Arrestin scaffolds NHERF1 to the $P2Y_{12}$ receptor to regulate receptor internalization

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Introduction: ADP plays a key role in regulating platelet function by activation of $P2Y_1$ and $P2Y_{12}$ G protein-coupled receptors (GPCRs). We have recently shown in a patient with mild bleeding that the PDZ-binding motif of the $P2Y_{12}$ receptor ($P2Y_{12}R$) is required for effective receptor traffic in human platelets (Nisar et al., 2011). For other GPCRs this motif is required for efficient receptor traffic via interaction with the PDZ-binding domain containing Na^+/H^+ Exchanger Regulatory Factors (NHERFs). Here we investigated the role of NHERF1 in $P2Y_{12}R$ regulation.
Methods: HA-tagged $P2Y_{12}R$ expressed in human 1321N1 astrocytoma cells and human platelets were both used in these studies. Protein interactions were investigated by co-immunoprecipitation and immunoblotting. Trafficking was studied using ELISA and confocal microscopy, to quantify and visualise receptor trafficking, respectively.
Results: Endogenous $P2Y_{12}R$ co-localised with NHERF1 in human platelets upon ADP stimulation. In addition the C-tail of the $P2Y_{12}R$ bound NHERF1 in platelet cell lysates. In 1321N1 cells $P2Y_{12}R$ interacted with NHERF1 in an agonist-dependent manner whilst siRNA knockdown of NHERF1 blocked $P2Y_{12}R$ internalisation (loss of surface receptor was 20.5±1.3% versus 5.4 ±1.9% following ADP treatment (10 μM; 30 min) in scrambled and NHERF1 siRNA knockdown cells respectively) but did not affect acute receptor signalling or desensitization. Interestingly removal of the PDZ-binding motif of the $P2Y_{12}R$ which reduces receptor internalization reduced agonist-independent NHERF1 / $P2Y_{12}R$ interaction but did not abolish agonist-dependent NHERF1 interaction. siRNA knockdown of β-arrestins which are also required for $P2Y_{12}R$ internalization (Mundell et al., 2006) did however reduce $P2Y_{12}R$/NHERF1 interaction. Further studies demonstrated that NHERF1 and β-arrestin are able to interact in cell lines and importantly $P2Y_{12}R$ activation increases the level of this interaction.
Conclusions: We report for the first time a novel interaction between NHERF1 and $P2Y_{12}$ and between NHERF1 and β-arrestin. This study is the first demonstration that NHERF proteins are required for agonist-dependent GPCR internalisation. Our data allow us to propose the following novel model of $P2Y_{12}R$ internalization. Prior to agonist stimulation there is a basal association between the $P2Y_{12}R$ and NHERF1 that requires the PDZ binding motif of this receptor. On receptor stimulation NHERF1 no longer directly interacts with the receptor but instead binds via β-arrestin where it plays an essential role in regulating receptor internalization. This study therefore suggests that arrestin can serve as an adaptor to promote NHERF1 interaction with a GPCR in order to facilitate effective NHERF1-dependent receptor internalization.

References

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