Frozen in Action: Capturing Vasopressin Receptors in Their Inactive State

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Since 2017, cryogenic electron microscopy (cryo-EM) has revolutionized GPCR structural biology by enabling crystallization-free high-resolution structural determination of agonist-bound active state GPCRs coupled to transducer proteins. However, determining GPCRs in apo and inhibitor-bound conformations by cryo-EM remains challenging. Nevertheless, the ability to apply cryo-EM to determine apo and inhibitor-bound GPCRs would open new pharmacological avenues for drug discovery. Nb6 recognizes and binds to the κ- opioid receptor (κOR) ICL3 in the inactive state. Grafting this loop into ICL3 of other class A receptors enables Nb6 to bind to these receptors in the inactive state (1). This strategy can also facilitate the determination of inactive state GPCR structures whereby Nb6 acts as a fiducial marker to facilitate cryo-EM particle alignment of GPCRs that have limited structural features outside of the receptor TM bundle that is embedded in the micelle of detergent-purified GPCRs. Here, we utilized this Nb6 strategy to determine structures of class A vasopressin receptors bound to non-peptide antagonists. Vasopressin receptor inhibitors have the potential for the treatment of many clinical conditions including chronic hyponatremia, heart failure, polycystic kidney disease, congenital X-linked nephrogenic disease, Raynauds disease, and regulation of social behaviors, such as autism spectrum disorders.

This research focuses on the vasopressin V1a receptor (V1aR), a key regulator of vascular tone and social behavior, to uncover the molecular basis of its function and pharmacology. Using cryo-electron microscopy, we determined the structure of V1aR in complex with the small-molecule antagonist relcovaptan (SR49059) and Nb6, capturing the receptor in an inactive state. The structure reveals a previously uncharacterized oligomeric state and a distinct extracellular loop 2 (ECL2) conformation. Complementary to this, engineered V1aR-κOR chimeras enabled live-cell BRET assays to characterize Nb6-sensitive conformational states and study the ligand-induced inactivation kinetics. Together, these approaches integrate structural biology, mutagenesis, and dynamic pharmacological assays to map the conformational landscape of V1aR. This work not only provides key mechanistic insights into vasopressin receptor regulation but also establishes a framework for structure-guided drug discovery and for probing GPCR dimerization and allosteric modulation in physiologically relevant contexts.

Reference

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