Doctoral Dissertation Defense Announcement

Natalia M. Mathieu

“Role of β-arrestin2 in the Regulation of Blood Pressure and Fluid Homeostasis in the Brain”

Candidate for Doctor of Philosophy in Physiology
Department of Physiology
School of Graduate Studies
Medical College of Wisconsin

Committee in Charge:
Curt D. Sigmund, PhD (Mentor)
Cecilia Hillard, PhD
Stephanie Watts, PhD
Justin Grobe, PhD
Aron Geurts, PhD
Pablo Nakagawa, PhD

Thursday, March 7, 2024
09:00 AM (CST)
Bolger Auditorium

Zoom Meeting ID: 925 6889 8220 Passcode: Z0q93Ved
https://mcw-edu.zoom.us/j/92568898220?pwd=WmxnMnhxaXdpVTJZQTMyRmx4MEZwQT09
Course Completed

Foundations in Biomedical Sciences I
Foundations in Biomedical Sciences II
Foundations in Biomedical Sciences III
Foundations in Biomedical Sciences IV
Techniques in Molecular and Cell Biology
Professional Development I
Organ Systems Physiology
Fundamentals in Neuroscience
Special Problems in Physiology
Biostatistics Health Science
Ethics and Integrity in Science
Graduate Human Physiology
Complement to Graduate Human Physiology
Current Topics – Physiology
Functional Genomics
Fundamental Practice Grant Writing
Current topics in Cardiovascular Biology
Research Ethics Discussion Series
Reading and Research
Doctoral Dissertation
Dissertation Summary

“Role of β-arrestin2 in the Regulation of Blood Pressure and Fluid Homeostasis in the Brain”

Hypertension (HTN) is a major risk factor for cardiovascular disease, renal failure, stroke, myocardial infarction, and death. The brain renin-angiotensin system (RAS) has been extensively studied for its role in blood pressure (BP) regulation. Angiotensin II (ANG) is the primary product of the RAS which exerts most of its physiological effects through the ANG type-1 receptor (AT1R). It is traditionally accepted that disproportionate ANG signaling within the brain can turn detrimental for a disease state like HTN, and these are primarily mediated through activation of Gαq/11. These effects are varied and include vasoconstriction, sodium reabsorption, aldosterone synthesis, water and salt intake, and sympathetic nervous system activation, altogether to elevate BP. The AT1R as most GPCRs also possess internal mechanisms intrinsic to the receptor to terminate G protein signaling. In particular, β-arrestin-mediated signaling of the AT1R terminates G protein activation by internalizing the receptor and therefore cause signal desensitization. Activation of this non-canonical pathway has gained a lot of attention as it is hypothesized to counterbalance maladaptive G protein signaling during disease. Utilizing a model of elevated brain RAS such as deoxycorticosterone (DOCA)-salt HTN, we previously examined the effects of an AT1R-specific β-arrestin biased agonist, TRV027. In this study, intracerebroventricular (ICV) infusion of TRV027 induced an aversion to saline and decreased BP in DOCA-salt HTN. This work, in combination with clinical studies linking the role of β-arrestin with multiple cardiovascular advantages has generated great interest in understanding how these benefits may be derived from the activation of ANG AT1R β-Arrestin pathway in the central nervous system. As such, the primary objective of my PhD thesis was to evaluate whether genetic deletion of β-arrestin from the whole animal to specific brain regions resulted in exacerbated responses to HTN.

To initially address this question, we employed a global mouse model deletion of β-arrestin1 (Arrb1) and β-arrestin2 (Arrb2) knockout (KO) mice. These animals were employed to evaluate drinking behavior and BP in response to DOCA-salt. Age- and sex-matched C57BL/6 mice served as controls. First, we measured intake of water and saline employing a two-bottle choice paradigm with and without DOCA. At baseline, Arrb2-KO mice exhibited a significant elevation in saline intake with no change in water intake. With DOCA treatment, Arrb2-KO mice exhibited a further increase in both saline and water intake. Second, we evaluated BP via telemetry in Arrb2-KO and C57BL/6 mice with and without DOCA. Arrb2-KO did not exhibit significant differences in BP before DOCA treatment when provided water alone, or when provided a choice of water and saline. However, Arrb2-KO exhibited a further pressor response to DOCA-salt. Together, these findings suggest that deletion of ARRB2 potentiates the pressor response to ANG. To further dissect what brain regions might be mediating such response, we develop a region-specific KO targeting the subfornical organ (SFO). The SFO is one of the primary ANG-sensing regions involved not only in BP regulation but also in hydromineral balance. We then employed Arrb2FLOX mice to induce deletion of Arrb2 from the SFO via ICV injection of adeno-associated virus (AAV)-CRE. Infection with ICV AAV-CRE primarily targeted the
SFO with few off-targets. Mice receiving ICV-AAV-Cre-GFP are denoted \textit{Arrb2}^{ICV-Cre}, and littermate controls receiving ICV-AAV-GFP are denoted \textit{Arrb2}^{Control}. First, BP was evaluated in response to ICV infusion of ANG. Consistent with global \textit{Arrb2}-KO results, \textit{Arrb2}^{ICV-Cre} mice also exhibited higher pressor response to ANG. Second, we measured intake of water and saline employing a two-bottle choice paradigm. At baseline, \textit{Arrb2}^{SFO-KO} mice exhibited a significant increase in saline intake compared to controls, also recapitulating the global KO results. We then challenged the animals to water- and sodium-depleted conditions to elevate levels of endogenous brain ANG. After water and sodium depletion, mice were subjected to the same two-bottle choice paradigm and fluid intake was recorded for 4 hours. Our results show that under water-depleted conditions, \textit{Arrb2}^{ICV-Cre} mice exhibit higher saline intake. And under sodium-depleted conditions \textit{Arrb2}^{ICV-Cre} mice exhibit higher saline and water intake. Collectively, selective \textit{Arrb2} deletion from the SFO increased saline intake and exacerbated the pressor response to endogenous or exogenous ANG levels, respectively.

In conclusion, our work indicates that, ARRB2 but not ARRB1 is involved in the regulation of blood pressure and fluid intake, and this response is in part mediated by ARRB2 functioning in the SFO. Stimulation of the AT$_1$R $\beta$-arrestin axis in the brain may represent a novel strategy to treat hypertension. Agonists of the ANG AT$_1$R which activate $\beta$-arrestin signaling in a biased manner lower arterial pressure in several models of HTN in rodents, and lower blood pressure in patients with a high index of renin-angiotensin activation. They have also been reported to be cardioprotective in preclinical models of heart failure and may have some benefit in heart failure patients that exhibit high BP. Further study is clearly warranted to assess the full range of cardioprotection and the mechanisms by which this occurs. The use of $\beta$-arrestin-biased agonists for GPCR (AT$_1$R included) remain a promising approach as it preserves the $\beta$-arrestin component thus limiting some G protein pathways, and theoretically at least may offer additional benefits over traditional receptor blockers, which block the receptor in its entirety.
EDUCATION

June 2019 – Present
Medical College of Wisconsin, Milwaukee WI
PhD Candidate, Department of Physiology.

Jan 2013 – Dec 2017
Universidad EIA-Universidad CES, Medellín, Colombia.
Bachelor of Science in Biomedical Engineering.

PROFESSIONAL EXPERIENCE

June 2018 – June 2019
Mayo Clinic, Rochester, MN
Post-Baccalaureate Research Fellow. Department of Biomedical Engineering and Physiology.

Jan 2017 – June 2017
Mayo Clinic, Rochester, MN
Undergraduate Research Fellow. Department of Biomedical Engineering and Physiology.

Jan 2017 – Dec 2017
Universidad EIA - Universidad CES, Medellín, Colombia
Undergraduate Research Fellow. Department of Biomedical Engineering.

GRANT FUNDING:

Jan 2022 – Dec 2023
Title: Role of Brain AT1R/B-Arrestin Signaling as a Regulator of Blood Pressure and Fluid Homeostasis
Source: American Heart Association Pre-Doctoral Fellowship
Mentor: Curt D Sigmund PhD
Amount: $64,072

HONORS AND AWARDS

Apr 2024
Pre-Doctoral Research Recognition Award. Water & Electrolyte Homeostasis Section, American Physiological Society

Apr 2023
Steven M. Horvath Research Recognition Award. Water & Electrolyte Homeostasis Section, American Physiological Society

Oct 2022
Cardio-renal Research Center Graduate Student Research Symposium Travel Award. University of Mississippi Medical Center

Sept 2022
Underrepresented Minorities Research Recognition Award. Council on Hypertension, American Heart Association

Apr 2022
Research Recognition Award. Water & Electrolyte Homeostasis Section, American Physiological Society
Nov 2021 Cardiovascular Center Research Retreat Poster Competition Winner. Medical College of Wisconsin
Sept 2021 Research Recognition Award. Trainee Advocacy Program Council on Hypertension, American Heart Association
June 2021 Graduate School of Biomedical Sciences Minority Scholarship, Medical College of Wisconsin.
Nov 2020 30th Annual Graduate School & Office of Postdoctoral Education Research Poster Winner. Medical College of Wisconsin
Dec 2017 Undergraduate thesis Cum Laude. Universidad EIA - Universidad CES.

NATIONAL COMMITTEE POSITIONS
2023 – Present Trainee Advocacy Committee. Council on Hypertension, American Heart Association

LOCAL INVOLVEMENTS, SERVICE, TEACHING AND MENTORSHIP
2022 – Present Instructor SUPREMES® (Students Understanding Principles of Research Education through Medicine, Engineering, and Science) Program Teaching. Medical College of Wisconsin & Marquette University
2023 Diverse Funding for Researchers Panelist. Graduate School Association, Medical College of Wisconsin
2021 – 2023 Student Health Science Conference Committee, Medical College of Wisconsin
2022 – 2023 Graduate School Science Policy Group, Medical College of Wisconsin
2022 – 2023 Graduate School Program Interview Volunteer. Medical College of Wisconsin
2022 – 2023 Physiology Student Representative. Medical College of Wisconsin
2021 – 2023 International Graduate Health Organization Co-Founder and Co-Lead. Medical College of Wisconsin
2022 – 2023 Student Wellness Committee. Medical College of Wisconsin, WI
2022 PhD Career Advice Panelist. Post-baccalaureate Research Education Program, Mayo Clinic, Rochester, MN
2020 – 2022 Graduate School Mentor. Medical College of Wisconsin

Undergraduate Students Mentored
2022 – Present Eden Tan, Brookfield High School, SUPREMES Program. Pursuing BS/MD at Brown University.
Summer 2023 Jacquelyn Wittman, Loyola University, Summer Program for Undergraduate Research. Pursuing MD at MCW.

PEER-REVIEWED BIBLIOGRAPHY FROM PHD

PUBLICATIONS


Currently Under Preparation


Full Bibliography – [Google Scholar](https://scholar.google.com)

**ABSTRACTS**

**National**


4. **Natalia M. Mathieu**, Pablo Nakagawa, Patricia Muskus, Daniel T. Brozoski, Justin L. Grobe, and Curt D. Sigmund. β-Arrestin2 Deficiency in the Brain Alters Blood Pressure Regulation. APS Summit 2023. Long Beach, CA


Institutional


3. Natalia M. Mathieu, Pablo Nakagawa, Justin L. Grobe, Curt D. Sigmund. Role of β-Arrestin in Mediating Drinking Response Downstream of the AT1R. Cardiovascular Center Research Retreat 2021. Milwaukee, WI.