Final Examination of

Allyson Catherine Marshall

For the Degree of

DOCTOR OF PHILOSOPHY
Physiology and Pharmacology
May 2014
Winston-Salem, NC

COMMITTEE IN CHARGE

Mark C. Chappell, PhD, Co-Advisor
Debra I. Diz, PhD, Co-Advisor
Robert N. Taylor, MD, Chair
Hossam A. Shaltout, PhD
Lisa K. Washburn, MD

WF Bio-Tech Place
575 N. Patterson Avenue
Auditorium, Room 151
Monday, March 31, 2014
1:00 p.m.
PROFESSORS IN CHARGE OF RESEARCH
Mark C. Chappell, PhD, Professor and Debra I. Diz, PhD, Professor

FIELDS OF GRADUATE STUDY
Major Subject: Physiology and Pharmacology, Neurobiology of Hypertension

SUMMARY OF DISSERTATION
ALTERATIONS IN THE CENTRAL RENIN ANGIOTENSIN SYSTEM IN A MODEL OF FETAL PROGRAMMING

The use of antenatal betamethasone (BM) is an approved therapy for women entering early preterm labor between 24 and 34 weeks gestation. The immediate effects of BM therapy are of benefit to the offspring, as fetal steroid exposure decreases infant mortality by accelerating lung development and activating the sympathetic nervous system. However, fetal steroid exposure may lead to elevated mean arterial pressure and decreased autonomic function in young adults. Baroreflex sensitivity for control of heart rate is an important marker of autonomic function and is reduced in conditions associated with hypertension. Indeed, sheep exposed in utero to BM develop decreased baroreflex sensitivity by 6-weeks of age and increased mean arterial pressure by 6-months of age. These changes in blood pressure and autonomic function are associated with alterations in components of the circulating and intra-renal renin-angiotensin systems (RAS) that shift the RAS to the pro-hypertensive peptide, angiotensin (Ang) II. In the brain, the Ang II AT₁ receptor opposes the beneficial actions of Ang-(1-7) at the Mas receptor for control of baroreflex sensitivity and blood pressure regulation. Our goal was to identify BM induced alterations in the central RAS that are consistent with functional data reporting increased pressure and decreased baroreflex function. This dissertation focuses on the RAS peptides, receptors, and enzymes that act as possible sites of BM induced alterations.

Ang-(1-7) tone was assessed in BM-exposed (BMX) and control animals by measuring angiotensin receptor expression, peptide levels, and processing enzymes at 0.5-years of age in the brain dorsal medulla containing the solitary tract nucleus, choroid plexus (ChP), and cerebral spinal fluid (CSF). We demonstrate reduced Ang-(1-7) tone in BMX offspring as shown by lower Mas receptor expression and an increased ratio of Ang II/Ang-(1-7) in the brain dorsal medulla, and decreased Ang-(1-7) peptide levels accompanied by increased Ang-(1-7) metabolism in the CSF. This increase in Ang-(1-7) metabolism is a result of increased angiotensin-converting enzyme (ACE) activity, and increased activity of an unidentified peptidase. Further studies investigate the Ang-(1-7) metabolising peptidase in the CSF and characterize the peptidase as a novel activity due to the unique inhibitor profile, substrate specificity, and optimal pH. We identify high activity of the Ang-(1-7) peptidase in the brain medulla and utilize this tissue as a source of activity for purification. After purifying the peptidase approximately 2000-fold, we confirm the unique inhibitor profile and substrate specificity originally reported in the CSF. These findings support the hypothesis that there is a novel Ang-(1-7) metabolising peptidase present in the CSF and brain medulla of sheep. Collectively, the findings in this dissertation support the hypothesis that in utero BM exposure shifts the central RAS towards the ACE-Ang II-AT₁ receptor axis and away from the ACE2-Ang-(1-7)-Mas receptor axis. Additionally, a novel peptidase involved in endogenous Ang-(1-7) regulation was uncovered and may play an important role in the development of pathological conditions involving central Ang-(1-7) peptide levels.
SCHOLASTIC VITAE

EDUCATION
2014 - PhD Candidate, Physiology and Pharmacology
Wake Forest University Health Sciences
Winston-Salem, NC

2010 - Bucknell University, BS in Cell Biology and Biochemistry, cum laude
Lewisburg, PA

PROFESSIONAL MEMBERSHIPS

ASPET, 2010 – Present
Cardiovascular Division Graduate Student Representative, 2013 - Present
Co-chair of symposium “Fetal Programming of Adult Cardiovascular Disease,” 2014

American Physiological Society, 2010 – Present
Cardiovascular Division Trainee Committee, 2013 – expected 2016
American Heart Association Council on High Blood Pressure, 2010 – Present
Volunteer at Annual Heart Walk, Tanglewood Park, NC, 2010, – Present
Winston Salem State Doctorate of Physical Therapy Guest Lecturer, 2013
Lecturer on Pharmacology for Physical Therapists & Applied Human Physiology
Orientation Aid, Integrative Physiology and Pharmacology Program, Wake Forest University
2011-2014

AWARDS
Featured Graduate Student Member in The Pharmacologist, American Society for
Pharmacology and Experimental Therapeutics (ASPET), March 2014
Finalist in Integrative Systems, Translational and Clinical Pharmacology Division Young
Investigator Oral Session, Experimental Biology, forthcoming in April 2014
ASPET Graduate Student Travel Award, 2013, 2014
Onsite Poster Competition Winner, American Heart Association Council on High Blood
Pressure Research, 2013
Honorable Mention, ASPET Best Abstract Competition, 2013
Alumni Travel Award Wake Forest University School of Medicine, 2012, 2013
PUBLICATIONS

Manuscripts


Chappell MC, **Marshall AC,** Alzayedneh EM, Shaltout HA, Diz DI. Update on the angiotensin converting enzyme 2-angiotensin (1-7)-mas receptor axis: Fetal programming, sex differences, and intracellular pathways. *Front Endocrinol (Lausanne).* 2014;4:1-13. PMID 24409169


Shaltout HA, **Marshall AC,** Rose JC, Chappell MC, Diz DI. Antenatal betamethasone exposure attenuates the role of angiotensin-(1-7) in the NTS for the baroreflex control of heart rate. *In preparation* as of March 2014

Published Abstracts


**Marshall AC,** Shaltout HA, Rose JC, Diz DI, Chappell MC. Antenatal Betamethasone Exposure Markedly Reduces the Levels of Ang-(1-7) in Cerebrospinal Fluid. *International Society of Hypertension.* 2013

**Marshall AC,** Shaltout HA, Rose JC, Diz DI, Chappell MC. Antenatal Betamethasone Exposure Markedly Reduces the Levels of Ang-(1-7) in Cerebrospinal Fluid. *Hypertension.* 2013; 177
