Comparative Studies of Intracellular Trafficking Pathways of Dopamine D_2 and D_3 receptors

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The regulation of G protein-coupled receptor is a complex process that involves various cellular events at different time frame, and the mechanism involved in receptor desensitization can be unique for each receptor type and the signal it mediates. The dopamine D_2 and D_3 receptors (D_2R and D_3R), which are potential targets for antipsychotic drugs, have a similar structural architecture and signaling pathway. Furthermore, in some brain regions they are expressed in the same cells, suggesting that differences between the two receptors might lie in other properties such as their regulation.

The mechanism underlying the intracellular trafficking of D_2R and D_3R was studied. Activation of D_2R caused GRK-dependent receptor phosphorylation, a robust translocation of β-arrestin to the cell membrane, and profound receptor internalization. The internalization of the D_2R was dynamin-dependent, suggesting that a clathrin-coated endocytic pathway is involved. In addition, the D_2R, upon agonist-mediated internalization, localized to intracellular compartments distinct from those utilized by the β2-adrenergic receptor. However, in the case of the D_3R, only subtle agonist-mediated receptor phosphorylation, β-arrestin translocation to the plasma membrane, and receptor internalization was observed. Interchange of the 2nd and 3rd intracellular loops of the D_2R and D_3R reversed their phenotypes, implicating these regions in the regulatory properties of the two receptors.

Interestingly, D_3R undergoes a more robust PKC-dependent sequestration than D_2R. PKC-dependent D_3R sequestration was dynamin-dependent but was not affected by co-expression of GRK, β-arrestin, or negative dorminant caveolin-1, or by perturbation of lipid raft. To locate receptor regions that determines PKC-dependent receptor sequestration, all possible phosphorylation sites (serine and threonine residues) in the 2^{nd} and 3^{rd} intracellular cytoplasmic loops of D_3R were grouped into 12 different motifs and they were mutated to alanine residues. Sequestration assays for mutant receptors identified serine residue at position 257 as critical phosphorylation site responsible for PKC-mediated D_3R phosphorylation and sequestration. YxxL endocytosis motif located between residue 252 and 255 has permissive roles for PKC-mediated D_3R sequestration. Actin binding protein 280 (filamin A), which is a PKC substrate and is known to interact with D_3R, was required for PKC-dependent D_3R sequestration. Our results show that PKC-dependent but not GRK-/β-arrestin-dependent pathway is responsible for D_3R sequestration, suggesting that any cellular events that alter cellular levels of PKC would determine D_3R regulation.