THE GRADUATE COLLEGE OF THE
UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

ANNOUNCES THE FINAL EXAMINATION OF

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FOR THE DEFENSE OF THE DOCTOR OF PHILOSOPHY DEGREE
GRADUATE COLLEGE
Department of Biochemistry and Molecular Biology

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https://ouhsc.zoom.us/j/93764730371
Password: 699306

Receptor engagement and G protein recruitment by the cardioprotective peptide Adrenomedullin 2/Intermedin

COMMITTEE IN CHARGE: Augen Pioszak, PhD; Rodger McEver, MD; Ann Louise Olson, PhD; Paul DeAngelis, PhD Mary Beth Humphrey, MD, PhD

ABSTRACT: The vasoactive peptide hormone adrenomedullin 2/intermedin (AM2/IMD) has a broad spectrum of actions including cardioprotective effects that are mediated by heterodimeric receptor complexes in humans composed of the calcitonin-like G protein-coupled receptor (CLR) and a receptor activity-modifying protein (RAMP1/2/3). CLR:RAMP complexes also mediate actions of the related calcitonin gene-related peptide (CGRP) and adrenomedullin (AM). Each agonist has a unique CLR:RAMP activation pattern and CGRP and AM exhibit selective binding to the extracellular domains (ECDs) of their preferred CLR:RAMP complexes. How AM2/IMD engages CLR:RAMP complexes is poorly understood and delineating the unique CLR:RAMP interactions for AM2/IMD compared to CGRP and AM is necessary to understand how the three agonists produce shared and distinct effects through CLR:RAMP complexes. We purified each CLR:RAMP ECD complex to examine peptide binding and found that AM2/IMD had a unique ECD-binding pattern preferring CLR:RAMP1/3 over CLR:RAMP2. Using X-ray crystallography we solved a 2.1 Å resolution structure of AM2/IMD bound to the CLR:RAMP1 ECD. AM2/IMD shared a similar binding pocket with CGRP and AM but adopted a unique triple β-turn structure. The structure was validated with binding studies and cell-based signaling assays using full-length receptors. To investigate receptor activation we developed a native PAGE assay to visualize detergent-solubilized fluorescently-labeled full-length CLR:RAMP complexes expressed in mammalian cells using adherent cultures or membrane preparations. Using this assay we determined CLR:RAMP binding affinities of agonists and engineered G_s protein surrogate (MiniG_s) that stabilizes the active receptor. The rank order of agonist affinities for CLR:RAMP:MiniG complexes indicated that transmembrane domain contacts along with RAMP-dependent selective ECD binding of the agonists produce CLR:RAMP binding preferences. Additionally, MiniG_s affinity for the agonist-saturated CLR was controlled by both the agonist and the RAMP, which we validated through rational design of peptide chimeras that behaved as predicted in cell-based assays. Our investigations demonstrated that unique CLR:RAMP selectivity of AM2/IMD stems from a combination of selective RAMP-modulated ECD binding of AM2/IMD and agonist- and RAMP-dependent control of G protein coupling. These findings lay a foundation for understanding how receptor interactions dictate distinct cellular responses, which is crucial for exploiting the effects of these hormones for drug development.