ANNOUNCING
Doctoral Dissertation Defense

Anna D. Manis

Candidate for Doctor of Philosophy in Physiology with a Concentration in Basic and Translational Sciences

Graduate School of Biomedical Sciences
Medical College of Wisconsin

Wednesday, December 15, 2021
9:00 AM CST

Zoom Meeting ID: 961 0073 4184
Passcode: dJYd61Va

https://mcw-edu.zoom.us/j/96100734184?pwd=RFpZVXRlWHJmK0wrSStoT3kwNTdaQT09

Committee in Charge:
Matthew R. Hodges, PhD (Advisor)
Alexander Staruschenko, PhD (Advisor)
Oleg Palygin, PhD
Wai-Meng Kwok, PhD
Gordon F. Buchanan, MD, PhD
Graduate Studies:

Biochemistry- Molecules to Cells
Medical Physiology
Complement to Medical Physiology
Classical and Molecular Genetics
Fundamentals of Neuroscience
Graduate Neuroanatomy
Special Problems in Physiology
Physiology Seminar
Reading and Research
Biostatistics
Ethics & Integrity in Science
Endocrine Regulation and Common Disease
Advanced Renal Physiology
Advanced Cardiovascular Physiology
Critical Reading in Respiratory Physiology
Physiological Genomics
Boundaries of Science and Medical Practice
Research Ethics Discussion Series
Integrating Pediatric Intensive Care Rounds
Doctoral Dissertation
Current Topics in Physiology
Dissertation:
The critical roles of Kir5.1 (Kcnj16) in cardiorenal and neurorespiratory physiology

Abstract:
Inwardly rectifying potassium (Kir) channels are expressed in nearly all organs and cell types and play critical roles in cellular function. Kir5.1 (encoded by the Kcnj16 gene) is a Kir channel subunit abundantly expressed in both the kidney and brain. A global knockout of Kcnj16 in the Dahl SS rat (SS\textsuperscript{Kcnj16\textminus\textminus}) was created to examine the contribution of Kir5.1 to neurological and renal physiology and pathophysiology.

In the kidney, Kir5.1 is highly expressed in the aldosterone-sensitive distal nephron, a major target for renin-angiotensin-aldosterone system hormones (RAAS), where it assembles with Kir4.1 (Kcnj10) to form a functional heterotetrameric channel. These channels contribute renal blood pressure control and have been implicated in salt-sensitive hypertension. We utilized SS\textsuperscript{Kcnj16\textminus\textminus} rats to investigate the relationship between Kir5.1 and RAAS function in the sensitivity of blood pressure to the dietary Na\textsuperscript{+}/K\textsuperscript{+} ratio. The knockout of Kcnj16 caused substantial elevations in plasma RAAS hormones (especially alternative axis angiotensin peptides) and altered the RAAS response to changing the dietary Na\textsuperscript{+}/K\textsuperscript{+} ratio. Blocking aldosterone with spironolactone caused rapid mortality in SS\textsuperscript{Kcnj16\textminus\textminus} rats, which was prevented by dietary K\textsuperscript{+} supplementation. These studies revealed that the knockout of Kcnj16 markedly altered RAAS regulation and function, suggesting Kir5.1 as a key regulator of the RAAS, particularly when exposed to changes in dietary sodium and potassium content. Further understanding of this mechanism may elucidate novel therapeutic targets or strategies for a subset of hypertensive patients.

In the brain, Kir channels are important for maintaining the neuronal resting membrane potential, thereby regulating excitability. Because mutations in KCNJ genes may alter neuronal excitability and have been linked to human seizure disorders, we hypothesized that SS\textsuperscript{Kcnj16\textminus\textminus} rats would exhibit neurological phenotypes including increased susceptibility to seizures. We found that SS\textsuperscript{Kcnj16\textminus\textminus} rats exhibited robust, reproducible tonic-clonic audiogenic seizures confirmed by electroencephalography. Repeated seizure induction altered behavior, exacerbated hypokalemia, and led to approximately 38\% mortality in male SS\textsuperscript{Kcnj16\textminus\textminus} rats. Dietary potassium supplementation did not prevent audiogenic seizures, but mitigated hypokalemia and prevented mortality induced by repeated seizures. These results reveal a distinct, non-redundant role for Kir5.1 channels in the brain, introduce a novel rat model of audiogenic seizures, and suggest yet to be identified mutations in KCNJ16 may cause or contribute to seizure disorders. Our next aim was to investigate the physiological consequences resulting from repeated seizures. We
hypothesized that repeated audiogenic seizures in SS*Kcnj16/-* rats would lead to progressively worsening cardiorespiratory suppression and associated brainstem pathology. Solitary GTCSs acutely led to apnea and decreased breathing frequency. When seizures were repeated for 10 days, breathing frequency became further suppressed leading to a suppression of total ventilation, and serotonergic immunoreactivity was reduced in brainstem respiratory nuclei. This data suggests that postictal respiratory suppression is exacerbated by the increasing number of total seizure exposures, and may be mediated by serotonergic dysregulation in cardiorespiratory brainstem nuclei. The progressive deterioration of ventilatory control with repeated seizures may provide physiological insight relevant to patients with uncontrolled epilepsy.

From this work, we can conclude that Kir5.1 plays a critical, nonredundant role in cardiorenal and neurorespiratory physiology. Specifically, Kir5.1 appears to contribute to vital life sustaining homeostatic systems including: RAAS balance, electrolyte homeostasis, blood pressure control, pH homeostasis, and neuronal excitability. KCNJ16 has historically been overlooked clinically, but due to modern advances, KCNJ16 mutations have recently begun to appear in clinical cases, showing severe neurological and renal phenotypes. Further work utilizing models like the SS*Kcnj16/-* rat will be required to fully understand the complex multi-system pathophysiology resulting from mutations in KCNJ16 and to evaluate potential therapeutics. Even with more consistent genetic screening in the future, direct Kir chanelopathies are rare. However, altered function of Kir5.1 containing channels is also likely present in more common diseases. Understanding the contribution of lesser-studied ion channel genes, like KCNJ16, to complex human diseases (e.g. idiopathic epilepsy and salt-sensitive hypertension) may unlock novel therapeutic targets and bring forth new treatment strategies.
CURRICULUM VITAE

Anna D. Manis
PhD Candidate
Staruschenko Lab & Hodges Lab

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Physiology Department
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Milwaukee, WI 53226
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manis.anna@gmail.com

Education:

Doctor of Philosophy – Physiology (in progress)  
*Concentration in Clinical and Translational Sciences 
Medical College of Wisconsin, Milwaukee, WI  
2016 – 2021

Bachelor of Science – Biochemistry and Molecular Biology  
*Minor in Neuroscience 
University of California, Davis, CA  
2007 – 2011

Work Experience:

Applied Molecular Transport, South San Francisco, CA 
Research Associate, November 2015 – June 2016

Afferent Pharmaceuticals, South San Francisco, CA 
Research Associate, September 2015 – February 2016

Kalamazoo Valley Community College, Kalamazoo, MI 
Teaching Assistant and Tutor in Psychology, June 2014 – February 2015

UC Davis Mouse Biology Program: Embryonic Stem Cell Lab, Davis, CA 
Staff Research Associate II, October 2012 - November 2013 
Staff Research Associate I, September 2011 - September 2012 
Laboratory Assistant, June 2009 - August 2011

Horwitz Lab, UC Davis Department of Neurobiology, Physiology and Behavior, Davis, CA 
Undergraduate Researcher, January 2010 - July 2011

UC Davis Medical Center: Child Life Department, Sacramento, CA 
Student Intern, January 2009 - September 2009

UC Davis: Department of Sociology, Science and Technology Studies, Davis, CA 
Research Assistant, December 2007 - June 2008
Publications:


Published Abstracts:


**Anna D Manis**, Vladislav Levchenko, Matthew R Hodges, Oleg Palygin, Alexander Staruschenko. Role of Kir4.1 (Kcnj10) in The Regulation of Salt-Induced Hypertension. J. Hypertens., 2020


**Anna D Manis**, Matthew R Hodges, Tengis S Pavlov, Alexander Staruschenko, Oleg Palygin. Knockout of Kcnj16 (Kir5.1) in Dahl Salt-Sensitive Rats Produces Seizure Phenotype. FASEB J., 2018

**Anna D Manis**, Gary C Mouradian Jr, Santiago Alvarez-Argote, Alexander Staruschenko, Oleg Palygin, Matthew R Hodges. Acute and Chronic Respiratory Effects from Repeated Audiogenic Seizures in SS<sup>Kcnj16-/-</sup> Rats. FASEB J., 2018
Oleg Palygin, Anna Manis, Vladislav Levchenko, Daria Zaika, Nicholas Burgraff, Aron Geurts, Matthew Hodges, Alexander Staruschenko. Kcnj10 (Kir 4.1) Knockout in Dahl SS Rats Determines the Expression of Kcnj10 and Kcnj16 Proteins in Brain and Kidney. FASEB J., 2018


**Awards and Honors:**

<table>
<thead>
<tr>
<th>Year</th>
<th>Award</th>
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<tr>
<td>2020</td>
<td>HTN New Investigator Travel Award for Hypertension 2020 Scientific Sessions</td>
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<td>2020</td>
<td>Finalist for APS Renal Section Predoctoral Excellence in Research Award EB2020</td>
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<td>2019</td>
<td>Awarded best trainee oral presentation at APS conference: Aldosterone and ENaC in Health and Disease: The Kidney and Beyond</td>
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<td>2019</td>
<td>MCW Graduate School Pre-Doctoral Travel Award</td>
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<td>2019</td>
<td>Awarded NIH F31 Predoctoral Fellowship</td>
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<td>2019</td>
<td>Selected as APS Graduate Student Ambassador 2019-2021 term</td>
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<tr>
<td>2019</td>
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<td>2019</td>
<td>APS Renal Section Research Recognition Award EB2019</td>
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<td>2019</td>
<td>Meritorious Research Travel Award; APS-Epithelial Transport Group; Experimental Biology</td>
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<td>2019</td>
<td>Respiration Section Trainee Poster Award, Experimental Biology</td>
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<td>2018</td>
<td>Advanced to PhD candidacy with highest scoring qualifying exam</td>
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<td>2018</td>
<td>MCW Graduate School Pre-Doctoral Travel Award 2018 Caroline Tum Suden APS Abstract Award, Experimental Biology</td>
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<td>2018</td>
<td>Cell and Molecular Section Chosen Speaker, Experimental Biology</td>
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<td>2018</td>
<td>Respiratory Section Chosen Speaker, Experimental Biology</td>
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<tr>
<td>2017</td>
<td>Acceptance into MCW’s Clinical and Translational PhD program</td>
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**Professional memberships, service, and other roles**

2020-present: American Heart Association Membership
2016-present: American Physiological Society Membership
2019-2021: Served as APS Graduate Student Ambassador
Feb 2021: Served as a judge at the USM Regional Science and Engineering Fair
2019-2020: MCW graduate school academic coach
April 2019: Volunteer science fair judge at Wauwatosa STEM Elementary School
Jan 2019: Participated in APS Professional Skills Training: Writing for Scientific Journals, Orlando FL
2018-2019: Committee for 2019 Student Choice Speaker Event, MCW Physiology Department
April 2018: Served as a judge for the Barbara A. Horwitz and John M. Horowitz Excellence in Undergraduate Research Awards
Research Presentations:

2021 Invited Postdoctoral Candidate Seminar- UCSF- Pearce Lab
“Knockout of Kcnj16 (Kir5.1) Produces Neurological, Respiratory, and Renal Dysfunction in the Dahl SS Rat”

2021 Invited Postdoctoral Candidate Seminar- Stanford University- Bhalla Lab
“Knockout of Kcnj16 (Kir5.1) Produces Neurological, Respiratory, and Renal Dysfunction in the Dahl SS Rat”

2021 MCW Graduate School Research Poster Session: Virtual Poster Presentation
“Role of Kir4.1 in the Regulation of Salt-Induced Hypertension”

2021 Experimental Biology: Oral and Poster Presentation
“Repeated Seizure Exposure in the SSKcnj16/- Rat Causes Progressive Respiratory Suppression and Associated Brainstem Pathology”

2020 Hypertension Scientific Sessions: Virtual Poster Presentation
“Role of Kir4.1 (Kcnj10) in the Regulation of Salt-Induced Hypertension”

2020 Experimental Biology: Poster Presentation (cancelled)
“Evidence of Progressive Brainstem Pathology after Repeated Seizure Exposure in a Novel Rat Model of SUDEP”

2020 Experimental Biology: Oral and Poster Presentation (cancelled)
“Role of Kir4.1 (Kcnj10) in the Regulation of Salt-Induced Hypertension”

2019 Pre-EB Meeting; Epithelial Transport Group: Oral Presentation
“Kir5.1 as a key regulator of neurological, respiratory, and renal functions”

2019 APS Renal Section Posters and Professors: Poster Presentation
“Kir5.1-Mediate Changes in Renin-Angiotensin-Aldosterone System Balance in Salt Sensitive Hypertension”

2019 Experimental Biology: Poster Presentation
“Kir5.1-Mediate Changes in Renin-Angiotensin-Aldosterone System Balance in Salt Sensitive Hypertension”

2018 MCW Cardiovascular Center Research Retreat: Poster Presentation
“Interaction of Kir5.1 with the renin-angiotensin-aldosterone system in electrolyte balance and blood pressure control”

2018 MCW Graduate School of Biomedical Sciences Poster Session: Poster Presentation
“Interaction of Kir5.1 with the renin-angiotensin-aldosterone system in electrolyte balance and blood pressure control”

2018 MCW Physiology Graduate Student Retreat: Oral Presentation
“Knockout of Kcnj16 (Kir5.1) produces Seizure Phenotype accompanied by Progressive Respiratory Dysregulation”

2018 Experimental Biology Cell and Molecular Section: Oral and Poster Presentation
“Knockout of Kcnj16 (Kir5.1) in Dahl Salt-Sensitive Rats Produces Seizure Phenotype”

2018 Experimental Biology Respiratory Section: Oral and Poster Presentation
“Acute and Chronic Respiratory Effects from Repeated Audiogenic Seizures in SSKcnj16/- Rats”

2018 MCW Graduate Student Association Symposium: Poster Presentation
“Knockout of Kcnj16 (Kir5.1) in Dahl Salt-Sensitive Rats Produces Seizure Phenotype”
2017 MCW Physiology Graduate Student Retreat: Oral Presentation
“Characterization of Behavioral Phenotype in Kcnj16/- Rat”

2017 MCW Physiology Department Seminar: Oral Presentation
“Characterization of Seizure Disorder Phenotype in Kcnj16/- Rat: A Structural Analysis of Brainstem Nuclei”

2016 MCW Physiology Department Seminar: Oral Presentation
“Angiotensin II Dosing Dilemma: in vivo vs. in vitro”

2011 UC Davis Undergraduate Research Conference: Oral Presentation
“Can Histamine Act on Multiple Hippocampal Regions to Prolong Hibernation Bout Duration?”

2011 Experimental Biology: Poster Presentation
“Cold enhances neuroprotection in hippocampal slices from hibernating syrian hamsters”

Funding

2019-present: NIH Predoctoral - F31 DK-122647
2017-2019: NIH Predoctoral - T32 HL 7852-21