Regulation of the Kinetics of Degradation of the Adrenomedullin I Receptor by Ubiquitination

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Adrenomedullin (AM) belongs to the calcitonin family of peptides, which also includes calcitonin gene-related peptide (CGRP), intermedin, amylin and calcitonin. AM participates in many biological functions and has profound effects on the cardiovascular system, promoting vasodilatation, angiogenesis and stabilization of the endothelial cell barrier. The latter function is important in pathological conditions such as sepsis. Indeed, AM has been shown to promote endothelial barrier function and is beneficial in animal models of sepsis. Although, the biology of AM is well known, much less is known about the molecular mechanisms controlling the trafficking and signalling of its receptor. AM receptors comprise the G protein-coupled receptor (GPCR), calcitonin receptor-like receptor (CLR) and a single transmembrane protein, receptor activity-modifying protein (RAMP). The three RAMP family members can all heterodimerize with CLR to form functional receptors. CLR•RAMP1 is a high affinity CGRP receptor, whereas CLR•RAMP2 and CLR•RAMP3 are high affinity AM receptors. Intermedin can also bind to the CLR•RAMP complexes with varying affinities. Unlike CLR•RAMP1, the molecular mechanisms that regulate the trafficking of CLR•RAMP2 are poorly understood. Therefore, we aimed to determine the mechanisms that regulate the post-endocytic trafficking of CLR•RAMP2. We investigated AM-induced trafficking of CLR•RAMP2 and found that unlike CLR•RAMP1, CLR•RAMP2 does not recycle back to the cell-surface, but instead is trafficked to lysosomes and degraded. The degradation of many GPCRs is regulated by a post-translational modification termed ubiquitination. To investigate the role of ubiquitination in the trafficking of CLR•RAMP2, we created a lysine-less mutant (CLRΔ9KR•RAMP2) and compared its post-endocytic sorting to CLR•RAMP2. The expression (at the cell-surface) and initial trafficking (to endosomes) and signalling (ERK1/2 activation) of CLR•RAMP2 and CLRΔ9KR•RAMP2 were similar. We observed that AM induced ubiquitination of CLR•RAMP2, but not CLRΔ9KR•RAMP2. However, this ubiquitination was not required to promote the trafficking of CLR•RAMP2 to lysosomes, as CLRΔ9KR•RAMP2 was also observed in lysosomes and was degraded to similar levels following long term exposure to AM (CLR, 7.5±3.5% and CLRΔ9KR, 5.4±1.9% of each unstimulated control; mean±SEM, 100 nM, 16 h). We then examined levels of CLR•RAMP2 and CLRΔ9KR•RAMP2 following a shorter exposure to AM and observed that CLR•RAMP2 was degraded at a much faster rate than CLRΔ9KR•RAMP2 (CLR, 64±3.5% and CLRΔ9KR, 109±10% of each unstimulated control; 100 nM, 4 h). Thus, our data suggest that ubiquitination of CLR•RAMP2 does not affect its fate, but does regulate the rate of degradation, perhaps through molecular mechanisms that recognise ubiquitinated proteins and facilitate the entry of CLR•RAMP2 into degradative compartments.