CGRP and adrenomedullin receptor activation mechanisms: two distinct areas of the transmembrane region of CLR with opposite effects on activation.

Activation of GPCRs involves conformational changes of the transmembrane (TM) helices. Inactive and active receptor conformations are stabilised by networks of interactions between the TM helices, often involving conserved motifs. This is best characterised with family A GPCRs, however a network of hydrophilic interactions between TM helices has recently been identified in the family B GLP-1 receptor [1]. The vasodilatory ligands CGRP and adrenomedullin (AM) bind and activate receptors consisting of a shared GPCR (CLR) and a single transmembrane accessory protein (RAMP1 or 2) [2]. To investigate the role of potential inter-helical interactions in these receptors, central facing amino acids were substituted with alanine and the effect on ligand-induced cAMP signalling was measured.

Point mutations were introduced into an N-terminal HA-tagged human CLR as described previously [3]. The receptors were transfected into Cos7 cells with RAMP1, 2 or 3 and cAMP was measured in response to increasing concentrations of CGRP (CLR/RAMP1) or AM (CLR/RAMP1, 2 or 3) by a LANCE assay [3].cAMP values were interpolated from a standard curve and data was analysed using an unpaired t-test. An ELISA detecting the HA tag was used to compare cell surface expression of the mutant receptors with WT [3]. A one way ANOVA was done on raw data. No significant changes were seen except for E348A which had a significantly reduced expression of 30% WT with all three RAMPs.

This identified a cluster of hydrophilic and hydrophobic residues in TM3, 5 and 6 essential for receptor activation. Specifically, alanine substitutions of N305 (TM5) and L345 (TM6) have dramatic effects on potency and efficacy and show both ligand and RAMP bias. Conversely, on the opposite side of the TM bundle a cluster of residues between TM2, 3 and 7 inhibit agonist-mediated receptor activation. Most notably, alanine substitution of H374 gave the CGRP receptor an AM receptor pharmacological profile when stimulated with AM. These results have identified two distinct and functionally opposing pockets of receptor activation within the CGRP and AM receptor TM regions that share similarities and differences with other family B GPCR interaction networks.

Table 1. pEC_{50} and Emax values for key alanine substitutions of the TM helices

CLR mutant	TM	R1 CGRP		R1 AM		R2 AM		R3 AM	
		pEC50 log difference	% WT Emax	pEC50 log difference	% WT Emax	pEC50 log difference	% WT Emax	pEC50 log difference	% WT Emax
N305A	5	2.48 ± 0.10****	62.10± 4.41	1.80 ± 0.26 ***	26.61± 2.49 **	N.C.	N.C.	1.52 ± 0.16 ****	30.69± 9.21 *
L345A	6	1.89 ± 0.10 ****	71.31± 4.80	0.95 ± 0.39	29.28± 0.68 *	N.C.	N.C.	1.11 ± 0.04 ****	19.51± 3.01 **
H374A	7	-0.27 ± 0.07 *	118.05± 6.72	-2.01 ± 0.10 ****	138.18± 9.47	-0.45 ± 0.09 *	110.68± 11.76	-0.20 ± 0.12	108.70± 5.75

Values are mean ± SEM. N ≥ 4. N.C. refers to no signalling curve produced. * p< 0.05, ** p < 0.01, p < 0.001 *** p<0.0001

This work was funded by the British Heart Foundation (PG/12/59/29795)

[1]. Wootten, D., et al., **Proc** Natl Acad Sci U S A, 2013. 110(13): p. 5211-6.

[2]. Woolley, M.J. and Conner, A.C., Curr Protein Pept Sci, 2013. 14(5): p. 358-74.

[3]. Woolley, M.J., et al., J R Soc Interface, 2013. 10(88): p. 20130589.