Insights into class B GPCR activation from structure-function analysis of the adrenomedullin N-terminus

Adrenomedullin (AM) is a 52 amino acid peptide, derived from endothelial cells and active in the vasculature. It activates a class B G protein-coupled receptor (GPCR), the calcitonin-like receptor. AM receptors are proposed as drug targets in a range of conditions, including cancer and heart failure. Thus, understanding the mechanisms of AM receptor activation is important for reaching this goal. A functional AM_1 receptor is an obligate heteromer of CLR with receptor activity-modifying protein 2 (RAMP2). The AM peptide is presumed to have two distinct components, as is the dogma for all class B GPCR ligands. The C-terminus binds to the extracellular domain of the receptor, while the N-terminus is responsible for receptor activation via the receptor extracellular loops and transmembrane bundle. AM contains a disulfide bond between residues 16-21, an unusual extension N-terminal to this (1-15), plus requires a C-terminal amide for high affinity binding. In an effort to characterise the peptide molecular signature that is necessary for receptor activation, we undertook an extensive analysis of AM and synthesised the following 28 AM-derived peptides.

AM_{15-52}	G15A AM_{15-52}	T22A AM_{15-52}	H27A AM_{15-52}	F33A AM_{15-52}	K38A AM_{15-52}
AM_{1-21}	R17A AM_{15-52}	V23A AM_{15-52}	Q29A AM_{15-52}	T34A AM_{15-52}	D39A AM_{15-52}
AM_{16-21}	F18A AM_{15-52}	Q24A AM_{15-52}	I30A AM_{15-52}	D35A AM_{15-52}	Δ35-39AM_{15-52}
AM_{15-30}	G19A AM_{15-52}	K25A AM_{15-52}	Y31A AM_{15-52}	K36A AM_{15-52}	
AM_{15-34}	T20A AM_{15-52}	L26A AM_{15-52}	Q32A AM_{15-52}	D37A AM_{15-52}	

As a primary screen we used HEK293S cells transiently transfected with HA-CLR and FLAG-RAMP2, and measured cAMP production in response to peptide. In addition, we conducted β-arrestin recruitment assays in CHO-K1 cells stably expressing these receptor components, using the Discoverx PathHunter assay. Selected analogues were used in radioligand binding assays with ^{125}I-AM_{13-52} and circular dichroism spectroscopy was also performed. Counter-screens were performed at AM_2 and CGRP receptors. The N-terminal extension of AM was not required for activity. Therefore we conducted an alanine scan of AM_{15-52} from positions 15 to 39. This revealed several key residues for receptor activation; F18A, T20A, L26A and I30A. These residues had effects on potency (>10-200 fold) and E_{max} (≥50% reduction). In addition, we tested a variety of fragments of AM. Most of them were inactive (at 10 or 100 μM), however AM_{15-34} was a full agonist, albeit with substantially reduced potency. A molecular model of the full length receptor with peptide bound suggested that the 15-34 region of the AM peptide contains the disulfide and also a short helix. We postulated that it may be possible to stabilise the AM_{15-34} peptide structure and enhance the potency of this fragment. Four variants were tested, with additional cyclisation on different turns of the short helix. However, potency was not enhanced. AM contains a string of charged amino acids (DKDKD), which act as a linker between our active fragment AM_{15-34} and extracellular binding portion of the peptide. To determine the importance of this region we generated a peptide with these residues removed (Δ35-39AM_{15-52}). This peptide had increased E_{max}, compared to AM_{15-52} but reduced potency (~100-fold). From the alanine scan data, the loss of K38 would appear to be the most detrimental.

Overall, these data reveal that the extreme N-terminus of AM is dispensable and that short fragments with full efficacy can be achieved. Furthermore, the mid-region of AM appears crucial to receptor activation, not solely the N-terminus as is the dogma for this peptide-receptor family.