Pirt, a TRPV1 Modulator, Is Required for Histamine-Dependent and -Independent Itch

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Introduction

The familiar sensation of itch has emerged as an important topic of research. Despite its great clinical significance, little is known about itch, especially in comparison to other sensory modalities [1]. In particular, efforts to investigate the molecular and cellular bases of itch sensation [2] are gaining momentum as pruritoception is still poorly characterized at these levels.

Previous work from our laboratory identified a novel gene encoding a membrane protein specifically expressed in sensory neurons [3]. Pirt, which is highly conserved among vertebrates, contributes to thermal nociception dependent upon the capsaicin receptor TRPV1 and regulates its physiology accordingly [3–5]. Pirt binds to both phosphatidylinositol (4,5)-bisphosphate (PIP2) and TRPV1 and is necessary for TRPV1-dependent activation of TRPV1 current. The sensory-specific expression pattern of Pirt and its ability to bind TRPV1 and other ion channels as well as PIP2 suggest additional modulatory functions and one promising candidate is pruritoception.

TRPV1 has previously been implicated in itch induced by histamine, the most commonly studied pruritogen. TRPV1−/− mice show a scratching deficit in response to histamine injection [6,7] and the presence of TRPV1 is required for proper activation of the histamine H1 receptor [6]. At a cellular level, TRPV1+ neurons play a major role in itch mediated by several pruritogens including, but not limited to, histamine [7,8]. How TRPV1 fits into the broader picture of histamine-dependent and -independent itch is uncertain. The possible role of Pirt in these processes, which we have identified as a critical modulator of TRPV1 function, has yet to be investigated.

Moreover, we have shown Pirt can bind other TRPs [3] and it may regulate other ion channels as well. Expression of Pirt is widespread among dorsal root ganglion (DRG) neurons, spanning the entire small- and medium-diameter population. Its functional interaction with PIP2 also hints at other roles in the physiology of sensory neurons. Working from the hypothesis that Pirt modulates TRPV1 and potentially other itch-associated proteins, we used the Pirt null mouse in conjunction with behavioral and imaging methods to examine the function of Pirt in the context of itch sensation.

Results

Pirt is essential for proper detection of histaminergic itch

We used a well-established assay to measure behavioral responses of mice to various itch-inducing compounds [9]. Histamine is known to generate a form of itch that is TRPV1-dependent [6,7], suggesting a role for Pirt in this process. As predicted, histamine produces itch in wild type mice (108 bouts of scratching) that is severely reduced in Pirt−/− littermates (12 bouts, Fig. 1A, B). In addition to producing a behavioral scratch response, many pruritogens can directly activate DRG neurons in vitro to generate intracellular calcium and/or electrophysiological responses. Histamine has been shown to directly activate DRG neurons and this response is decreased in the presence of a TRPV1 antagonist [6,10]. The percentage of neurons responding to histamine is significantly reduced in DRG cultures from Pirt−/− mice (5.3%) compared to wild type controls (9.8%, Fig. 1C). Histamine trifluoromethyl toluidide (HTMT), an agonist selective for the histamine H1 receptor [11], can also induce scratching, and this is strongly attenuated in Pirt−/− mice (91 bouts in WT vs.

Abstract

Itch, or pruritus, is an important clinical problem whose molecular basis has yet to be understood. Recent work has begun to identify genes that contribute to detecting itch at the molecular level. Here we show that Pirt, known to play a vital part in sensing pain through modulation of the transient receptor potential vanilloid 1 (TRPV1) channel, is also necessary for proper itch sensation. Pirt−/− mice exhibit deficits in cellular and behavioral responses to various itch-inducing compounds, or pruritogens. Pirt contributes to both histaminergic and nonhistaminergic itch and, crucially, is involved in forms of itch that are both TRPV1-dependent and -independent. Our findings demonstrate that the function of Pirt extends beyond nociception via TRPV1 regulation to its role as a critical component in several itch signaling pathways.
Pirt plays a crucial role in nonhistaminergic forms of itch

While histamine is a classical itch mediator that has proven fruitful for studying pruritoception, it is now clear nonhistaminergic types of itch exist that signal through separate pathways [1]. This is of great clinical significance as many pathological forms of itch are refractory to antihistamine treatment [14]. We next investigated the involvement of Pirt in nonhistaminergic itch.

The antimalarial drug chloroquine (CQ) commonly produces itch as a side effect [15,16] and this cannot be ameliorated by antihistamines [17,18]. CQ also induces itch when injected into mice and is transduced in a histamine-independent manner by the G protein-coupled receptor (GPCR) MrgprA3 [19]. Pirt mutant mice show a severe reduction in the behavioral itch response to CQ (38 bouts in KO vs. 184 in WT, Fig. 2A, B). Accordingly, fewer DRG neurons from Pirt KO mice respond to CQ (6.1%) compared to WT neurons (9.5%, Fig. 2C).

Serotonin (5HT) is a well-known neurotransmitter that can also produce itch [20,21]. We tested the response of Pirt KO mice to alpha-methyl-serotonin (α-Me-5HT), a 5HT derivative that acts as a selective agonist for the 5HT2 family of receptors and is able to induce a scratching response [7,22]. WT mice scratch more than twice as often (186 bouts) as Pirt mutants (82 bouts, Fig. 2D, E). Interestingly, DRG neurons in culture do not exhibit Ca2+ responses to α-Me-5HT, while responses to 5HT itself are unchanged in neurons from Pirt KO mice (5.0% for WT against 4.9% in KO, Fig. 2F). This suggests an indirect mechanism of itch generated by α-Me-5HT, e.g. activation of 5HT receptors in skin [23] or mast cells [24].

The peptide Ser-Leu-Ile-Gly-Arg-Leu-NH2 (SLIGRL), derived from the sequence of protease-activated receptor 2 (PAR2), which exhibits self-activation [25], produces a nonhistaminergic form of itch [26]. In vitro, we saw a marked reduction in the number of Pirt KO DRG neurons responsive to SLIGRL (2.4% of WT neurons vs. 0.5% in KO, Fig. 3A). We observed a decrease in the number of scratching bouts upon SLIGRL injection (148 bouts in WT vs. 92 in KO, Fig. 3B), but this difference was not statistically significant (p = 0.16). This may be due to the more variable scratching response to SLIGRL in comparison to other pruritogens.

One of the most potent itch mediators is endothelin-1 (ET-1). This peptide is perhaps best known for its vasodilator activity [27], but it has also been shown to produce itch in human subjects [28,29]. ET-1 induces a robust behavioral itch response in mice through interaction with the endothelin A receptor [30,31]. We found that Pirt KO mice (85 bouts) showed greatly reduced scratching in comparison with WT mice (269 bouts, Fig. 3C, D). We did not observe DRG neuron activation by ET-1 in our in vitro
preparation, suggesting ET-1 may mediate itch indirectly, e.g. through action at mast cells [32,33]. Alternatively, there may be a neuronal response we are unable to detect by Ca\textsuperscript{2+} imaging as other work suggests ET-1 may directly activate peripheral fibers in rats and human subjects [34,35].

Thus, beyond a critical role for Pirt in histaminergic itch, deficits in the response to several histamine-independent pruritogens tested, i.e. CQ, \(\alpha\)-Me-5HT, and ET-1, demonstrate that Pirt is involved in nonhistaminergic itch as well. Importantly, these results go beyond our initial hypothesis of Pirt regulating TRPV1-dependent itch as CQ [7,36], 5HT/\(\alpha\)-Me-5HT [6,7], and ET-1 [7] all induce behavioral itch responses that do not require TRPV1. This establishes a definite role for Pirt in itch sensation that is separate from, and potentially in addition to, its modulation of TRPV1 function. While Pirt was identified for its regulation of TRPV1 activity and related effects on temperature and pain sensation [3], these results hint at a broader mechanism of action such as interactions with other ion channels or plasma membrane components (see Discussion).

**Pirt contributes to mast cell-dependent itch**

Besides itch that entails direct activation of peripheral sensory neurons by pruritogens, itch-inducing compounds can also operate indirectly. Activity through secondary cell types such as keratinocytes or mast cells [37] can lead to release of substances that act directly upon DRG neurons to produce itch sensation. These indirect effects can be in addition to peripheral activation, e.g. CQ appears to generate itch via activation of MrgprA3+ DRG neurons as well as degranulation of mast cells [19].

One allergy model of itch makes use of the egg protein ovalbumin, an otherwise innocuous molecule that induces an immune response when co-injected with an adjuvant; subsequent injection of ovalbumin alone causes scratching [38]. We tested Pirt KO mice in this allergy model and found they exhibit significantly less scratching compared to WT (154 bouts vs. 115 bouts in KO, Fig. 4A, B). As mast cells are thought to play a critical role in the allergy response, we also tested SASH mice, which lack mast cells due to a chromosomal inversion affecting c-kit gene function [39]. They show drastically reduced scratching (30 bouts) in response to ovalbumin injection, verifying the role of mast cells in allergy-associated itch.

Another way to test mast cell-dependent itch is by inducing degranulation to release pruritogens. Compound 48/80 is an established mast cell degranulator that has been shown to produce itch in mice upon intradermal injection [9,40]. Pirt KO mice in this allergy model and found they exhibit significantly less scratching compared to WT (154 bouts vs. 115 bouts in KO, Fig. 4A, B). As mast cells are thought to play a critical role in the allergy response, we also tested SASH mice, which lack mast cells due to a chromosomal inversion affecting c-kit gene function [39]. They show drastically reduced scratching (30 bouts) in response to ovalbumin injection, verifying the role of mast cells in allergy-associated itch.

**Pirt displays specificity for different categories of itch**

These results lead to the question of how broadly Pirt functions in itch and whether it is involved in all types of pruritogenesis. We tested another compound, formalin, which has long been used to model pain responses [41] although recent evidence suggests its acute effect may be pruritogenic [42,43]. Interestingly, Pirt mice show scratching responses that do not differ significantly from WT controls (429 bouts in WT vs. 367 in KO, Fig. 5A, B). Formalin is known to directly activate the TRPA1 channel, which is also
required for the compound’s pain behavior [44,45]. Earlier work suggests Pirt is not involved in TRPA1-dependent physiological or behavioral, i.e. formalin-induced pain, responses [3]. Consistent with these findings, we found Pirt is not compulsory for all varieties of itch and reveals a degree of specificity in its function.

We also did not detect apparent changes in Pirt mice with respect to spontaneous itch behavior. Injection of saline vehicle produced minimal scratching in either WT or mutant mice (Fig. 5D). This suggests Pirt contributes to evoked scratch responses without affecting spontaneous itch.

Discussion

We used behavioral and imaging methods to study the role of Pirt, a recently identified regulator of TRPV1 [3], in itch sensation. Large reductions in the response to histamine and HTMT in mutant mice show a clear role for Pirt in histaminergic itch. Nonhistaminergic itch as induced by CQ, α-Me-5HT, and ET-1 is also markedly decreased. Unexpectedly, although we observed a cellular change in the response to SLIGRL, the behavioral deficits in the KO were not significant. This may be explained by the high variability of behavioral responses obtained with this pruritogen.

Notably, these results allude to a mechanism that is independent of TRPV1, which does not participate in itch mediated by several compounds that produce scratching deficits in Pirt KO mice. This opens up a number of possibilities as to how Pirt functions mechanistically in itch sensation. Other TRP channels such as TRPV3 and TRPV4 have been implicated in itch [46–48] and Pirt may interact with these as it does with TRPV1. Beyond the TRP family, other ion channels found in DRG neurons may contribute to itch sensation, and furthermore, PIP2 has been linked to ion channel function in many contexts [49]. This provides a possible explanation if Pirt does not directly modify channel activity (see below). PIP2 and phosphoinositide signaling as a whole are also involved in other means of ion channel regulation such as membrane targeting and vesicle trafficking [49]. A recent study [50] suggests direct binding of TRPV1 to PIP2 may not be Pirt-dependent. However, the lack of a Pirt effect on TRPV1 is likely due to the concentration of capsaicin used in the study, i.e. 0.8–1.0 μM. We found a positive effect of Pirt only when a higher capsaicin concentration, i.e. 5 μM, was used to activate TRPV1 [3]. Moreover, as the deficits in Pirt mice are not limited to TRPV1-dependent forms of itch, we do not expect PIP2 modulation of TRPV1 by Pirt to completely account for the various Pirt−/− phenotypes.

Considering many compounds are known to exert their pruritogenic effects by acting as ligands for GPCRs, including endothelin receptors, Mrgprs, and the histamine H1 receptor, Pirt may modulate some aspect(s) of the GPCR signaling cascade. The
extensive network of the GPCR pathway presents various opportunities for regulation. For example, the enzyme phospholipase Cβ3 (PLCβ3) has been shown to be critical for several varieties of itch and likely acts downstream of particular GPCRs that serve as itch receptors [7,11]. Thus, one possible mechanism is that Pirt modulates hydrolysis of PIP2 by PLCβ3. A recent study [51] has shown that chronic changes in PIP2 levels affect TRPV1 and TRPA1 activity in a concentration- and cell type-dependent manner, suggesting other sensory neuron factors like Pirt are important for PIP2-mediated TRP regulation. Specific G proteins, including both α and βγ subunits, may also have distinct roles in itch transduction [36,52].

Another study [36] involving our laboratory found that TRPA1 is required for itch induced by CQ but not histamine. While seemingly contradictory, these results do not preclude roles for both Pirt and TRPA1 in detecting CQ itch. Pirt does not contribute to direct activation of TRPA1 by mustard oil or formalin, but because CQ generates itch by first directly activating MrgrpA3 followed by downstream TRPA1 activation, the overall process can accommodate functions for both Pirt and TRPA1. Although it does not directly modify TRPA1 activity, Pirt may modulate PIP2 and/or other aspects of the G protein signaling pathway that constitutes CQ itch transduction.

Pirt−/− neurons show diminished Ca2+ responses to several compounds we tested. While these results are consistent with impaired signaling of these pruritogens, it remains to be determined whether the deficits are directly linked to itch. For example, histamine elicits responses in a relatively broad population in DRG that is probably not limited to the neurons required for detecting itch. Work from our laboratory and others [1,2] has indicated the existence of itch-sensing neurons in the DRG that are a subset of nociceptive neurons and would produce itch sensation upon activation. It would be worthwhile to examine if the reduction in histamine responsiveness found in Pirt−/− neurons is restricted to itch-sensing neurons, e.g. the MrgrpA3-expressing population described recently [19,36]. This can also be addressed by asking whether the targeted removal of Pirt from these purported itch-sensing DRG neurons produces cellular and behavioral deficits akin to those in the Pirt global KO mouse.

Our results provide further evidence that multiple molecular (TRPV1-dependent and -independent) and cellular (direct and indirect activation of sensory fibers) pathways are involved in itch signaling and demonstrate that Pirt plays a role in many of them. We have shown the presence of Pirt is indispensable for proper itch sensation. How it fits into this process mechanistically remains to be determined and may elucidate the different signaling pathways required to produce all varieties of itch. As Pirt is found in over 80% of DRG neurons [3], it is probable that it serves functions beyond thermal pain and now itch. Its mechanism of action in these contexts may shed light on other somatosensory processes to which Pirt contributes.

At the same time, the specific expression pattern of Pirt, which is limited to sensory neurons, indicates its potential use as a target for itch therapeutics. There are a number of mouse models to...
investigate various aspects of medical itch [53–58] that can be utilized to see if Pirt plays a role in any facet of itch in its various pathological manifestations such as inflammatory, dermatitis, etc. Our results establish a critical role for Pirt in sensing itch and generate new questions to advance knowledge of itch from both basic science and clinical perspectives.

Materials and Methods

**Pirt**

The mice were generated as previously described [3]. Briefly, the entire *Prt* coding region was replaced with an EGFP-IRES-rTA-ACN targeting construct to produce a null allele. Heterozygotes were mated to produce *Pirt* / mice (KO) and *Pirt* / littermates (WT).

**DRG neuron cell culture**

DRGs were dissected from all spinal levels of 3–8 week old mice. These were collected in ice cold DH10 medium (90% DMEM/F-12, 10% FBS, 100 U/ml penicillin, 100 µg/ml streptomycin, Gibco) and digested at 37°C in enzyme solution (5 mg/ml dispase, 1 mg/ml collagenase type I in HPBS without Ca<sup>2+</sup> or Mg<sup>2+</sup>, Gibco). After trituration and centrifugation, cells were resuspended in DH10 medium supplemented with NGF (20 ng/ml) and GDNF (25 ng/ml) and plated on glass coverslips coated with poly-D-lysine (0.5 mg/ml) and laminin (10 µg/ml). These were cultured in an incubator (95% O<sub>2</sub> and 5% CO<sub>2</sub>) and used for Ca<sup>2+</sup> imaging after 18–24 h.

**Ca<sup>2+</sup> imaging**

Neurons were loaded with fura-2-acetoxymethyl ester (Molecular Probes) for 30 min in the dark at room temperature. Cells were washed and imaged using 340 and 380 nm excitation to detect intracellular free Ca<sup>2+</sup>. Neurons were tested for responses to each compound in at least four independent experiments with a minimum of 100 cells analyzed each time.

**Itch behavior assay**

Mice tested were 2- to 3-month old males that had been backcrossed to C57Bl/6 mice for at least ten generations. Pruritogens were dissolved in a 50 µl volume of saline and subcutaneously injected into the nape of the neck. Bouts of scratching behavior were counted for 30 min immediately afterward with one bout defined as continuous scratching movements by the hindpaws directed at the area near the injection site.

The allergy model [38] was performed as follows: 50 µg ovalbumin dissolved in phosphate-buffered saline, with 2 mg aluminum hydroxide gel, was administered intraperitoneally, twice at two week intervals. Two weeks after the second sensitization, 50 µg ovalbumin dissolved in saline was administered in the same manner as other pruritogens and scratching behavior was quantified. All behavioral tests were performed by an experimenter blind to genotype and were done under the protocol approved by the Animal Care and Use Committee of Johns Hopkins University School of Medicine (Protocol number: MO09M534; Approval date: 07/22/2010).

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Figure 5. Pirt is not involved in all types of itch. (A, B) Pirt WT (n = 10) and KO (n = 8) mice display comparable formalin-induced (5%) itch behavior. (C) Ca<sup>2+</sup> responses to formalin (0.02%) are unaltered in Pirt mutant DRG neurons (n = 6 per genotype). (D) Saline vehicle induces a similar baseline, i.e. spontaneous, scratching response in *Pirt* / (n = 5) and WT (n = 7) mice.

doi:10.1371/journal.pone.0020559.g005
Acknowledgments

X.D. is an Early Career Scientist of the Howard Hughes Medical Institute. K.N.P., was a Graduate Research Fellowship of the National Science Foundation.

Author Contributions

Conceived and designed the experiments: KNP QL BJU XD. Performed the experiments: KNP QL SM. Analyzed the data: KNP QL SM BJU XD. Contributed reagents/materials/analysis tools: KNP QL SM BJU XD. Wrote the paper: KNP XD.

References

1. Patel KN, Dong X (2010) An itch to be scratched. Nature 463: 334–339.
2. Patel KN, Dong X (2011) Itch: cells, molecules, and circuits. ACS Chem Neurosci 2: 17–25.
3. Kim AV, Tang Z, Liu Q, Patel KN, Maag D, et al. (2008) Pirt, a phosphoinositide-binding protein, functions as a regulatory subunit of TRPV1. Cell 133: 473–485.
4. Caterina MJ, Schumacher MA, Tomimaga M, Rosen TA, Levine JD, et al. (1997) The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389: 816–824.
5. Caterina MJ, Leffler A, Mahmberg AB, Marin WJ, Trailon J, et al. (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Nature 408: 306–311.
6. Shinn WS, Tak MH, Lee MH, Kim M, Kim M, et al. (2007) TRPV1 mediates histamine-induced itching via the activation of phospholipase A2 and 12-lipoxygenase. J Neurosci 27: 2331–2337.
7. Imamachi N, Park GH, Lee H, Anderson DJ, Simon MI, et al. (2009) TRPV1-expressing primary afferents generate behavioral responses to pruritogens via multiple mechanisms. Proc Natl Acad Sci USA 106: 11330–11335.
8. Kim SJ, Park GH, Kim D, Lee J, Min H, et al. (2011) 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 USA 108: 3371–3376.
9. Kuraishi Y, Nagasawa T, Hayashi K, Satoh M (1995) Scratching behavior induced by pruritogenic but not allogeneic agents in mice. Eur J Pharmacol 275: 229–233.
10. Kim BM, Lee SH, Shinn WS, Oh U (2004) Histamine-induced Ca(2+) influx via the PLA2/C6/lipoxygenase/TRPV1 pathway in rat sensory neurons. Neurosci Lett 361: 159–162.
11. Han SK, Mancino V, Simon MI (2006) Phospholipase C beta 3 mediates serotonin-induced mechanical hyperalgesia. Cell 135: 1352–1365.
12. Bell JK, McQueen DS, Rees JL (2004) Involvement of histamine H4 and H1 receptors in scratching induced by histamine receptor agonists in Balb C mice. Br J Pharmacol 142: 374–380.
13. Dunford PJ, Williams KN, Desai PJ, Karlsson L, McQueen D, et al. (2007) Histamine H1 receptor agonists are superior to traditional antihistamines in the attenuation of experimental pruritus. J Allergy Clin Immunol 119: 176–183.
14. Twycross R (2003) Itch: scratching more than the surface. QJM 96: 7–26.
15. Matsushima H, Yamada N, Matsue H, Shimada S (2004) The effects of endothelin-1 on degranulation, cytokine, and growth factor production by skin-derived mast cells. Eur J Immunol 34: 1910–1919.
16. Petz M, Lammel V, Gibbs BF, Maurer M (2006) Inflammatory murine skin responses to UV-B light are partially dependent on endothelin-1 and mast cells. J Pathol 169: 815–822.
17. Gokin AP, Fareed MU, Pan HL, Hans G, Schirchitz GR, et al. (2001) Local injection of endothelin-1 produces pain-like behavior and excitation of nociceptors in rats. J Neurosci 21: 3566–3566.
18. Namer B, Hallgrens M, Orstavik K, Schmidt R, Weidner C, et al. (2008) Endothelin 1 activates and sensitizes human C-nociceptors. Pain 137: 41–49.
19. Wilson SR, Grekohl B, Birók-Fischer A, La QP, Patel KN, et al. (2011) TRPA1 is required for histamine-independent, mas-related G-protein coupled receptor-mediated responses to serotonin. J Pharmacol Exp Ther 336: 306–316.
20. Steinhoff M, Bienstock J, Schmelz M, Maurer M, Wei E, et al. (2006) Neurophysiological, neuroimmunological, and neuroendocrine basis of pruritus. J Invest Dermatol 126: 1705–1718.
21. Hashimoto Y, Arii I, Tanaka M, Nakaai S (2005) Prostaglandin D2 inhibits IgE-mediated scratching by suppressing histamine release from mast cells. J Pharmacol Sci 98: 90–93.
22. Yamazaki M, Tsujimura T, Morii E, Isozaki K, Onoue H, et al. (1994) Kit gene is expressed by skin mast cells in embryos but not in puppies of Wsh/Wsh mice: age-dependent abolishment of e-kit gene expression. Blood 83: 3509–3516.
23. Sugimoto Y, Unakoshi K, Nojiri N, Kamei C (1998) Effects of histamine H1 receptor antagonists on compound 48/80-induced scratching behavior in mice. Eur J Pharmacol 351: 1–5.
24. Duboisson D, Denniss SG (1977) The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brain stem stimulation in rats and cats. Pain 4: 161–174.
25. Ross SE, Mardini AR, McCorr AE, Zarwinski J, Cohen S, et al. (2010) Loss of inhibitory interneurons in the dorsal spinal cord and elevated itch in Bhlhb5 mutant mice. Nature 456: 498–498.
26. Akiyama T, Carstens MI, Carstens E (2010) Differential itch- and pain-related behavioral responses and µ-opioid modulation in mice. Acta Derm Venereol 90: 575–581.
27. Macpherson LJ, Xiao B, Kwan KY, Petrus MJ, Dubin AH, et al. (2007) An ion channel essential for sensing chemical damage. J neurosci 27: 11412–11415.
28. McNamara CR, Mandel-Brehm J, Bautista DM, Siemens J, Deranian KL, et al. (2007) An ion channel essential for sensing chemical damage. J Neurosci 27: 11412–11415.
29. Chung MK, Lee H, Mizuno A, Suzuki M, Caterina MJ (2004) TRPV3 and TRPM5 mediate the sodium influx via TRPV4 mediate warmth-evoked currents in primary mouse keratinocytes. J Biol Chem 279: 21369–21375.
47. Biró T, Tóth BI, Marincsák R, Dobrosi N, Géczy T, et al. (2007) TRP channels as novel players in the pathogenesis and therapy of itch. Biochim Biophys Acta 1772: 1004–1021.
48. Yoshioka T, Imura K, Asakawa M, Suzuki M, Oshima I, et al. (2009) Impact of the Gly573Ser substitution in TRPV3 on the development of allergic and pruritic dermatitis in mice. J Invest Dermatol 129: 714–722.
49. Ufret-Vincenty CA, Klein RM, Hua L, Angueyra J, Gordon SK (2011) Localization of the PIP2 sensor of TRPV1 ion channels. J Biol Chem 286: 9688–9698.
50. Han SK, Dong X, Hwang JI, Zylka MJ, Anderson DJ, et al. (2002) Orphan G protein-coupled receptors MrgA1 and MrgC11 are distinctively activated by RF-amide-related peptides through the Galpha q/11 pathway. Proc Natl Acad Sci USA 99: 14740–5.
51. Miyamoto T, Nojima H, Shinakado T, Nakahashi T, Kuraishi Y (2002) Itch-associated response induced by experimental dry skin in mice. Jpn J Pharmacol 88: 285–92.
52. Takano N (2003) Analysis of the spontaneous scratching behavior by NC/Nga mice: a possible approach to evaluate antipruritics for subjects with atopic dermatitis. Eur J Pharmacol 471: 223–228.
53. Nojima H, Cuellar JM, Simons CT, Carstens MI, Carstens E (2004) Spinal c-fos expression associated with spontaneous biting in a mouse model of dry skin pruritus. Neurosci Lett 361: 79–82.
54. Takano N, Acari I, Kurachi M (2006) A method to induce stable atopic dermatitis-like symptoms in NC/Nga mice housed with skin-lesioned mice. Br J Dermatol 154: 426–30.
55. Yatsuzuka R, Inoue T, Jiang S, Nakano Y, Kamei C (2007) Development of new atopic dermatitis models characterized by not only itching but also inflammatory skin in mice. Eur J Pharmacol 565: 225–31.
56. Pereira U, Misery L (2010) Experimental models of itch. In: Misery L, Stander S, eds. Pruritus. London: Springer. pp 51–59.