ROLES OF THE C-TERMINAL DOMAIN IN SIGNAL TRANSDUCTION, DIMERISATION, DESENSITISATION AND INTERNALISATION OF THE $P2Y_1$ RECEPTOR

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The $P2Y_1$ receptor for ATP is coupled through a $G_{q/11}$ protein to phospholipase C. We have examined by sequence truncations the essentiality of its intracellular C-terminal domain for several activities of this receptor.

The human P2Y1 sequence of 373 amino acids was terminated at position 354 (construct $P2Y_1$-T1) or at position 334 ($P2Y_1$-T2), covering almost all of the tail. These proteins were N-terminally tagged with the Myc epitope, giving Myc-$P2Y_1$, Myc-$P2Y_1$-T1 and Myc-$P2Y_1$-T2, and expressed separately in 293 cells: reaction with fluorescent anti-Myc antibody showed in confocal microscopy that all three are then localised to the cell membrane, with no obvious difference. The Ca^{2+} transients evoked by the $P2Y_1$ receptor agonist 2-methylthio-ADP (2-MeSADP) gave essentially identical EC_{50} values at 37°C and maxima for Myc-$P2Y_1$ (EC_{50}: 2.8 nM $\pm$ 4.5, n=3) and Myc-$P2Y_1$-T1 (EC_{50}: 3.7 nM $\pm$ 3.6, n=3), but EC_{50} for Myc-$P2Y_1$-T2 was 6.9-fold weaker (EC_{50}: 25.3 nM $\pm$ 16.2, n=3). Downstream signalling was tested at the level of extracellular-signal-regulated kinase (ERK): in phosphorylation of ERK2 in intact cells, EC_{50} for Myc-$P2Y_1$-T1 was 38.2 nM $\pm$ 25.6 (n=3) at 37°C, not significantly different from Myc-$P2Y_1$ (EC_{50}: 46.7 nM $\pm$ 28.6, n=3) value, and 2.9-fold weaker for Myc-$P2Y_1$-T2 (EC_{50}: 109.6 nM $\pm$ 38.9, n=3). Also, the intact $P2Y_1$ receptor is very sensitive to desensitisation at 37°C: 10 min exposure to 100 nM 2-MeSADP fully prevented a second response to up to 10 μM 2-MeSADP in both Ca^{2+} and ERK transductions, a desensitisation reversed fully after 8 min wash. This behaviour was again fully retained after the T1 or T2 truncations.

In contrast, other properties were lost thereby. When the living cells expressing the full length Myc-tagged $P2Y_1$ receptors were exposed to 10 μM 2-MeSADP at 37°C, confocal Z-scanning showed that the surface $P2Y_1$ receptors were fully lost within 50 min by internalisation, as shown when they were labelled by fluorescent anti-Myc antibody either pre- or post-treatment with the agonist. With the T1 as well as the T2 truncation this loss was fully prevented. Further, we have previously shown by fluorescence resonance energy transfer (FRET) that the $P2Y_1$ receptor forms a homo-dimer, induced by agonist exposure. Dimer formation here was measured by FRET on living cells expressing Myc-$P2Y_1$, which was ~ equi-labelled by either fluorescein or cyanine 3 (Cy3) conjugated to anti-Myc antibody. Acceptor (Cy3) photobleaching at 543 nm for 50 min produces, in the presence of agonist, a progressive increase in the fluorescein emission peak, a measure of FRET due to the dimers present. However, repeats of this reaction using instead Myc-$P2Y_1$-T1 or Myc-$P2Y_1$-T2 consistently showed no detectable FRET.

We conclude that the C-terminal tail of the $P2Y_1$ receptor is not involved in its desensitisation and only secondarily affects the transduction, but carries essential determinants of its homo-dimerisation and of its agonist-driven internalisation. The latter two processes may therefore be linked.

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