THE GRADUATE COLLEGE OF THE
UNIVERSITY OF OKLAHOMA HEALTH SCIENCES CENTER

ANNOUNCES THE FINAL EXAMINATION OF

# Jason M. Booe

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

June 30, 2020, 12:00 Noon
Location: https://ouhsc.zoom.us/j/95744875237
Password: 557278

## *Modulation of CGRP and Adrenomedullin Peptide Selectivity by Receptor Activity-Modifying Proteins*

COMMITTEE IN CHARGE: Augen Pioszak, Ph.D. (chair), Paul DeAngelis, Ph.D., Rodger McEver, M.D., Jialing Lin, Ph.D. and William Hildebrand, Ph.D.

ABSTRACT: The receptors for the peptides adrenomedullin (AM) and calcitonin gene-related peptide (CGRP) are important drug targets for cardiovascular disease and migraine, respectively. Their receptors are heterodimers composed of a class B1 G protein-coupled receptor (GPCR), the calcitonin receptor-like receptor (CLR), and one of three receptor activity-modifying proteins (RAMP1-3) that determine peptide binding preference. CGRP prefers RAMP1:CLR while AM prefers RAMP2:CLR and RAMP3:CLR. The primary driver of selectivity is the interaction of RAMP and CLR via their extracellular domains (ECDs). Crystal structures of CGRP analog-bound RAMP1:CLR and an AM-bound RAMP2:CLR ECD complexes provided insights into how AM and CGRP engage their cognate receptor. Beyond this, knowledge of how RAMP ECDs determine CGRP and AM peptide selectivity is poorly understood. Further, peptide therapeutics for these clinically important receptors are lacking as their short half-lives limit their utility as drugs.

In this work, I use structural, biochemical, and pharmacological approaches to probe the mechanism by which RAMP ECDs determine AM and CGRP peptide selectivity. Rational, structure-guided design of AM and CGRP variants and an AM-based combinatorial peptide library approach revealed key determinants of selectivity and identified novel, affinity-enhancing peptide substitutions. A suite of novel peptide variants was described with enhanced affinity and/or altered selectivity. Crystal structures of select AM variants bound to RAMP1:CLR and RAMP2:CLR ECD complexes revealed the structural basis for their affinity-enhancing effect and supported a dual role for RAMP function in altering CGRP and AM peptide selectivity through both direct RAMP-peptide contacts and allosteric effects. AM and CGRP agonists and antagonists were developed with enhanced pharmacological activity, including picomolar affinity antagonists and sustained signaling agonists. These novel peptides will be valuable pharmacological tools for studying RAMP:CLR receptors and may function as novel peptide therapeutics to treat RAMP:CLR-related diseases.