Journal Club

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Warming up the Cold Reception at a TRPM8 Function

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Review of Andersson et al. (<http://www.jneurosci.org/cgi/content/full/27/12/3347>)

The transient receptor potential (TRP) family of cation-permeable channels responds to mechanical, thermal, and chemical stimuli originating from inside and outside the cell (Clapham, 2003). The TRP (melastatin)-8 (TRPM8) channel is expressed in a subpopulation of temperature-sensing dorsal root ganglion (DRG) neurons and facilitates calcium influx at temperatures <25°C (Peier et al., 2002). TRPM8 is also expressed in sensory nerves in deep tissues that encounter little temperature variation, suggesting that TRPM8 is also regulated by a temperature-independent mechanism, perhaps through an endogenous agonist. Vanden Abeele et al. (2006) demonstrated that lysophospholipids (LPLs), which are produced by phospholipase A₂ (PLA₂), are able to prolong the open time of TRPM8 channels and that downregulation of PLA₂ inhibits channel activity and TRPM8-mediated responses. In their recent publication in *The Journal of Neuroscience*, Andersson et al. (2007) further investigated the role of PLA₂ and its products on TRPM8 channel activity.

The authors applied PLA₂ inhibitors to Chinese hamster ovary (CHO) cells over-

expressing TRPM8 to monitor increases in intracellular calcium evoked by cold or the chemical agonists icilin and menthol (Fig. 1). The nonselective PLA₂ inhibitor *N*-(p-aminocinnamoyl)anthranilic acid (ACA) abolished the responses to cold and icilin and decreased the response to menthol [Andersson et al. (2007), their Fig. 1 (<http://www.jneurosci.org/cgi/content/full/27/12/3347/F1>)], and E-6-(bromomethylene)-tetrahydro-3-(1-naphthalenyl)-2*H*-pyran-2-one (BEL), a selective inhibitor of the calcium-insensitive PLA₂ (iPLA₂), produced similar results. However, the selective inhibitor of the cytosolic PLA₂ (cPLA₂), cPI [*N*-{(2*S*,4*R*)-4-(biphenyl-2-ylmethyl-isobutyl-amino)-1-[2-(2,4-difluorobenzoyl)-benzoyl]-pyrrolidin-2-ylmethyl}-3-[4-(2,4-dioxothiazolidin-5-ylidenemethyl)-phenyl] acrylamide HCl], had no obvious effect on [Ca²⁺]_i responses [Andersson et al. (2007), their Table 1 (<http://www.jneurosci.org/cgi/content/full/27/12/3347/T1>)]. These findings suggest that the activation of TRPM8 is not regulated by all PLA₂ proteins but is specifically controlled by the iPLA₂ subset.

Andersson et al. (2007) also examined the effects of PLA₂ inhibition on native TRPM8 in cultured DRG neurons. The cultures were heterogeneous, with ~11–12% of the cells responding to cold stimulation, ~7% to menthol, and ~9% to icilin. Cold-sensitive neurons can be divided into two subpopulations: menthol sensitive and menthol insensitive. The menthol-insensitive population has a lower temperature threshold and does not express TRPM8 (Babes et al., 2004). In

agreement with the experiments in CHO cells, treatment with ACA or BEL reduced the number of neurons that responded to cold or menthol and nearly abolished response to icilin [Andersson et al. (2007), their Table 2 (<http://www.jneurosci.org/cgi/content/full/27/12/3347/T2>)]. The similarity in the proportions of cold-sensitive, menthol-insensitive neurons in normal DRG cultures and cold-sensitive neurons remaining after inhibition of PLA₂ (~4%) is consistent with TRPM8 expression only in the menthol-sensitive subpopulation regulated by PLA₂.

iPLA₂ breaks glycerophospholipids into polyunsaturated fatty acids (PUFAs) and LPLs; thus, the authors examined the effects of these compounds on TRPM8. The authors treated TRPM8-expressing CHO cells with LPLs of different head groups and acyl chain lengths and measured [Ca²⁺]_i responses and single-channel activity. Zwitterionic lysophosphatidylcholine (LPC), and anionic lysophosphatidylserine and lysophosphatidylglycerol all produced robust [Ca²⁺]_i responses, whereas a sphingolipid, sphingosyl-phosphorylcholine, had a modest effect [Andersson et al. (2007), their Fig. 5 (<http://www.jneurosci.org/cgi/content/full/27/12/3347/F5>)]. LPC produced single-channel current activities comparable with menthol, albeit after a longer delay [Andersson et al. (2007), their Fig. 6 (<http://www.jneurosci.org/cgi/content/full/27/12/3347/F6>)]. This latency suggests that LPC acts intracellularly, requiring time to translocate to the

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cytoplasm. Like menthol, LPC appeared to potentiate the effect of cooling in a dose-dependent manner, as shown by increases in current amplitude, $[Ca^{2+}]_i$ response, and temperature activation threshold [Andersson et al. (2007), their Fig. 7 (<http://www.jneurosci.org/cgi/content/full/27/12/3347/F7>)]. The authors clearly showed that LPLs, rather than iPLA2 itself, were responsible for these responses, because ACA had no effect when applied in conjunction with LPL. The LPL response was dependent on TRMP8 because extracellular calcium was required, and application of these lipids was ineffective on untransfected cells.

PUFAs are the other product of glycerophospholipid cleavage by PLA₂ activity and are known to activate a number of TRP channels. Therefore, it was expected that these compounds would mimic the effect of LPLs on TRMP8 activity. However, treatment with arachidonic acid, eicosapentaenoic acid, or docosahexaenoic acid nearly abolished $[Ca^{2+}]_i$ response to icilin and cold stimulation in CHO cells and reduced the response to menthol [Andersson et al. (2007), their Figs. 3 (<http://www.jneurosci.org/cgi/content/full/27/12/3347/F3>), 4 (<http://www.jneurosci.org/cgi/content/full/27/12/3347/F4>)]. The authors showed that equimolar concentrations of PUFA and LPL (as would be generated by the cleavage of glycerophospholipids) had a net activation effect on TRMP8, but they suggested that cellular concentrations of PUFAs might be varied to regulate TRMP8 activity.

Figure 1. Overview of TRPM8 regulation, as proposed by Andersson et al. (2007). TRPM8 channels are activated by cold and chemical agonists, like icilin and menthol, producing an influx of Ca^{2+} . These channels are also modulated by endogenous lipid agonists derived from glycerophospholipids via iPLA₂. PUFAs act as negative modulators, whereas LPLs act as positive modulators. At equimolar concentrations of LPLs and PUFAs, there is net activation of TRPM8 channels, so inhibitors of iPLA₂, such as ACA and BEL reduce TRPM8 activity.

Andersson et al. (2007) have demonstrated that the endogenous products of iPLA₂ are essential for regulation of TRPM8 (Fig. 1), which may explain why these thermosensitive ion channels are present in sensory nerves that rarely experience temperatures $<25^{\circ}C$. However, the mechanism by which PUFAs and LPLs act to regulate TRPM8 needs additional study, because it is still unclear whether these lipid modulators act directly by binding to TRPM8 channels or indirectly by interacting with other proteins.

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