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Evidence that human $P2Y_1$ and $P2Y_{12}$ receptors form heterodimers

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Introduction: $P2Y_1$ and $P2Y_{12}$ receptors belong to the class A family of transmembrane GPCRs that are activated by endogenous nucleotides[1]. There is growing evidence that many GPCRs, including P2Y receptors, can exist as dimers or higher-order oligomers[2]. For example, $P2Y_{12}$ and PAR4 receptors were recently reported to dimerise[3]. Our previous studies indicated that $hP2Y_1$ and $hP2Y_{12}$ receptors may form a functional heterodimer with novel pharmacological and signalling properties[4]. The aim of this project was, therefore, to characterise the physical interaction between $hP2Y_1$ and $hP2Y_{12}$ receptors.

Method: tSA201 cells were transfected or co-transfected with $hP2Y_1$ and $hP2Y_{12}$ receptors, tagged with HA or a fluorescent protein. Cellular localisation and co-localisation of the receptors were determined using confocal microscopy. Transfected cells were cultured in the absence or presence of the N-glycosylation inhibitor tunicamycin (2.0 µg/ml) for 16 hours to determine the role of N-glycosylation in receptors expression. Receptor cell surface expression was quantified using ELISA. To investigate physical interaction between the two P2Y subtypes, co-immunoprecipitation was performed using anti-HA-agarose beads followed by immunoblotting with anti-GFP, anti-HA then alpha-Tubulin antibodies.

Result: Following transfection on their own or together, both receptors were localised mainly at the cell membrane, and this was unaffected by tunicamycin. Co-immunoprecipitation confirmed that $P2Y_1$ and $P2Y_{12}$ receptors associate physically. Each subtype enhanced the other's surface expressions. In particular, expression of the $P2Y_{12}$ receptor more than doubled that of $P2Y_1$ receptors at the cell surface.

Conclusion: These results show that $P2Y_1$ and $P2Y_{12}$ receptors are physically associated at the cell membrane and that they enhance each other's cell surface expressions. These results are consistent with our previous data indicating that $P2Y_1$ and $P2Y_{12}$ receptors form a functional heteromer.

References:

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