Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol8Issue1abst137P.pdf

Agonist-independent constitutive trafficking of the $P2Y_{12}$ purinoceptor - relevance in platelets?

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The platelet $P2Y_{12}$ purinoceptor plays a critical role in haemostasis with the level of surface expression of receptor having an important effect on platelet responsiveness. Work from our laboratory has shown that $P2Y_{12}$ purinoceptor responsiveness is rapidly and reversibly modulated in human platelets (Mundell et al., 2008) with ADP stimulation promoting receptor internalization and subsequent recycling required to maintain receptor responsiveness. Although agonist-induced internalisation of $P2Y_{12}$ is a relatively well characterised process there has been little investigation into constitutive, agonist-independent internalisation.

n these studies we used both Chinese Hamster Ovary (CHO) cells stably transfected with N-terminal haemaglutanin (HA)-tagged $P2Y_{12}$ receptor as previously described (Mundell et al., 2008) or human platelets. In CHO cells changes in surface receptor expression were assessed by an ELISA assay or immunofluorescent microscopy as is standard in our laboratory (Mundell et al., 2008). In human platelets endogenous $P2Y_{12}$ surface receptor expression was assessed by immunofluorescent microscopy in fixed platelets making use of a $P2Y_{12}$ specific N-terminal antibody.

In our initial studies we found that the $P2Y_{12}$ purinoceptor internalises in the absence of agonist in human platelets. Further studies in CHO cells revealed significant agonist-independent internalization both by ELISA (17.2% ±3.4 of total surface receptor (n=5)) and immunofluorescent microscopy. In cell lines blockade of arrestin (by dominant negative mutant) and dynamin (by Dynasore - 80μM) resulted in a reduction in internalisation of up to 30% of total surface receptor number (Arrestin: control 31.9% ±9.2, DNM 8.7% ±6.2; Dynamin: control 16.8%, Dynasore -14.6%). Interestingly, unlike agonist-induced internalisation, the constitutive process is not dependent upon the presence of an intact PSD-95/drosophila discs large/ZO-1 (PDZ) binding motif found at the extreme C-terminus of the receptor. This motif is the site of mutations which result in abnormal bleeding phenotypes in patients. Further study is ongoing to dissect the particular region of the C-terminus which regulates the process of constitutive internalisation as well as further characterization of the interacting partners involved.

In conclusion we have discovered that the $P2Y_{12}$ purinoceptor receptor internalises in human platelets and CHO cells in an agonist-independent manner. The functional significance of this process in relation to both $P2Y_{12}$ purinoceptor signalling and platelet function is now under investigation.

Mundell, S.J., J.F. Barton, M.B. Mayo-Martin, A.R. Hardy and A.W. Poole (2008) Rapid resensitization of purinergic receptor function in human platelets. *J Thromb Haemost*. 6:1393-404.

Work supported by BHF Studentship to IGS