M. Sc. Liquan Yang

Theme: Role of Phospholipase D2 in agonist-induced Endocytosis of Opioid Receptors

Abstract

Opioids are classically associated with effects such as analgesia, respiratory depression, and addiction, which are mediated via interaction with specific G protein-coupled opioid receptors. Opioid receptor endocytosis which occurs after agonist exposure is one important regulation process of opioid signaling. Our group has previously demonstrated that μ-opioid receptor (MOR) interacts with phospholipase D2 (PLD2) and that MOR-mediated activation of PLD2 is essential for the induction of MOR endocytosis. Here we found that delta-opioid receptor (DOR) also physiologically interacts with and agonist-dependently activates PLD2 in a PKC-independent manner in transfected HEK293 cells. As revealed by quantitative internalization assays and confocal microscopy studies, coexpression of PLD2 strongly enhanced the extent and rate of agonist-induced DOR endocytosis, whereas inhibition of PLD2 by expression of a catalytically inactive mutant PLD2 (K758R) significantly attenuated DPDPE-induced DOR endocytosis. Similarly, the inhibition of PLD2-mediated phosphatidic acid (PA) synthesis with 1-butanol blocked DOR endocytosis. These observations suggest that PLD2 activity is required for agonist-induced DOR endocytosis and that PA plays a crucial role. PA and diacylglycerol (DAG) can be converted to each other by PA phosphohydrolase and DAG kinase. Inhibition of PA phosphohydrolase, which inhibits the dephosphorylation of PA to DAG, attenuated both DPDPE-induced DOR endocytosis and DAMGO-induced MOR endocytosis. Conversely, inhibition of DAG kinase increased agonist-induced endocytosis of both receptors. Furthermore, addition of a DAG analog DOG which is a synthetic cell-permeable DAG with short chain fatty acids remarkably augmented agonist-stimulated DOR and MOR endocytosis, whereas inhibition of protein kinase C (PKC) did not influence agonist-induced opioid receptor endocytosis. These findings indicate that PA-derived DAG is involved in agonist-induced opioid-receptor endocytosis in a PKC-independent way. We also revealed that PLD2 activity and the subsequent PA-derived DAG are required for opioid receptor-mediated p38 MAPK activation which is involved in DOR and MOR endocytosis.

Taken together, PLD2 activity regulates agonist-induced δ- and μ-opioid receptor endocytosis, which involves the conversion of its product PA to DAG, and the following activation of P38 kinase.