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 β2 adrenergic receptor (β2AR) 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 Cβ. We determined the rate of PtdInsP2 degradation using the PH domain of phospholipase Cδ1 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 β2AR, 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 β2AR 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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