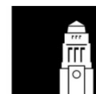

UNIVERSITY OF LEEDS

This is a repository copy of *Itchy channels and where to find them*.

White Rose Research Online URL for this paper:
http://eprints.whiterose.ac.uk/116263/

Version: Accepted Version

Article:

Gamper, N orcid.org/0000-0001-5806-0207 (2017) Itchy channels and where to find them. The Journal of Physiology, 595 (11). pp. 3257-3259. ISSN 0022-3751

https://doi.org/10.1113/JP274199

© 2017 The Authors. The Journal of Physiology © 2017 The Physiological Society. This is the peer reviewed version of the following article: "Gamper, N. (2017), Itchy channels and where to find them. J Physiol", which has been published in final form at http://doi.org/10.1113/JP274199. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

Reuse

Unless indicated otherwise, fulltext items are protected by copyright with all rights reserved. The copyright exception in section 29 of the Copyright, Designs and Patents Act 1988 allows the making of a single copy solely for the purpose of non-commercial research or private study within the limits of fair dealing. The publisher or other rights-holder may allow further reproduction and re-use of this version - refer to the White Rose Research Online record for this item. Where records identify the publisher as the copyright holder, users can verify any specific terms of use on the publisher's website.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk/

Itchy Channels and where to find them

Nikita Gamper[1,2]

[1]School of Biomedical Sciences, Faculty of Biological Sciences, University of Leeds, Leeds, UK

[2]Department of Pharmacology, Hebei Medical University, Shijiazhuang, China

Correspondence should be sent to:

Nikita Gamper
School of Biomedical Sciences,
Faculty of Biological Sciences,
University of Leeds, Leeds LS2 9JT, UK
Email: n.gamper@leeds.ac.uk

Itch (pruritus) is an unpleasant sensory experience that triggers a desire to scratch. It is induced by activation of a subset of cutaneous C fibers in response to environmental irritants and some endogenous mediators (such as histamine). As a somatic sensation itch is closely related to pain. Indeed, itch-mediated afferents are mostly classed as nociceptors, moreover, high concentrations of pruritogenic substances (*e.g.* histamine) produce pain, while some pain-inducing agents (*e.g.* capsaicin) can produce itch when applied topically to the skin (reviewed in (Lee *et al.*, 2016)). As with the pain, acute physiological itch is a healthy reaction as it informs the organism of harmful conditions and helps to eliminate them (*e.g.* by removing parasites or potentially damaging agents from the skin). Yet, chronic itch developing in some pathologies (*e.g.* atopic dermatitis) can be an excruciating disease.

Recent years saw swift progress in understanding of itch circuits and deciphering molecular mechanisms of itch signaling. Thus, histaminergic and non-histaminergic itch have been identified as distinct phenomena and the receptors of several non-histaminergic pruritogens have been discovered. Particularly, members of Mas-related G protein-coupled receptors (Mrgprs): MrgprA3, MrgprC11 and MrgprD were identified as itch-mediating receptors for chloroquine (Liu *et al.*, 2009), pruritic peptide BAM8-22 (Liu *et al.*, 2009) and β-alanine (Shinohara *et al.*, 2004), respectively. The Mrgpr-expressing primary afferents were, thus, identified as 'itch' afferents (Liu *et al.*, 2009; Liu & Dong, 2015).

Intense current research is focused on the elucidation of how G protein coupled receptors (GPCR) of itch generate receptor potential in the peripheral endings of itch afferents. The major emphasis thus

far was on the members of transient receptor potential (TRP) channel family, TRPV1 and TRPA1 (see excellent review by Kittaka & Tominaga (2017)). Histamine receptors expressed in sensory neurons (H1 and H4) and some Mrgprs couple to $G_{q/11}$ signalling cascade involving activation of phospholipase C (PLC) and hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) into diacylglycerol (DAG) and inositol trisphosphate (IP_3). DAG-mediated branch of this signalling cascade may involve activation of protein kinase C and phospholipase A_2, while IP_3 branch results in release of Ca^{2+} from the IP_3-sensitive intracellular stores and modulation of plenitude of downstream Ca^{2+}-sensitive targets (Gamper & Rohacs, 2012). Number of these pathways were implicated in pruritogen-induced activation of TRPV1 and TRPA1 with the assumption that these, in turn, produce depolarization of itch nerve endings sufficient to trigger action potentials (Kittaka & Tominaga, 2017). However, due to inherent difficulties with directly assessing and controlling the excitability of nerve endings, the majority of these studies used indirect approaches, such as electrophysiological and imaging experiments with the dissociated sensory neurons in culture in combination with genetic models and behavioural tests.

In the new study published in the current issue of the Journal of Physiology, Ru and colleagues developed an improved skin-nerve preparation in which electrophysiological recordings from intact itch afferents is combined with fast, reliable and repeatable subcutaneous application of drugs (Ru *et al.*, 2017). In this preparation dorsal side of the mouse skin is dissected out along with the blood vessels (subscapsular artery and its branches) and the nerves (with their associated T7-T10 dorsal root ganglia). The presence of the subscapsular artery allowed to overcome slow diffusion of the externally applied drugs through the skin using intra-arterial injections. The accuracy of the intra-arterial drug delivery has been carefully verified with dye injections.

Using this preparation it became possible to directly test the effect of genetic deletion or pharmacological inhibition of TRPV1, TRPA1 and other candidate ion channels on the excitation of itch afferent's peripheral endings in in response to pruritogen application to the skin. This powerful approach delivered a number of clear-cut but rather unexpected results. Thus, the authors showed that histamine and non-histaminergic agonists (chloroquine, BAM8-22 and ovalbumin) excite largely the same subpopulation of TRPA1- and TRPV1-expressing C-fibers (~25% of total cutaneous C fibers). This is at odds with previous observation that selective silencing of trigeminal histaminergic fibers does not significantly affect non-histaminergic itch and *vice versa* (Roberson *et al.*, 2013). This discrepancy may reflect differences between spinal and trigeminal systems and requires further investigation.

But a real surprise came when the responses of itch C fibers from trpa1$^{-/-}$ or trpa1$^{-/-}$/trpv1$^{-/-}$ double-knockout mice were investigated. Astoundingly, deletion of TRPA1, or TRPA1 together with TRPV1,

had no effect on the itch afferent excitation by chloroquine, histamine or ovalbumin. Moreover, regarding the excitation of itch afferents by chloroquine, the following conditions were also without an effect: i) the double deletion of TRPC3 and TRPC6; ii) the pharmacological inhibition of TRPA1 on the trpc3$^{-/-}$/trpc6$^{-/-}$ background; iii) the pharmacological inhibition of TRPC3/TRPC6 on the trpa1$^{-/-}$/trpv1$^{-/-}$ background. Even a broad-spectrum TRP channel blocker ruthenium red, which inhibits most of the sensory TRP channels, was without an effect. In these experiments the absence of the TRPA1 and/or TRPV1 channel activity in the fiber has been confirmed with allyl isothiocyanate (AITC) and capsaicin, respectively. Evidence presented herein argues strongly against the important role of TRP channels in the itch signal initiation at the level of free nerve endings of the cutaneous itch fibers, which is in stark contrast to the body of previous literature on the role of TRP channels in itch (see (Kittaka & Tominaga, 2017) for review).

The authors suggested that TRPA1/TRPV1 channels expressed somewhere else within the itch circuitry (but not at the itch nerve endings) might be important for the scratch reflex. This could account for the reduced scratching seen in some studies on TRPV1 and TRPA1 KO animals (Shim *et al.*, 2007; Wilson *et al.*, 2011). Additionally, it is possible that some degree of TRP channel inhibition/desensitization could have been produced by the surgical procedure (e.g. due to excessive activation of mechanosensitive fibres or due to the tissue damage-induced release of some excitatory compounds). In this case the TRP channel contribution to the pruritogen-induced itch fibre excitation could have been underestimated. It has to be noted though that some measures to prevent desensitisation have been taken by Ru and colleagues (*e.g.* a cyclooxygenase inhibitor has been included in the media throughout). In addition, most recorded itch afferents responded robustly to AITC and capsaicin, indicating that active TRPA1 and TRPV1 channels were present.

Notably, in contrast to the previous study (Wilson *et al.*, 2011), Ru and colleagues found no reduction in behavioural itch response to chloroquine in trpa1$^{-/-}$ mice. Moreover, chloroquine-induced inward current and excitability in the DRG neuron somata were also unaffected by TRPA1 deletion (again, *cf.* (Wilson *et al.*, 2011)). Thus, the discrepancy with earlier studies cannot at present be explained solely by the 'upstream' effects of TRPA1/TRPV1 in CNS or by some easily tractable technical issues. Clearly, further research will be required to resolve this controversy.

If not TRP, what is the mechanism for the pruritogen-induced activation of itch fiber endings? A somewhat similar uncertainty about the role of TRPV1 channels surrounded the mechanisms of nociceptive fiber excitation by the inflammatory mediator bradykinin (reviewed by Petho & Reeh, (2012)). Search for additional players resulted in the identification of a TRPV1-independent signalling

cascade whereby bradykinin B_2 receptors, acting via the $G_{q/11}$-PLC pathway, produced simultaneous activation of depolarizing Ca^{2+}-activated Cl^- channel TMEM16A and inhibition of hyperpolarizing M-type K^+ (KCNQ) channels (Liu *et al.*, 2010). Ru and colleagues tested if a similar mechanism could be at play in the GPCR-induced activation of itch fibers. Consistent with the involvement of the $G_{q/11}$-PLC pathway, chloroquine-induced C fiber excitation was almost abolished in the PLCβ3 knock-out mice. Furthermore, pharmacological inhibition of TMEM16A with the selective inhibitor MONNA reduced both, the chloroquine-induced action potential discharge from the itch fibers *in vitro* and the chloroquine-induced scratching behaviour *in vivo*.

Although these pharmacological experiments do not offer the same level of conclusiveness as the data obtained in knock-out mice (a limitation acknowledged by the authors), they indeed suggest that at least some chloroquine-induced itch can be attributed to the GPCR-induced activation of Ca^{2+}-activated Cl^- channels in the itch fiber endings. Interestingly, M channel inhibition with the selective blocker XE991 did not induce itch fiber activity *in vitro*, however, the *in vivo* effect of XE991 has not been tested.

The excitatory action of the Cl^- channel TMEM16A in sensory afferents, suggested in this (Ru *et al.*, 2017) and previous (Liu *et al.*, 2010; Cho *et al.*, 2012; Jin *et al.*, 2013; Takayama *et al.*, 2015) studies is at odds with known inhibitory action of another Cl^- channel abundantly expressed in sensory afferents, the $GABA_A$ channel (see *e.g.* (Takkala *et al.*, 2016; Du, 2017). The reasons for this apparent discrepancy are currently unknown but could involve distinct densities and localization (e.g. peripheral endings *vs.* more proximal parts of the fiber) of these channels.

Addressing the conundrums highlighted by the interesting study of Ru and colleagues will require a concerted effort of researchers studying somatosensory system and is likely to bring an important next step to our understanding of peripheral mechanisms of itch and pain.

References

Cho H, Yang YD, Lee J, Lee B, Kim T, Jang Y, Back SK, Na HS, Harfe BD, Wang F, Raouf R, Wood JN & Oh U. (2012). The calcium-activated chloride channel anoctamin 1 acts as a heat sensor in nociceptive neurons. *Nat Neurosci* **15,** 1015-1021.

Du X, Hao, H., Yang, Y., Huang, S., Wang, C., Gigout, S., Ramli, R., Li, X., Jaworska, E., Edwards, I., Deuchars, J., Yanagawa, Y., Qi, J., Guan, B., Jaffe, D.B., Zhang, H., Gamper, N. (2017). Local GABAergic Signaling within Sensory Ganglia Controls Peripheral Nociceptive Transmission. *J Clin Invest* **In press**.

Gamper N & Rohacs T. (2012). Phosphoinositide sensitivity of ion channels, a functional perspective. *Subcell Biochem* **59,** 289-333.

Jin X, Shah S, Liu Y, Zhang H, Lees M, Fu Z, Lippiat JD, Beech DJ, Sivaprasadarao A, Baldwin SA, Zhang H & Gamper N. (2013). Activation of the Cl- Channel ANO1 by Localized Calcium Signals in Nociceptive Sensory Neurons Requires Coupling with the IP3 Receptor. *Sci Signal* **6,** ra73.

Kittaka H & Tominaga M. (2017). The molecular and cellular mechanisms of itch and the involvement of TRP channels in the peripheral sensory nervous system and skin. *Allergol Int* **66,** 22-30.

Lee JS, Han JS, Lee K, Bang J & Lee H. (2016). The peripheral and central mechanisms underlying itch. *BMB reports* **49,** 474-487.

Liu B, Linley JE, Du X, Zhang X, Ooi L, Zhang H & Gamper N. (2010). The acute nociceptive signals induced by bradykinin in rat sensory neurons are mediated by inhibition of M-type K^+ channels and activation of Ca^{2+}-activated Cl^- channels. *J Clin Invest* **120,** 1240-1252.

Liu Q & Dong X. (2015). The role of the Mrgpr receptor family in itch. *Handb Exp Pharmacol* **226,** 71-88.

Liu Q, Tang Z, Surdenikova L, Kim S, Patel KN, Kim A, Ru F, Guan Y, Weng HJ, Geng Y, Undem BJ, Kollarik M, Chen ZF, Anderson DJ & Dong X. (2009). Sensory neuron-specific GPCR Mrgprs are itch receptors mediating chloroquine-induced pruritus. *Cell* **139,** 1353-1365.

Petho G & Reeh PW. (2012). Sensory and signaling mechanisms of bradykinin, eicosanoids, platelet-activating factor, and nitric oxide in peripheral nociceptors. *Physiol Rev* **92,** 1699-1775.

Roberson DP, Gudes S, Sprague JM, Patoski HA, Robson VK, Blasl F, Duan B, Oh SB, Bean BP, Ma Q, Binshtok AM & Woolf CJ. (2013). Activity-dependent silencing reveals functionally distinct itch-generating sensory neurons. *Nat Neurosci* **16,** 910-918.

Ru F, Sun H, Jurcakova D, Herbstsomer RA, Meixong J, Dong X & Undem BJ. (2017). Mechanisms of pruritogen-induced activation of itch nerves in isolated mouse skin. *J Physiol*.

Shim WS, Tak MH, Lee MH, Kim M, Kim M, Koo JY, Lee CH, Kim M & Oh U. (2007). TRPV1 mediates histamine-induced itching via the activation of phospholipase A2 and 12-lipoxygenase. *J Neurosci* **27,** 2331-2337.

Shinohara T, Harada M, Ogi K, Maruyama M, Fujii R, Tanaka H, Fukusumi S, Komatsu H, Hosoya M, Noguchi Y, Watanabe T, Moriya T, Itoh Y & Hinuma S. (2004). Identification of a G protein-coupled receptor specifically responsive to beta-alanine. *J Biol Chem* **279,** 23559-23564.

Takayama Y, Uta D, Furue H & Tominaga M. (2015). Pain-enhancing mechanism through interaction between TRPV1 and anoctamin 1 in sensory neurons. *Proc Natl Acad Sci U S A* **112,** 5213-5218.

Takkala P, Zhu Y & Prescott SA. (2016). Combined Changes in Chloride Regulation and Neuronal Excitability Enable Primary Afferent Depolarization to Elicit Spiking without Compromising its Inhibitory Effects. *PLoS computational biology* **12,** e1005215.

Wilson SR, Gerhold KA, Bifolck-Fisher A, Liu Q, Patel KN, Dong X & Bautista DM. (2011). TRPA1 is required for histamine-independent, Mas-related G protein-coupled receptor-mediated itch. *Nat Neurosci* **14,** 595-602.