Distribution Agreement

In presenting this thesis as a partial fulfillment of the requirements for a degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis in whole or in part in all forms of media, now or hereafter now, including display on the World Wide Web. I understand that I may select some access restrictions as part of the online submission of this thesis. I retain all ownership rights to the copyright of the thesis. I also retain the right to use in future works (such as articles or books) all or part of this thesis.

Heeju Jasmine Lim April 12, 2022

Sex Differences on N-methyl-D-aspartate Receptor Expression along the Rodent Nephron

By

Heeju Jasmine Lim

Cesar Romero, M.D., Ph.D.
Adviser

Department of Biology

Cesar Romero, M.D., Ph.D.
Adviser

Kate O'Toole, Ph.D.

Committee Member

Jose Soria, Ph.D.

Committee Member

Kevin Corrigan, Ph.D.

Committee Member

2022

Sex Differences on N-methyl-D-aspartate Receptor Expression along the Rodent Nephron

By

Heeju Jasmine Lim

Cesar Romero, M.D., Ph.D.
Adviser

An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Department of Biology

2022

Abstract

Sex Differences on N-methyl-D-aspartate Receptor Expression along the Rodent Nephron

By Heeju Jasmine Lim

Background: N-methyl-D-aspartate (NMDA) receptors are calcium channels gated by glutamate and glycine. In the kidneys, epithelial NMDA receptors (NMDAr) induce renal vasodilation. Previous data suggest that estrogen supplementation increases renal vasodilation induced by amino acids in aged rats. However, the mechanism of this increase is not known. Additionally, there is no data showing if there is a sex difference in NMDAr expression in kidney. We hypothesized that there is a sex difference in renal NMDAr expression and that it is dependent on Estrogen.

Methods: We evaluated the renal expression of NMDAr type 1 in mouse kidney epithelial cells by immunohistochemistry and immunofluorescence, and in total kidney by Western blot. We also evaluated the expression of NMDAr on a continuous cultured epithelial cell line with characteristics of renal collecting duct principal cells (mpkCCD), with and without the presence of estradiol (50nM) in the media for 24 hours.

Results: NMDAr were found to be present along the nephron, in both cortical and medullar regions, with higher expression on the juxtamedullary and medullary regions. Under confocal microscopy, we confirmed the expression of NMDAr in AQP2 positive cells. Western blot analysis showed an increased expression of NMDAr type 1 in female mouse kidney in comparison with males (3.34±1.6 vs. 0.95±0.3 AU, p=0.01). Twenty-four-hour mpkCCD cell incubation with estrogen increases NMDAr expression (2.3±1.3 vs. 0.9 ±0.58 AU, p=0.05).

Conclusion: There is a sex difference in kidney NMDAr expression which is related to the presence of estrogen. Sex difference in Kidney NMDAr may explain the differences in renal physiologies and disease susceptibilities between males and females.

Sex Differences on N-methyl-D-aspartate Receptor Expression along the Rodent Nephron

By

Heeju Jasmine Lim

Cesar Romero, M.D., Ph.D.
Adviser

A thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
of the requirements of the degree of
Bachelor of Science with Honors

Department of Biology

2022

Acknowledgements:

I would first like to thank and acknowledge my adviser, Dr. Cesar Romero for his unwavering support and guidance throughout my thesis process and for providing wonderful opportunities and a welcoming space in which to learn and grow. I would also like to thank Truyen D. Pham and Delaney C, Abood of the Wall lab, Auriel Mossey of the Eaton lab, and the Sands-Klein lab for their technical support and advice. I would like to thank my thesis committee, Dr. Kate O'Toole, Dr. Jose Soria, and Dr. Kevin Corrigan for their time and energy in providing helpful feedback and creating a community of support throughout this process. Lastly, I give my greatest thanks to my friends and my family: my father Insik Lim, my mother Miyeun Jung, and my younger sister Heeeun Lim for their constant love and support throughout my life.

Table of Contents:

Index of Figures and Tables:

Chapter 1: Introduction

Hypertension or high blood pressure (BP) is a global cause of myocardial infarction, heart failure, stroke, and the need of hemodialysis. The kidney is vital in the maintenance of the water-electrolyte balance as it deals with body fluid volume which influences the blood pressure. Thus, sodium chloride retention is associated with increased blood pressure. Vasodilation mechanisms in the kidney eliminate sodium chloride, water, and other solutes. For instance, when renal vasodilation is impaired in the kidney, patients tend to develop hypertension, such as in cases of renal arterial stenosis or kidney vasculitis[1]. At present, the direct causes of hypertension are still unknown in more than 90% of the cases[2]. In women, hypertension is less prevalent before menopause, and it becomes dramatically more prevalent post-menopause[3]. This phenomenon is not well understood, but a role for the sexual hormone has been proposed[4].

N-methyl-D-aspartate (NMDA) receptors are calcium channels that activate via glutamate and glycine binding. The NMDAr are non-specific cation channel that allow Ca^{2+} (and Na^{2+}) into the cell[5]. NMDAr have three subunits: GluN1, GluN2, and GluN3. We know thus far that NMDA receptors are tetramers where two subunits must be GluN1[5]. GluN2 is subclassified in a, b, c, and d according to kinetic properties. GlutN3 is subclassified in a and b. It has been shown that NMDAr are expressed in the kidney, but with limited information about its localization. NMDAr play a role in hemodynamics and BP regulation by inducing renal vasodilation[6] dependent on an autoregulatory mechanism occurring in the connecting duct called connecting tubule-glomerular feedback (CNTGF)[7]. CNTGF is part of the autoregulatory kidney mechanisms that integrate tubular and vascular functions. When high sodium is detected in the CNT by the epithelial sodium channel (ENaC), the epithelial cells of the CNT release prostaglandins and epoxy-acids which act locally as active vasodilators over the afferent arteriole. The consequence of that vasodilation is

the increase in the glomerular blood flow and glomerular filtration, which generate more sodium-chloride filtration (tubular load), induce the excretion of salt and water, and decrease blood pressure [8]. Preliminary data have shown that the inhibition of CNTGF may induce hypertension[9].

We believe that the loss of renal vasodilation is associated with an impairment of sodium excretion and consequently an increase in BP[7]. Thus, changes in NMDAr expression may increase or decrease CNTGF in the kidney and induce change in BP. It is not known whether there is a sex difference in NMDAr expression and whether this sex difference explains the low hypertension prevalence in young women and the loss of protection from hypertension in the postmenopausal period.

Sex-specific hormones have been found to have different effects on renal function. It has been found that estrogen, the female-specific sex hormone, has protective effects against hypertension [10]. In females, the loss of estrogen (oophorectomy or menopause) is associated with loss of the renal functional reserve and hypertension[11]. However, the mechanism associated with menopausal hypertension is unknown. Additionally, the role of estrogen on kidney NMDAr is not known. Our main hypothesis is that there is a sex difference in renal NMDAr expression and regulation, especially in the connecting duct that is dependent on estrogen which may explain those differences. The goal of this thesis is to identify the localization of the obligate subunit GluN1, to observe whether there is a sex difference in NMDAr expression and determine whether estrogen plays a role in the said sex difference. Identifying the localization and expression of the different subunits along the nephron would enable further study of the role that each play in renal function and sex differences. Comparing the differences in sex- specific hormones and their interactions with NMDAr would be beneficial in creating sex-specific therapies for hypertension.

Chapter 2: Localization of NMDAr Along the Nephron

Nephron Anatomy

The kidneys play an important role in the filtration of blood. It removes waste and toxins from the bloodstream through urine. The necessary nutrients are returned to the bloodstream. The nephron is the smallest functional unit of the kidney. There are on average approximately 900,000 to 1 million nephrons in each human kidney [12]. As shown in Figures 1 and 2, each nephron consists of the Bowman's capsule which envelops the glomerulus, the proximal convoluted tubule, loop of Henle, distal convoluted tubule, and the connecting tubule which empties into the collecting ducts. The glomerulus is a bed of capillaries responsible for the filtration of blood. It returns necessary nutrients back into the blood and removes the waste [13]. The proximal convoluted tubule (PCT) leads to the Loop of Henle where extracellular fluid is regulated [14]. The Loop of Henle leads to the distal convoluted tubule (DCT) which leads to the connecting tubule (CNT) and the cortical collecting duct (CCD). The CNT is of particular interest in this paper because Connecting tubular glomerular feedback (CNTGF), a vasodilator mechanism, is anatomically localized in this region.

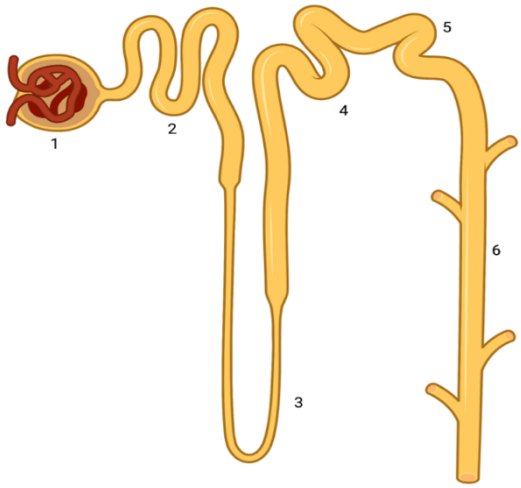


Figure 1. One unit of the nephron. (1) glomerulus / Bowman's capsule, (2) proximal convoluted tubule (PCT), (3) loop of Henle, (4) distal convoluted tubule (DCT), (5) connecting tubule (CNT), (6) cortical collecting duct. Image created from BioRender.

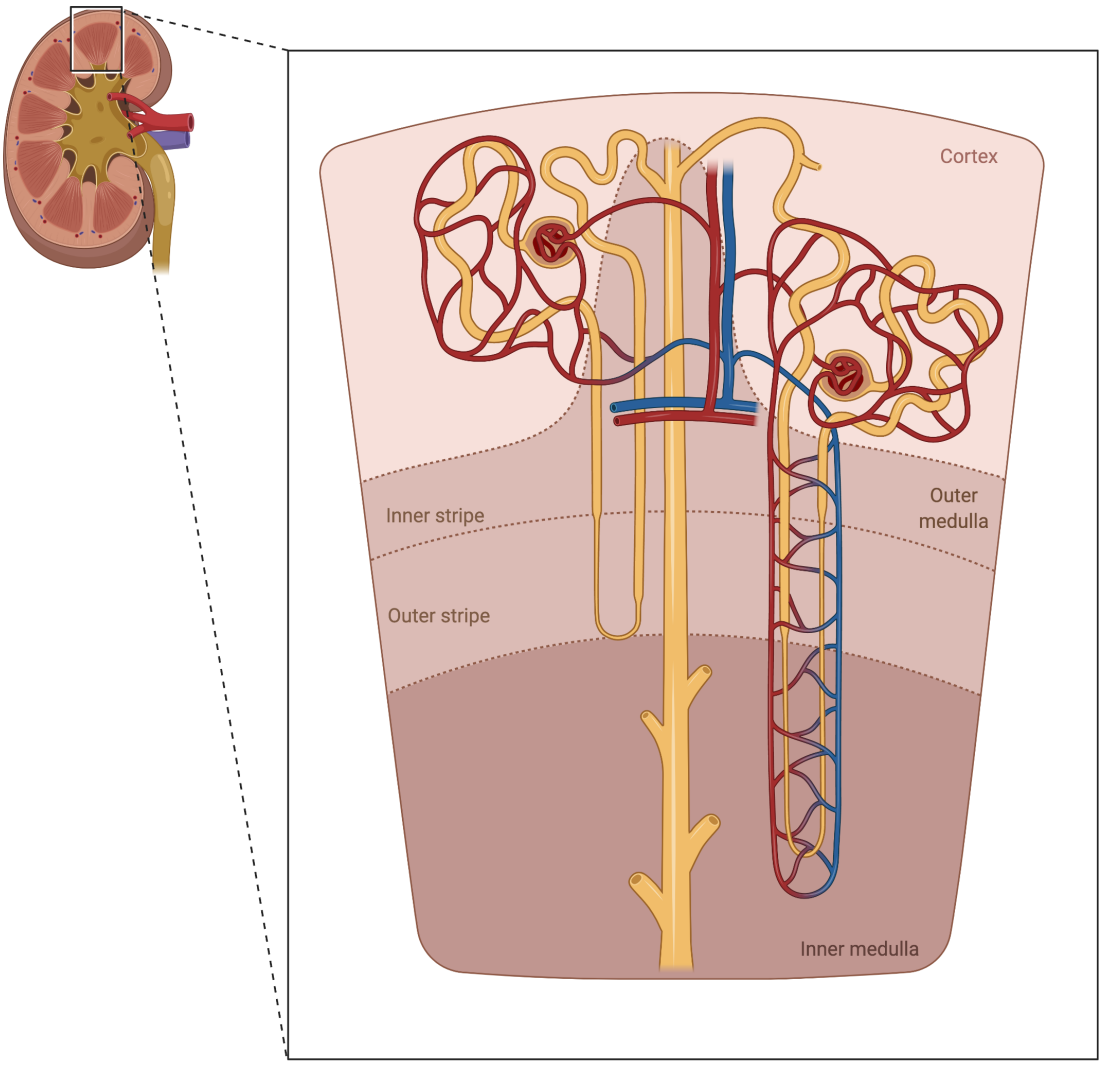


Figure 2. Two nephrons depicted within a cross-section of the kidney. The glomeruli are in the renal cortex while the loop of Henle and collecting ducts dip down into the renal medulla. *Image created from BioRender.*

NMDAr Structure and Function

NMDAr are transmembrane proteins that have four subunits. The functional NMDAr is comprised of two obligate GluN1 (NR1 or *grin1*) subunits. For the NMDAr to be functional, the other two subunits must consist of either two GluN2 (NR2 or *Grin2*) subunits or one GluN2 and one GluN3 (NR3 or *Grin3*) subunits [15]. As shown in Figure 3, upon glutamate and glycine binding to their respective sites, the receptor activates and brings calcium ions into the cell. NMDAr are expressed along the nephron. Our goal was to confirm the general expression of NMDAr as well as identify the localization along specific parts of the nephron.

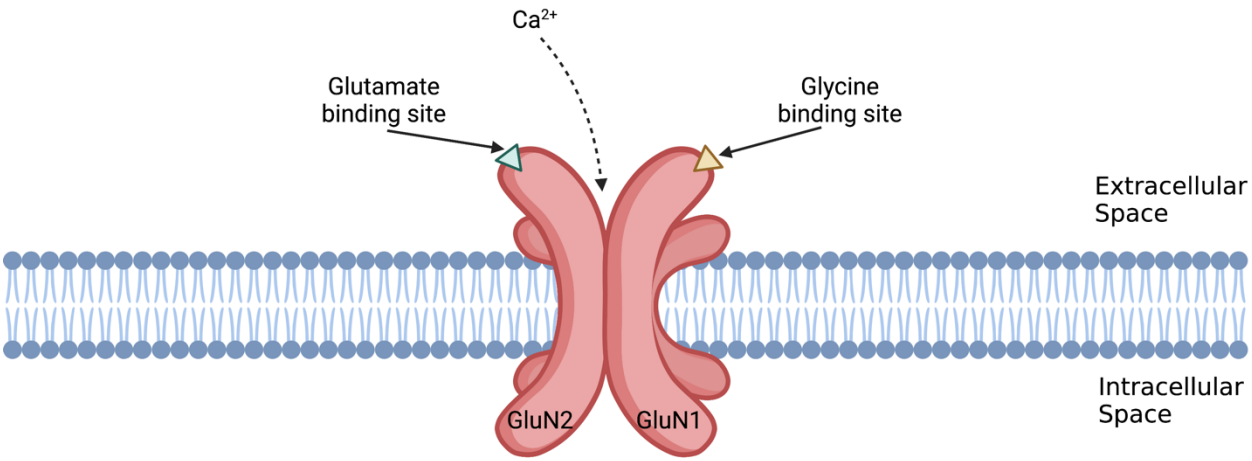


Figure 3. Model of NMDAr's general structure across a phospholipid bilayer. The tetramer is comprised of two GluN1 subunits and two GluN2 subunits or one GluN2 and one GluN3 subunits. *Image created from BioRender.*

Localization of NMDAr

Observing the colocalizations of the protein of interest (NMDAr) to other known proteins would indicate the localization of NMDAr. ENaC has been known to be expressed in the CNT and CCD and has been found to be expressed in the DCT2 [16]. The subunit ENaCα is obligate and therefore is present in every known localization of ENaC. The colocalization of NMDAr and ENaC would confirm the presence of NMDAr in specific parts of the nephron. Aquaporins (AQP) are membrane proteins that aid in the transfer of water and small molecules. Aquaporin 2 (AQP2) is an isoform that is an antidiuretic hormone (ADH)-regulated water channel. AQP2 plays an important role in water reabsorption in the kidney. It has been found to be expressed in the CNT and CCD of the nephron [17]. In the same logic as the colocalization of ENaC, the colocalization of NMDAr with AQP2 would confirm the presence of NMDAr in the CNT and CCD.

The localization of NMDAr is important to allow insight into the areas to target in studying their role in blood pressure regulation. It would also enable further studies in mechanisms through other known proteins such as the ENaC. There are two known renal blood flow regulation mechanisms which take place in the macula densa (MD) and the CNT. The connecting tubule is the final sodium chloride balance regulation point of the nephron [7]. The CNTGF is a positive feedback mechanism that increases sodium excretion [7]. The presence of this glomerular feedback and its known roles with ENaC makes the CNT a target region of which to study NMDAr expression.

Methods

RNA expression in micro dissected tubule segments: To explore the RNA expression along the different tubule segments of the nephron we used the NHI-web tool resource where full-length

RNA-seq transcriptomes of 14 micro dissected segments of mice kidney tubule have been provided [18]. The online tool has been validated and compared with previous data bases and protein expression [18]. We explored RNA expression on each different tubule sub segments of the *Grin1, Grin 2a, Grin2b, Grin2c, Grin2d, Grin3a* and *Grin3b* genes for each NMDAr subunits. RNA-seq read are expressed as logarithmic transformed transcripts per million (TPM). https://esbl.nhlbi.nih.gov/MRECA/Nephron/

Mouse Kidney Retrieval: We harvested kidney samples from 9–12-week mice under normal sodium diet. Under gaseous anesthesia, kidneys were perfused with a mixture of saline and heparin (anticoagulant). An incision in the right atrium was made as to not burst the heart. 4% formalin was used to fix the tissue and was injected through the left ventricle. The kidneys were harvested without the renal capsule and later processed according to specific protocols. The harvested kidney was sent to be cut and fixed onto glass slides by the Winship Pathology Lab.

Immunohistochemistry (IHC): To identify the localization of NMDAr, we conducted IHC experiments to observe glass slides via microscopy. The primary antibody binds to the antigen of interest (NMDAr). The secondary antibody binds to the primary and is attached to a signal amplification system (Peroxidase). The diaminobenzidine (DAB) turns the signals into brown staining. For the primary antibody on the IHC experiments, we have found that the best final concentration of the NMDAr type 1 receptor (StressMarq, S308-48) is 1/5000. For the secondary antibody, we used a horse anti-mouse attached to Avidin/Biotin Systems, Mouse on Mouse (M.O.M.) Systems Immunodetection Kit (Vector, BMK-2202). Hematoxylin nucleus counterstaining was used as well as DAB.

Immunofluorescence (IF): IF experiments were conducted to observe colocalization of NMDAr with ENaC and AQP2. Three antigens were detected: NMDAr, AQP2 and ENaC. Trivial signaling

was blocked by 1% bovine serum albumin (BSA) to specify the signals made by the NMDAr and AQP2. For IF, we determined that the best primary antibody concentration was 1/50 (StressMarq, S308-48) and the secondary antibody, goat-anti mouse, attached to Alexafluor 640 fluorophore (Sigma) was 1/400. AQP2 primary antibody concentration was 1/250 and secondary concentration was 1/200. ENaC primary antibody concentration was 1/400 and secondary concentration was 1/200.

Frozen kidney tissue was fixed with acetone and blocked with albumin 1%. The samples were incubated overnight at 4ºC with the primary antibody. Secondary antibody, attached with a fluorophore was incubated with the sample for two hours at room temperature. After washing, nuclear staining was performed using DAPI. The images were observed through Zeiss confocal microscope and the Zen software.

Results

NMDAr RNA expression: RNA expression of NMDAr was found in most of the tubule segments. Figure 4 and Table 1 show the RNA expression of the *Grin1* gene, which codes for the GlutN1 subunits. Except for the downstream section of the PT (PTS2-3) and the medullary section of the thick ascending limb of Henle (MTAL), NMDAr type 1 is expressed in all the subsegments of the tubules. There is higher expression in the region localized in the juxtamedullary and medullary regions. Figure 5 shows a heat map with the relative expression of the other NMDAr subunits. Most of the subunits are expressed in the medullary region of the kidneys. In the cortex, the first segments of the proximal tubules (PTS1) have various expressions of all the subunits except for the *Grin2c*. In the CNT and CCD, our regions of interest, only *Grin2d* and trace amounts of *Grin3a* and *b* are expressed.

Table 1. *Grin* gene expression along the 14 renal segments of the nephron

Gene_symbol	PTS1	PTS2	PTS3	DTL1	DTL2	DTL3	ATL	MTAL	CTAL	DCT	CNT	CCD	OMCD	IMCD
Grin1	0.4	0	0	5.9	2.2	3.6	2.9	0	0.1	0.1	0.1	0.2	2.3	0.6
Grin2a	0.2	0	0	3.4	0.6	0.7	0.6	0	0	0	0	0	0.4	0.1
Grin2b	0.3	0	0	2.5	2.3	1.5	1.6	0	0.1	0	0	0	1.2	0.7
Grin2c	0	0	0	0.1	0	0.1	0.3	0	0.1	2	0	0	0	0
Grin2d	0.2	0	0	0.6	0.3	0.3	0.2	0.9	0.4	0.2	0.2	0.3	0.8	0.5
Grin3a	0.4	0	0	5.5	2	2	4.5	0	0.1	4	0.1	0.1	1.6	1.1
Grin3b	0.9	1.6	1	1.6	0.7	1.1	0.8	0.2	0.1	0	0.1	0.1	0.6	0.6

Proximal Tubule Segment 1,2, 3 (PTS1,2,3), Descending Thin Limb 1,2,3 (DTL 1,2,3), Ascending Thin Limb (ATL), Medullar Thick Ascending Limb (MTAL), Cortical Thick Ascending Limb (CTAL), Distal Convoluted Tubule (DCT), Connecting Tubule (CNT), Cortical Collecting Duct (CCD), Outer Medullary Collecting Duct (OMCD), Inner Medullary Collecting Duct (IMCD).

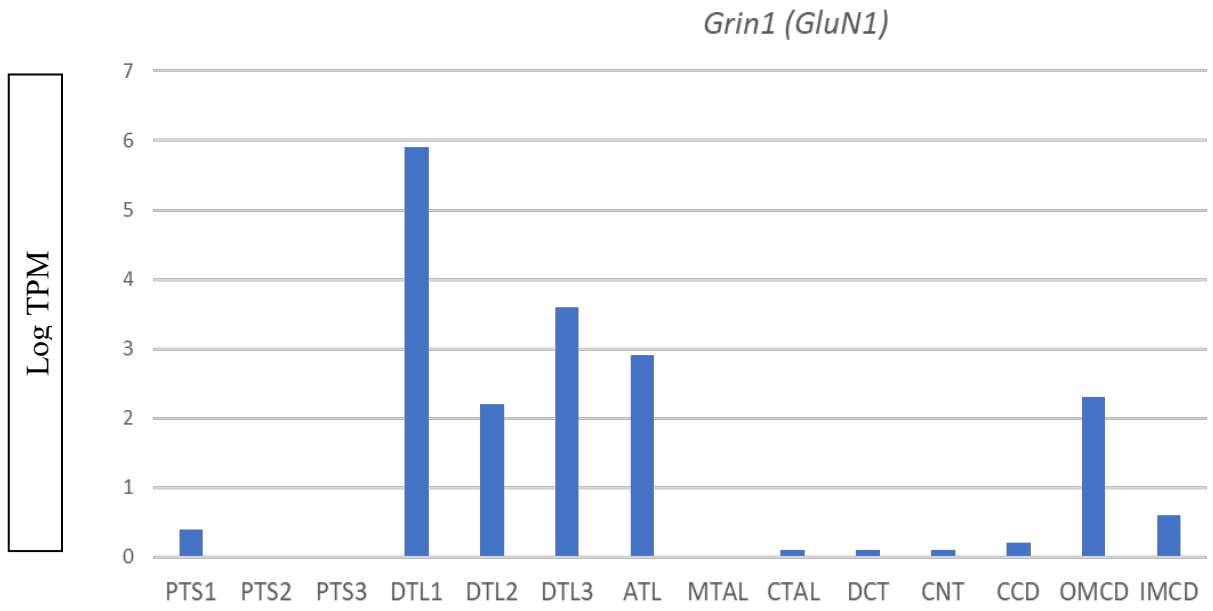


Figure 4. *Grin1* expression along the 14 renal segments of the nephron. The higher NMDAr type 1 receptor expression is found in the medullary regions of the nephrons.

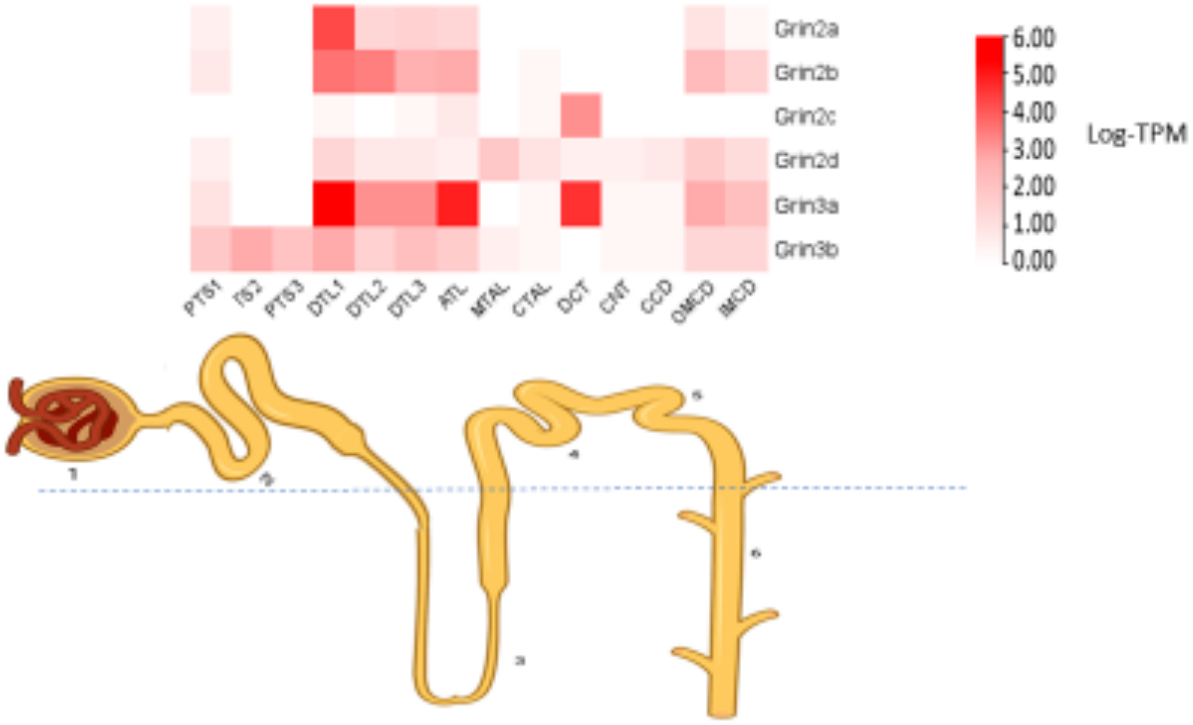


Figure 5. RNA expression of NMDAr subunits in 14 renal segments of the nephron. Higher expression of NMDAr subunits is found in the medullary region. *Grin2D* is detected in the CNT/CCD region.

NMDAr protein expression: The IHC shows that there is a general expression of NMDAr in the total kidney. As shown in Figure 6, the brown staining, indicative of presence of NMDAr type 1 (GlutN1), is shown on the cortex and the medulla. The staining is the most prevalent in the transitional juxtamedullary regions and outer medulla of the kidney as was predicted with the RNA expression. Figure 7 shows the expression in the cortex and medulla in higher magnification.

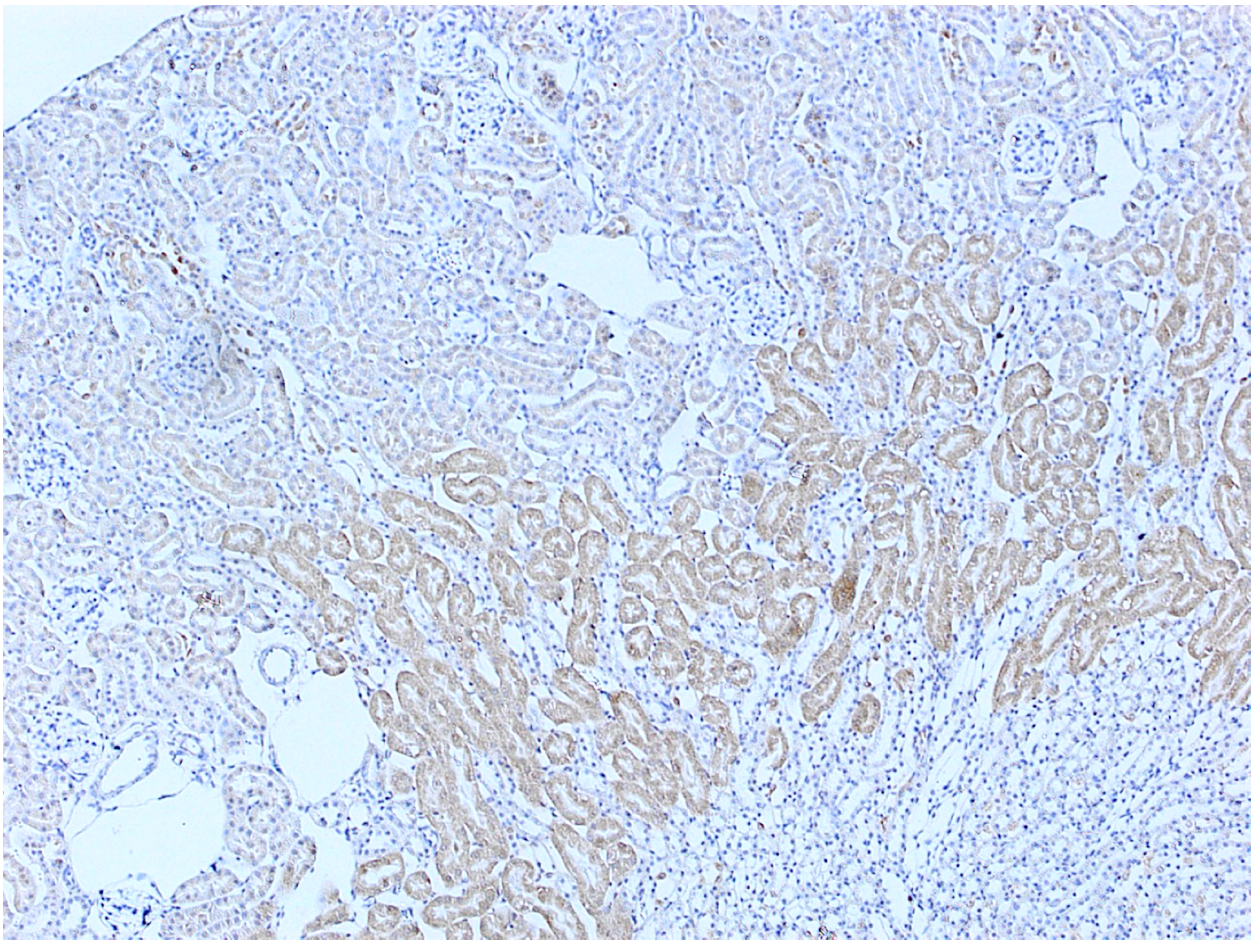


Figure 6: 100x magnification of a male rodent kidney. The brown staining indicates NMDAr GluN1 localization while the blue staining indicates the nucleus localization.

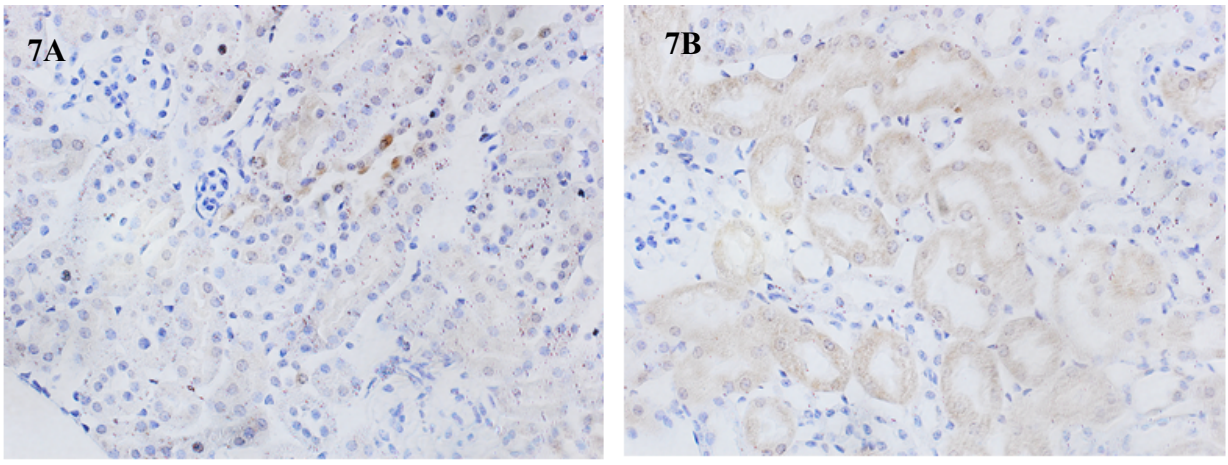


Figure 7. 200x magnification of the expression of NMDAr GluN1 in (A) cortex and (B) medulla.

NMDAr presence on CNT and CCD: To further validate the expression of NMDAr in our region of interest we performed co-localization studies between GlutN1 and markers of the CNT/CCD segments. Using confocal microscopy, we evaluated the presence of GlutN1 (red) and AQP2 (green) in the kidney. Figure 8 shows that there is an overlap in the GlutN1expression (8A) and AQP2 (8B), indicating the presence of the GlutN1 in CNT/CCD region. The merge of this overlap is showed in the figure 8D, confirming the RNA results were *Grin1* was expressed in CNT/CCD region.

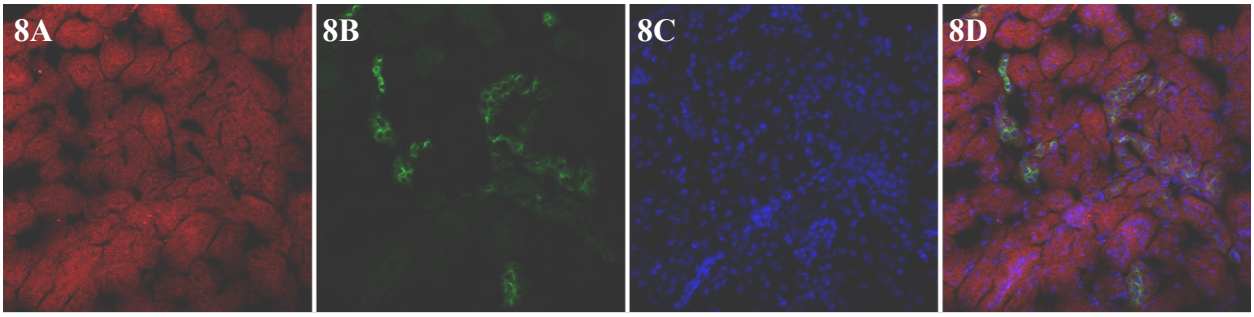


Figure 8. NMDAr overlap with AQP2 expression indicating colocalization. Fig. 8A NMDAr expression. Fig. 8B AQP2 expression. Figure 8C DAPI or nuclei expression. Figure 8D Merged imaging of all three immunofluorescence

Additionally, we explored the co-localization between ENaC, a principal cell marker in CNT/CCD, with GlutN1. Figure 9 shows the expression and co-localization of ENaC (green) with GlutN1 (red). Figure 9C confirms the presence of GlutN1 in principal cells (yellow). We also documented the presence of GlutN1 on ENaC negative cells from CNT/CCD which may correspond to intercalated cells (arrow).

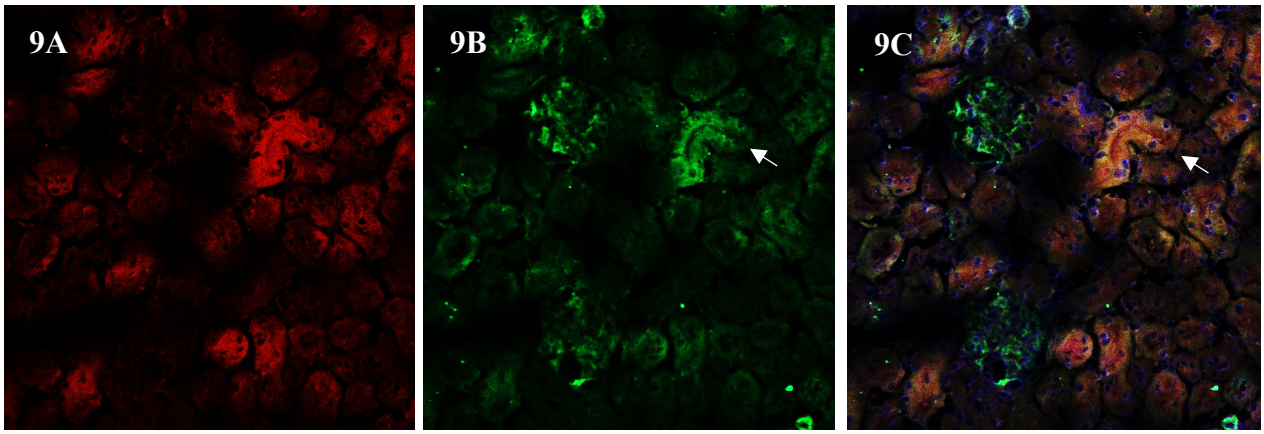


Figure 9: NMDAr colocalize with ENaC. Fig. 9A NMDAr expression. Fig. 9B ENaC expression. Figure 9C Merged image. Arrow indicate ENaC negative cells, which may correspond to intercalated cells, which also express GlutN1.

Chapter 3: Sex Differences in NMDAr Expression

Sex Differences in Hypertension

Sex differences in hypertension prevalence has been studied in human and animal models[19]. It has been found that women under the age of 45 are more protected from hypertension than men of the same age range and post-menopausal women[19]. Figure 10 illustrates this sex difference across different ages according to the NHANES study. Although the reason is unclear, it is evident that there is a difference in susceptibility to hypertension based on sex. Because higher NMDAr expression may correlate to lower blood pressure, we hypothesize that female mice will have a higher expression of NMDAr than male mice. Understanding the role of NMDAr and their sex differences would allow for a more thorough understanding of the mechanisms and help to create sex-specific therapies for BP-related diseases.

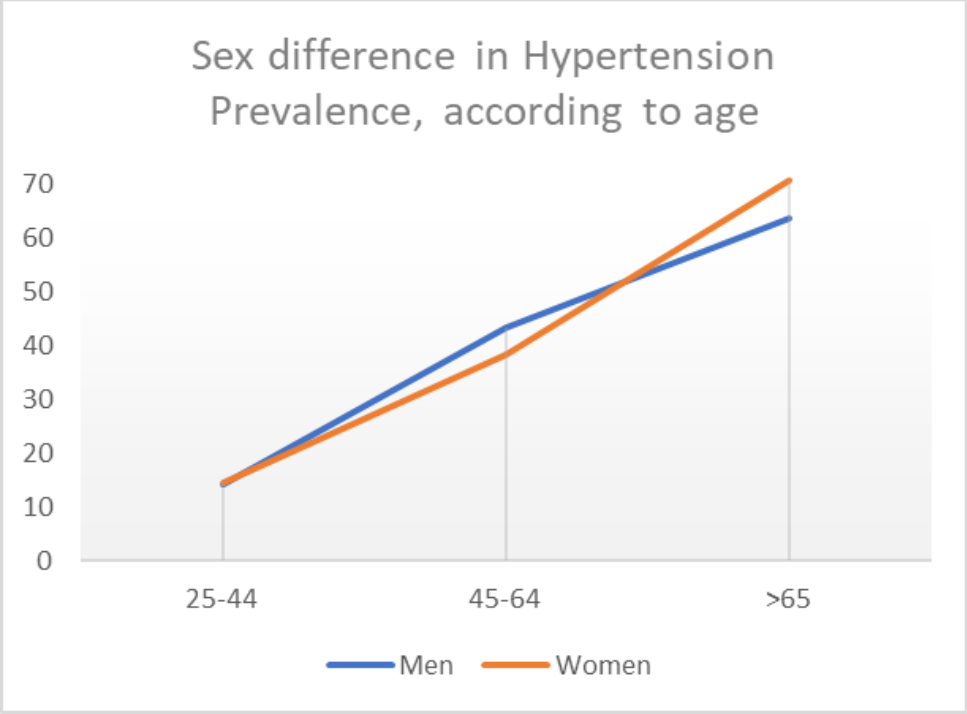


Figure 10: Prevalence of hypertension between male and female population of increasing age. The prevalence is lower for female participants until 45 to 64 years of age at which point the rate increases past the male population. Modified from NHANES 2011-2016 Am J Epidemiol. 2019 Dec; 188(12): 2165–2174)

Methods

Immunohistochemistry (IHC): IHC protocols were repeated to evaluate the differences between male and female mice in NMDAr expression.

Western blots (WB): For comparison of GluN1 expression between male and female mice, we used western blots. WBs are used to separate and identify proteins. Via gel electrophoresis, the mixture of proteins was separated by molecular weight. Mice kidney tissues were processed to generate a cell lysate. Once the cell lysis was complete, we ran the samples through gel electrophoresis with a weighted control ladder to identify the proteins within the tissue. After membrane transfer, the protein was blotted, and the expression amount was quantified and compared between sexes.

Statistics: Values are expressed as mean± standard differences (SD). T-tests were used to compare the mean differences between the two groups in the western blot experiments. GraphPad Prism software v8.1.1 was used for graphs and statistic calculations.

Results

Difference in NMDAr Expression Between Male and Female Mice: Comparing the NMDAr expression in female mice versus male mice we found dramatic differences in the staining of the kidneys with stronger signaling in the female samples. This was true in the cortex as well as the medulla. As shown in Figures 11 and 12, the NMDAr density is higher in the female mice regardless of whether it is in the cortex or medulla. We also observed that in male, the receptors tend to localize in the basolateral membrane (arrow), while in female the NMDAr expression was uniform in all the subcellular spaces. Figure 13 shows the sex difference quantification using western blot. We found that on average, female mice have a significant five-fold increase in NMDAr expression. These results demonstrate that there is a sex difference in NMDAr expression

in the kidney, where female kidneys have a higher expression in both cortical and medullary regions.

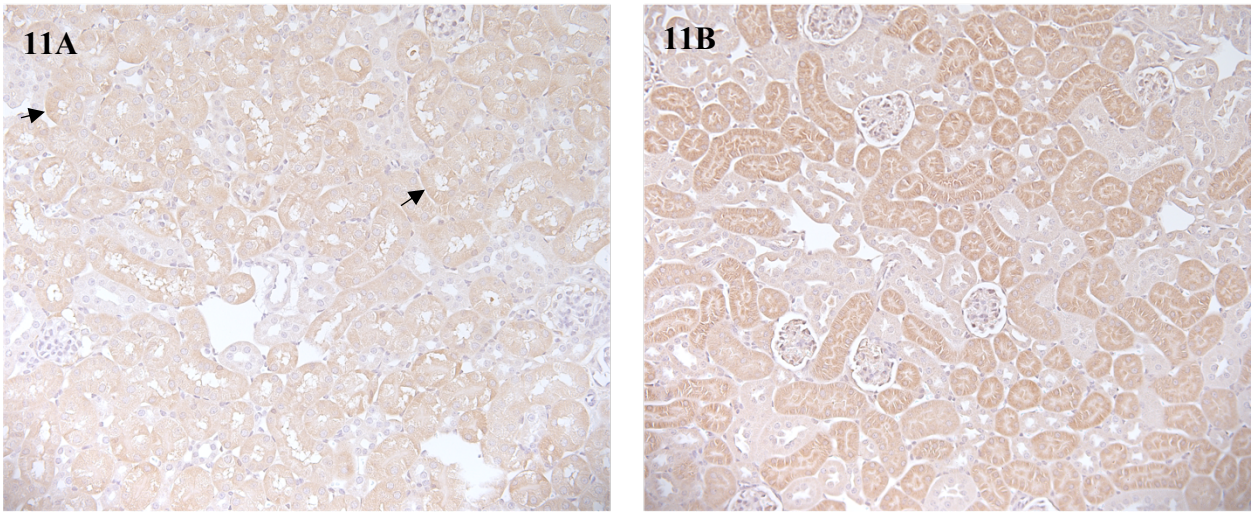


Figure 11. Higher NMDAr expression in female mice (11B) than male mice (11A) in the renal cortex in higher magnification (200x). Arrows indicate the tendency to localize in the basolateral membrane in males, whereas in females, the distribution was homogenous across the subcellular regions.

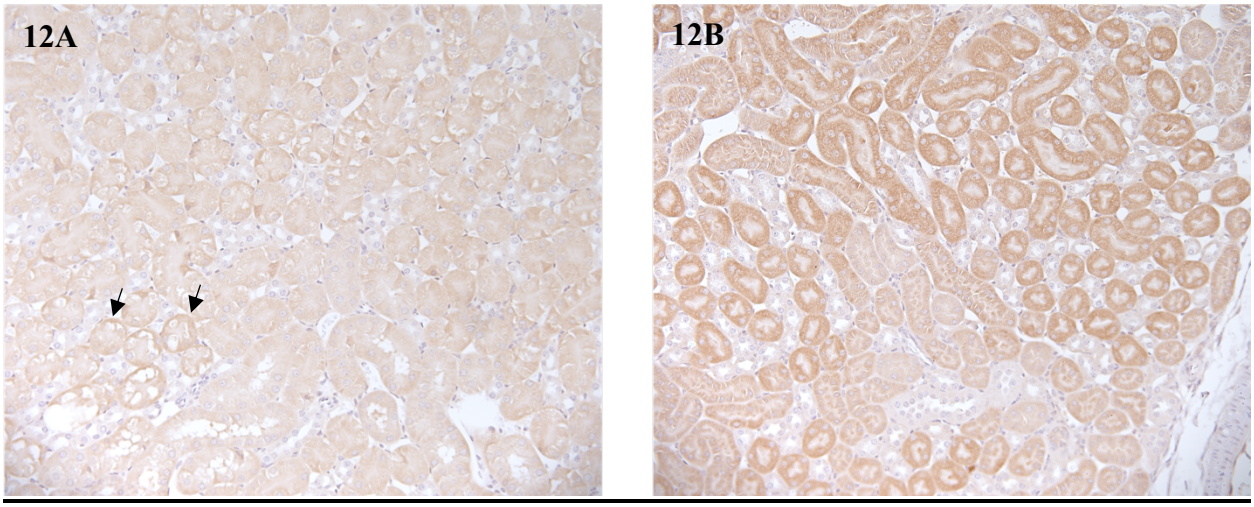
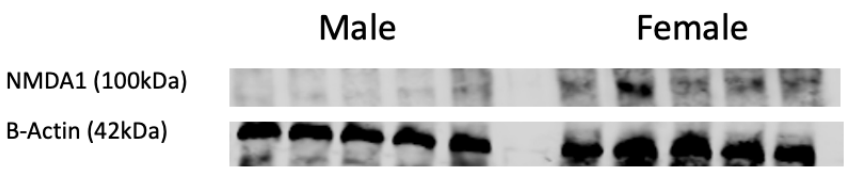


Figure 12. Higher NMDAr expression in female mice (12B) than male mice (12A) in the renal medulla (200x). Arrows indicate the tendency to localize in the basolateral membrane in male, whereas in female the distribution was homogenous across the subcellular regions.

13A



13B

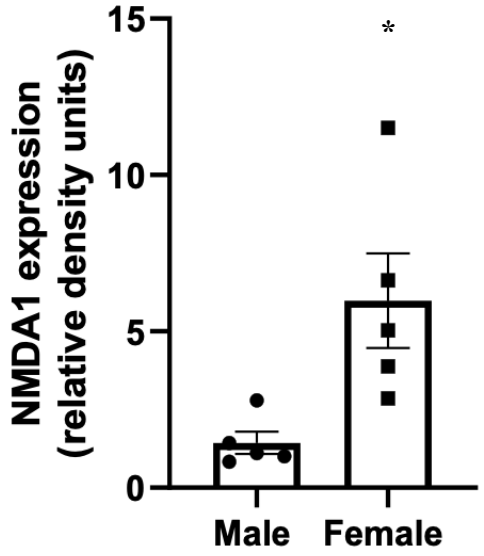


Figure 13. Sex difference of NMDAr expression. Quantitative analysis of NMDAr expression using western blot. Figure 13A shows the relative density of NMDAr expression related to housekeeping protein beta-actin. Figure 13B shows an average of a 5-fold increase of NMDAr expression in females. n=10. * p=0.01.

Chapter 4: Role of Estrogen in NMDAr Expression

Role of Estrogen in the Kidney

Women who are child-bearing ages are more protected from HTN while post-menopausal women are more susceptible to hypertension [19]. As hypertension is the leading risk factor for cardiovascular disease in women, the role of estrogen in blood pressure regulation is critical. Estrogen plays a role in the cardiovascular protective mechanisms in women [19]. Additionally, estrogen plays a role in the vasodilation function of the kidney [11]. After observing differences in NMDAr expression between male and female mice, we wanted to know the effect of estrogen. To explore that, we used a continuous cell line from the principal cell of the mouse CNT/CCD region (mpkCCD cells). They were obtained from collecting ducts that express ENaC and AQP2 which is useful in evaluating the transporters. The processes observed parallel the processes that take place in the CCD. We treated these cells with Estrogen or vehicle (DMSO) and compared the NMDAr expression.

Methods

Estrogen Treatment: The estrogen regulation of NMDAr within female mice was studied via western blots. Cell culture of mpkCCD cells were treated with 17b-estradiol (50nM) or vehicle (DMSO) for 24 hours. The estrogen samples and vehicle samples were run through western blots with a positive control (homogenized female kidney).

Statistics: Values are expressed as mean± standard differences (SD). T-Test was used to compare the mean differences between the two groups in the western blot experiments. GraphPad Prism software v8.1.1 was used for graphs and statistic calculations.

Results

NMDAr Expression in Estrogen-Treated Cells: In treating mpkCCD cells with 17-beta estradiol for 24 hours, we were able to run the WB experiments to quantify the protein density. The quantification shown in Figure 14 suggests that there is a 1.6-fold increase on average where estradiol treated cells have higher NMDAr expression than vehicle treated cells.

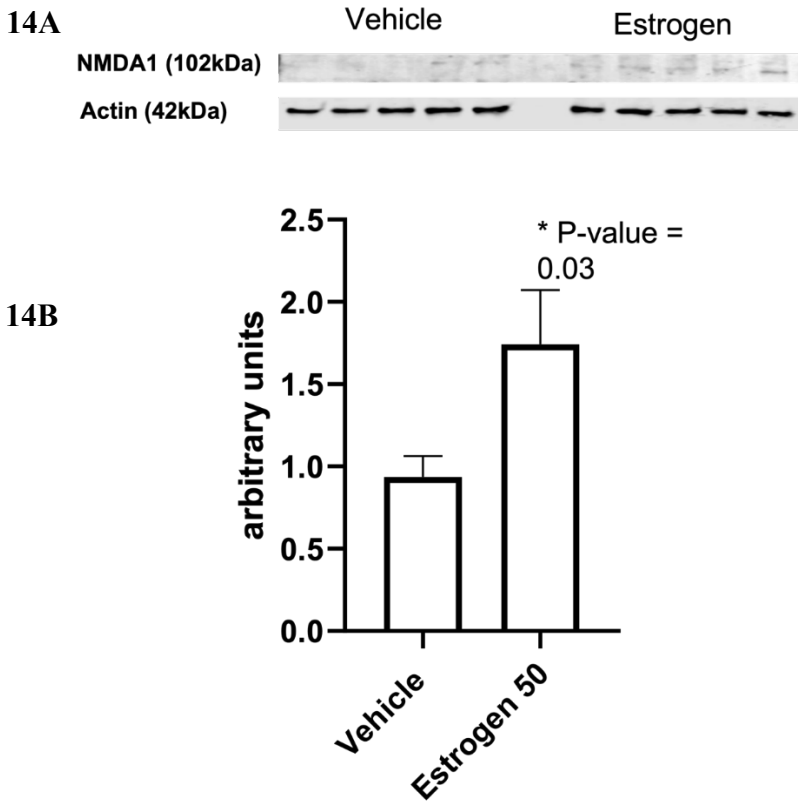


Figure 14. Effects of estrogen on NMDAr. Quantitative analysis of NMDAr expression using western blot. Figure 14A shows the relative density of NMDAr expression related to housekeeping protein beta-actin. Figure 14B shows a 1.6-fold increase of NMDAr expression after 24-hour treatment with Estrogen (50nM). n=10. p=0.03.

Chapter 5: Discussion

In observing the NMDAr expression along the nephron of mice, we found that NMDAr are expressed in most of the tubular segments. We confirmed that NMDAr is expressed in the connecting tubule and collecting duct of the rodent nephron. We also found that there is a difference in NMDAr expression based on sex, where females have the higher expression and that the difference was mediated by estrogen. These findings could contribute to uncovering sex differences in renal physiology and may provide partial explanations of why women have lower prevalence of hypertension than males during child-bearing ages but then are less protected after menopause. Targeting NMDAr could be useful in creating specific BP therapies for postmenopausal women.

The suggested interplay of NMDA r and estrogen has been observed in the brain[20]. In an original investigation, Mehta *et al.* observed that arsenic trioxide (As_2O_3)-regulated behavior deficits (such as anxiety) downregulated the estrogen receptor (ERα) which also downregulated NMDAr 2B and brain-derived neurotropic factors (BDNF) [20]. This suggests that there could be a correlation between ER expression and NMDAr expression. Such findings suggests that there could be similar mechanisms in other organs in which NMDAr are expressed, such as the kidney. We do not know at this point the physiological relevance of NMDAr in females, but we can speculate that NMDAr are important for renal vasodilation, and that NMDA receptors may be important in renal blood regulation during the pregnancy.

Unfortunately, we are not able to provide functional results about the consequences of this sex differences which is a limitation of our study. Future work will explore the impact of this sex difference on renal blood flow measurements. The second limitation to our study is that we did not explore the estrogen effects *in vivo.* We did observe the estrogen-mediated difference within cells;

however, animal models would have strengthened the importance of this finding. We did also not explore which estrogen receptors are mediating the NMDAr expression change, which will be explored in future work.

This thesis demonstrates the localization of NMDAr in tubular sub segments of the kidneys and the presence of a sex difference in NMDAr, where female have higher NMDAr expression. These sex difference may be related to the presence of estrogen.

References

1. Chade, A.R., *Small Vessels, Big Role: Renal Microcirculation and Progression of Renal Injury.* Hypertension, 2017. **69**(4): p. 551-563.
2. Omura, M., et al., *Prospective study on the prevalence of secondary hypertension among hypertensive patients visiting a general outpatient clinic in Japan.* Hypertens Res, 2004. **27**(3): p. 193-202.
3. Foti, K., et al., *Hypertension Awareness, Treatment, and Control in US Adults: Trends in the Hypertension Control Cascade by Population Subgroup (National Health and Nutrition Examination Survey, 1999-2016).* Am J Epidemiol, 2019. **188**(12): p. 2165-2174.
4. Sandberg, K. and H. Ji, *Sex differences in primary hypertension.* Biol Sex Differ, 2012. **3**(1): p. 7.
5. Bozic, M. and J.M. Valdivielso, *The potential of targeting NMDA receptors outside the CNS.* Expert Opin Ther Targets, 2015. **19**(3): p. 399-413.
6. Deng, A., et al., *Vasodilatory N-methyl-D-aspartate receptors are constitutively expressed in rat kidney.* J Am Soc Nephrol, 2002. **13**(5): p. 1381-4.
7. Romero, C.A. and O.A. Carretero, *A Novel Mechanism of Renal Microcirculation Regulation: Connecting Tubule-Glomerular Feedback.* Curr Hypertens Rep, 2019. **21**(1): p. 8.
8. Romero, C.A. and O.A. Carretero, *Tubule-vascular feedback in renal autoregulation.* Am J Physiol Renal Physiol, 2019. **316**(6): p. F1218-f1226.
9. Romero, C., et al., *OS 21-02 CONNECTING TUBULE-GLOMERULAR FEEDBACK (CTGF) IN RENAL HEMODYNAMICS AND BLOOD PRESSURE REGULATION AFTER UNILATERAL NEPHRECTOMY (UNx).* Journal of Hypertension, 2016. **34**: p. e235.
10. Xue, B., A.K. Johnson, and M. Hay, *Sex differences in angiotensin II- and aldosterone-induced hypertension: the central protective effects of estrogen.* Am J Physiol Regul Integr Comp Physiol, 2013. **305**(5): p. R459-63.
11. Nielsen, C.B., et al., *Decreases in renal functional reserve and proximal tubular fluid output in conscious oophorectomized rats: normalization with sex hormone substitution.* J Am Soc Nephrol, 2003. **14**(12): p. 3102-10.
12. Bertram, J.F., et al., *Human nephron number: implications for health and disease.* Pediatr Nephrol, 2011. **26**(9): p. 1529-33.
13. Brenner, B.M., J.L. Troy, and T.M. Daugharty, *The dynamics of glomerular ultrafiltration in the rat.* J Clin Invest, 1971. **50**(8): p. 1776-80.
14. Marcoux, A.A., et al., *Anatomophysiology of the Henle's Loop: Emphasis on the Thick Ascending Limb.* Compr Physiol, 2021. **12**(1): p. 3119-3139.
15. Paoletti, P., C. Bellone, and Q. Zhou, *NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease.* Nat Rev Neurosci, 2013. **14**(6): p. 383-400.
16. Mistry, A.C., et al., *The sodium chloride cotransporter (NCC) and epithelial sodium channel (ENaC) associate.* Biochem J, 2016. **473**(19): p. 3237-52.
17. Takata, K., et al., *Localization and trafficking of aquaporin 2 in the kidney.* Histochem Cell Biol, 2008. **130**(2): p. 197-209.

18. Chen, L., C.L. Chou, and M.A. Knepper, *A Comprehensive Map of mRNAs and Their Isoforms across All 14 Renal Tubule Segments of Mouse.* J Am Soc Nephrol, 2021. **32**(4): p. 897-912.
19. Yang, X.P. and J.F. Reckelhoff, *Estrogen, hormonal replacement therapy and cardiovascular disease.* Curr Opin Nephrol Hypertens, 2011. **20**(2): p. 133-8.
20. Mehta, K., et al., *Resveratrol attenuates arsenic-induced cognitive deficits via modulation of Estrogen-NMDAR-BDNF signalling pathway in female mouse hippocampus.* Psychopharmacology (Berl), 2021. **238**(9): p. 2485-2502.