A Novel Role for NHERF proteins in P2Y1 and P2Y12 Receptor Internalization

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BACKGROUND: ADP plays a key role in regulating platelet function by activation of P2Y1 and P2Y12 GPCRs. We have recently shown that P2Y receptors rapidly internalize and resensitize in human platelets (Mundell et al., 2008) however the mechanisms underlying these processes are not fully elucidated. Both the P2Y1 and P2Y12 receptors possess a type 1 PDZ ligand in their C terminal tail. For other GPCRs this motif is required for efficient receptor recycling via interaction with Na+/H+ Exchanger Regulatory Factors (NHERFs). Here we investigated the role of NHERF1 and 2 in P2Y receptor regulation.

METHODS: Human platelets and HA-tagged P2Y1 and P2Y12 receptors expressed in human 1321N1 astrocytoma cells were used in these studies. Protein interactions were investigated by co-immunoprecipitation. ELISA and confocal microscopy were used to quantify and visualise receptor trafficking, respectively.

RESULTS: Endogenous P2Y12 receptor co-localised with NHERF1 in human platelets upon ADP stimulation. In addition the C-tail of the P2Y1 and P2Y12 receptor bound NHERF1 found in platelet lysates. In 1321N1 cells P2Y1 and P2Y12 receptors interacted with NHERF1 in an agonist-dependent manner. siRNA-inhibition of NHERF1 blocked P2Y1 and P2Y12 internalization but did not affect acute receptor signalling or desensitization. NHERF2 inhibition selectively blocked P2Y12 internalization. Deletion of P2Y receptor PDZ ligands, like NHERF protein knockdown also resulted in attenuated internalization. However mutant receptors lacking the PDZ motif retained the ability to interact with NHERF1. Interestingly, β-arrestins which are also required for P2Y12 internalization (Mundell et al., 2006), failed to co-immunoprecipitate with P2Y12 following removal of the PDZ ligand. Interestingly we also demonstrated that NHERF1 and β-arrestin are able to interact.

CONCLUSIONS: We report an interaction between NHERF1 and P2Y12 and between NHERF1 and β-arrestin. This study is the first demonstration that NHERF proteins are required for GPCR internalization. These data suggest that formation of a super-complex consisting of NHERF1, β-arrestin and P2Y12 is required for agonist-induced internalization. These findings may have important implications for alternative methods of manipulating P2Y12 receptor activity and are now under further investigation in NHERF1 knock-out mice.

REFERENCES: Mundell et al. 2006, Mundell et al. 2008