Receptor Component Protein (RCP) as an regulator of agonist bias at the CGRP receptor

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Introduction RCP is a 148 amino acid, 17 kDa peripheral membrane protein expressed in numerous immortalized cell lines (1). It is found *in vivo* in the brain, spinal cord, the uterus as well as in vasculature. It is required for efficient coupling of the calcitonin gene-related peptide (CGRP) receptor to production of cAMP via Gαs, the stimulatory G protein. Functional CGRP receptors consist of a 7-transmembrane domain receptor, the calcitonin receptor-like receptor (CLR). This requires an accessory protein, receptor activity modifying protein 1 (RAMP1) for ligand binding and receptor expression (2). RCP is a third component of the receptor. It appears to physically associate with the receptor, interacting with its second intracellular loop (ICL2) (3). In this study, we have examined the role of RCP on a variety of signalling pathways that are activated by the CGRP receptor.

Methods HEK293T cells were transfected with 10 nM siRNA (Sigma) to knock down endogenous RCP expression (Fig 1), and were transfected 48 h later with CLR and RAMP1 as described previously (4). Cells were stimulated with human alpha CGRP (10^{-14} to 10^{-6}M) and cAMP (LANCE kits, Perkin Elmer), pERK and pAtk-1 (CISBIO kits) were determined. Intracellular calcium was measured via FURA-4AM. Concentration-response curves were analysed via PRISM 7 according to the operational model to obtain log(tau) and log(K_A), from which bias plots were constructed (4). All experiments were repeated in triplicate.

Results CGRP stimulated cAMP production (pEC_{50} 10.5±0.12), pAtk (pEC_{50} 8.5±0.24) and pERK phosphorylation (pEC_{50} 9.44±0.27) and increased intracellular calcium (pEC_{50} 6.8±0.20). There were no differences in the responses in the presence of siRNA against RCP to pAtk, pERK or calcium, but for cAMP, the Emax was reduced by 57% and the pEC_{50} was reduced to 9.6±0.18 (Fig 1). Cell surface expression of CLR was unaffected as determined by ELISA (135±35% of control). Homology modelling suggested that RCP interacts with the αB-αC loop of Gαs that shows little homology to other G proteins.

Conclusions RCP appears to be an allosteric modulator of CLR that specifically influences agonist bias by selectively enhancing coupling to Gs. This work was supported by BBSRC grants BB/M000176/1 and BB/M00015X/1.

References

1) Dickerson, I.M., (2013) *Curr Protein Pept Sci* 14, 407-15
2) McLatchie, L.M. *et al*., (1998) *Nature* 393, 333-339
3) Egea S.C. and Dickerson I.M. (2012) *Endocrinology* 153,1850-60
4) Weston C. *et al*., *J. Biol Chem.* (2016) 291, 21925-21944

Figure 1 Actions of RCP

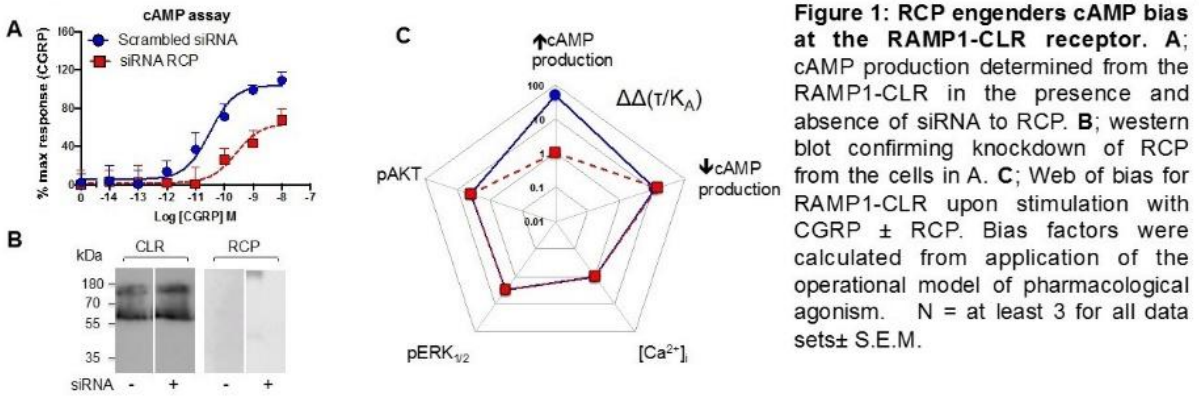

Figure 1: RCP engenders cAMP bias at the RAMP1-CLR receptor. A; cAMP production determined from the RAMP1-CLR in the presence and absence of siRNA to RCP. **B**; western blot confirming knockdown of RCP from the cells in A. **C**; Web of bias for RAMP1-CLR upon stimulation with CGRP ± RCP. Bias factors were calculated from application of the operational model of pharmacological agonism. N = at least 3 for all data sets± S.E.M.