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DISSERTATION

"Regulation of the cold-sensing TRPM8 and the heat-sensing TRPV1 ion channels by cellular signaling pathways"

by

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ABSTRACT

The sensory neurons of dorsal root ganglia (DRG) express the cold-sensing Transient Receptor Potential Melastatin 8 (TRPM8) and the heat-sensing Transient Receptor Potential Vanilloid 1 (TRPV1) ion channels. Both TRPM8 and TRPV1 require the plasma membrane phospholipid phosphatidylinositol 4,5-bisphosphate [$PI(4,5)P_2$ or PIP_2] for activity.

TRPM8 is important for both physiological temperature detection and cold allodynia. Activation of G-protein coupled receptors (GPCRs) by pro-inflammatory mediators inhibits TRPM8. It was proposed that this inhibition proceeds via direct binding of $G_{\alpha q}$ to the channel, and that a decrease in cellular levels of $PI(4,5)P_2$ does not contribute to channel inhibition.

We found that supplementing the whole cell patch pipette with $PI(4,5)P_2$ reduced inhibition of TRPM8 by activation of $G_{\alpha q}$-coupled receptors in mouse DRG neurons. Stimulating the same receptors activated Phospholipase C (PLC) and decreased plasma membrane $PI(4,5)P_2$ levels. Co-expression of a constitutively active $G_{\alpha q}$ protein that does not couple to PLC inhibited TRPM8 activity, and in cells expressing this protein decreasing $PI(4,5)P_2$ levels using a voltage sensitive 5'-phosphatase induced a stronger inhibition of TRPM8 activity than in control cells. Our data indicate that upon GPCR activation, $G_{\alpha q}$ binding reduces the apparent affinity of TRPM8 for $PI(4,5)P_2$ and thus sensitizes the channel to inhibition induced by decreasing $PI(4,5)P_2$ levels.

TRPV1 detects high temperatures and provides the sensation of burning heat and pain. Interestingly, the TRPV1 agonist capsaicin has been used as a topical treatment of chronic pain due to its effect on TRPV1 desensitization. It was shown in our lab that Ca^{2+} influx through TRPV1 activates PLC hydrolyzing $PI(4,5)P_2$, which leads to channel desensitization. It is well known that the PIP_2 hydrolysis product diacylglycerol (DAG) activates protein kinase C (PKC), which phosphorylates and positively regulates TRPV1. Our hypothesis is that the DAG/PKC pathway may limit capsaicin-induced TRPV1 desensitization.

We show that $PI(4,5)P_2$ decreases and DAG transiently forms in the plasma membrane upon capsaicin-induced TRPV1 activation. Inhibition of DAG kinase I, which phosphorylates DAG into phosphatidic acid, potentiates this capsaicin-induced DAG increase and TRPV1 activity. Our data indicate that DAG formed during TRPV1 activation is rapidly phosphorylated by DAG kinase, which limits its effect on TRPV1.