Abstract: Receptor activity modifying protein 1 (RAMP1) forms a complex with calcitonin receptor-like receptor (CLR) to produce the receptor for calcitonin gene-related peptide (CGRP). RAMP1 has two main roles. It facilitates the cell-surface expression of CLR. It is also essential for the binding of CGRP to the receptor. It seems likely that Y66, F93, H97 and F101, amongst other residues, form a binding site for CLR. These cluster together on the same face of the extracellular portion of RAMP1, probably close to where it enters the plasma membrane. Residues at the other end of RAMP1 are most likely to be involved in CGRP recognition, although it is currently unclear how they do this. Within this area, W74 is important for the binding of the nonpeptide antagonist, BIBN4096BS, although it does not seem to be involved in the binding of CGRP itself. It has been shown that there is an epitope within residues 23-60 of CLR that are essential for RAMP recognition. Under some circumstances, changes in the expression of RAMP1 can alter the sensitivity of cells to CGRP, demonstrating that regulation of its levels may be of physiological or pathophysiological importance.
INTRODUCTION

RAMPs and CGRP Receptors

Receptor activity modifying proteins (RAMPs) owe their discovery to the search for calcitonin gene-related peptide (CGRP) receptors. In the early 1990s, a new G-protein coupled receptor (GPCR) was cloned from rat and human sources.1-3 This receptor showed around 50% sequence identity to the related calcitonin receptor and so was named calcitonin receptor-like receptor (CRLR; this is now abbreviated to CLR).4 However, when expressed in most cells it was not activated by any known ligand, except for one clone of HEK293 cells where it responded to CGRP.5 It was hypothesised that these cells expressed some kind of cofactor that allowed CLR to bind CGRP. This was proven to be the case in 1998 when RAMP1 was cloned and shown to interact with CLR to give a CGRP receptor.6 In the same study, RAMPs 2 and 3 were shown to produce receptors for adrenomedullin (AM); these receptors are now known as AM1 and AM2 receptors.4 The AM2 receptor shows significant affinity for CGRP.6 Similarly, CGRP receptors also bind AM with a reasonably high affinity. Subsequently the RAMPs were also shown to interact with calcitonin receptors to give receptors for amylin,7,8 known as the AMY1, AMY2 and AMY3 receptors. Of these, the AMY1 and AMY3 also show significant ability to interact with CGRP.8 They will not be considered in this chapter.

Initially, the complex between the CLR and RAMP1 was known as the CGRP1 receptor, reflecting concerns about possible CGRP receptor heterogeneity.4 Subsequently this issue has been resolved so that the complex is now simply known as the CGRP receptor.9

CLR and CGRP

CLR is an example of a family B GPCR; these have large N-termini, characterised by three sets of conserved disulphide bonds. The N-termini of these receptors bind the C-termini of their cognate ligands. The N-termini of the ligands interact with the transmembrane domains of the receptors and it is this interaction which activates the receptor.10 CGRP is a 37 amino acid peptide; CGRP8-37 is an antagonist and these are the residues which are most likely to interact with the N-terminus of CLR when it is complexed to RAMP1. There is no structure available for the N-terminus of CLR. However, it is unlikely to show much departure from those defined for a number of allied GPCRs.11-14 There seems to be a common pattern with alpha-helices at the extreme N- and C-termini of the extracellular domain, with two or three short beta-sheets between them.