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GRADUATE SCHOOL

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
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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 (AT_1R). 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 $G\alpha_{q/11.}$ 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 AT_1R as most GPCRs also possess internal mechanisms intrinsic to the receptor to terminate G protein signaling. In particular, β-arrestin-mediated signaling of the AT_1R 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 AT_1R-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 AT_1R 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 the beneficial effects of TRV027 may be mediated by ARRB2, but not ARRB1 associated with the AT_1R during DOCA-salt.

We next asked whether deficiency of ARRB2 sensitizes the response to ANG within the brain due to loss of Gαq termination signaling. To evaluate this, we subjected *Arrb2*-KO mice to acute BP responses to ICV infusion of ANG. Although no differences at baseline BP were observed, *Arrb2*-KO mice exhibited greater maximal BP after ANG. This result suggests that deletion of ARRB2 potentiates the pressor response to ANG. To further dissect what brain regions might been 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 *Arrb2*^{FLOX} 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 *Arrb2*$^{ICV\text{-}Cre}$, and littermate controls receiving ICV-AAV-GFP are denoted *Arrb2*^{Control}. First, BP was evaluated in response to ICV infusion of ANG. Consistent with global *Arrb2*-KO results, *Arrb2*$^{ICV\text{-}Cre}$ mice also exhibited higher pressor response to ANG. Second, we measured intake of water and saline employing a two-bottle choice paradigm. At baseline, *Arrb2*$^{SFO\text{-}KO}$ 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, *Arrb2*$^{ICV\text{-}Cre}$ mice exhibit higher saline intake. And under sodium-depleted conditions *Arrb2*$^{ICV\text{-}Cre}$ mice exhibit higher saline and water intake. Collectively, selective *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_1R β-arrestin axis in the brain may represent a novel strategy to treat hypertension. Agonists of the ANG AT_1R which activate β-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 β-arrestin-biased agonists for GPCR (AT_1R included) remain a promising approach as it preserves the β-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.

Curriculum Vitae

Natalia M. Mathieu

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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
2018 – 2019	Undergraduate Professional Mentor Program. University of Minnesota-Mayo Clinic, Rochester, MN

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

1. Gopika SenthilKumar*, **Natalia M. Mathieu***, Julie K. Freed, Curt D Sigmund and David D. Gutterman. Addressing the Decline in Graduate Students' Mental Well-Being. Editorial - Am J Physiol Heart. 2023 Aug 25. *co-first author
2. Connie C. Grobe, John J. Reho, Daeleon Brown-Williams, Alisha Ziegler, **Natalia M. Mathieu**, Samuel Lawton, Eva Fekete, Daniel T. Brozoski, Kelsey Wackman, Colin Burnett, Pablo Nakagawa, Curt D. Sigmund, Jeffrey L. Segar and Justin L. Grobe. Cardiometabolic Effects of DOCA-salt in Mice Depend on Ambient Temperature. Hypertension. 2023 Sep;80(9):1871-1880.
3. **Natalia M. Mathieu***, Eva M. Fekete*, Patricia C. Muskus*, Daniel T. Brozoski, Ko-Ting Lu, Kelsey Wackman, Javier Gomez, Shi Fang, John J. Reho, Connie C. Grobe, Ibrahim Vazirabad, Gary C. Mouradian, Jr, Matthew R. Hodges, Jeffrey L. Segar, Justin L. Grobe, Curt D Sigmund and Pablo Nakagawa. Genetic Ablation of Prorenin Receptor in the Rostral Ventrolateral Medulla Influences Blood Pressure and Hydromineral Balance in Deoxycorticosterone-Salt Hypertension. Function (Oxf). 2023 Aug 7;4(5):zqad043. *co-first author
4. Kirthikaa Balapattabi, Yavuz Yavuz, Jingwei Jiang, Guorui Deng, **Natalia M. Mathieu,** McKenzie L. Ritter, Megan A. Opichka, John J. Reho, Pablo Nakagawa, Lisa L. Morselli, Gary C. Mouradian, Deniz Atasoy, Huxing Cui, Matthew R. Hodges, Curt D. Sigmund and Justin L. Grobe. Angiotensin AT1A receptor G protein signal switching in Agouti-related peptide neurons mediates resting metabolic rate adaptation during prolonged obesity. Cell Rep. 2023 Aug 2;42(8):112935.
5. **Natalia M. Mathieu**, Pablo Nakagawa, Justin L. Grobe and Curt D. Sigmund. Insights Into the Role of Angiotensin-II AT1 Receptor-Dependent β-Arrestin Signaling in Cardiovascular Disease. Hypertension. 2023 Nov;79(11):2480-2492. ***Invited Submission.***
6. **Natalia M. Mathieu**, Pablo Nakagawa, Connie C. Grobe, John J. Reho, Daniel T. Brozoski, Ko-Ting Lu, Kelsey Wackman, McKenzie L. Ritter, Jeffrey Segar, Justin L. Grobe and Curt D. Sigmund. β-Arrestin-2 Deficiency Alters Fluid Homeostasis and Blood Pressure Regulation. Hypertension. 2022 Nov;79(11):2480-2492.
7. Vanessa Oliveira, John J. Reho, Kirthikaa Balapattabi, McKenzie Ritter, **Natalia M. Mathieu,** Megan A. Opichka, Ko-Ting Lu, Connie C. Grobe, Sebastiao D. Silva, Jr., Kelsey K. Wackman, Pablo Nakagawa, Jeffrey L.Segar, Curt D. Sigmund, and Justin L. Grobe. Chronic intracerebroventricular infusion of angiotensin II causes dose- and sex- dependent effects upon intake bahviors and energy homeostasis in C57BL/6J mice. Am J Physiol Regul Integr Comp Physiol. 2022 Oct 1;323(4):R410-R421.
8. Vanessa Oliveira, Ruth A Riedl, Kristin E. Claffin, **Natalia M. Mathieu**, McKenzie Ritter, Kirthikaa Balapattabi, Kelsey K. Wackman, John J. Reho, Daniel T. Brozoski, Donald A. Morgan, Huxing Cui, Kamal Rahmouni, Colin M.L. Burnett, Pablo Nakagawa, Curt D. Sigmund, Lisa L. Morselli and Justin L. Grobe. Melanocortin MC4R receptor is required for energy expenditure but not blood pressure effects of angiotensin II within the mouse brain. Physiol Genomics. 2022 Jun 1;54(6):196-205.
9. Mario Zanaty, Fernando A.C. Seara, Pablo Nakagawa, Guorui Deng, **Natalia M. Mathieu**, Kirthikaa Balapattabi, Sadashiva S. Karnik, Justin L. Grobe, Curt D. Sigmund. B-Arrestin-Biased Agonist Targeting the Brain AT1R (Angiotensin II type 1 Receptor) Increases Aversion to Saline and Lowers Blood Pressure in Deoxycorticosterone Acetate-Salt Hypertension. Hypertension. 2021 Feb; 77(2):420-431.

Currently Under Preparation

1. **Natalia M. Mathieu,** Pablo Nakagawa, Eden E. Tan, John J. Reho, Daniel T. Brozoski, Patricia Muskus, Ko-Ting Lu, Kelsey K. Wackman, Justin L. Grobe, and Curt D. Sigmund. Genetic Deletion of β-Arrestin-2 from the Subfornical Organ and Nuclei Surrounding the Lateral Ventricle Alters Fluid Homeostasis and Blood Pressure Regulation. *Hypertension.*
2. **Natalia M. Mathieu,** Pablo Nakagawa, Eden E. Tan, Patricia Muskus, Justin L. Grobe, and Curt D. Sigmund. Divergent Neuronal Circuitry Controlling Water and Saline Intake from Mice Carrying Genetic Deletion of β-Arrestin-2 from the Subfornical Organ and Nuclei Surrounding the Lateral Ventricle.
3. Pablo Nakagawa, Javier Gomez, Éva M. Fekete, Patricia C. Muskus, Carie Boychuk, Ana Hantke-Guixa, Michelle Xie, Azeez Ganiyu, Daria Golosova, **Natalia M. Mathieu**, Ko-Ting Lu, Kelsey K. Wackman, Daniel T. Brozoski, Gary C. Mouradian, Matthew R. Hodges, Jeffrey L. Segar, Justin L. Grobe, Curt D. Sigmund. Definitive Evidence for the Identification and Function of Renin-Expressing Cholinergic Neurons in the Nucleus Ambiguus. *Hypertension.*

Full Bibliography – Google Scholar

ABSTRACTS

National

1. Alyssa M. Madison, Connie C. Grobe, **Natalia M. Mathieu**, John J. Reho, Curt D. Sigmund, Justin L. Grobe and Jeffrey Segar. Antagonism of the Angiotensin AT1 Receptors in the Brain Ameliorates Metabolic Dysfunctions Programmed by Early-life Sodium Restriction. Midwest Society for Pediatric Research 2023. Chicago, IL.
2. **Natalia M. Mathieu**, Pablo Nakagawa, Patricia Muskus, Daniel T. Brozoski, Justin L. Grobe, and Curt D. Sigmund. β-Arrestin2 Deficiency in the Subfornical Organ Alters Blood Pressure. AHA Council on Hypertension 2023. Boston, MS. ***Podium Presentation***
3. Eva M. Fekete, Patricia Muskus, **Natalia M. Mathieu**, Daniel T. Brozoski, Javier Gomez, Gary Mouradian, Matthew R. Hodges, Justin L. Grobe, Curt D. Sigmund and Pablo Nakagawa. Conditional Deletion of Renin in the Mouse Brain Results in Cardiovascular and Respiratory Abnormalities in Response to Hypoxia or Autonomic Blockade. AHA Council on Hypertension 2023. Boston, MS.
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
5. **Natalia M. Mathieu**, Pablo Nakagawa, Justin L. Grobe and Curt D. Sigmund. Non-Canonical AT1R/β-Arrestin2 Activation in Brain as a Regulator of Blood Pressure. AHA Council on Hypertension 2022. San Diego, CA. ***Podium Presentation – Plenary Session.***
6. **Natalia M. Mathieu**, Pablo Nakagawa, Justin L. Grobe and Curt D. Sigmund. Role of β-Arrestin as a Regulator of Fluid Homeostasis and Blood Pressure. Experimental Biology 2022. Phyladelphia, PA. ***Podium Presentation.***
7. Pablo Nakagawa, Daniel Brozoski, **Natalia M. Mathieu**, Ko-Ting Lu, Javier Gomez, John Reho, Shi Fang, Jeffrey Segar, Justin L. Grobe, and Curt D. Sigmund. Deletion of Prorenin Receptor in the Rostral Ventrolateral Medulla Results in Biphasic and Sex-Dependent Pressor Responses in Deoxycorticosterone Acetate-salt Hypertension. Experimental Biology 2022. Phyladelphia, PA.

8. **Natalia M. Mathieu**, Pablo Nakagawa, Justin L. Grobe, Curt D. Sigmund. Non-Canonical AT1R/ β-Arrestin Activation in Brain as a Regulator of Fluid Homeostasis and Blood Pressure. AHA Council on Hypertension 2021. **e-Podium Presentation.**
9. Pablo Nakagawa, Daniel Brozoski, **Natalia M. Mathieu**, Ko-Ting Lu, Javier Gomez, John Reho, Shi Fang, Jeffrey Segar, Justin L. Grobe, and Curt D. Sigmund. Biphasic and Sex-Dependent Roles of the Prorenin Receptor in the Rostral Ventrolateral Medulla in Mice Subjected to Deoxycorticostirone Acetate-salt Hypertension. Council of Hypertension Virtual Meeting 2021.

Institutional

1. **Natalia M. Mathieu**, Pablo Nakagawa, Justin L. Grobe and Curt D. Sigmund. β-Arrestin2 Deficiency in the Subfornical Organs Alters Blood Pressure and Fluid Homeostasis. 33rd Annual Graduate School & Office of Postdoctoral Education 2020. Medical College of Wisconsin.
2. **Natalia M. Mathieu**, Pablo Nakagawa, Justin L. Grobe, Curt D. Sigmund. Non-Canonical AT1R/β-Arrestin Signaling as a Regulator of Fluid Homeostasis and Blood Pressure. Cardiovascular Center Research Retreat 2023. Milwaukee, WI. ***Podium Presentation.***
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.
4. **Natalia M. Mathieu**, Pablo Nakagawa, Justin L. Grobe and Curt D. Sigmund. Role of β-Arrestin in mediating drinking response. 30th Annual Graduate School & Office of Postdoctoral Education 2020. Medical College of Wisconsin.