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"DIFFERENTIAL ROLES OF PHOSPHOINOSITIDES IN TRANSIENT RECEPTOR POTENTIAL CHANNEL REGULATION"

by
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Thursday, March 10th, 2022
10:30 A.M.

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ABSTRACT

Transient Receptor Potential (TRP) channels mediate a significant proportion of our sensory experience. Many members of this protein family are molecular sensors that can be activated by a variety of stimuli including heat, acidity, mechanical stress, and natural agonists. The members of this protein family are polymodal, mostly non-selective Ca^{2+} permeable cation channels that are involved in a variety of physiological processes such as thermo-sensation, pain and Ca^{2+} homeostasis. Their activity is regulated by cellular factors including signaling pathways that cause degradation of phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$) via phospholipase C activation. Like many ion channels, $PI(4,5)P_2$ and other forms of phosphoinositides play important roles in the regulation of TRP channel function. However, the nature of this regulation and the lipid-protein interactions are diverse between the TRP family members and not well understood until recently. Here in this thesis dissertation, we aimed to explore the regulation of TRPV1, TRPV5 and TRPV6 channels by phosphoinositides.

TRPV5 and TRPV6 are highly selective for Ca^{2+} and are critical for epithelial Ca^{2+} transport. Their activity is regulated by Ca^{2+}-induced feedback mechanisms. Mice with genetic deletions had disrupted ~~the~~ Ca^{2+} homeostasis and male infertility. Recently, loss of function mutations in TRPV6 have been identified in patients suffering from chronic pancreatitis. While being constitutively active, activity of TRPV5 and TRPV6 heavily depends on $PtdIns(4,5)P_2$. These channels are also known to be inhibited by the calcium sensing protein calmodulin. However, the mechanism of these regulations was not fully understood yet. In the first part, we aimed to ~~determine~~ identify the structural determinants of TRPV5 and TRPV6 channels and $PI(4,5)P_2$ interaction. We identified the proximal N terminal residue R302 and the S4-S5 linker residue K484 as primary contacts for $PI(4,5)P_2$ ~~on~~ in TRPV5 and TRPV6. Also, we reported that calmodulin inhibits the channel by blocking the pore through direct interactions with the pore forming W583 residue.

In the second part, we aimed to reveal the regulation of the heat- and capsaicin-activated TRPV1 by phosphoinositides. TRPV1 is the founding member of the vanilloid subfamily and its regulation by phosphoinositides is complex and controversial. In the TRPV1 cryoEM structure, an endogenous phosphoinositide was detected in the vanilloid binding site, and phosphoinositides were proposed as competitive antagonists. This model is difficult to reconcile with $PI(4,5)P_2$ being a well-established positive regulator of TRPV1. To resolve this controversy, we proposed that phosphoinositides regulate TRPV1 via functionally distinct binding sites. Consistently, our experimental approach confirmed that phosphatidylinositol (PtdIns), when co-applied with $PI(4,5)P_2$, decreased capsaicin-induced TRPV1 currents in excised inside-out patch clamp recordings. This negative effect of PtdIns depended on the concentrations of both capsaicin and PtdIns, supporting the competitive binding of the two compounds to ~~the~~ overlapping sites. On the other hand, in the absence of $PI(4,5)P_2$, PtdIns partially stimulated TRPV1 currents presumably by binding to the activating site when the vanilloid site was occupied by saturating levels of capsaicin. Overall, we showed that PtdIns has dual effects on TRPV1 currents by acting as a positive or negative modulator. Our two binding site model proposes that each site with distinct functions can become accessible for PtdIns depending on the concentration of capsaicin.