

**P013** Agonist-dependent consequences of proline to alanine substitution in the transmembrane helices of the calcitonin receptor

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Transmembrane (TM) proline (P) residues form functionally important kinks in family-A G protein-coupled receptors (GPCRs). In family-B GPCRs, only VPAC1 and calcitonin receptor-like receptor (CL) TM prolines have been studied; these show parallels with family-A receptors. We determined the function of these residues in the human calcitonin receptor, a close relative of CL. TM prolines were individually mutated to alanine (A) and the receptors transfected into Cos-7 cells. Salmon and human calcitonin-induced cAMP responses were measured and homologous competition binding experiments performed with both agonists. P246A, P249A and P280A were wild-type in terms of human calcitonin-induced cAMP activation. P326A and P336A had reduced function (165 and 12-fold, respectively). Human calcitonin binding was not detectable for any mutant receptor in membranes but in whole-cells, binding was detected for all mutants apart from P326A. Salmon calcitonin activated all receptors comparably to wild-type although  $B_{\max}$  values were significantly reduced for all mutants apart from P326A. In summary, P326 and P336 appear important for calcitonin receptor function and are likely involved in generating receptor conformations appropriate for agonist binding and receptor activation. However, agonist-specific effects were observed; this may be evidence for distinct conformations of the human calcitonin receptor.