

**P021** The role of the N-terminus of the CL receptor in binding CGRP

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Calcitonin-gene related peptide (CGRP) is a 37 amino acid neuropeptide which acts as a potent vasodilator. The CGRP receptor consists of a heterodimer of a family B G-protein coupled receptor, called the calcitonin receptor-like receptor (CL) and a single-pass transmembrane protein known as the receptor activity modifying protein 1 (RAMP1). Little is known of the residues within the CL receptor needed for the binding and activation of the receptor by CGRP. In this study, the extreme N-terminus was investigated by means of an alanine scan. **Residues from S31 to Q58** (excluding Q33, I34, K51 and I52) were replaced by alanine, either individually or in pairs. Point mutants were introduced into CLR by the Stratagene Quik Change mutagenesis kit. The ability of CGRP to stimulate cAMP production in the mutated receptor was measured following transfection into Cos 7 cells. The results showed that there were two distinct “hotspots” required for CGRP mediated-cAMP production, centred on N39K40 and Y49C48, where the mutations significantly decreased the  $pEC_{50}$  for CGRP. Molecular modelling suggests that the first region may be involved in an interaction with RAMP1 whereas the other could stabilise the structure of the N-terminus of the CL receptor. This work was funded by the BBSRC and the British Heart Foundation (PG 15278)