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DISSERTATION

"REGULATION OF PIEZO2 CHANNELS BY Gi-PROTEIN COUPLED RECEPTORS AND IDENTIFICATION OF NOVEL INHIBITORS OF TRPV5 CHANNELS"

by

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Friday, August 28th, 2020
11:00 A.M.
WebEx
Meeting number (access code): 120 715 6554
Meeting password: mxXuJPCB683

ABSTRACT

Mechanically activated Piezo2 channels are key players in somatosensory touch, but their regulation by cellular signaling pathways is poorly understood. Piezo2 channels are non-selective cation channels highly expressed in dorsal root ganglion (DRG) neurons. DRG neurons express a variety of G-protein-coupled receptors that modulate the function of sensory ion channels. Gi-coupled receptors are generally considered inhibitory, as they usually decrease excitability. Paradoxically, activation of Gi-coupled receptors in DRG neurons sometimes induces mechanical hypersensitivity, the mechanism of which is not well understood. Therefore, for the first part of this thesis, we decided to investigate the potential role of Gi-coupled receptors in the regulation of Piezo2 channels. Our findings show that activation of Gi-coupled receptors potentiates mechanically activated (MA) currents in DRG neurons and heterologously expressed Piezo2 channels, but inhibits Piezo1 currents in heterologous systems in a Gβγ-dependent manner. Pharmacological inhibition of kinases downstream of Gβγ, phosphoinositide 3-kinase (PI3K) and mitogen-activated protein kinase (MAPK), also abolishes the potentiation of Piezo2 currents. In addition, local injection of sumatriptan, an agonist of the Gi-coupled serotonin 1B/1D receptors, increases mechanical sensitivity in mice, and the effect is abolished by inhibiting PI3K and MAPK, suggesting an indirect mechanism of action of Gβγ to sensitize Piezo2 channels.

For the second part of this thesis, we explored the regulation of the Ca^{2+}-selective transient receptor potential vanilloid 5 (TRPV5) channels by exogenous modulators. TRPV5 channels act as rate limiting factor for transcellular reabsorption of calcium in the kidney, which make them crucial players for renal calcium homeostasis. Using a combination of structure-based virtual screening (SBVS) technology, whole-cell electrophysiology and cryo-electron microscopy (cryo-EM), we identified three novel inhibitors of TRPV5 channels: ZINC9155420 and ZINC05626366 and ZINC17988990. ZINC17988990, which is a derivative of ZINC9155420, showed potency in the nanomolar range (~100 nM) and a remarkable specificity for TRPV5 over TRPV6 and other TRP channels. The ZINC17988990-bound TRPV5 structure revealed that ZINC17988990 binds TRPV5 between the intracellular S1-S4 bundle and the TRP helix. Mutagenesis of some of the residues at this binding pocket shifted the original IC_{50} to mid-micromolar range. Moreover, ZINC9155420-bound TRPV5 structure showed that this compound binds TRPV5 at the interface between the S4-S5 linker of one monomer and the S6 helix of an adjacent monomer and mutagenesis experiments of residues at that region in the pocket confirmed the binding site.

Taking this together, this thesis illustrates 1) a novel and indirect intracellular mechanism of Gβγ to sensitize Piezo2 currents and increase mechanosensitivity after Gi-coupled receptor activation and 2) the identification of novel TRPV5-exogenous inhibitors that may serve as basis for the development of potential drugs to treat kidney conditions associated with dysregulation of calcium homeostasis and reabsorption.