

The role of native signal peptides in trafficking RAMP2 to the plasma membrane

Kelly M. Witt¹, Philip R. Brauer², Peter W. Abel¹, and Margaret A. Scofield¹

¹*Department of Pharmacology, Creighton University School of Medicine, 2500 California Plaza, Omaha, NE 68178;*

²*Department of Biomedical Sciences, Creighton University School of Medicine, 2500 California Plaza, Omaha, NE 68178*

Receptor activity-modifying protein 2 (RAMP2) forms a heterodimer with the calcitonin receptor-like receptor (CL) to produce an adrenomedullin receptor. Recently, splice variants in the signal peptide region of RAMP2 have been identified in pufferfish and rats (*FASEB J* **20** 437.7). The splice variant rat RAMP2b has a 26 amino acid deletion in a highly hydrophobic region of the signal peptide. Our goal was to determine whether the rat RAMP2b trafficked to the plasma membrane. We transiently co-transfected COS-7 cells with an N-terminal HA-tagged CL and either the wild-type RAMP2, containing the intact signal peptide, or the RAMP2b. Forty-eight hours after transfection, non-permeabilized live cells were immunostained with HA and RAMP2 primary antibodies to determine if epitopes of the proteins were present on the outer surface of the plasma membrane. A RAMP2 mutant, lacking the signal peptide, was also constructed and tested. Our studies showed that both the rat RAMP2 and RAMP2b trafficked to the cell membrane in the presence or absence of CL, but that the RAMP2 mutant without the signal peptide did not reach the plasma membrane, even though it was detected inside the cells.

Work was presented at Experimental Biology April 29, 2007