-emotional stress, and in psychosomatic or psychiatric diseases [2–9]. In short, "itch" is one of the central themes of medicine and the life sciences.

Both clinicians and neurophysiologists, therefore, must be able to differentiate between different types of pruritus. Given that pruritus can be initiated by localized or systemic as well as by peripheral or central stimuli (resulting in either localized or generalized pruritus), the following itch categories have been suggested, based on partly mechanism-based (i.e. neurophysiologically defined) and partly operational (i.e. clinically relevant) definitions [2,4,9]:

- Pruriceptive itch: peripherally induced pruritus arising from skin 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, thyroid dysfunction.
- Neuropathic itch: pruritus due to a primary neurological disorder of the central or peripheral nervous systems, such as it can be induced by certain brain tumors, multiple sclerosis, peripheral neuropathy (e.g. postherpetic pruritus), nerve compression or irritation (e.g. notalgia paresthetica, brachioradial pruritus).
- Psychogenic itch: pruritus is related to psychological or psychiatric disorders such as parasitophobia, obsessive–compulsive disorder, leading to "neurotic excoriations".

This review focuses on the first itch category (pruriceptive itch), i.e. the characteristics of pruritus originating from the skin.

### 1.3. Theories of itch

From a neurophysiological point of view, numerous questions arise when one tries to define the exact mechanism of itch among the plethora of distinct sensory physiological phenomena. These include, for example:

- Is itch a type of pain, or just a "pain in the neck"?
- In other words, does pruritus "function" as a sub-modality of pain or is it rather a separate sensory entity?
- Actually, what is the exact neurophysiological relationship between itch and pain?
- Accordingly, do we really possess a specific sensory pathway for itch, or does itch "use" defined elements of the nociceptive system?

In order to properly answer these questions, it helps to begin by systemically reviewing competing theories of itch.

According to the "intensity theory" of itch, low level activation of nociceptors induces pruritus, whereas higher stimulation rate provokes pain [10]. Following this theory, itch represents quasi a submodality of pain and is based on a specific pattern of action potentials running via nociceptive pathways; hence, itch may result from the combination of other primary sensory signals [2,7]. However, application of low concentrations of algogens generally does not cause itch, just less intense pain, and stimulation of afferent nerves induces either pain or pruritus. Moreover, increasing the stimulation frequency elevates the intensity of pain or itch, but no transition from one sensory modality to the other is recognized [5]. These arguments clearly challenge the validity of the "intensity theory".

It is well known, however, that pain inhibits itch [5,6,8,11,12]. Actually, the itch-induced scratching response, in itself, is a motoric activity to induce pain and, hence, to alleviate itch. This has encouraged the development of an itch hypothesis that mirrors the "gate-control theory" of pain [8]. Indeed, numerous painful signals (such as noxious heat and cold, mechanical, chemical or electrical stimuli) – via the activation of specific nociceptive pathways and spinal cord as well as higher CNS structures – have already been experimentally shown to inhibit itch. This strongly suggests a central mode of action [5,11,13–15]. Interestingly, certain agents that effectively induce analgesia (e.g. μ-opioid receptor agonists) may indeed *reduce* the *inhibition of itch by pain*, thus enhancing pruritus [16,17]. Conversely, the application of μ-opioid antagonists (e.g. to patients with cholestatic itch) can exert antipruritic effects; however, the reduction of itch is accompanied by the induction of pain [18] and withdrawal-like reactions [19]. It appears, therefore, that besides similar patterns in sensation characteristics, a definite antagonistic interaction does exist between itch and pain.

Recent landmark findings have helped to establish a novel hypothesis for the generation of pruritus; i.e. the "specificity or selectivity theory" of itch [4], and introduced the novel *pruriceptive system* dedicated for peripheral and central itch processing. As detailed below, the pruriceptive system contains specific as well as non-specific sensory afferents, itch-inducing mediators (collectively referred to as pruritogens), and itch-sensitive spinal projection neurons as well as higher CNS structures. It is noteworthy, however, that the individual components of the pruriceptive system are strikingly similar (if not identical) to those of the classical, long-established nociceptive system. This provides sound neurophysiological explanations for the intimate relationship between itch and pain, as delineated above.

### 1.4. The pruriceptive system

#### 1.4.1. Pruriceptive primary afferent fibers

The skin is highly innervated with a dense network of sensory afferents. Among the unmyelinated C-afferent axons (also referred to as C-nociceptors), approximately 80% are mechano-sensitive polymodal nociceptors, which respond to mechanical, thermal (heat), and chemical stimuli [11,20,21]. The remaining approximately 20% do not respond to mechanical stimulation ("silent nociceptors") and are activated by chemical stimuli [20,22].

Among the mechano-insensitive human C-fibers, a certain subset of afferents (comprising approximately 20% of the mechano-heat-insensitive class of C-fibers) has been discovered

and characterized which have a strong and sustained response to histamine (one of the most "popular" mediators of itch, see below) in parallel to the itch ratings of human subjects [23–28]. However, the histamine-sensitive fiber subclass cannot account for all aspects of itch, especially when pruritus is induced mechanically or without the characteristic flare reaction (axon-reflex erythema). Thus, other subgroups of primary afferents (most probably mechano-heat sensitive ones) must be involved in the generation of pruritus [29]. This diversity of afferent "itch fibers" would nicely explain the various "submodalities" of pruritus experienced by patients, similar to those of pain [30,31]. Nevertheless, these intriguing data strongly suggest that there is a *specific* or – since pruriceptors also respond to capsaicin and mustard oil, i.e. characteristic activators of mechano-heat sensitive afferents (for details, see below) [32] – rather a *highly selective* neuronal pathway for itch.

*1.4.2. Spinal pruriceptive projection neurons and higher CNS structures involved in central processing of itch*

This concept has been further strengthened by the identification of a distinct, histamine-responsive, and mechano-insensitive class of dorsal horn (lamina I) neurons in the cat [12]. Importantly, the time course of these responses was perfectly paralleled to that of itch sensation in humans. Interestingly, in contrast to nociceptive second-order spinothalamic neurons, the itch-specific projection neurons do not exhibit spontaneous activity. It is proposed that the lack of spontaneous activity may be generated by an active (tonic) inhibition exerted by pain-processing neurons [11]. Therefore, suspension of the activity of the "itch-inhibitory" nociceptive pathway might provoke pruritus by a pure spinal mechanism — i.e. without any activation of pruriceptive afferents of the skin (as seen e.g. in neuropathic and neurogenic pruritus) [2–9].

The itch-selective spinal neurons form a distinct pathway projecting from lamina I of the spinal cord to the ventrocaudal part of nucleus medialis dorsalis of the thalamus which has projections to higher CNS structures. The various sensory components of itch have been therefore attributed to activation of certain brain areas: the sensory-discriminative component in the primary somatosensory cortex; the motor component of the goal-directed scratching in the premotor and supplementary motor cortex; the affective and motivational components in the anterior cingulate [12,33–37] and insular cortex. Of great importance, all these brain areas are also involved in pain processing [38] suggesting that no clear-cut itch-specific CNS centers exist. Thus, differences between central pain and itch processing – such as the lack of activation of the secondary somatosensory cortex areas and predominant activation of the ipsilateral motor areas in itch – most likely result from a different activation pattern of basically identical CNS centers [36].

*1.4.3. Pruritogens — Mediators of itch*

As we have seen above, during the induction of pruriceptive itch by defined noxi or skin diseases, the itch-selective afferent fibers are stimulated by various chemical agents, collectively referred to as pruritogens. These molecules are mostly released from various intracutaneous cell types which are in close proximity to or even in direct physical contact with sensory nerve endings [39,40]. The released pruritogens then are able to stimulate and/or sensitize the itch-selective sensory afferents resulting in action potential firing of the neurons and hence initiating itch.

However, upon activation by noxious and/or pruritogenic stimuli, certain neuropeptides (such as substance P [SP] and calcitonin gene related peptide [CGRP]) are "anti-dromically" released from cutaneous pruriceptors, which may act on specific neuropeptide receptors expressed on a large variety of non-neuronal cell types [39,40]. This serves to profoundly modulate (i.e., mostly to further enhance!) the release of pruritogens, thus generating (positive) regulatory loops. Hence, the established bi-directional sensory neuron–non-neuronal cell network lies at the central core of pruritus pathogenesis [39–41].

In the following sections, we therefore shall briefly discuss a (highly selected) list of pruritogens and their related pruritogenic mechanisms (Table 1). The key message here is that the "army" of itch-inducing agents appreciated to-date contains many more pivotal players than the usual "suspect", histamine; and that these agents (not surprisingly at all, see above) are also capable of inducing pain, depending on the actual peripheral or central conditions [3]. However, due to the "extreme" overlapping of the nociceptive and pruriceptive systems, it is of current debate whether (1) are there any pruritogens which have no effect on the nociceptors; (2) can pruritogens activate pruriceptors (without other stimuli) but only sensitize the nociceptors; and (3) why do the below mediators sometimes cause itch but sometimes cause pain?

*1.4.3.1. Histamine.* Although histamine is the best-known pruritogen [42–44], its pivotal function in the pathogenesis of itch is rather controversial. Endogenously, histamine is mostly released from activated mast cells and epidermal keratinocytes, and acts on specific H-receptors [39–41,45,46]. Accordingly, histamine-induced skin reactions (the "classical triad" of itch, wheal and flare) in humans were shown to be efficiently prevented by inhibitors of histamine $H_1$ receptors, also located on sensory nerves [45,47]. However, small doses of histamine that fail to produce itch are still sufficient to produce edema and erythema upon intracutaneous injection. Furthermore, antagonists of the $H_1$ (and/or $H_2$) have often found ineffective as antipruritic drugs, for example in the prototypic pruritic disease AD [48].

Recently, however, additional H receptors have been discovered, and at least one of them ($H_4$) was shown to function as an "itch receptor" in mice [49]. These latter data encourages one to design new clinical trials to revisit the relative importance of histamine in the pathogenesis of pruritus. Similarly, further studies are required to clarify those findings that $H_1$ and $H_2$ receptors, which are also expressed on keratinocytes, were implicated in the regulation of epidermal barrier [50], the dysregulation of which contributes to the development of pruritus in dry skin conditions or in such diseases as AD and psoriasis [6].

Table 1
Selected pruritogens and their effects[a]

| Pruritogen | Cellular action | Source | Functions related to itch |
|---|---|---|---|
| Acetylcholine | Nicotinic and muscarinic acetylcholine receptors | Nerves, epidermal keratinocytes, lymphocytes, melanocytes | –Mediates itch in AD patients |
| Bradykinin | Bradykinin ($B_1$, $B_2$) receptors | Endothelial cells, immunocytes | –Induces pain but also pruritus<br>–$B_2$ receptor antagonists reduce itch<br>–Sensitizes sensory afferents for various chemical stimuli (e.g. histamine)<br>–Pruritogenic in AD |
| Calcitonin gene-related peptide (CGRP) | CGRP-receptors | Sensory nerve fibers | –Induces pruritus intradermally<br>–Prolongs SP-induced itch latency injection (inhibitory effect?)<br>–Increased CGRP in afferent fibers in itchy skin diseases |
| Endothelins | Endothelin (ET) receptors | Endothelium, mast cells | –Induces burning itch<br>–Degraded by chymase via $ET_A$-receptor activation |
| Histamine | Histamine ($H_{1-4}$) receptors | Mast cells, epidermal keratinocytes | –Induces itch via $H_1$ in humans and also via $H_4$ in mice<br>–In humans, $H_1$ antagonists alleviate itch<br>–$H_1$ and $H_2$ receptors regulate epidermal barrier integrity |
| Interleukins (IL) | IL receptors | Immune cells | –IL-2 induces itch in cancer patients<br>–IL-2 sensitizes sensory afferents<br>–Mice overexpressing IL-4 in the epidermis spontaneously develop itch<br>–Elevated IL-6-like immunoreactivity was found in nerve fibers of patients with prurigo nodularis<br>–Mice overexpressing IL-31 in T-cells develop itch<br>–IL-31 is increased in an atopic mouse model as well as in AD |
| Leukotrienes | Leukotriene receptor | Mast cells, epidermal keratinocytes | –Leukoriene $B_4$ induces itch in mice<br>–Leukoriene $B_4$ is involved in the SP- and nociceptin-mediated induction of itch |
| NGF and neurotrophins (NT) | Specific receptors<br>TrkA : NGF<br>TrkB : NT-4<br>TrkC : NT-3 | Epidermal and hair follicle keratinocytes, fibroblasts, mast cells | –NF, NT-3 and NT-4 acutely sensitize sensory afferents, and up-regulate neuronal neuropeptides and TRPV1<br>–NGF and NT-4 are enhanced in AD<br>–NGF induces tryptase release from mast cells<br>–NGF and NT-4 induces sprouting of sensory afferents resulting in chronic sensitization |
| Prostaglandins | Prostanoid receptors | Mast cells, epidermal keratinocytes | –$PGE_1$ and $PGE_2$ potentiate the action of histamine<br>–$PGE_1$ lowers itch threshold<br>–$PGE_2$ evokes pruritus in AD |
| Proteases, Kallikreins | Partly by proteinase-activated receptors (PARs, tryptic enzymes) | Keratinocytes, endothelial cells, mast cells, platelets | –Massive itch behavior in mice overexpressing epidermal kallikrein 7<br>–Tryptase and $PAR_2$ are increased in AD<br>–Tryptase induces inflammation and itch by a neurogenic mechanism via $PAR_2$ |
| Substance P (SP) | Tachykinin (neurokinin, NK) receptors | Sensory nerve fibers | –Primes and activates mast cells to release histamine<br>–Also induces the release of various pruritogens (kinins, cytokines) from endothelial cells, keratinocytes, and immune cells |

[a] Listed in alphabetical order.

*1.4.3.2. SP and other neuropeptides.* Upon activation, pruriceptors induce the release of SP [23,51] which was suggested to indirectly induce pruritus via the activation of non-neuronal skin cells expressing cognate receptors. Indeed, intradermal application of SP activates mast cells to release histamine which, in turn, induces itch [42–44]. In addition, mast cells can also release a plethora of (mostly) inflammatory mediators which induce further liberation of SP from peripheral nerve endings, leading to more mast cell activation [39–41,46]. SP may also trigger the release of other pruritogens from various other cell types such as endothelial cells (kinins, endothelins), keratinocytes (nitric oxide), and immunocytes (cytokines) [52–55] (see also next paragraphs).

In contrast, the role of the other main sensory neuron-derived neuropeptide, CGRP, in the induction of itch is still rather controversial. Namely, CGRP was shown to modulate itch and inflammation [56,57]. In contrast, CGRP was found to prolong SP-induced itch latency suggesting an inhibitory effect of CGRP on SP-induced itching [58]. Interestingly, however, increased levels of CGRP were found in nerve fibers of pruritic diseases such as AD, nummular eczema [55], and prurigo nodularis [59].

*1.4.3.3. Inflammatory mediators as pruritogens — Peripheral sensitization of itch by arachidonic acid derivatives, kinins, and interleukins.* It has long been recognized that acute or chronic inflammation of the skin sensitizes the nociceptive sensory afferents resulting in inflammatory hyperalgesia [60]. It was also known that numerous inflammatory mediators released from mast cells (tryptase, tumor necrosis factor-α [TNF-α], prostaglandins, leukotrienes), keratinocytes (prostanoids, nerve growth fact [NGF] and other neurotrophins), endothelial cells (kinins, endothelins), or immune cells (interleukins) also lower the threshold for pruritic stimuli, and thus cause peripheral itch sensitization [3,4,6,61]. These agents may trigger pruritus by either binding to their specific receptors of the pruriceptive sensory afferents or by inducing non-specific mechanisms leading to elevated sensitivity of these neurons to other pruritogens [62].

For example, prostaglandins (especially prostaglandin $E_1$ and $E_2$) were shown to potentiate histamine-induced itch independently of the liberation of mast cell-derived histamine [63,64]. In addition, pretreatment of human skin with prostaglandin $E_1$ significantly lowered itch threshold [65] whereas prostaglandin $E_2$ evoked pruritus both in controls and in AD patients [66]. Similarly, intradermal injection of another eicosanoid, leukotriene $B_4$ induced scratching in mice [67]. Furthermore, it was also suggested the SP-evoked itch (at least in part) is due to the production prostaglandins and leukotrienes [68].

The inflammatory mediator bradykinin (which induces pain via activation of bradykinin $B_2$ receptors located on the nociceptors) can also induce itch [24,69,70]. Furthermore, bradykinin induces the release of histamine from mast cells [71]), augments the responses to subsequent histamine application [72,73], and sensitizes sensory afferents for various chemical stimuli [72]. Administration of bradykinin to the skin also elevates the release of SP, CGRP, and prostaglandin $E_2$ [56] hence may significantly amplify the activity of the pruritogenic cellular network. Finally, a recent report has also shown that bradykinin is a potent pruritogen in AD as well [74].

Certain members of the large family of inflammatory immune cell-derived interleukins (IL) are also implicated in the pathogenesis of pruritus. For example, recombinant IL-2 (administered to cancer patients) was shown to induce pruritus [75]. Moreover, IL-2 induces itch by activation of a subpopulation of cutaneous C-fibers that are chemosensitive to histamine and bradykinin [76,77] suggesting that bradykinin may enhance the itch-inducing effect of IL-2 [78]. Furthermore, transgenic mice overexpressing IL-4 in the epidermis spontaneously developed a pruritic inflammatory skin disease resembling human AD [79]. Accordingly, elevated IL-6-like immunoreactivity was found in nerve fibers of patients with prurigo nodularis [80]. In addition, it was demonstrated that transgenic overexpression of the novel cytokine IL-31 in T-lymphocytes induces severe pruritus and dermatitis in mice [81]. In good accord with these data, expression of IL-31 was found to be increased in an atopic mouse model [82] – as well as in patients with AD [83] – and was shown to correlate to itch behavior [84]. These results unambiguously argue for a special role for IL-31 in pruritus and that IL-31 and its signaling pathway may represent a novel target for antipruritic therapy [85].

*1.4.3.4. Proteases and their receptors.* Serine proteases (trypsin, chymotrypsin, chymase), generally regarded as "weapons of (protein) mass destruction", are capable of inducing inflammation, pain, and pruritus [86–88]. Among their G protein-coupled, metabotropic proteinase-activated receptors (PAR) [89,90], $PAR_2$ has been shown to play a key role in the pathophysiology of itch [91]. Importantly, functional $PAR_2$ is expressed on sensory afferents which release neuropeptides upon stimulation by tryptase [92,93]. With respect to itch, it was also observed that the levels of the endogenous $PAR_2$ agonist tryptase as well as $PAR_2$ were markedly increased on primary afferent fibers of lesional skin of AD patients. Additionally, keratinocyte-derived $PAR_2$ was also upregulated in the epidermis of AD patients [94] and hence may mediate pruritus induced by endogenous (trypsins, kallikreins) or exogenous proteases (bacteria, house-dust mite). In perfect agreement with these findings, intracutaneous injection of specific $PAR_2$ agonists provoked sustained and prolonged itch in such patients. Moreover, inhibition of tissue kallikreine (tryptic enzyme) suppressed itch [95] and kallikreine activity was also increased in pruritic papular eruptions [96]. Since the concentration of histamine was not changed in AD, these results suggest that the activation of the novel protease-$PAR_2$ pathway on cutaneous sensory nerves might be more important than histamine for the transmission of itch responses in AD (and probably in other skin diseases).

*1.4.3.5. NGF and certain neurotrophins — Acute and chronic itch sensitizers.* Neurotrophins (such as NGF and neurotrophin-4), which are produced and released by keratinocytes, mast cells, and fibroblasts of the skin [97], were originally described to play crucial roles in cutaneous nerve development and regeneration [98]. It was also shown, however, that NGF is highly overexpressed in inflamed and injured tissues and that (as an inflammatory mediator) it initiates acute sensitization and sprouting (leading to chronic sensitization) of C-type afferent fibers via the activation of specific TrkA receptors [98–101]. Indeed, NGF upregulates the expression of pruritogenic neuropeptides (SP, CGRP) and certain receptors (e.g. transient receptor potential vanilloid-1 [TRPV1], see below) involved in itch, induces degranulation of mast cells, and was found to be pruritogenic when administered therapeutically [1,21,27,29,102,103,105]. In addition, serum levels of NGF and SP were found to be increased in AD patients [104] and these substances were shown to induce the release of the pruritogenic mediator tryptase [97]. Also, mast cells and keratinocytes produce high levels of NGF in AD which can be stimulated by histamine [105]. Moreover, increased NGF expression was detected in prurigo nodularis [106] and also in pruritic lesions of patients with psoriasis [107], similarly to the overexpression of neurotrophin-4 in lesional skin of AD patients [108]. Taken together, these results are clearly in favor of important roles of neurotrophins in the pathophysiology of itching.

## 2. TRP channels — "TRiPping" novel players in pruritus pathogenesis

### 2.1. Why TRP channels?

Recently, numerous lines of novel evidence suggest the involvement of new "players" in the pathogenesis of pruriceptive itch. Among these, special attention has been attracted to the transient receptor potential (TRP) superfamily [109–112], especially to the thermosensitive ones [113]. Here is, why:

- ➢ As shown above, itch is a characteristic sensory modality with specific/selective CNS processing → TRP channels mostly function as "cellular sensors" of e.g. temperature, osmotic concentration, and taste.
- ➢ Skin-originating itch requires an orchestration of bidirectional sensory neuron — non-neuronal cell network → TRP channel are ideally suited to this task, since they are equally expressed by both sensory neuronal structures and non-neural cell types of the skin.
- ➢ The wide array of pruritogens may not only act on specific receptors but also on various new, previously unappreciated, targets → such as the TRP channels.
- ➢ During the pharmacological treatment of pruritus, certain agents may target novel molecules → such as the TRP channels.
- ➢ As was revealed during anti-itch therapy using physical methods, the itch pathway is clearly sensitive to temperature changes → such as numerous TRP channels.

On this background, the following paragraphs offer an "exotic TRiP" to the fascinating, sometimes "cloudy", yet steadily unfolding "realm" of the TRP family, and discuss their putative roles in itch pathogenesis. Along the way, we provide novel perspectives on the potential use of TRP ligands in itch management (Table 2).

### 2.2. TRPV1 — A thermosensitive channel with a central role in the pathogenesis and therapy of itch

#### 2.2.1. TRPV1 — A "hot and spicy" target of capsaicin on sensory neurons

TRPV1, as key peripheral integrator of pain sensation, was originally described on C-type nociceptive sensory neurons [114,115] as a molecular target for capsaicin, the pungent vanilloid ingredient of hot chili peppers [116]. The activation of this non-specific cation channel (permeable to e.g. sodium and calcium) first excites these neurons by initiating ionic fluxes and concomitant action potential firing resulting in the induction of pain *in vivo* [115,116]. Upon activation by TRPV1 agonists (e.g. during capsaicin-treatment of the skin), these neurons release their neuropeptide content (efferent function of the sensory afferents, see above) which, by acting on the numerous neighboring cell populations, leads to the initiation of neurogenic inflammation [46,90,117]. Afterwards, prolonged stimulation of TRPV1 induces desensitization (and, depending on the actual concentration of the agonist, cell death) of the sensory afferents [114,115] which, at least in part, is due to intracellular calcium accumulation and the depletion of neuropeptides. Hence, prolonged TRPV1 activation results in the suspension of the interplay between skin sensory neurons and non-neuronal cells [2–4,6,8,9,114,115].

#### 2.2.2. "Endovanilloids" — The "itch connection"

In addition to capsaicin, TRPV1 can also be activated and/or sensitized by numerous endogenous substances collectively referred to as "endovanilloids" [118]. The receptor was first shown to be directly stimulated by low-threshold (>43 °C) heat and acidosis [114]. Later, several other molecules were also described to indirectly act on the TRPV1, i.e. via the activation on their specific receptors and initiating various intracellular signaling pathways. These stimuli are for example the bradykinin [119,120], ATP [121], lipoxygenase products [120,122], prostaglandins [123], histamine [124], various neurotrophins (such as NGF, neurotrophin-3 and-4) [101,119], TNF-α [125], pro-inflammatory chemokines [126], activation of $PAR_2$ by proteases [127,128] or activation of metabotropic receptor-coupled hydrolysis of phosphatidylinositol 4,5-bisphosphate ($PIP_2$) by phospholipase C (PLC) [129]. Most of the above substances and mechanisms, released/activated chiefly during inflammation, shift the activation threshold (>43 °C) of TRPV1 ("to the left") towards more physiological temperatures hence leading to the acute or chronic sensitization of the TRPV1-expressing nociceptive C-afferents (inflammatory thermal "heat/hot" hyperalgesia) [115,130]. Taken together, these findings imply that TRPV1 is indeed a central integrator molecule in the pain pathway [115,131].

It is very important to note, however, the above endogenous substances may not only act as algogenic chemical substances but are also recognized as effective pruritogens (see in Table 1). In addition, the itch-selective sensory afferents also respond to the TRPV1-activator capsaicin suggesting that TRPV1 might also be expressed on the pruriceptor subunit of mechano-insensitive fibers [4]. Finally, it has also been shown that increases in skin temperature or dysregulation of the pH of the skin (another significant stimuli of TRPV1) can also effectively modulate itch sensation in human [6,15,132]. Therefore, if one takes a closer look to the above "special features" of TRPV1 in relation to the neurophysiology of itch, one may also propose that TRPV1 can also function as a "central integrator" molecule in the itch pathway [3,6,8].

#### 2.2.3. TRPV1 on non-neuronal cell types of the skin — Other "itchy" findings to explore

Recent "hot" findings further highlighted the neurophysiological importance of TRPV1 signaling in itch. Namely, TRPV1 channels were described on numerous non-neuronal cell types [133–136] including, of greatest importance, human skin epidermal keratinocytes, dermal mast cells, dendritic cells, and various keratinocyte populations of the hair follicle [137–143]. In addition, it was also proven that the activation of TRPV1 – besides markedly affecting proliferation, differentiation, and apoptosis – results in the release of such cytokines and mediators (e.g. various ILs, prostaglandins, growth factors)

Table 2
Functions and potential therapeutic significance of thermosensitive TRP channels and their endogenous or exogenous modulators in itch

| Receptor | Expression in the skin | Effects of endogenous modulators—physiological role in itch | Effects of exogenous modulators — potential role in the therapy of itch |
|---|---|---|---|
| TRPV1 | –Sensory neurons<br>–Mast cells<br>–Sebocytes<br>–Epidermal keratinocytes<br>–Hair follicle keratinocytes<br>–Langerhans cells<br>–Smooth muscle<br>–Sebocytes | –Activated at T > 43 °C and acidosis<br>–Activated/sensitized by pruritogenic substances such as histamine, bradykinin, eicosanoids, ATP, TNF-α, chemokines, prostaglandins, NGF, neurotrophins, and tryptases<br>–Activated/sensitized by the PLC-dependent hydrolysis of $PIP_2$<br>–Also activated by the endocannabinoid anandamide<br>–Mediates inflammatory thermal "hot" hyperalgesia<br>–Regulates epidermal barrier integrity and homeostasis | –Capsaicin and RTX excites and then desensitizes TRPV1 expressing sensory neurons resulting in depletion of neuropeptides<br>–Capsaicin inhibits experimental and disease-related itch<br>–Camphor, which alleviates itch in pruritic diseases, transiently activates then strongly desensitizes TRPV1<br>–Allicin, the pungent ingredient of garlic, activates TRPV1<br>–Noxious thermal stimuli (50 °C) inhibit histamine-induced itch<br>–TRPV1 activation by capsaicin affects epidermal and follicular proliferation, differentiation, and apoptosis and release of cytokines, ILs, and growth factors<br>–TRPV1 is increased in epidermal keratinocytes of prurigo nodularis patients |
| TRPV2 | –Sensory neurons<br>–Mast cells | –Activated at T ≥ 52 °C<br>–Activation of TRPV2 on mast cells by thermal stimuli results in a pro-inflammatory degranulation event | –Noxious thermal stimuli (50 °C) inhibit histamine-induced itch |
| TRPV3 | –Sensory neurons<br>–Epidermal keratinocytes | –Activated at T ≥ 33 °C, also on epidermal keratinocytes<br>–Sensitized by arachidonic acid and related unsaturated fatty acids<br>–Augmented by histamine and bradykinin in a PLC-dependent manner | –Eugenol, thymol, and carvacrol (from oregano, clove, and thyme) activate and sensitize TRPV3<br>–Eugenol releases IL-1α from keratinocytes via TRPV3<br>–Camphor activates TRPV3 |
| TRPV4 | –Sensory neurons<br>–Epidermal keratinocytes<br>–Mast cells | –Activated at T 27–42 °C<br>–Activated by anandamide and arachidonic acid via the productions of lipid peroxidation products as eicosanoids<br>–Regulates epidermal barrier integrity and homeostasis | –Not known |
| TRPM8 | –Sensory neurons | –Activated at T ≤ 28 °C<br>–Metabotropic receptor-coupled hydrolysis of $PIP_2$ inhibits its activity | –Menthol, eucalyptol, and icilin activate TRPM8 (and induce sensation of cooling)<br>–Cooling, menthol, and icilin inhibit experimental or disease-related itch |
| TRPA1 | | –Activated by bradykinin, arachidonic acid, and prostaglandins<br>–Activated by a PLC-dependent mechanisms<br>–Its noxious (T ≤ 17 °C) cold sensitivity is controversial<br>–Its role in inflammatory thermal "cold" hyperalgesia is controversial | –Allyl isothyocyanate (alkaloid of e.g. mustard oil, wasabi, and horseradish) and cinnamaldehyde (substance isolated from cinnamon oil) activates TRPA1<br>–Allicin, the pungent ingredient of garlic, activates TRPA1<br>–Carvacrol activates TRPA1<br>–Menthol inhibits TRPA1<br>–Camphor inhibits TRPA1 |

from these non-neuronal cells that were shown to participate in induction of itch sensation [133–136,141–143].

These novel findings, therefore, invite an attractive hypothesis: According to this, algogenic and pruritogenic substances may therefore not only target TRPV1-expressing sensory neurons but also e.g. TRPV1-expressing mast cells and keratinocytes, and, hence, significantly modulate the proposed neuronal–non-neuronal interaction network to initiate and augment itch [3,6,8]. In fact, the importance of keratinocyte-specific TRPV1 was strengthened by presenting that expression of the receptor was dramatically increased in epidermal keratinocytes of prurigo nodularis patients [141].

### *2.2.4. Therapeutic implications — The vanilloid-TRPV1 system to mitigate itch*

*2.2.4.1. Capsaicin and related exovanilloids.* The generally accepted basis for the therapeutic application of capsaicin to mitigate pain and itch is the well-appreciated densensitizing effect of this vanilloid [115,116,144,145]. Namely, prolonged or repeated vanilloid application results in a depletion of neuropeptides such as SP in the C-type neurons, hence suspending the interplay between skin sensory neurons and other cell populations of the skin [2–4,6,8,9,146]. Indeed, topical capsaicin effectively prevents histamine-induced itch under experimental conditions [147]. In addition, capsaicin cream (0.025–0.075%) is widely used as an antipruritic agent in several pruritic dermatoses, ranging from prurigo nodularis and notalgia paresthetica via pruritus ani, to hemodialysis-related and uremic pruritus, where a cascade-like augmentation of the intercellular skin network "into a vicious itch circle" (which can be effectively interrupted by capsaicin administration) is clearly documented [2,9,144,146,148].

The most notorious clinical limitation of capsaicin application is the TRPV1-coupled acute excitation of the sensory C-afferents, which results in a marked burning sensation

[2,9,116,144,145]. However, if one takes into account that pain inhibits itch via central mechanisms, one might also speculate: What if the effectiveness of capsaicin to terminate itch is (at least in part) due to its (undesired) pain-inducing properties? Unfortunately, systematic studies that address this intriguing question have not yet been published (e.g. by measuring the itch behavior of TRPV1-KO mice). Nevertheless, at least for now, the need of repeated and prolonged capsaicin application protocols (which fully deplete neuropeptide content of sensory afferents) to efficiently alleviate itch [8,144,146,148] argues against this hypothesis and suggest a peripheral action of capsaicin in its function as an anti-itch agent.

Considering that pruritic patients usually poorly tolerate capsaicin-induced pain, very often resulting in a greatly reduced patient compliance [149], another important therapeutic achievement, therefore, would be to find and/or synthetically design such TRPV1 agonists which cause only *minor* receptor *excitation* (i.e. pain), but still possess a *significant desensitization power* (thus suppressing itch). Perhaps, the most promising candidate to start-off this important search for better anti-pruritic agents is resiniferatoxin (RTX), another natural product of *Euphorbia resinifera* (a cactus-like plant) [116,117,150]. Intriguingly, this TRPV1 agonist exerts a threefold higher potency to induce desensitization (i.e. to treat pain and presumably itch) than excitation (i.e. to induce pain) [116]. Furthermore, future clinical trials should also systemically analyze the *in vivo* effects of TRPV1 *antagonists* (such as capsazepine or iodo-RTX) [151,152] to suppress itch.

*2.2.4.2. Cannabinoids and TRPV1 — The challenge of getting the skin "high", rather than "itchy".* Another endogenous, arachidonic acid metabolism-derived substance, anandamide (N-arachidonoylethanolamine) connects TRPV1-mediated signaling to the cannabinoids and their receptors (CB-R), another very intriguing endogenous system for the modulation of both pain and itch [153]. CB-Rs and TRPV1 show a marked co-localization pattern on primary afferents [154–157]. On these sensory structures, the stimulation of CB-Rs by various (endo- and exo-) cannabinoids effectively inhibits pain [158,159] — similar to the effects seen with TRPV1-mediated desensitization (see above). The intimate relationship between the two systems is also supported by the observation that the endocannabinoid anandamide, depending on its concentration and other local factors (such as e.g. acidosis in inflammation) [160], may also stimulate TRPV1-mediated signaling (e.g. intracellular calcium elevations) acting as an "endovanilloid" [118,153,158].

Various synthetic exocannabinoids, applied either intradermally or topically, also effectively prevent both histamine-induced [161] and disease-related [162,163] pruritus. Since this effect is accompanied by a decreased neuropeptide release from the sensory endings [161], this suggests that the TRPV1 pathway may also be involved in mediating the antipruritic action of cannabinoids. Moreover, CB-R1 and CB-R2 have recently been identified on epidermal keratinocytes, along with numerous other non-neuronal cell types of human skin (in part, in co-localization with TRPV1!) [157,164]. On keratinocytes, the activation of CB-R2 resulted in the release of β-endorphin [165], an analgesic and antipruritic proopiomelanocortin product. This suggests the involvement of CB-R-mediated signaling in the neuronal–non-neuronal cellular networks that generate itch.

Collectively, these finding strongly argue for that – just as the TRPV1-coupled vanilloid system – the cannabinoid/CB-R signaling mechanisms not only participate in nociception, but also in the modulation of pruriception. If this concept is corroborated by further experimental evidence, the co-administration of TRPV1 and CB-R agonists becomes another very promising, novel pharmacological anti-itch strategy. This approach would not only augment our anti-pruritic armamentarium (to additively alleviate itch), but may also serve to greatly reduce or even eliminate the ill-tolerated burning sensation caused by capsaicin. As a support for this argument, CB agonists have already been shown to prevent acute excitation induced by the TRPV1 agonist capsaicin [166,167]. Alternatively, some CB agonists were shown to mildly activate TRPV1 causing desensitization of the channel and hence leading to a reduced activation by capsaicin [168].

*2.2.4.3. Physical methods to target TRPV1 — "Burning itch with fire"?.* It is ancient knowledge among "pruritologists" [2,4] that whereas cooling inhibits the itch sensation, warming of the skin aggravates pruritus. Recent findings, however, seem to challenge (at least in part) this popular paradigm. Namely, Yosipovitch and coworkers [15] have shown that stimulation of human skin by noxious thermal stimuli (close to 50 °C), surprisingly, markedly inhibited the histamine-induced itch (and hyperemia). Although the exact mechanism and its potential therapeutic implications of this intriguing effect remain to be determined, the phenomenon itself strongly suggests that TRPV1 expressed on sensory neurons and/or non-neuronal cells (as well as other noxious heat-activated TRP channels such as TRPV2, see below) are directly involved in the antipruritic efficacy of such a "thermal physiotherapy".

### *2.3. Other thermosensitive TRP channels as novel players in pruritus*

The profound temperature-dependence of itch sensation has also drawn attention to numerous other thermosensitive TRP channels (besides TRPV1), which have recently been identified as molecular sensors of temperature changes [109–113]. Each of these distinct cellular temperature sensors, which belong to the TRPV, TRPM, and TRPA subfamilies, is specialized to detect well-defined temperature spectra (some are stimulated by heat, others by cold), thus enabling the body to obtain temperature "readings" over almost the entire relevant temperature range faced by mammals [109–113].

*2.3.1. TRPV2, TRPV3, and TRPV4 — More "hot" is coming to fight itch*

Similar to TRPV1 (which is activated at ≥43 °C), the TRPV2, TRPV3, and TRPV4 (originally described as an osmosensor [169]) channels also function as cellular temperature sensor molecules, since all are activated by increasing tem-

peratures, from the innocuous to the noxious range [109–113]. In the context of skin-related sensory modalities, TRPV3 (activated at ≥33 °C) [170–172] and TRPV4 (with an activation range of 27–42 °C) [173,174] have attracted special attention.

However, the expression of these channels on sensory neuronal structures is very controversially described in the literature. Namely, whereas TRPV3 was clearly identified in human and monkey dorsal root ganglia (DRG) [170,172], its presence was not found in rat or mouse DRG [171]. In contrast, TRPV4 was described in mouse DRG [169] and on free nerve endings of mouse skin [175]. Moreover, in human and monkey DRG, TRPV3 is expressed on the small diameter neuron population (similar to TRPV4 in mouse DRG [175]), and, of importance, in co-localization with TRPV1 [170,172]. In addition, using a heterologous expression system, it was also postulated that TRPV3 subunits form heteromultimeric structures by interacting with TRPV1 monomers and therefore may act as signal co-transducers and/or regulators of TRPV1-mediated signaling [170]. However, to further complicate this issue, another group (using another expression system) failed to identify homo- or heteromeric assembly of TRPV1 and TRPV3 subunits [176].

Of great importance, however, previous studies unambiguously argue for that TRPV3 and TRPV4 are highly expressed in skin epidermal keratinocytes [171,172,177,178], which, as was detailed above, also express TRPV1 [137–141]. Moreover, it was shown that these channels expressed by keratinocytes are functional, since they mediate heat-activated membrane currents [177,178]. This is most strongly supported by the finding that mice lacking TRPV3 have prominent deficits in their response to both innocuous (mostly TRPV3 and TRPV4 range) and painful heat (rather TRPV1 range) [179] — underscoring the concept that TRPV3 is crucially involved in cutaneous temperature sensation.

Although we possess only very limited data (see below) on whether or not the activation of TRPV3 and TRPV4 results in mediator release from keratinocytes (e.g. of pro-inflammatory mediators, algogens, and pruritogens), the sensitivities of the channels to certain chemicals and pharmacological agents implicate their putative roles in the neurophysiology of itch. Namely, several plant-derived skin sensitizers and allergens (and, thus, potentially itch-inducing substances) that cause a "warm" skin sensation (such as eugenol, thymol, and carvacrol — major components of oregano, savory, clove and thyme) strongly activate and sensitize TRPV3 [180]. In addition, the activation of TRPV3 by eugenol on mouse keratinocytes results in the release of (pro-inflammatory) IL-1α which may augment the skin-sensitizing effect of this alkaloid [180].

It also deserves further consideration that the activities of both channels (TRPV3, TRPV4) are potentiated by recognized inflammatory pruritogens (similarly to the sensitization of TRPV1, see above). Indeed, TRPV4 is activated by arachidonic acid and its derivative, the endocannabinoid anandamide via the generation of lipid peroxidation products such as eicosanoids [181,182]. Similarly, TRPV3 is sensitized by arachidonic acid and related unsaturated fatty acids [183], while the effects of the culinary ingredients listed above are augmented by histamine and bradykinin in a metabotropic receptor-PLC-dependent manner [180]. Moreover, both TRPV4 and TRPV1 (similar to the pruritogenic histamine $H_1$ and $H_2$ receptors [52]) were shown to play important roles in skin permeability barrier homeostasis [184], the dysregulation of which represents a major stimulus for the onset of itch [6].

In light of these data, it is of great clinical significance that camphor (an aromatic and, when applied to human skin, mostly pungent phytoproduct derived from the *Cinnamonum camphora* tree) is a potent activator of TRPV3 [171,172,179]. Camphor has long been applied to alleviate itch in certain pruritic dermatoses (such as contact irritant dermatitis) [185]. Interestingly, camphor also transiently activates TRPV1 (albeit to lesser extent than capsaicin), but then strongly desensitizes this vanilloid receptor thereby suspending the excitation of TRPV1-expressing sensory afferents [186]. Moreover, camphor also inhibits TRPA1 [187] which data collectively suggest a possible synergistic interplay between TRPV1, TRPV3, and TRPA1 to mediate the antipruritic (as well as analgesic) [188] effect of camphor (see also below).

Finally, another heat-sensitive channel, TRPV2, deserves mentioning. The molecule was first described on capsaicin- and proton-insensitive, medium to large diameter primary nociceptive sensory neurons and is activated by high-threshold (≥52 °C) noxious thermal stimuli [189]. Keeping in mind the key role of mast cells in the initiation of itch [3,40], it is important to note that mast cells also express TRPV2 (along with TRPV4 and TRPV1) [134,140,141,190]. Intriguingly, the activation of mast cells by physical and thermal stimuli can result in a pro-inflammatory degranulation event which depends on the activity of protein kinase A-related signaling [190] — one of the chief mechanisms in initiating the sensitization of nociceptors and pruriceptors [123,191]! Furthermore, we have already seen above that TRPV2 (besides TRPV1) may also participate in mediating the antipruritic therapeutic effect of noxious heat stimuli [15]. Clearly, further studies are urgently needed to define the itch-related "non-thermosensor" roles of these "hot" channels. However, taken together, the available evidence already suggests that they closely resemble TRPV1 with respect to cell-specific expression (e.g. on sensory neurons and various non-neuronal cell types of the skin), receptor activation, sensitization, and signal transduction characteristics, and warrant careful exploration in pathogenesis and clinical management of itch.

### 2.3.2. TRPM8 — A "cool" anti-itch agent

TRPM8 is another thermosensitive member of the TRP family [109–113]. In contrast to the heat-sensitivity of the TRPV1–V4 channels, however, this calcium-permeable channel is activated by *decreasing* temperatures, down to the range of innocuous (i.e. not painful) cold or coolness (≤28 °C) [192,193]. Moreover, again in contrast to TRPV1 [129], the metabotropic receptor-coupled hydrolysis of $PIP_2$ inhibits the activity of TRPM8 [194]. Consistent with these data, TRPM8 is selectively expressed on a certain subpopulation of A and C-type sensory afferents which is distinct from TRPV1-expressing nociceptors [195]. Besides cooling, TRPM8 is highly activated

by menthol, eucalyptol, and the "super-cooling agent", icilin [192,193,196]. These agents shift the activation threshold of TRPM8 towards room temperature and, thus, sensitize the TRM8-expressing sensory afferents. This induces the characteristic "cool" sensation when these substances are applied to the skin or mucosal surfaces.

In this context, it is important to recall that skin cooling effectively inhibits itch, as has been demonstrated in numerous trials [4,15,197]. Like physical skin cooling, menthol also alleviates experimental [197] or disease-related [185,198,199] pruritus. Importantly, menthol, besides stimulating TRPM8, also inhibits TRPA1 [187] which may also contribute to its effect to mitigate itch (see also below). Moreover, the super-cooling agent icilin (which is 200–400 times more potent than menthol in activating TRPM8) [192,196], when applied as 2% ointment, reduced the degree of excoriations by 55%–60% in a *hairless* rat model of scratching, provoked by a magnesium deficient diet [8]. These findings strongly argue in favor of the concept that TRPM8 offers an excellent target receptor for mediating the antipruritic (as well as the analgesic) effects of the above chemical and physical tools.

### 2.3.3. TRPA1 — A "pungent ic(e)ing on the cake"

TRPA1 was originally also described as a cold-sensitive, calcium-permeable, non-selective cation channel [200], activated at the noxious cold temperature range (≤17 °C) [109–113]. Recent findings, however, suggest that – based on its localization, ligand sensitivity, and its relation to inflammation – this "ice-cold" channel is more closely related to the "hot" TRPV channels than to the "cool" TRPM8.

Namely, TRPA1 was found on TRPV1-positive sensory nociceptive (and pruriceptive) afferents, yet not on the subset of A and C-fibers which express TRPM8 (see above) [195,200]. Of further importance, pungent human skin irritants (i.e. potential pruritogenic stimuli) such as allyl isothyocyanate (alkaloids contained e.g. in mustard oil, wasabi, and horseradish, which induce skin pain and inflammation similar to the TRPV1-activating capsaicin) and cinnamaldehyde (a substance isolated from cinnamon oil) markedly and specifically activated TRPA1 [201,202]. In addition, recognized algogenic and pruritogenic inflammatory mediators such as bradykinin, arachidonic acid, and prostaglandins as well as activation of PLC were also shown to effectively activate TRPA1 [202]. This must be expected to shift the activation temperature-threshold of TRPA1 to more "physiological" temperature ranges — similar to the effect seen with excitatory actions of these substances on TRPV1, TRPV3, and TRPV4 (see above).

Therefore, in light of the above (i.e. TRPA1 was activated by noxious cold and, at the same time, by agents causing pungent "hot" sensation), Bandell et al. have proposed that TRPA1 may serve as a molecular model explaining how noxious cold may paradoxically cause painful burning [202]. In support of this hypothesis, in animal models using either TRPA1-targeted antisense or knockout technologies, it was shown that TRPA1 indeed participates in noxious cold sensation [203] and in the development of cold hyperalgesia induced by either inflammation or nerve injury [204,205].

Intriguingly, however, in parallel to the above studies, other groups were unable to prove the (noxious) cold-sensitivity of TRPA1 in cell cultures [201,206]. Similarly, Bautista et al. have shown that TRPA1 knockout mice exhibit normal response to cold stimuli [207]. However, these mutant animals displayed pronounced deficits in nociceptor excitation and, of great importance, in thermal pain hypersensitivity (heat hyperalgesia) evoked by the inflammatory mediator bradykinin [203,207] — similar to findings observed with TRPV1-deficient mice [130]! It may be postulated, therefore, that algogenic and (since sensitization also occurs for itch [3]) pruritogenic inflammation operates as the common activation mechanism for TRPV1 and TRPA1 (and, quite probably, for other heat-sensitive TRPV channels, as well). Indeed, using knockout animals, it was shown that both TRPA1 and TRPV1 are required for the bradykinin-induced thermal hyperalgesia [207] which, at the cellular level, involves the stimulation of TRPA1 following activation/sensititation of TRPV1 [207] or, alternatively, the sequential co-activation of the two channels "the other way around" [168,208].

Naturally, further studies are to be performed to unambiguously reveal the exact role of TRPA1 in temperature sensation and the molecular and functional relationship of TRPA1 and TRPV1. Nevertheless, the "exciting" complexity and controversy of the "story" of above TRP channel may provide an attractive explanation for how the distinct painful "cold" and painful "hot" sensory modalities can travel alongside each other so intimately.

### 2.3.4. Temperature-sensitive TRP channels establish a basic syntax and molecular substrate of nociception and pruriception — The "molecular psychophysics" of itch and pain sensation

The data presented above, on the one hand, clearly demonstrate the potential neurophysiological roles of defined temperature-sensitive TRP channels in the pathogenesis (and possibly in the therapy) of pruritus. On the other hand, the differential activation pattern of these molecules by various skin-irritating agents may also help to understand the extreme variability of sensations experienced by patients when such substances are applied to human skin.

We propose that temperature-sensitive TRP channels establish a basic syntax and molecular substrate of nociception and – due to the intimate relationship of pain and itch – for pruriception, as well (Table 3). Following this novel working hypothesis (which can also be referred to as the "molecular psychophysics" of itch and pain sensation), these sensory categories can, then, be distinguished and re-defined, which will have to be taken into account when analyzing the pathogenesis and management of itch:

- "Warm only" sensation (i.e. without pungency) — Stimuli inducing a pure sensation of "warmth" (without any "burning pain" component) may exclusively act on TRPV3 (and, perhaps, also on TRPV4). The prototypic compound for such sensation is eugenol (ingredient of clove oil), which selectively activates TRPV3 without affecting the activity of other thermosensitive TRP channels [180].

➢ "Cool only" sensation (i.e. without pungency) — Stimuli inducing a "pure coolness" sensation (without any "burning pain" component) may exclusively act on TRPM8. Such a compound is menthol which selectively activates TRPM8 [192,193,200]. However, it also inhibits TRPA1 [187] which suggest that the "pure coolness" sensation induced by menthol (via TRPM8) can be "fine-tuned" by eliminating the "burning" component (via inhibiting TRPA1-mediated signaling).

➢ "Painful burning" sensation (i.e. pungency) — Within this category, based on experimental findings and on purely theoretical speculations, the following subcategories can be defined:

- It is well established that stimuli which may exclusively act on TRPV1 (e.g. capsaicin) induce pungency with a characteristic "hot" component ("Hot painful burning" sensation).
- Therefore, it would be feasible to propose that stimuli which may exclusively act on TRPA1 would induce pungency with a characteristic "cold" component. However, since the "noxious" cold-sensitivity of TRPA1 is very controversially described (see above) and, furthermore, since none of the recognized TRPV1-acting agents such as allyl isothyocyanate and cinnamaldehyde induce cold sensation but rather evoke "Hot painful burning", the existence of the "Cold painful burning" sensation is rather questionable.
- However, it is a common experience that allicin, the pungent ingredient of garlic and capsaicin, the pungent ingredient of hot chili peppers cause distinct pungent sensations upon consumption (yet they both initiate cutaneous irritation and edema). Namely, the ingestion of garlic induces pungency without (or much less) characteristic "hot" (or "cold") component. Since allicin was shown equally activate TRPA1 and TRPV1 [209,210], it can be speculated that simultaneous activation of TRPA1 and TRPV1 would result in a different sensation compared to those experienced upon selective involvement of these channels. Therefore, an "Unspecified painful burning only" sensation subcategory is proposed for such agents and/or mechanisms which may co-stimulate TRPV1 and TRPA1.

Naturally, "promiscuous" combinations of TRP channel activation and/or inhibition by certain substances may result in even more complex sensations, as they are sometimes reported by patients [2,8]. An intriguing example for such a multimodal (context-dependent?) inducer of itch/pain/burning sensations is the spice-ingredient, carvacrol (contained in oregano) which, besides activating TRPV3 (see above), also stimulates TRPA1 [180]. In perfect agreement with these cellular data, carvacrol, when applied to the skin or mucosal surfaces, not only produces "warm only" sensation but is also described as "pungent" and painful [180].

In this complex framework of receptor-mediated sensations under the "itch umbrella", camphor is probably the "trickiest" among the better-known, clinically long-employed compounds. This skin irritant potently activates TRPV3 [171,172,179]. Subsequently, however, it first rapidly activates, and then strongly desensitizes TRPV1 [186]. To add a further level of complexity, camphor also inhibits TRPA1-mediated signaling [186,187]. According to our "molecular psychophysics" hypothesis introduced above, these mixed cellular effects on various TRP channel targets may perfectly explain the *in vivo* sensation "package" induced by skin-applied camphor; i.e. its ability to induce "warm" (TRPV3 activation) as well as transient "burning hot" (activation of TRPV1) sensations followed by the well-known analgesia (desensitization of TRPV1 and inhibition of TRPA1) [188]. Taken together, the presented "triple" mechanism may also provide a rational molecular basis for the well-

Table 3
Temperature-sensitive TRP channels establish a basic syntax and molecular substrate ("molecular psychophysics") of nociception and pruriception

| Prototypic agent | Effect on TRP channels | Sensory phenomenon |
|---|---|---|
| Eugenol | –Activates TRPV3 | –"Warm only" sensation (i.e. "pure warmness" without any "burning pain" component) |
| Menthol | –Activates TRPM8<br>–Inhibits TRPA1 | –"Cool only" sensation (i.e. "pure coolness" without any "burning pain" component) |
| Capsaicin | –Activates, then desensitizes TRPV1 | –"Hot painful burning" sensation (i.e. pungency with a characteristic "hot" component)<br>–Usually followed by analgesia |
| Allyl isothyocyanate, cinnamaldehyde | –Activates TRPA1 | –"Hot painful burning" sensation (i.e. pungency with a characteristic "hot" component)<br>–The existence of a "Cold painful burning" sensation is to be determined |
| Allicin | –Activates TRPA1 | –"Unspecified painful burning only" sensation (i.e. pungency without characteristic "hot" or "cold" components) |
| Carvacrol | –Activates TRPV1<br>–Activates TRPV3<br>–Activates TRPA1 | Mixed sensation:<br>–"Warm only" sensation<br>–"Painful burning" sensation |
| Camphor | –Activates TRPV3<br>–Activates, then desensitizes TRPV1<br>–Inhibits TRPA1 | Mixed sensation:<br>–"Warm only" sensation<br>–"Painful burning" sensation<br>–Followed by analgesia |

documented efficacy of camphor to alleviate itch in certain pruritic diseases (such as contact irritant dermatitis) [185]. Furthermore, it could also explain the great inter-individual variations in a patient's response to cutaneous camphor administration which might reflect constitutive differences in the expression levels of and cross-signaling between distinct TRP channels.

### *2.4. Other non-thermosensitive TRP channels — Distant connections to pruritus*

Although very little concrete information is as yet available on these *non-thermosensitive* TRP channels, certain members of the TRPC and TRPM subfamilies [109–113] can be envisaged to contribute to the pathogenesis of pruritus.

#### *2.4.1. TRPCs — Itch modulation via altered keratinocyte differentiation?*

TRPC channels are key regulators of intracellular calcium homeostasis in a wide array of different cells types [109–113]. Importantly, numerous TRPC channels (TRPC1, TRPC4, TRPC5, TRPC6, TRPC7) are expressed by epidermal or mucosal keratinocytes [211–213]. Their expression levels significantly fluctuate in a differentiation-dependent manner, suggesting a role for these TRPC channels in the control of keratinocyte differentiation [211–213]. Indeed, TRPC1 has already been implicated in the calcium-induced terminal differentiation of human keratinocytes *in vitro* [214].

Moreover, TRPC1 is overexpressed in epidermis of patients with Darier's disease (DD) (or keratosis follicularis), a genodermatosis which is induced by a genetic defect of the sarco(endo)plasmic reticulum $Ca^{2+}$-ATPase (SERCA2), and which causes a severe, sometimes intensely pruritic epithelial differentiation disorder [215,216]. TRPC1-mediated $Ca^{2+}$ influx is 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. Therefore, it is at least conceivable that a TRPC1-mediated keratinization defect contributes to a – puritogenic! – disruption of the epidermal barrier, and thus indirectly to itch pathogenesis.

#### *2.4.2. TRPM6 and TRPM7 — Itch and the magnesium connection*

Finally, two channels of the TRPM subfamily (i.e. TRPM6 and TRPM7) may also affect itch pathogenesis. These molecules are recognized as key regulators of cellular magnesium homeostasis [109–113]. Since, in the rat, low-dietary magnesium leads to lowered serum $Mg^{2+}$, resulting in universal dermatitis and intense scratching behavior [217], it is tempting to ask whether a signaling dysfunction in the "magnesium-controlling" members of the TRPM subfamily can actually induce pruritogenic pruritus. That this line of thought is not entirely speculative is suggested by the intriguing clinical report that the pruritus of uremic patients completely disappeared after decreasing the concentration of magnesium $Mg^{2+}$ in the dialysate [218].

## 3. Conclusions, future perspectives

Here, we have reviewed pruritogenic stimuli, pruritogens and selected neural elements and processing centers involved in pruritus pathogenesis and the generation of the itch sensation. Focusing on recent neurophysiological concepts that explain pruriceptive pruritus and on the role of TRP channels in itch pathogenesis, we have sketched the long path "pruritology" has traveled to move, at long last, much closer to developing intelligent combination strategies for more effective pruritus management [3,4,6,8]. However, we still lack a single, easily applicable, well-tolerated, and universally effective pharmacological "itch killer".

This underscores the vital clinical importance of identifying novel candidate anti-pruritic agents that target defined molecular itch pathogenesis pathways. In this review, our aim was to specifically highlight the promise that individual members of the fascinating TRP channel family hold in this respect. Though the available information already has invited the proposal of a novel molecular syntax of various itch-related sensory qualities (which border on pain, warm, and cold sensations or incorporate elements thereof), evidently, much more extensive *in vitro* and *in vivo* studies are required to explore the exact roles of TRP channels in the neurophysiology and future therapy of itch.

Suffice it, therefore, we conclude this "exotic TRiP" into the "dominion" of experimental, theoretical, and clinical "pruritology" by defining a few "itchy frontiers" in pruritus research:

- Identification of physiological roles of TRP channels expressed on non-neuronal cells of human skin during inflammation and its related production of pruritogens.
- Definition of relationship of TRP channels to peripheral and central itch processing (sensitization, desensitization).
- Determination of the expression patterns of individual TRP channels in various pruritic dermatoses.
- Investigation of itch-related behavior of TRP channel gene-deficient mice.
- *In vivo* evaluation of "combination therapies", using substances that target different TRP channels.
- Identification, synthesis, and *in vitro* as well as *in vivo* evaluation of novel TRP-channel-acting pharmacological agents, partly based on sensations defined by the concept of "molecular psychophysics".

## Acknowledgements

Grants, supports: Writing of this article was supported in part by Hungarian research grants (OTKA T049231 and K063153, ETT 480/2006) for T. Bíró and by a grant from Deutsche Forschungsgemeinschaft to R. Paus (Pa 345/11-2).

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