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Insight on novel mechanisms mediating the generation of inflammatory pain in somatosensory neurons

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Intracellular Ca^{2+} signalling at plasma membrane-endoplasmic reticulum junctions of somatosensory neurons

Intracellular Ca^{2+} signalling is essential to the regulation of many biological mechanisms, which have distinct functional roles. The promiscuity of Ca^{2+} signalling represents a challenge for fine signalling transduction, and this appears particularly evident in somatosensory neurons, which are capable of generating and relaying signal related to well-defined, but discrete, sensory information, yet express numerous Ca^{2+} channels. To overcome this specificity issue, Ca^{2+} signalling is often restricted to subcellular compartments (nanodomains) – specialised regions within a cell where Ca^{2+} undergoes local modulation, allowing fine spatiotemporal tuning.

Endoplasmic reticulum (ER)-plasma membrane (PM) junctions are an example of such nanodomains, where physical proximity between these two membranes is critical for store operated Ca^{2+} entry (SOCE). In mammalian cells, SOCE complexes have of two main components: (1) the ER Ca^{2+} sensor stromal interaction protein 1 (STIM1), and (2) the pore-forming subunits of the Ca^{2+} release-activated Ca^{2+} channel (CRAC) protein 1–3 (Orai1-3). Activation of heterotrimeric Gq coupled receptor triggers phospholipase C enzyme activation, subsequent formation of inositol,1,4,5, triphosphate (IP_3), and release of Ca^{2+} from the ER; this leads to a drop in ER Ca^{2+} levels, which leads to STIM1 oligomerisation. Finally, via protein-protein interaction, STIM1 opens Orai1 channels on the plasma membrane, allowing SOCE (Woo *et al.* 2018). Various adaptor proteins are required to stabilize PM-ER junctions and to allow SOCE, among which are junctophilins (JPH).

The role of junctophilins in store operated Ca^{2+} entry and inflammatory mechanisms in sensory neurons

The JPHs are a family of junctional membrane complex-associated proteins whose C- and N-termini are situated in the PM and ER, respectively. Thus, they form junctional complexes between these two membrane compartments. There are four junctophilin isoforms (JPH1–4) displaying different tissue specificity of expression. While JPH3–4 are ubiquitously expressed in the central nervous system (CNS), JPH expression in dorsal root ganglia (DRG) has not yet been explored in depth.

ER-PM junctions exist in DRG somatosensory neurons, and functional SOCE complexes have also been reported in these neurons (Gemes *et al.* 2011). SOCE has been associated with pain elicited by pro-inflammatory mediators such as bradykinin (BK). BK acts via its B_2 receptors (B_2R) to excite peripheral neurons by inhibiting M-type K^+ channels and activating Ca^{2+}-dependent Cl^- channels (Brown and Passmore, 2010) (Liu *et al.* 2010). The role of junctophilins in this process has not previously been elucidated.

JPH4 is the predominant JPH isoform in rat DRGs and colocalises with SOCE components Orai1 and STIM1

In a recent publication in *The Journal of Physiology*, Hogea *et al.* (2021) characterised the role of junctophilins in SOCE in DRG somatosensory neurons and investigated their potential involvement in inflammatory pain *in vitro* and *in vivo*. The authors first aimed to identify which isoforms of the JPH, STIM and Orai families were expressed in primary sensory neurons. Utilising immunohistochemistry and western blot analyses, they found that three JPH isoforms – JPH1, JPH3 and JPH4 – were expressed in neonatal male rat DRG neurons. JPH4 was the predominant isoform, found to be expressed in 76% of analysed DRG neurons. It was significantly more abundant than the other isoforms and seemingly preferentially located at the PM, prompting the group to focus on this isoform for further experiments. Immunohistochemistry also confirmed the expression of STIM1-2, as well as Orai1-3 in rat DRG neurons, with STIM 1 and Orai1 being the predominant isoforms, expressed in 54% and 72% of DRG neurons, respectively. Interestingly, the authors showed that upon ER Ca^{2+} store depletion, JPH4 highly co-localised with STIM1 and Orai1, essential for SOCE in DRG neurons. JPH4, STIM1 and Orai1 were found to be expressed uniformly across neurons of different somatic sizes. JPH4 expression was found in 75% and 84% of neurons labelled for NF200 and TRPV1, respectively. It should be noted that not all polymodal nociceptors express TRPV1, and furthermore TRPV1 is also expressed in some Aδ fibres. This overlap was not addressed and other markers for different sensory neuron modalities were not used in this study.

JPH4 senses ER Ca^{2+} depletion and is required for BK-induced SOCE in rat DRGs

Moving forward, the group investigated the role of JPH4 in GPCR activation-mediated store depletion in primary somatosensory neurons. Using proximity ligation assay and super-resolution imaging, they confirmed co-localization of JPH4 with STIM1 and Orai1, and that – similarly to the established clustering of STIM1 and Orai1 – ER Ca^{2+} store depletion stimulated recruitment of JPH4 to this cluster as well. Immunohistochemical and western blot analyses following siRNA-mediated JPH4 knockdown revealed that JPH4 was in fact necessary for efficient clustering of STIM1 and Orai1 after BK treatment. This is in agreement with previous literature showing JPH1–2 dependence of STIM1-Orai1 ER clustering in skeletal myocytes and CNS (Takeshima *et al.* 2015).

To clarify JPH4 role in Ca^{2+} signalling, the authors performed a set of *in vitro* calcium imaging experiments. First, they demonstrated that SOCE could be measured in cultured DRG neurons. Stimulating DRGs with BK in the absence of extracellular Ca^{2+} induced a robust increase in cytoplasmic Ca^{2+}, depleting ER stores; Ca^{2+} was then added back to the extracellular solution, inducing a second Ca^{2+} transient – this was prevented

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by treating DRGs with SOCE inhibitors, indicating that the increase in cytoplasmic Ca^{2+} during the Ca^{2+} add-back involved the SOCE complex. Next, they showed that in JPH4-silenced neurons the initial BK-induced Ca^{2+} transient was maintained, but the increase in cytoplasmic Ca^{2+} was significantly attenuated when Ca^{2+} was added back to the extracellular solution, suggesting that JPH4 plays a functional role in SOCE. The authors then questioned if this apparent JPH4 involvement in replenishing ER Ca^{2+} stores would affect recurrent GPCR-mediated Ca^{2+} signalling. Since prolonged or repeated GPCR stimulation can lead to decreased responsiveness to an agonist treatment, the ER Ca^{2+} stores were first depleted via different GPCRs – purinergic P2Y receptors – by stimulating DRG neurons with the P2Y agonist ATP in the absence of extracellular Ca^{2+}. Subsequently, Ca^{2+} was added back to the extracellular solution, and the neurons were stimulated with BK; interestingly, the release of Ca^{2+} from the ER in response to BK was attenuated by 40% in JPH4-silenced neurons. These experiments suggest that not only JPH4 is required for STIM1-Orai1 clustering upon ER Ca^{2+} store depletion, but that by impairing SOCE, JPH4 knockdown ultimately decreases DRG sensitivity during repeated GPCR stimulation.

Finally, to determine whether JPH4 is involved in pain perception, Hogea *et al.* (2021) delivered cholesterol-conjugated JPH4 siRNA to the lumbar region (L5-L6) of adult rats to silence JPH4 gene expression and performed behavioural experiments. Intraplantar injection of BK induces inflammation and nocifensive behaviour in rodents. The authors demonstrated that while normal mechanical and thermal sensitivity was maintained in rats with decreased JPH4 expression, the time-span of nocifensive behaviour significantly decreased in these animals. Overall, this experiment corroborates the molecular findings, suggesting that JPH4 is implicated in peripheral BK-mediated inflammatory pain.

Final remarks

Whilst the authors present strong evidence of their hypothesis regarding the involvement of JPH4 in inflammatory mechanisms at PM-ER nanodomains, the use of other *in vivo* models such as knockout mice or the inclusion of female animals could have strengthened these findings and clarified whether these mechanisms are conserved across the two sexes or in different species. Moreover, whilst DRG cells bodies are of growing interest in the context of nociception input regulation, it would have been valuable to investigate these JPH4-mediated mechanisms at nerve terminals – the loci of inflammatory excitation. However, given (1) the presence of functional BK receptors in the periphery as demonstrated by BK-induced pain response *in vivo*, (2) the expression of ER in proximal C-fibres, and (3) the reduction of duration of nocifensive behaviours *in vivo* upon JPH4 knockdown in rats' lumbar region, it is reasonable to speculate that the observed effects are also relevant for the peripheral portions of somatosensory nerves. To conclude, the work of Hogea *et al.* (2021) has successfully provided the first evidence of the role of the junctophilin isoform JPH4 in SOCE and inflammatory pain mediation in peripheral somatosensory neurons of male rats.

References

Brown DA & Passmore GM (2010). Some new insights into the molecular mechanisms of pain perception. *J Clin Invest* **120**, 1380–1383.

Gemes G, Bangaru ML, Wu HE, Tang Q, Weihrauch D, Koopmeiners AS, Cruikshank JM, Kwok WM & Hogan QH (2011). Store-operated Ca^{2+} entry in sensory neurons: functional role and the effect of painful nerve injury. *J Neurosci* **31**, 3536–3549.

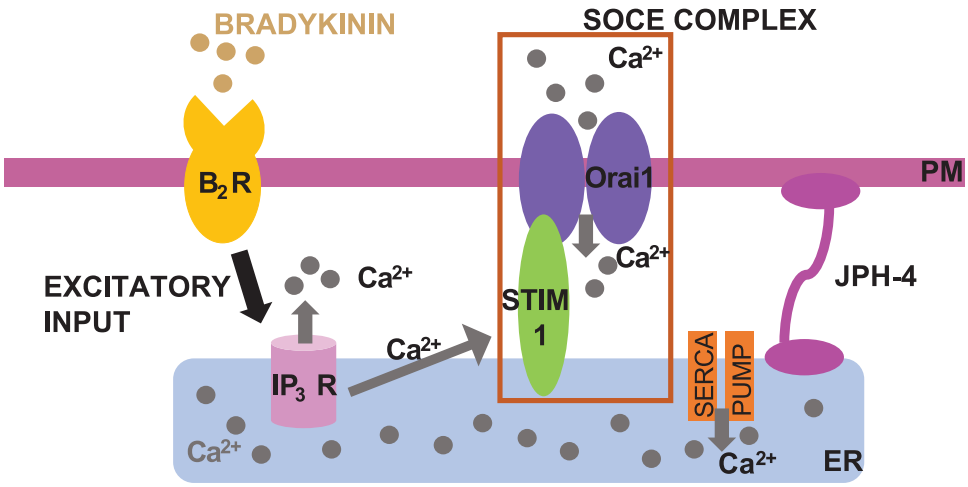

Figure 1. The role of JPH4 in stabilising ER-PM junction and mediating bradykinin-initiated store operated Ca^{2+} entry in rat somatosensory neurons
Schematic diagram of store operated Ca^{2+} entry in rat somatosensory DRG neurons. The activation of Gq coupled B_2 receptors by bradykinin triggers phospholipase C enzyme activation, ultimately leading to the formation of second messenger inositol,1,4,5, triphosphate (IP_3), and release of Ca^{2+} from the ER to the cytoplasmic space, with subsequent decrease of Ca^{2+} in the ER. This drop in ER Ca^{2+} level is sensed by STIM1, leading to its oligomerisation. Finally, STIM1 via protein-protein interaction opens Orai1 channels on the plasma membrane, allowing SOCE (Woo *et al.* 2018). The mechanism requires close proximity of PM-ER, which the authors suggest is dependent upon JPH4 expression in rat DRGs.

Hogea A, Shah S, Jones F, Carver CM, Hao H, Liang C, Huang D, Du X & Gamper N (2021). Junctophilin-4 facilitates inflammatory signalling at plasma membrane-endoplasmic reticulum junctions in sensory neurons. *J Physiol* **599**, 2103–2123.

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.

Takeshima H, Hoshijima M & Song LS (2015). Ca^{2+} microdomains organized by junctophilins. *Cell Calcium* **58**, 349–356.

Woo JS, Srikanth S & Gwack Y (2018). Modulation of orai1 and STIM1 by cellular factors. In *Calcium Entry Channels in Non-Excitable Cells*, eds Kozak JA & Putney JW Jr, pp. 73–92.Boca Raton, FL, USA.

Additional information

Competing interests

No competing interests declared.

Author contributions

A.F. and G.T.: conception or design of the work; drafting the work or revising it critically for important intellectual content; final approval of the version to be published; agreement to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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calcium signalling, junctophilins, pain, PM-ER junctions