In hypertension, reduced activation of Piezo1 channels attenuates acetylcholine release from the endothelium to cause female pudendal artery dysfunction

Hypertension is a common condition that negatively impacts sexual function in women by affecting the vascular health of genital tissues, impairing sexual arousal. The vascular endothelium plays a key role in this process. Endothelial cells release nitric oxide in response to acetylcholine (ACh), causing vasodilation and lowering blood pressure. However, the source of ACh for endothelial cells, which lack direct cholinergic innervation, is not fully understood, though it's known that endothelial cells can synthesize and release ACh in response to flow-induced calcium changes. A key mechanosensor in endothelial cells, Piezo1, responds to shear stress and is crucial for regulating vascular tone and blood pressure. Despite evidence that endothelial dysfunction contributes to female sexual dysfunction (FSD), the specific role of Piezo1 and non-neuronal ACh signaling in regulating blood flow to female genital tissue under hypertensive conditions remains unexplored. The central hypothesis of this research is that reduced Piezo1 activation by flow-mediated shear stress in hypertension leads to impaired synthesis and release of ACh from the endothelium. This diminished ACh release, acting in an autocrine or paracrine manner, results in attenuated endothelium-dependent relaxation of the pudendal artery (PA), reduced blood flow to the genital region, and contributes to FSD. The study has two main objectives:1. To determine the role of impaired Piezo1 activation and expression in endothelial dysfunction and reduced flow-mediated relaxation of the PA in hypertensive BPH/2J mice. 2. To investigate the role of impaired Piezo1-mediated shear stress responses in regulating ACh synthesis and release, and its contribution to flow-mediated dilation and PA dysfunction in hypertensive BPH/2J mice. This study will investigate PA dysfunction in female hypertensive BPH/2J mice for the first time. If successful, it will be the first to elucidate the relationship between Piezo1 and the synthesis and release of non-neuronal ACh in response to flow-mediated shear stress in PA, which may be involved in increasing blood flow to the genital region and improving female sexual function. Additionally, the study will explore how reductions in the expression or activity of Piezo1, as well as enzymes involved in the synthesis and release of ACh (such as ChAT and VAChT), are linked to hypertensive FSD. These findings could reveal new therapeutic targets for FSD in hypertension.

In hypertension, reduced activation of Piezo1 channels attenuates acetylcholine release from the endothelium to cause female pudendal artery dysfunction

1. Specific Aims and Hypotheses

Hypertension is a common condition that can negatively impact sexual function in women [1]. It affects vascular health, leading to structural and functional alterations in the vasculature supplying the clitoris and vagina. As result, sexual arousal is impaired [2]. The vascular endothelium plays a crucial role in maintaining vascular tone and regulating blood pressure. Interestingly, vascular endothelial cells respond to the neurotransmitter acetylcholine (ACh) by releasing nitric oxide (NO), leading to vasodilation and a decrease in blood pressure [3]. Since most arteries and endothelial cells do not have cholinergic innervation, the cellular source of ACh for these cells is not well understood [4,5]. However, numerous studies have demonstrated that the components necessary for the synthesis, release, and degradation of ACh are present in endothelial cells [6–12]. According to previous research, endothelial cells secrete non-neuronal ACh as an autocrine signal following Ca^{2+} responses triggered by flow [13]. Among the mechanosensors found in endothelial cells, Piezo1 is a major player, activated by shear stress and membrane stretch, and is essential for regulating vascular tone and blood pressure [14]. Despite evidence that endothelial dysfunction contributes to female sexual dysfunction (FSD), the specific role of Piezo1 and non-neuronal ACh signaling in regulating blood flow to female genital tissue under hypertensive conditions remains unexplored. Understanding this interplay could provide novel therapeutic targets for improving sexual health in hypertensive women. The overall objective of this proposal is to investigate the role of impaired Piezo1 activation and expression in endothelial dysfunction and reduced flow-mediated relaxation of the pudendal artery (PA) in hypertensive BPH/2J mice, focusing on how altered Piezo1-mediated shear stress responses affect ACh synthesis and release, and their contribution to vascular dysfunction and clitoral dysfunction. The central hypothesis is that reduced Piezo1 activation by flow-mediated shear stress in hypertension leads to impaired synthesis and release of ACh from the endothelium. This diminished ACh release, acting in an autocrine or paracrine manner, results in attenuated endothelium-dependent relaxation of the PA, reduced blood flow to the genital region, and contributes to FSD. The following aims will address this hypothesis:

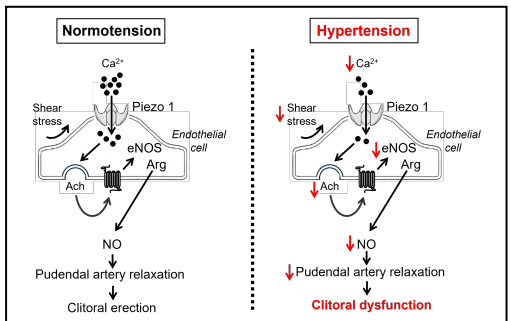

Figure 1: Clitoral dysfunction in hypertensive BPH/2J mice is associated with impaired Piezo1 function. The mechanism involved in this hypertensive FSD may be related to impaired Piezo1 function, which, when activated by shear stress, results in reduced synthesis and release of acetylcholine (ACh). This reduction in ACh leads to decreased relaxation of the pudendal artery (PA), contributing to FSD.

Specific aim 1 will test the hypothesis that impaired activation and expression of Piezo1 in the endothelium of hypertensive BPH/2J mice lead to endothelial dysfunction and reduced flow-mediated relaxation of the PA, contributing to vascular dysfunction in these mice.

Objective: To determine the role of impaired Piezo1 activation and expression in endothelial dysfunction and reduced flow-mediated relaxation of the PA in hypertensive BPH/2J mice.

Rationale: By investigating how impaired Piezo1 activation and expression affect endothelial function and vascular relaxation in hypertensive BPH/2J mice, this aim will provide critical insights into the mechanisms underlying vascular dysfunction and FSD in this model.

Specific aim 2 will test the hypothesis that impaired Piezo1-mediated shear stress responses in the endothelium of hypertensive BPH/2J mice lead to decreased synthesis and release of ACh, resulting in reduced flow-mediated dilation and endothelial dysfunction in the PA, contributing to vascular dysfunction and sexual dysfunction in these mice.

Objective: To investigate the role of impaired Piezo1-mediated shear stress responses in regulating ACh synthesis and release, and its contribution to flow-mediated dilation and PA dysfunction in hypertensive BPH/2J mice.

Rationale: By identifying these mechanisms, we can better understand the endothelial dysfunction associated with hypertension that impacts FSD and develop targeted therapies to restore normal blood flow to the genital region, improving sexual health.

2. Background & Preliminary Data

2.1 Background

Despite FSD having a high prevalence, estimated to range from 40% to 70%[15], and being characterized by disturbances in sexual desire, arousal, painful intercourse, and difficulty achieving orgasm[16], FSD remains less studied than the extensively researched male sexual dysfunction[1]. FSD is a multifactorial condition, with several underlying etiologies identified, including vasculogenic, neurogenic, hormonal/endocrine, and psychogenic factors[16].

Healthy genital vascularization in women is essential for vasodilation and increased blood flow, which are key to vaginal lubrication and clitoral erection, both critical components of female sexual arousal. The internal PA plays a crucial role in supplying blood to the clitoris and vagina. Inadequate blood supply to these areas contributes to the vasculogenic component of FSD[17,18]. Moreover, endothelial dysfunction can impair genital blood flow during phases of sexual function that require proper arterial perfusion, particularly sexual arousal[19]. While the link between cardiovascular disease (CVD) and FSD remains controversial, some evidence suggests a higher prevalence of FSD in women with CVD compared to the general population [19,20].

Endothelial cells lining the inner surface of blood vessels are continuously subjected to different mechanical forces[21]. As well, the endothelium is equipped with many mechanosensors that convert mechanical stimuli into intracellular biochemical signals, allowing it to modulate its own behavior or communicate with surrounding cells in response to mechanical stress [22]. Among the mechanosensors, Piezo1 emerges as a key player, being expressed in both endothelial and vascular smooth muscle cells. Piezo1 channels are activated by shear stress and stretch/membrane tension, and they play a significant role in regulating vascular tone and blood pressure [14]. The activation of the Piezo1 channel in endothelial cells triggers calcium influx through Piezo1, resulting in increased intracellular calcium levels. This calcium influx activates endothelial NO synthase, leading to the production of NO and promoting dilation [23]. In hypertension, disrupted laminar flow reduces shear stress[24], impairing Piezo 1 channel-mediated calcium influx leading to reduced eNOS activation. This diminishes NO release, contributing to endothelial dysfunction, vasoconstriction, and hypertension progression[25].

Interestingly, endothelial cells also respond to the neurotransmitter ACh, which leads to the release of NO and promotes vasodilation. However, the source of ACh for vascular endothelial cells remains unclear, as most arteries and endothelial cells lack direct cholinergic innervation [4,5]. Additionally, ACh is not expected to reach endothelial cells directly through the vessel wall or bloodstream. This is because autonomic nerves are typically located on the adventitial side of blood vessels, and ACh would need to pass through the basal lamina to reach the endothelial cell membrane where M3 muscarinic ACh receptor is located. Furthermore, circulating ACh in the bloodstream is rapidly inactivated by cholinesterase, making persistent activation of endothelial cells by ACh unlikely [5,11,26]. Endothelial cells, however, contain all the components necessary for the synthesis, release, and degradation of ACh. This suggests that, under certain conditions, these cells may produce endogenous ACh. This endogenous ACh could then activate receptors on adjacent endothelial cells, contributing to the regulation of local vascular tone [12]. Several studies have demonstrated the presence of ChAT [10,11], VAChT [12], choline transporter [27], and acetylcholinesterase [28,29] in endothelial cells, suggesting the existence of a non-neuronal cholinergic system in these cells [6–9].

A previous study has shown that endothelial cells release non-neuronal ACh as an autocrine signaling molecule in response to flow-mediated Ca^{2+} responses, revealing a novel function for this classical neurotransmitter in endothelial mechanotransduction. However, the potential involvement of the cholinergic system in the increased blood flow is still under discussion [13]. Moreover, the mechanisms by which ACh release is activated by changes in

flow (shear stress) remain unclear. We propose that Piezo1, the principal mechanosensor of shear stress in endothelial cells, is activated by flow-mediated shear stress on the endothelium. This activation can lead to an increase in intracellular calcium, which stimulates the production and release of ACh. The released ACh can act in an autocrine or paracrine manner, inducing flow-mediated vascular dilation.

In this project, we plan to investigate the role of impaired Piezo1 activation in endothelial dysfunction and its contribution to FSD in hypertensive BPH/2J mice. Specifically, we will explore how disrupted Piezo1-mediated shear stress responses affect ACh synthesis and release, leading to reduced flow-mediated dilation and vascular dysfunction in the PA. Our hypothesis is that reduced Piezo1 activation by flow-mediated shear stress in hypertension leads to impaired synthesis and release of ACh from the endothelium. This diminished ACh release results in attenuated endothelium-dependent relaxation of the PA, reduced blood flow to the genital region, and contributes to FSD. By understanding these mechanisms, the project seeks to uncover potential targets for therapies that can restore normal blood flow to the genital region and improve sexual health in women with hypertension.

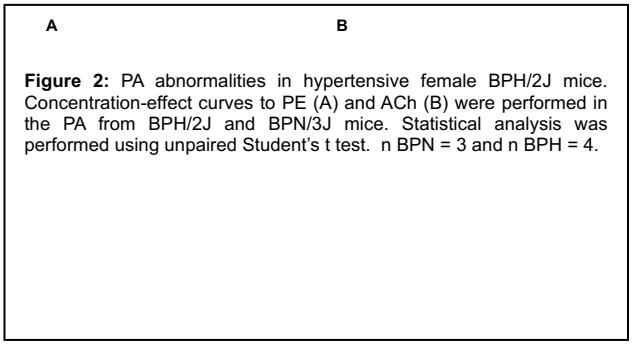

Figure 2: PA abnormalities in hypertensive female BPH/2J mice. Concentration-effect curves to PE (A) and ACh (B) were performed in the PA from BPH/2J and BPN/3J mice. Statistical analysis was performed using unpaired Student's t test. n BPN = 3 and n BPH = 4.

Figure 3: Yoda2-induced relaxation was reduced in PA from hypertensive female BPH/2J mice. Statistical analysis was performed using unpaired Student's t test. n BPN = 3 and n BPH = 4.

2.2 Preliminary Data

Phenylephrine (PE)-induced contraction was reduced in the PA of female hypertensive mice (BPH/2J), while ACh-induced relaxation was not significantly different between strains, although it trended toward being reduced in hypertensive mice (Figure 2). Piezo1 function is impaired in female PA from BPH/2J, leading to reduced relaxation when this channel is activated by Yoda2, a novel Piezo1 activator (Figure 3). Our group reports, for the first time, the co-localization of Piezo1 and VAChT in the endothelial cells of the aorta (Figure 4). This finding suggests a potential interaction between these two components within these cells. Further studies will be performed in female PA to investigate this interaction. Relaxations induced by Yoda2 were partially inhibited by acetylethylcholine mustard, an inhibitor of ChAT in PA from C57BL/6 mice (Figure 5). This suggests that ACh, produced as a result of ChAT activity, may contribute to some of the relaxation effects induced by Piezo1 stimulation through Yoda2.

Figure 4: Immunofluorescence representative image of Piezo1 and VAChT expression in aorta from C57BL/6 mice.

Figure 5: Inhibition of choline acetyltransferase (ChAT) by acetylethylcholine mustard attenuates the vascular relaxation induced by Yoda2 in PA from C57BL/6 mice. Statistical analysis was performed using an unpaired Student's t-test. n=4

3. Experimental Design

Specific aim 1 will test the hypothesis that impaired activation and expression of Piezo1 in the endothelium of hypertensive BPH/2J mice lead to endothelial dysfunction and reduced flow-mediated relaxation of the PA, contributing to vascular dysfunction in these mice. *Methods:* **1)** To evaluate PA dysfunction, concentration-response curves to different contractile and relaxant drugs will be performed in PA isolated from BPH/2J and BPN/3J mice using a wire myograph. **2)** To evaluate flow-mediated dilation, we will use a pressure myograph to analyze changes in arterial diameter in PA from BPH/2J and BPN/3J

mice in response to alterations in flow conditions. **3)** To measure the expression of Piezo1 in endothelial cells from hypertensive female BPH/2J mice, we will use Western blotting, immunofluorescence, and RT-qPCR. **4)** To evaluate Piezo1 function in the PA, concentration-response curves to the Piezo1 activator will be performed using tissues isolated female from BPH/2J and BPN/3J mice.
Anticipated results: Based on preliminary data, we expect to observe vascular and Piezo1 dysfunction in the PA of female hypertensive mice. Additionally, we anticipate reduced flow-mediated dilation and lower expression of Piezo1 in the PA of these mice.
Potential problems/alternative approaches: Yoda2's actions might be confounded by off-target effects. To verify the specificity of drug action, we can use additional approaches, such as employing other Piezo1-specific agonists (e.g., Yoda1) or antagonists (e.g., Dooku1) to validate our findings.
Specific aim 2 will test the hypothesis that impaired Piezo1-mediated shear stress responses in the endothelium of hypertensive mice lead to decreased synthesis and release of ACh, resulting in reduced flow-mediated dilation and endothelial dysfunction in the PA, contributing to vascular dysfunction and sexual dysfunction in these mice. *Methods:* **1)** To compare the expression of ChAT and VAChT in endothelial cells from PA of hypertensive and normotensive mice, we will perform Western blotting, immunofluorescence, and RT-qPCR. **2)** To evaluate flow-mediated dilation in arteries from hypertensive and normotensive mice in the presence of pharmacological inhibitors of Piezo1, type 3 muscarinic receptor, ChAT and VAChT, using a pressure myograph. **3)** To measure ACh levels using ELISA in arterial rings from hypertensive and normotensive mice exposed to shear stress with a pressure myograph. **4)** To confirm Piezo1's role in regulating ACh release under shear stress, we will knockdown Piezo1 in cultured endothelial cells using Piezo1 siRNA. Shear stress in these cells will be induced using a FlexCell Streamer Device, and ACh levels will be measured using ELISA. Additionally, we will utilize endothelium-specific Piezo1 knockout (KO) mice to evaluate flow-mediated dilation in arteries from both control and Piezo1 KO mice using a pressure myograph and measure ACh levels in these arterial rings exposed to shear stress.
Anticipated results: We expect to find reduced expression of ChAT and VAChT in endothelial cells from PA of hypertensive mice. Similarly, we anticipate lower levels of ACh when Piezo1 is activated by shear stress in arterial rings from hypertensive mice.
Potential problems/alternative approaches: One potential limitation is the lack of a selective inhibitor for Piezo1. To address this, we will use Piezo1 siRNA in endothelial cells and endothelium-specific Piezo1 KO mice. In addition, if our hypothesis is incorrect, we will explore the role of other shear stress-responsive channels in the synthesis and release of ACh in endothelial cells, such as TRPV4 channels, which are involved in inducing vasodilation.
Statistical design and data analysis: Results will be presented as mean ± SEM. Standard statistical procedures, including paired and unpaired Student's t-tests, analysis of variance (ANOVA), and non-linear regression, will be performed using GraphPad Prism. In all cases, p-values less than 0.05 will be considered statistically significant. The Bonferroni correction will be applied for multiple comparisons among groups.

4. Significance

This study will investigate PA dysfunction in female hypertensive BPH/2J mice for the first time. If successful, it will be the first to elucidate the relationship between Piezo1 and the synthesis and release of non-neuronal ACh in response to flow-mediated shear stress in PA, which may be involved in increasing blood flow to the genital region and improving female sexual function. Additionally, the study will explore how reductions in the expression or activity of Piezo1, ChAT, and/or VAChT are linked to hypertensive FSD. These findings could reveal new therapeutic targets for FSD in hypertension.

5. Timeline

The proposed study is estimated to take two years to complete. The two aims of the project will be carried out over this 2-year period.

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Authored by: Raiana dos Anjos Moraes, PhD
Position: Postdoctoral Fellow
Institution: University of South Carolina School of Medicine
Mentors: Fernanda Priviero, PhD, and R. Clinton Webb, PhD

Diversity, Equity, and Inclusion (DEI) in women's sexual health

My project directly addresses issues of Diversity, Equity, and Inclusion (DEI) in women's sexual health by focusing on the intersection of underrepresented groups in science, particularly in the context of female sexual dysfunction. As a Latin woman from Brazil, I belong to a group that is historically underrepresented in scientific research. Through the ISSWSH Research Grant, this project aims not only to contribute valuable insights into female sexual dysfunction but also to promote greater inclusivity and equity in the field of women's sexual health. Furthermore, my research fosters DEI by providing an opportunity for the development and training of underrepresented individuals in the field of sexual health research. As a woman from an underrepresented background in science, I am committed to serving as a role model for other Latin and minority women pursuing careers in research. By advancing my own career through this research, I hope to inspire and mentor others from similar backgrounds to pursue research in women's health, ultimately contributing to a more diverse and inclusive scientific community. It is important to note that female sexual dysfunction remains significantly less studied than its male counterpart, despite sexual health being a fundamental aspect of well-being for both men and women. This disparity in research creates a gap in our understanding of the physiopathological mechanisms that may be altered in female sexual function. As a woman scientist from an underrepresented background, I am uniquely positioned to contribute to this field, where female voices and perspectives are still limited. My research will expand the investigation into these mechanisms, helping to identify novel therapeutic targets and ultimately improving sexual health care for women. The support of the ISSWSH Research Grant would not only advance this crucial research but also further my career, allowing me to contribute meaningfully to the improvement of sexual health outcomes for women worldwide.

CAREER DEVELOPMENT PLAN

Prior research experience

My academic training in cardiovascular physiology, pharmacology, and sexual dysfunction, during both my Master's and Ph.D. studies, has provided me with a strong background in vascular smooth muscle and endothelial function in health and disease. I was mentored by Dr. Darizy Flavia Silva in Brazil. Additionally, I worked as a Research Associate at the University of South Carolina, studying the roles of TRPM8 and TRPA1 channels in female and male pudendal artery function and dysfunction under the supervision of Dr. Clinton Webb.

Research in Progress in the Sponsor's Laboratory

Ongoing research is focused on understanding the cell signaling mechanisms underlying smooth muscle dysfunction, endothelial dysfunction, and erectile and clitoral dysfunction in diabetic and hypertensive conditions. Experimental approaches include in vivo and in situ measures of sexual function, pharmacology, standard muscle bath procedures, organ perfusion, cell culture, instillation of antibodies and siRNA, transgenic mouse models, protein and mRNA expression, cell imaging, measurement of enzyme activities, ion flux measurements, and radiotelemetry devices for blood pressure monitoring.

Plans to develop the Applicant's research independence

I will have complete responsibility for conducting this research. I will work closely with Dr. Clinton Webb, interacting daily to discuss experimental design, data analysis, and the scientific interpretation of the proposed work. In Dr. Webb's laboratory, I will develop this project alongside expert investigators in this field, including Dr. Fernanda Priviero.
During my postdoctoral training, my goals are to publish peer-reviewed manuscripts, present abstracts at regional, national, and international conferences, and enhance my research and presentation skills. I also aim to develop research collaborations and professional connections. I will participate weekly in research meetings and seminars within the Department of Cell Biology and Anatomy and the Cardiovascular Translational Research Center. Furthermore, I will meet monthly with the department chair to discuss career objectives and other aspects of my training at the University of South Carolina.

Development of technical skills

I will have the opportunity to learn new technical skills, including breeding tissue-specific knockout animals, small interfering RNAs (siRNAs) knockdown, PCR and using our recently acquired streamer shear stress device for cell culture. Additionally, our core facility provides training with experts on all available equipment, allowing me to fully utilize the resources and capabilities offered by the core facility.

Relationship of the Research Training Plan to the Applicant's Career Goals

My long-term professional aspiration is to become an independent investigator in an academic setting. Through this research, I aim to advance my career and inspire and mentor others to pursue research in women's health. It is important to note that female sexual dysfunction remains significantly less studied than its male counterpart, despite sexual health being a fundamental aspect of well-being for both men and women. This disparity in research creates a gap in our understanding of the physiological and pathological mechanisms that may be altered in female sexual function. As a woman scientist, I am committed to contributing to this field, where female voices and perspectives are still limited.

Assessment of the Applicant

By the end of each year, I will be expected to attend at least two scientific meetings and submit at least one original research paper or review article as the first author. Additionally, I will mentor one student, if available, and submit at least one grant proposal. These objectives will help ensure my progress and contribution to the field while advancing my career goals.

BUDGET

Award Term:
July 1, 2025 – June 30, 2026

Award Amount:
1 grant of up to $7,500.00

DIRECT COSTS

Period 1:

Stipend: $3,750.00 for research supplies (antibodies, reagents, chemicals, and general lab supplies).

Period 2:

Stipend: $3,750.00 for research supplies (antibodies, reagents, chemicals, and general lab supplies).

Total cost (2 periods): $7,500.00

SCOPE AND SOURCE OF FUNDS AVAILABLE TO THIS PROJECT

The project will be supported by multiple funding sources:

1. Institute for Cardiovascular Research: Intramural funding from the Office of the Vice President for Research, University of South Carolina (2023-2027).
2. NIH RO1 DK-132948 Multi-PI Grant (2022-2026).

Verified,

R. Clinton Webb, Ph.D.
Director, Institute on Cardiovascular Disease Research
Director, Cardiovascular Translational Research Center
Professor, Department of Cell Biology and Anatomy

SmartState Endowed Chair in Biofabrication
Department of Biomedical Engineering
Molinaroli College of Engineering and Computing