

## **THE EFFECT ON CGRP-BINDING OF MUTATIONS TO THE HYDROPHILIC RESIDUES WITHIN THE FIRST TRANSMEMBRANE REGION OF HUMAN CALCITONIN RECEPTOR-LIKE RECEPTOR (CRLR)**

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The molecular basis of type II GPCR-ligand interactions has been studied extensively via the use of site-directed point mutations. The mutation of hydrophilic residues within the transmembrane regions of the secretin-receptor, for example, led to a marked reduction in ligand-affinity. This approach has been used to investigate the role of hydrophilic residues within the transmembrane domains of CRLR. The four hydrophilic amino acids within the first transmembrane domain of CRLR, (H128, S131, S134, and S138) were mutated to alanine. The genes encoding the mutant and wild-type CRLRs contained within the pcDNA3- mammalian expression vector were transiently transfected into an available RAMP1-transformed HEK293 cell-line. Point mutations were created using the Stratagene Quick-change Mutagenesis method. Transient transfections were prepared according to the Clontech CalPhos Mammalian Transfection System. Transfected cells were homogenised and CGRP-affinity measured via the competition for <sup>125</sup>I-human CGRP by unlabelled human CGRP. All four mutants within the first transmembrane domain of human CRLR displayed *in vitro* CGRP-binding affinities which were comparable to wild-type. Average IC<sub>50</sub> values were 3.84 nM for wild-type CRLR and 1.29, 5.96, 4.66, and 13.4 nM for the CRLR mutants, H128A, S131A, S134A, and S138A, respectively. It is likely that hydrophilic residues within the transmembrane domains of CRLR will be involved in the recognition or action of CGRP. This study suggests that individual residues such as this within the first transmembrane helix of CRLR are not crucial for CGRP-recognition or RAMP1 interaction. We are currently investigating the remaining helices.