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The Quantitative Optical Modulation of Dopamine Receptor-Mediated Endocytosis Using an Optogenetic System

Authors : Qiaoyue Kuang, Yang Li, Mizuki Endo, Takeaki Ozawa

Abstract : G protein-coupled receptors (GPCR) are the largest family of receptor proteins that detect molecules outside the cell and activate cellular responses. Of the GPCRs, dopamine receptors, which recognize extracellular dopamine, are essential to mammals due to their roles in numerous physiological events, including autonomic movement, hormonal regulation, emotions, and the reward system in the brain. To precisely understand the physiological roles of dopamine receptors, it is important to spatiotemporally control the signaling mediated by dopamine receptors, which is strongly dependent on their surface expression. Conventionally, chemical-induced interactions were applied to trigger the endocytosis of cell surface receptors. However, these methods were subjected to diffusion and therefore lacked temporal and special precision. To further understand the receptor-mediated signaling and to control the plasma membrane expression of receptors, an optogenetic tool called E-fragment was developed. The C-terminus of a light-sensitive photosensory protein cyptochrome2 (CRY2) was attached to β-Arrestin, and the E-fragment was generated by fusing the C-terminal peptide of vasopressin receptor (V2R) to CRY2's binding partner protein CIB. The CRY2-CIB heterodimerization triggered by blue light stimulation brings β-Arrestin to the vicinity of membrane receptors and results in receptor endocytosis. In this study, the E-fragment system was applied to dopamine receptors 1 and 2 (DRD1 and DRD2) to control dopamine signaling. First, confocal fluorescence microscope observation qualitatively confirmed the light-induced endocytosis of E-fragment fused receptors. Second, NanoBiT bioluminescence assay verified quantitatively that the surface amount of E-fragment labeled receptors decreased after light treatment. Finally, GloSensor bioluminescence assay results suggested that the E-fragment-dependent receptor light-induced endocytosis decreased cAMP production in DRD1 signaling and attenuated the inhibition effect of DRD2 on cAMP production. The developed optogenetic tool was able to induce receptor endocytosis by external light, providing opportunities to further understand numerous physiological activities by controlling receptor-mediated signaling spatiotemporally.

Keywords : dopamine receptors, endocytosis, G protein-coupled receptors, optogenetics
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