

P022 Identification of residues required for high affinity CGRP binding on RAMP1 by means of RAMP1-RAMP3 chimeras.

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Calcitonin-gene related peptide (CGRP) is a 37 amino acid neuropeptide. The CGRP receptor is a heterodimer of a family B G-protein coupled receptor, calcitonin receptor-like receptor (CLR) and a single-pass transmembrane protein, receptor activity modifying protein 1 (RAMP1). The N-terminus of RAMP1 probably forms a helical bundle that associates with the N-terminus of CLR to produce a binding pocket for the C-terminus of CGRP. CLR can also interact with a second RAMP, RAMP3. The two proteins have very similar structures but the CLR/RAMP3 heterodimer has a significantly lower affinity for CGRP than the RAMP1-containing complex. We have exploited this difference to map the regions of RAMP1 required for high affinity CGRP binding by producing chimeras between the two RAMPs. A series of RAMP1 constructs were produced where 4 amino acids from RAMP3 were systematically substituted along the entire sequence. These were transfected into Cos-7 cells with CLR and their ability to respond to CGRP was assayed via measurement of CGRP-mediated cAMP production. The constructs revealed that at least two main areas of RAMP1 were needed for high affinity CGRP binding; from T62 to A70 and from N86 to F93. This work was funded by the BBSRC.