4th International Meeting on Calcitonin Gene-Related Peptide (CGRP)
TheScientificWorld (2001) 1(S1), 8
ISSN 1532-2246; DOI 10.1100/tsw.2001.405

STRUCTURAL DETERMINANTS IN THE CALCITONIN RECEPTOR-LIKE RECEPTOR (CRLR) IMPORTANT FOR CGRP AND ADRENOMEDULLIN (AM) RECEPTOR FUNCTION OF CRLR/RECEPTOR-ACTIVITY-MODIFYING PROTEIN (RAMP) 1 AND CRLR/RAMP2 HETERODIMERS

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Cell surface protein cross-linking, coimmmunoprecipitation, and confocal microscopy identified CRLR/RAMP1-, CRLR/RAMP2-, and calcitonin receptor isotype 2 (CTR2)/RAMP1 heterodimers as CGRP-, AM-, and CGRP/amylin receptors, linked to cAMP production. Along these lines, effects of structural alterations in the N-terminal extracellular domain of the human CRLR on cell surface expression as well as the association with RAMP and CGRP or AM have been investigated.

Site-directed mutagenesis identified Asn^{60} and Asn^{112} as N-glycosylation sites. N-glycosylation was important for cell surface expression of CRLR/RAMP complexes, but not for ligand specificity. Substitution of Asn^{117} by Asp, on the other hand, maintained normal CRLR function and N-glycosylation, but Asn^{117} to Thr, Ala, Gln, or Pro mutations revealed inactive mutant CRLR/RAMP1 heterodimers at the cell surface.

Substitution of the N-terminal 18 amino acids of the CRLR by the corresponding CTR2 domain did not affect RAMP1-dependent CGRP receptor function, but RAMP2-mediated AM binding was abolished and the EC_{50} of AM stimulating cAMP accumulation was raised over 100-fold.

In conclusion, differential interactions of CGRP and AM have been identified in the N-terminal extracellular region (1-118) of the human CRLR.