

The Effect of Phosphatidylinositol 4,5-bisphosphate Depletion on the Internalization of G Protein-coupled Receptors

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Phosphatidylinositol 4,5-bisphosphate (PtdInsP₂) has been shown to be critical for many endocytic processes including the internalization of G protein-coupled receptors (GPCRs). Our aim in this study was to compare the effect of different plasma membrane PtdInsP₂ depletion methods on GPCR internalization.

We used bioluminescence resonance energy transfer (BRET) to follow the internalization of the luciferase-tagged β_2 adrenergic receptor (β_2 AR) in HEK 293 cells. To reduce PtdInsP₂ levels, we applied either the rapamycin-inducible recruitment of a 5-phosphatase domain to the plasma membrane, or a truncated form of type 1 angiotensin receptor (AT₁R) which activates phospholipase C β . We determined the rate of PtdInsP₂ degradation using the PH domain of phospholipase C δ_1 which binds PtdInsP₂ specifically, and found it to be comparable for the two depletion methods. While PtdInsP₂ depletion by our rapamycin-based system inhibited the internalization of β_2 AR, PtdInsP₂ depletion by AT₁R had no effect on it, measured by the same method.

Our data suggest that the effect of plasma membrane PtdInsP₂ depletion on the internalization of β_2 AR can be different depending on the method by which the lipid is degraded. Further investigation is needed to determine whether this discrepancy is due to degradation of distinct PtdInsP₂ pools of the plasma membrane or other factors are responsible for it.

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