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Characterisation of $P2Y_2$ receptors in human vascular endothelial cells using AR-C118925XX, a potent and selective $P2Y_2$ antagonist

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Introduction: P2Y receptors are a family of eight G protein-coupled receptors that mediate the actions of endogenous nucleotides, such as uridine 5'-triphosphate (UTP) (1,2). The physiological functions of many of the subtypes are unclear due to the limited selectivity and low potency of most currently available antagonists. A putative $P2Y_2$ antagonist, AR-C118925XX, has recently become available, so the aims here were to quantify the action of AR-C118925XX at recombinant $P2Y_2$ receptors and then to determine the role of native $P2Y_2$ receptors in the actions of UTP in human vascular endothelial cells.

Method: EAhy926 cells, immortalised human umbilical vein endothelial cells (3), and 1321N1 cells stably expressing recombinant human $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_{11}$ or rat $P2Y_6$ receptors, were grown on glass coverslips. Following incubation with the Ca^{2+}-sensitive dye, Cal-520AM (5 µM), they were placed in a fluorimeter and intracellular Ca^{2+} measured. Cells were superfused continuously and agonists were applied in the superfusate (ADP-$P2Y_1$; UTP-$P2Y_2$, EAhy926; ATP-$P2Y_4$, $P2Y_{11}$; UDP-$P2Y_6$), before and after 5 min superfusion with AR-C118925XX. Where appropriate the Hill equation was fitted to the data, and antagonist potency calculated using the Gaddum-Schild equation or a Schild plot.

Results: UTP (10nM-3µM) evoked a concentration-dependent rise in intracellular Ca^{2+} in 1321N1 cells expressing recombinant $P2Y_2$ receptors (EC_{50}=54nM, 95% cl=43-67nM, n=5). AR-C118925XX (10nM-1µM), produced a progressive rightward shift in the UTP concentration-response curve, with no effect on maximum response (n=6 each). Schild analysis gave a pA_2=8.30 and slope=0.985. In contrast, AR-C118925XX (1µM), a concentration 200x greater than its K_B at $P2Y_2$ receptors, had no effect at recombinant $P2Y_1$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$ receptors (n=5 each). UTP (100nM-30µM) also increased intracellular Ca^{2+} in EAhy926 endothelial cells in a concentration-dependent manner (EC_{50}=680nM, 95% cl=506-912nM, n=5). AR-C118925XX (30nM), shifted the UTP curve rightwards (EC_{50}=7.6µM, 95% cl. 4.3-13.2µM, n=5), with no decrease in maximum response. Gaddum-Schild analysis gave a K_B=3.0nM (95% cl=1.3-4.6nM).

Conclusion: These data show that AR-C118925XX is a potent and selective $P2Y_2$ antagonist, which enabled us to identify $P2Y_2$ receptors as the P2Y subtype that mediates UTP-evoked increases in intracellular Ca^{2+} in human endothelial cells. Currently, AR-C118925XX is the only selective $P2Y_2$ antagonist available and so will be invaluable in identifying the physiological functions of other native $P2Y_2$ receptors.

References:

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