Negative feedback inhibition of Ca^{2+} influx during P_{2Y2} receptor activation

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G protein-coupled receptors transduce extracellular stimuli via G proteins to intracellular effectors resulting in the activation of second messenger signalling cascades mediated by Ca^{2+} or cAMP. In HT29 human colonic epithelial carcinoma cells, the activation of the M_3 muscarinic receptor by carbachol (CCh) results in a Ca^{2+} response with a characteristic prolonged plateau phase that occurs due to Ca^{2+} influx following activation of store-release sensitive channels in the plasma membrane. In contrast, the Ca^{2+} response following the activation of the P_{2Y2} purinergi receptor shows no plateau phase (Cummins *et al*., 2000). Both of these responses are pertussis toxin insensitive and mediated by G proteins of the G_q family. These data suggest that during P_{2Y2} activation, the lack of a plateau phase may result from the inhibition of Ca^{2+} influx. The small G proteins Rac and Cdc42 are known to be involved in Ca^{2+} signalling pathways (Peppelenbosch *et al*., 1996; Djouder *et al*., 2000) and protein kinase C (PKC), a known target of Cdc42 (Slater *et al*., 2001), has also been reported to regulate the Ca^{2+} signalling pathway (Petersen & Berridge, 1994; Lee *et al*., 1997). The aim of this present study was to investigate the roles of Rac, Cdc42 and PKC in the inhibition of Ca^{2+} influx during P_{2Y2} receptor activation.

Standard Fura-2 imaging techniques were used to monitor changes in intracellular Ca^{2+} concentration in HT29 cells. Ca^{2+} influx was monitored using the rate of quenching of the Fura-2 signal by exogenous Mn^{2+}. Replication-deficient adenoviruses expressing the cDNA encoding either wild type (wt), dominant negative (dn) or constitutively active (ca) mutants of Rac, Cdc42 and PKCα were created using standard techniques (Cummins *et al*., 2000).

In HT29 cells exposed to UTP, the rate of Mn^{2+} influx as measured by Fura-2 quenching was 66% of the influx rate in response to CCh, indicating that the lack of a plateau phase during UTP exposure was due to reduced influx. When HT29 cells were infected with adenoviruses expressing dnRac or dnCdc42, the rates of Mn^{2+} influx increased to those observed with CCh stimulation. Furthermore, when the cells were infected with caRac, Mn^{2+} influx induced by CCh was reduced to below control levels. These indicate the involvement of both Rac and Cdc42 in the negative feedback inhibition of UTP mediated Ca^{2+} influx. Co-infection of dnCdc42 and caRac returned Mn^{2+} influx to the levels observed in control cells with UTP stimulation, indicating that Rac is upstream of Cdc42.

In HT29 cells infected with an adenovirus expressing wtPKCα, there was no change in the rates of Mn^{2+} influx in the presence of UTP. In contrast, when the cells were infected with a dnPKCα adenovirus, the rate of Mn^{2+} influx was increased to the levels observed with CCh, indicating a role for PKCα in the control of Ca^{2+} influx in these cells. To determine whether PKCα was acting via the same signalling pathway as Rac/Cdc42, HT29 cells were treated with Toxin B, an inhibitor of Rac/Cdc42, and dioleylglycerol, an activator of PKC. This resulted in an inhibition of Mn^{2+} influx to levels similar to that observed with UTP, indicating that the effect of blocking Rac/Cdc42 could be overcome by activating PKC, demonstrating that PKCα is downstream of Rac/Cdc42. This study shows that the main difference in the muscarinic and purinergic Ca^{2+} responses in HT29 cells, is due to a Ca^{2+} influx negative feedback pathway that is activated by P_{2Y2} receptors.

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