P-2.35

Domain-specific Binding Affinities of Adpr and 2'-deoxy-adpr Suggest Sequential Activation of the Calcium Channel Trpm2

Tatiana Kupriianova [1], Thorben Thalacker [2], Lucas Defelipe [1], Ralf Fliegert [2], Maria Garcia Alai [1]

[1] European Molecular Biology Laboratory, Hamburg, Germany

[2] University Medical Centre Hamburg–Eppendorf, Hamburg, Germany

TRPM2 is a non-selective Ca^{2+}-permeable cation channel important for immune response, temperature sensation, insulin secretion, and pH balance. Its activation can also contribute to neurodegeneration, stroke, and diseases like bipolar disorder, diabetes, and Alzheimer's. Despite its significance, the regulatory mechanisms of TRPM2 activation are not well understood. This study investigates ligand interactions with human TRPM2. TRPM2 activation requires ADP-ribose (ADPR) binding to two domains: MHR1/2 and NUDT9H. The ADPR derivative, 2'-deoxy-ADPR (dADPR), induces 10.4-fold stronger whole-cell currents, making it a superagonist. We measured the binding affinities of ADPR and dADPR to MHR1/2 and NUDT9H using Nano Differential Scanning Fluorimetry (nDSF) and Isothermal Titration Calorimetry (ITC). MHR1/2 showed higher binding affinity (Kd: ADPR = 0.51 μM, dADPR = 0.21 μM) compared to NUDT9H (Kd: ADPR = 192 μM, dADPR = 124 μM). ITC confirmed the lower affinity of NUDT9H (Kd: ADPR = 167 μM, dADPR = 165 μM). No significant difference in binding affinity was observed between ADPR and 2'-deoxy-ADPR, suggesting that the enhanced current is due to structural changes rather than stronger binding. MHR1/2 binds ligands more easily than NUDT9H, suggesting sequential activation. Similar Kd values for ADPR and 2'-deoxy-ADPR indicate that structural effects drive superagonist potency.