4-PHENYLBUTRATE, SALT SENSITIVE HYPERTENSION, AND CKD

THE EFFECT OF THE ER STRESS INHIBITOR, 4-PHENYLBUTYRATE, ON CHRONIC KIDNEY DISEASE IN A MODEL OF SALT SENSITIVE HYPERTENSION

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TITLE: The effect of the ER stress inhibitor, 4-phenylbutyrate, on chronic kidney disease in a model of salt sensitive hypertension

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ABSTRACT

ER stress in the kidney is associated with proteinuria. Clinical studies have linked proteinuria with the progression of chronic kidney disease (CKD) at all stages of GFR decline. We hypothesized that treatment with a chemical chaperone, 4-phenylbutyrate (4-PBA), would reduce the severity of CKD and proteinuria in salt sensitive hypertension. The differences in renal pathology between salt sensitive and insensitive hypertension when animals were fed an 8% NaCl (HS) diet were assessed. The Dahl salt sensitive (Dahl S) rat was used as a model of salt sensitive hypertension, while the spontaneously hypertensive rat (SHR) was used as a model of salt insensitive hypertension. The myogenic response of the arcuate artery was studied to determine whether the differences in renal pathology between these models of hypertension was due to an effect of salt on myogenic constriction. Myogenic constriction displayed salt sensitivity in the Dahl S as there was a significant reduction in blood vessel constriction with increasing intralumenal pressures. Myogenic constriction was reduced, but not completely abolished in the SHR with high salt (HS), providing a possible explanation of why this model of hypertension does not develop an equivalent level of renal damage with blood pressure increase as the Dahl S rat. ER stress induction with tunicamycin in arcuate arteries from normotensive animals resulted in an attenuation of the myogenic response. Myogenic constriction was protected from tunicamycin induced ER stress with 4-PBA. 4-PBA treatment (1g/kg/day) in HS fed Dahl S ameliorated proteinuria, renal intratubular protein casts, and renal fibrosis. This correlated with a protection of myogenic constriction and integrity of the glomerular filtration barrier. This suggests that myogenic constriction of the renal

vasculature is an important mechanism to protect against salt sensitive hypertension-induced proteinuria. Further, that high salt feeding may inhibit this protective mechanism by inducing ER stress in the renal blood vessels.

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4-PBA	4-phenylbutyrate
α-SMA	α- smooth muscle actin
ATF6	Activated by transcription factor 6
BN13	Dahl S background with a chromosome 13 substitution from the Brown Norway rat
CHOP	C/EBP homologous protein
CKD	Chronic kidney disease
CV	Cardiovascular
CVD	Cardiovascular disease
Dahl S	Dahl salt sensitive
DBP	Diastolic blood pressure
ECM	Extracellular matrix
EMT	Epithelial to mesenchymal transition
ER	Endoplasmic reticulum
ESRD	End-stage renal disease
GBM	Glomerular basement membrane
GFB	Glomerular filtration barrier
GFR	Glomerular filtration rate
GRP78	78 kDa glucose-regulated protein
HBSS	Hank's buffered salt solution
HS	High salt (8% NaCl)
IGS	Imerslund-Gräsbeck syndrome

IRE1	Inositol-requiring enzyme 1
MCP-1	Monocyte chemoattractant protein
MDRD	Modification of Diet in Renal Disease
NS	Normal salt (0.4% NaCl)
PAS	Periodic Schiff acid
PERK	PKR-like ER protein kinase
PKD	Polycystic kidney disease
PSR	Picro-sirius red
ROS	Reactive oxygen species
SBP	Systolic blood pressure
SHR	Spontaneously hypertensive rat
TEM	Transmission electron microscopy
TGF	Tubuloglomerular feedback
TGF-β1	Transforming growth factor -β1
TUDCA	Tauroursodeoxycholate
UPR	Unfolded protein response
UUO	Unilateral ureteral obstruction
WKY	Wistar-Kyoto
XBP-1	X-box-binding protein-1

DECLARATION OF ACADEMIC ACHIEVEMENT

Vessel studies were performed with the assistance of Chao Lu.

1.0 INTRODUCTION

1.1 General Overview

Chronic kidney disease (CKD) is an important chronic health concern with increasing incidence and prevalence of greater than 10% in the Canadian population (Arora et al., 2013). CKD is of particular concern since it is an important risk factor for the morbidity and mortality associated with cardiovascular disease (CVD) (Arora et al., 2013) and can progress to end stage renal disease (ESRD), which is a life threatening illness. ESRD requires renal replacement therapy, either dialysis or kidney transplant. Dialysis is a very costly but necessary treatment for ESRD, however, it does not prevent the development of CVD associated with CKD and has a negative influence on patient quality of life (Molsted, Prescott, Heaf, & Eidemak, 2007). Kidney transplant produces much better outcomes for patients, improved quality of life, reduced risk of cardiovascular disease and lower mortality (Tonelli et al., 2011). However, the low availability of kidneys for transplant limits patients' options and has created long waiting lists where patients suffering from ESRD remain on dialysis until a kidney becomes available. Therefore, strategies to reduce the rate of progression of CKD to ESRD are important to reduce the cost and harm associated with long-term maintenance on dialysis. We have chosen to examine CKD caused by hypertension and proteinuria, important risk factors for the progression of CKD to ESRD, to determine novel therapeutic approaches to modify these risk factors and thereby limit the progression of CKD.

1.2 Chronic Kidney Disease

Chronic kidney disease (CKD) is defined as kidney damage, as indicated by pathological changes, or glomerular filtration rate (GFR) of less 60 ml/min/1.73m^2 for 3 or more months (Johnson, Feehally, & Floege, 2003). The GFR is the total volume of fluid filtered through the kidney from the glomerular capillaries to Bowman's capsule per unit time. The force driving GFR is the net filtration pressure within the glomerulus. This net filtration pressure is the product of the difference in hydrostatic pressure between the glomerular capillary lumen and that of Bowman's space and the difference in oncotic pressure between these two spaces. This is referred to as Starling's forces and they dictate the GFR of the kidney. The Starling forces include the hydrostatic force, the force pushing plasma through the glomerular filtration barrier (GFB), as well as the oncotic force, which is the osmotic force due to proteins retained in the blood resisting filtration.

There are 5 stages of CKD based on GFR (Table 1), with stages 1 and 2 representing stages in which GFR is considered normal (60-90 ml/min/1.73m^2) but there is evidence of renal damage (microalbuminuria/proteinuria, or hematuria). Late stage CKD, stages 3-5, are characterized by moderate to severe declines in GFR that begin to interfere with the ability of the kidney to remove waste products of metabolism from the blood. This occurs due to the loss of nephrons from the filtration process through various forms of damage and when progressive leads to the inability of the kidney to perform its function. ESRD requires renal replacement therapy, either in the form of dialysis or transplantation.

Table 1: Stages of chronic kidney disease (CKD). Adapted from (Levey et al., 2003)

Stage	Description	GFR ($mL/min/1.73m^2$)	Related terms
1	Kidney damage with normal or increased GFR	≥ 90	Albuminuria, proteinuria, hematuria
2	Kidney damage with mild decrease of GFR	60-89	Albuminuria, proteinuria, hematuria
3	Moderate decrease of GFR	30-59	Chronic renal insufficiency, early renal insufficiency
4	Severe decrease of GFR	15-29	Chronic renal insufficiency, late renal insufficiency, pre-ESRD
5	Kidney failure	< 15	Renal Failure, uremia, ESRD

1.2.1 GFR measurements

GFR is measured by the clearance of markers from the plasma and the rate of their appearance in ultrafiltrate or urine. Ideal markers for GFR are small enough to be freely filtered through the GFB, and is neither reabsorbed nor secreted by the renal tubules (Rosner & Bolton, 2006). Ideal endogenous markers are ones that are produced at a constant rate in the body. Exogenous markers may also be used if they are safe, inexpensive, and physiologically inert allowing their addition to the plasma intravenously.

Approximately 2% of total creatine, a compound important for energy supply, is non-enzymatically converted to creatinine, which diffuses out of cells and is excreted by the kidneys (Wyss & Kaddurah-Daouk, 2000). As creatinine is produced in the body at a constant rate and is not reabsorbed by renal tubules, it is commonly used as an endogenous marker to calculate GFR. Creatinine clearance is calculated using both urine and plasma creatinine levels in order to determine the excretion rate of creatinine. When renal function becomes severely impaired, plasma creatinine levels are markedly elevated. However, creatinine clearance overestimates GFR as the renal tubules secrete creatinine. The Modification of Diet in Renal Disease (MDRD) equation estimates GFR using 4 variables: serum creatinine, age, sex, and ethnicity (Levey et al., 1999; Levey et al., 2003; Rosner & Bolton, 2006). However, this equation cannot be extended to a large number of ethnicities. Although there have been studies to determine the ethnic coefficients for a variety of races, including Chinese, Japanese, and Korean (Imai et al., 2007; Lee et al., 2010; Y. C. Ma et al., 2006).

An exogenous marker that may also be used to determine GFR is inulin. Inulin is a plant polysaccharide that is not produced by the body, freely filtered by the kidneys, and neither secreted nor reabsorbed (Rosner & Bolton, 2006). These properties of inulin are why it is considered the gold-standard of GFR measurement (Woitas et al., 2000). However, GFR measurement by inulin clearance is labour-intensive and therefore used infrequently in the clinical setting.

1.2.2 Risk factors of CKD

Diabetes is the leading risk factor for CKD, accounting for 30%-40% of CKD cases ("KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease," 2007; Whaley-Connell et al., 2009). Damage to the microvasculature of the kidney as well as the retina are common in diabetic patients. A major characteristic of diabetic nephropathy is microalbuminuria. Changes in the hemodynamic factors within the vessels may cause glomerular hyperfiltration which is present in diabetic nephropathy (Mogensen, 1986; Zatz & Brenner, 1986).

Polycystic kidney disease (PKD) is a genetic disorder that is characterized by formation of cysts in the kidneys. PKD is the most common genetic cause of ESRD (Igarashi & Somlo, 2007). There are two major forms of PKD: autosomal dominant PKD (ADPKD) and autosomal recessive PKD (ARPKD). ADPKD is the more common type and is usually diagnosed during adulthood, while ARPKD is usually found in newborns and infants (Grantham & Slusher, 2003).

Low nephron number at birth has also been linked to a greater risk of renal disease and renal failure (Hoy, Hughson, Singh, Douglas-Denton, & Bertram, 2006; Luyckx & Brenner, 2010). Aboriginals from Australia have higher rates of renal disease and hypertension and this has been linked to the reduced nephron number found in this population (Hoy et al., 2006). This may also explain why low birth weight is associated increased risk of proteinuria and albuminuria in adults (Hoy, Wang, VanBuynder, Baker, & Mathews, 2001; Painter et al., 2005).

Hypertension is the second leading risk factor for CKD and an important factor in the progression of CKD (Fox et al., 2004; Klag et al., 1996; Muntner et al., 2004; Wuhl & Schaefer, 2011). Achieving blood pressure targets in CKD patients with and without diabetes results in slower progression to ESRD (Bakris et al., 2003; Wuhl & Schaefer, 2011). Antihypertensive drugs have been shown to slow the progression of proteinuria as well as the decline of GFR in CKD (Peterson et al., 1995).

1.3 Hypertension

Hypertension is defined as the sustained elevation of blood pressure above 140/90 mmHg. Blood pressure is the product of cardiac output and total peripheral resistance. Cardiac output is influenced by plasma volume since venous return will determine cardiac output if the heart maintains normal function. Total peripheral resistance is the resistance that is generated by all of the vasculature to blood flow and plays an active role in maintaining blood pressure due to tonic vascular tone in the resistance blood vessels (Johnson et al., 2003). There are two forms of hypertension: essential and secondary

hypertension. Essential hypertension accounts for 95% of all hypertensive cases and involves all cases that are not secondary to various known disorders such as hyperaldosteronism (Carretero & Oparil, 2000).

Secondary hypertension has an identifiable cause such as medications, hyperaldosteronism (Conn's syndrome), and cancers of the adrenal medulla such as pheochromocytoma. Medications such as Non-steroidal anti-inflammatory drugs (NSAIDs) have also been shown to increase blood pressure by increasing sodium reabsorption and thereby causing sodium retention (Brater, 1999). Hyperaldosteronism is characterized as the excess production of aldosterone by the adrenal glands. Aldosterone is a hormone that acts on the distal tubules of the kidney to increase sodium reabsorption, and therefore leads to water retention and volume expansion. Pheochromocytoma is a tumour within the adrenal medulla. The tumour results in large secretions of catecholamines, particularly norepinephrine and results in overstimulation of the sympathetic nervous system, excess blood vessel constriction and an increase in total peripheral resistance resulting in hypertension. Some risk factors of essential hypertension include obesity, insulin resistance, and salt sensitivity.

1.3.1 Salt sensitivity

Although there are a variety of factors that increase the risk of hypertension, high sodium intake has been identified as a risk factor (Sacks et al., 2001). There are certain populations prone to salt-sensitive hypertension, including the African American population. African Americans also have a higher incidence of progression to ESRD

(Coresh, Astor, Greene, Eknoyan, & Levey, 2003; Weiner et al., 2004). However, there are also certain populations, such as the Caucasian population, that tend to be salt insensitive and have lower incidence of CKD (Luft et al., 1991). A reduction in the renal autoregulatory response associated with a high sodium diet may result in glomerular exposure to increased arterial pressure and may account for increased CKD seen with salt sensitive hypertension. This effect has been demonstrated in the Dahl salt-sensitive hypertensive (Dahl S) rat, a frequently used model of CKD. In the Dahl S rat, artificial mechanical renal blood vessel constriction, mimicking renal blood flow autoregulation, was used to block high pressure from reaching the kidney and was found to reduce the severity of CKD (Mori et al., 2008).

High dietary sodium is linked to increases in proteinuria and increased progression of renal disease (Cianciaruso et al., 1998; Vegter et al., 2012). In one study of non-diabetic CKD patients, high sodium intakes limited the effectiveness of angiotensin-converting enzyme (ACE) inhibitors at preventing CKD progression. High sodium intake was linked to increased risk for ESRD in these patients (Vegter et al., 2012). In a study of patients with type I diabetes, urinary sodium excretion had a non-linear relationship with all-cause mortality and ESRD (Thomas et al., 2011).

A double-blind randomized crossover trial observing the effects of dietary sodium in non-dialyzed, non-transplanted CKD patients was performed to observe the effects of sodium reduction in these patients (McMahon et al., 2013). During periods of low sodium, there was a significant decrease in blood pressure by 10/4 mmHg (SBP/DBP) over 24 hours and a reduction of extracellular volume. Significant reductions in

proteinuria and albuminuria were also observed during periods of reduced sodium. The effect of sodium on proteinuria and albuminuria was found to be independent of change in BP. This may be due to the direct effects of dietary sodium on glomerular structure or function (McMahon et al., 2013). Sodium may have a direct effect on blood vessels, as sodium reduction has been linked to reduced oxidative stress, inflammation and endothelium dysfunction (Al-Solaiman, Jesri, Zhao, Morrow, & Egan, 2009; McMahon et al., 2013; Rugale, Delbosc, Cristol, Mimran, & Jover, 2003).

1.3.2 Animal models

The two hypertensive animal models that will be used for the purposes of this Master's project are the spontaneously hypertensive rat (SHR) and the Dahl salt sensitive (Dahl S) rat. The animal model controls respectively, are the Wistar-Kyoto (WKY) rat and a consomic strain on the Dahl S background with a chromosome 13 substitution from the normotensive Brown Norway rat (BN13).

In the absence of external factors, the SHR develops high blood pressure spontaneously; some reports have observed the development of significant BP difference between the SHR and WKY by 3 weeks of age (Lais, Rios, Boutelle, DiBona, & Brody, 1977). However, other reports have found no significant difference between strains until 4 weeks of age (Dickhout & Lee, 2000).

The Dahl S rat develops hypertension upon salt loading and demonstrates large amounts of renal injury, while the SHR develops little to no renal injury (Ishimitsu et al., 1992; Karlsen, Andersen, Leyssac, & Holstein-Rathlou, 1997; Raij, Azar, & Keane, 1985;

Siegel et al., 2004). This provides evidence that although high blood pressure is a risk factor for CKD, it does not necessarily lead to CKD in all circumstances. Dahl S rats are clinically relevant models of salt sensitive hypertension as they exhibit similar pathologies as hypertensive African Americans (Cowley et al., 2001) as this population is 5 times more likely to progress to ESRD (Hsu, Lin, Vittinghoff, & Shlipak, 2003; Norris et al., 2006).

Using the Dahl S background, salt-induced hypertension and severity of proteinuria was corrected by substitution of chromosome 13 from the normotensive Brown Norway genetic background (Cowley et al., 2001). Therefore, to observe differences in the myogenic response with chromosome 13 substitution, we utilized BN13 animals as controls. Studies have been performed to elucidate the genes on chromosome 13 responsible for the attenuation of salt-sensitive hypertension (Hoagland et al., 2004; Moreno et al., 2011). One study found a difference in the formation of 20-hydroxyeicosatetraenoic acid (20-HETE) due to the reduced expression of cytochrome P450 in the Dahl S compared to the BN13 (Hoagland et al., 2004). 20-HETE is a potent vasoconstrictor and has been found to play a role in vasoconstriction in both renal and cerebral arteries (Ge et al., 2013; Harder et al., 1994; Imig et al., 1996; Y. H. Ma et al., 1993). 20-HETE is important for the myogenic response to properly constrict with increases in luminal pressures. Cytochrome P450 catalyzes arachidonic acid with 20-HETE as the primary metabolite. Inhibition of cytochrome P450, reduced the myogenic response in canine and mouse renal arcuate arteries (Kauser et al., 1991).

1.4 The Brenner Hypothesis

According to the Brenner Hypothesis, unfavorable hemodynamics of the glomerulus can have a negative impact on the kidney (Brenner, Lawler, & Mackenzie, 1996; Brenner, Meyer, & Hostetter, 1982; Hostetter, Olson, Rennke, Venkatachalam, & Brenner, 1981). Increased filtration and pressure entering the glomerulus can lead to barotrauma of the glomerular filtration barrier. This will allow protein to enter the nephron, causing further injury to the kidney (Tryggvason & Pettersson, 2003). The presence of protein has been linked to increased interstitial fibrosis and increased progression to ESRD (Hemmelgarn et al., 2010; Hsu, Iribarren, McCulloch, Darbinian, & Go, 2009). Therefore, it is important to protect the kidney from barotrauma due to hyperfiltration, the kidney autoregulates renal blood flow by constricting the renal blood vessels leading to the glomerulus, and dysfunctional autoregulation can result in hyperfiltration of the kidney. Hyperfiltration in individual glomeruli can be caused by reduced renal mass through nephron loss (Hostetter, Olson, Rennke, Venkatachalam, & Brenner, 2001) or impaired autoregulation of renal blood flow (Van Dokkum, Alonso-Galicia, Provoost, Jacob, & Roman, 1999; van Dokkum, Sun, Provoost, Jacob, & Roman, 1999).

The ability of small vessels to constrict as a response to increased luminal pressure is called the myogenic response. The myogenic response works to decrease the hydrostatic pressure of the capillary entering the glomerulus. In response to stretching of blood vessels as a result of increased luminal pressure, small arteries constrict in order to increase the resistance within the vessel. The myogenic response is an inherent property

of vascular smooth muscles and is mediated through the $G_{q/11}$-couple receptors which act as mechanosensors. $G_{q/11}$-couple receptors are activated upon stretch, which eventually results in signaling transient receptor potential cation channels (TRPC) in a G-protein and phospholipase C dependent pathway (Mederos y Schnitzler et al., 2008).

1.5 Proteinuria

Damage to the GFB can result in large molecules including proteins that are usually retained in the plasma passing into the nephron. This occurs when these molecules are able to pass through the fenestrated endothelium of the glomerular capillary, transverse the glomerular basement membrane, and the slit diaphragm between the podocyte foot processes into the nephron. Proteinuria is the abnormal presence of protein in the urine and is an early sign of renal disease. It is considered a cause as well as a consequence of renal disease (Zandi-Nejad, Eddy, Glassock, & Brenner, 2004). Protein that is filtered into the nephron is reabsorbed by epithelial cells of the proximal tubules, thereby removing a large proportion of protein from the final urine. Two endocytic receptors are responsible for the uptake of filtered proteins. They are megalin and cubilin. Megalin, also known as lipoprotein receptor-related protein-2 (LRP2) and glycoprotein-330 (gp330), is a 600-kDa transmembrane protein that belongs to the LDL-receptor family. Megalin is a multi-ligand receptor, binding to a large variety of proteins including albumin. Megalin reabsorbs albumin by direct binding, or by mediating the actions of cubilin. Cubilin is a 460-kDa protein that contains an NH2-terminal region that appears to anchor the protein to the membrane. Similar to megalin, cubilin is an albumin binding

protein, and mediates its reabsorption. Increased production rates of membrane proteins, such as megalin and cubilin, in the ER may be due to greater demand for protein reabsorption in the proximal tubule may result in proteins misfolding. When there is a greater demand for these reabsorptive proteins, as in instances of proteinuria, endoplasmic reticulum (ER) stress may occur.

1.5.1 Importance of proteinuria in the progression of CKD

The presence of protein has been linked to increased interstitial fibrosis and progression to end stage renal disease (ESRD) (Bianchi, Bigazzi, & Campese, 1999; Hemmelgarn et al., 2010; Hsu et al., 2009). At all stages of eGFR, higher levels of proteinuria were associated with increased mortality, myocardial infarction, and progression to ESRD (Hemmelgarn et al., 2010). In a 25-year study, researchers determined that the presence of proteinuria was the most potent risk factor for the progression to ESRD (Hsu et al., 2009). In addition, the level of proteinuria was found to be a strong predictor of the progression to ESRD in African American patients with hypertensive CKD (Lea, Greene, Hebert, & et al., 2005). Excessive amounts of protein in the nephron, possibly due to damage to the GFB, can form protein casts within the tubules. These protein casts can result in the obstruction of the tubule, causing further damage and nephron loss (Bertani, Cutillo, Zoja, Broggini, & Remuzzi, 1986; Bertani, Rocchi, Sacchi, Mecca, & Remuzzi, 1986; Zandi-Nejad et al., 2004).

1.6 Endoplasmic reticulum stress

Endoplasmic reticulum (ER) stress is believed to play a role in cardiac hypertrophy (Dickhout, Carlisle, & Austin, 2011), β-cell dysfunction (Wu & Kaufman, 2006), liver disease (Malhi & Kaufman, 2011) and progressive damage to blood vessels (Dickhout et al., 2005; Kassan et al., 2012).The ER is a crucial organelle for the synthesis and proper folding of membrane resident and secretory proteins (Dickhout & Krepinsky, 2009). Cells can experience ER stress with an accumulation of unfolded proteins in the ER and can respond to this stress by activation of the unfolded protein response (UPR). There are three known signal transduction pathways in the UPR that respond to the accumulation of unfolded proteins; these pathways are activated by transcription factor 6 (ATF6) cleavage, inositol-requiring enzyme 1 (IRE1) phosphorylation, and PKR-like ER protein kinase (PERK) phosphorylation (Figure 1).

ATF6, IRE1, and PERK are ER transmembrane proteins that bind to the protein-folding chaperone, 78 kDa glucose-regulated protein (GRP78). GRP78 has a greater affinity to unfolded proteins in the ER lumen than to the ER transmembrane proteins where it is normally bound (Dickhout & Krepinsky, 2009; S. Luo, Mao, Lee, & Lee, 2006). When there are increased levels of unfolded proteins in the ER lumen, dissociation of GRP78 from ATF6, IRE1, and PERK results in UPR activation. Due to the role of GRP78 in the activation of the UPR, it has been called a master regulator of the UPR (Dickhout & Krepinsky, 2009; Wu & Kaufman, 2006). However, further study has demonstrated that IRE1 may also be activated directly by interacting with misfolded proteins (Pincus et al., 2010). ATF6 cleavage and eIF2α phosphorylation mediated

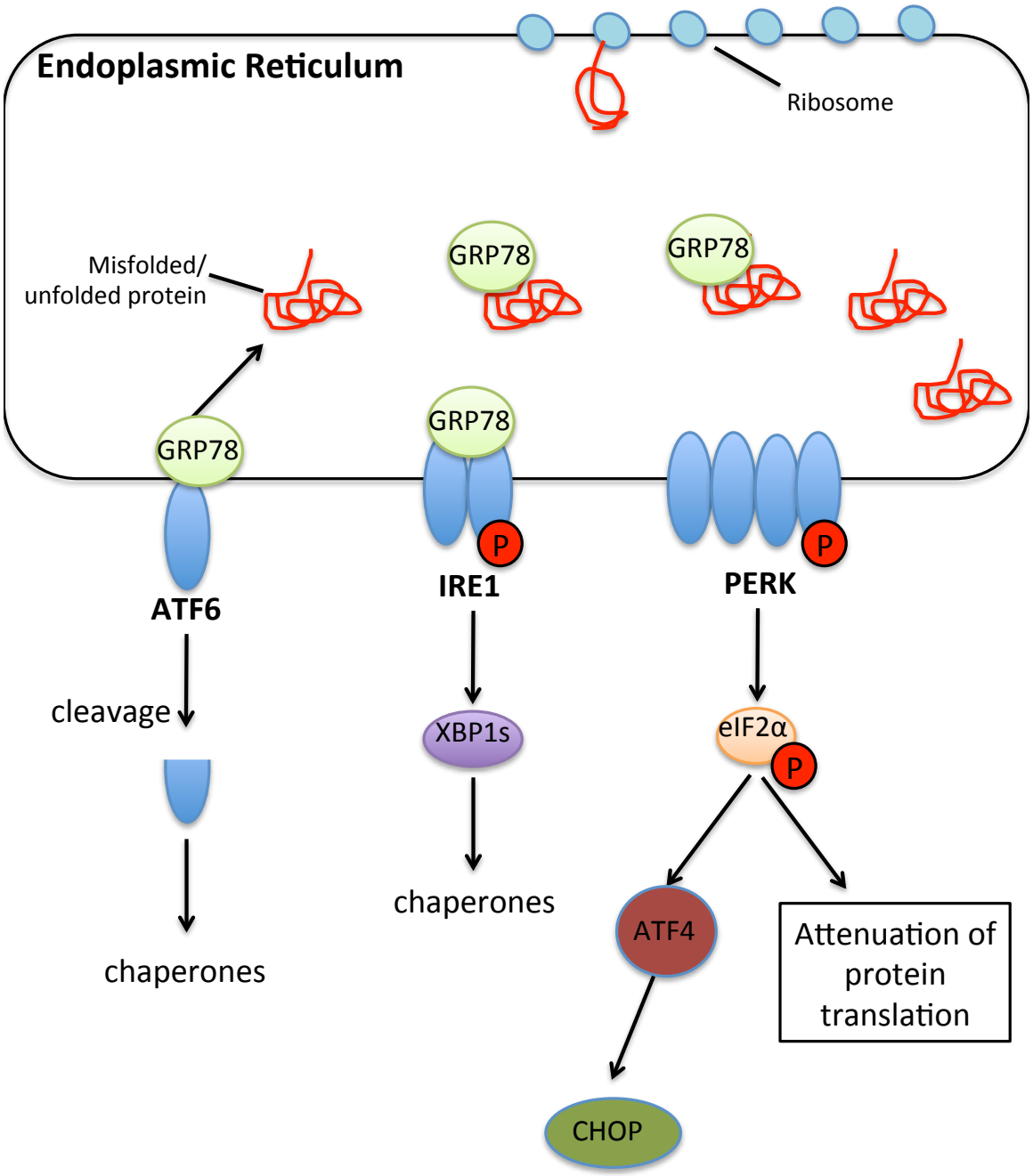

Endoplasmic Reticulum
Ribosome
Misfolded/
unfolded protein
GRP78
GRP78
GRP78
GRP78
P
P
ATF6
IRE1
PERK
cleavage
XBP1s
eIF2α
P
chaperones
chaperones
ATF4
Attenuation of
protein
translation
CHOP

Figure 1. The three signal transduction pathways of the unfolded protein response (UPR). GRP78 has a high affinity for unfolded proteins, resulting in its dissociation from ATF6, IRE1, and PERK. Dissociation of GRP78 results in cleavage of ATF6, phosphorylation of IRE1 and PERK, activating a branch of the UPR. UPR activation results in upregulation of chaperones and proapoptotic factors, such as CHOP. Concept taken from (Dickhout & Krepinsky, 2009).

increases in the transcription of ATF4 play a role in transcriptional induction of ER stress response genes. IRE1 activation leads to the cleavage of mRNA encoding the transcription factor called X-box-binding protein-1 (XBP-1). Spliced XBP-1 results in the upregulation of many genes involved in the UPR, including GRP78, a prosurvival factor. The PERK pathway involves the phosphorylation of the α-subunit of eukaryotic translation initiation factor-2 (eIF2) (Tabas & Ron, 2011). Another ER stress-response element, C/EBP homologous protein (CHOP), a pro-apoptotic factor is also upregulated in the PERK pathway.

1.6.1 Findings relating proteinuria to ER stress

Various studies have demonstrated a relationship between proteinuria and the induction of ER stress (Cybulsky, 2010; Inagi, Nangaku, Onogi, et al., 2005; Kimura, Jin, Ogawa, & Aoe, 2008; Lindenmeyer et al., 2008; Ohse et al., 2006). Kidney biopsies from diabetic nephropathy patients who have established proteinuria and hyperglycemia have shown the expression of ER stress response genes (Lindenmeyer et al., 2008). One study examined mice expressing a mutant form of GRP78. This form of GRP78 lacked the KDEL sequence, preventing its return to the ER from the Golgi, and therefore did not completely eliminate GRP78 function (Kimura et al., 2008). These mice developed renal tubular injury with aging, which was accelerated with protein-overload. While *in vitro*, immortalized proximal tubule cells exposed to albumin in cell culture demonstrated ER stress (Cybulsky, 2010; Ohse et al., 2006). Podocyte injury due to misfolded protein accumulation was associated with ER stress in the megsin transgenic rat model (Inagi,

Nangaku, Onogi, et al., 2005). In this case, ER stress in the podocytes induced injury to podocytes leading to proteinuria (Inagi, Nangaku, Usuda, et al., 2005).

1.6.2 ER stress inhibitor, 4-phenylbutyrate

4-Phenylbutyrate (4-PBA) is an FDA approved drug as an ammonia scavenger for urea cycle disorders in children (Maestri, Brusilow, Clissold, & Bassett, 1996). 4-PBA can act as a low molecular weight chemical chaperone for misfolded proteins (Perlmutter, 2002; Ronald C. Rubenstein & Zeitlin, 2000). 4-PBA has been shown to facilitate protein traffic to the plasma membrane as well as prevent aggregation of mutant proteins, thereby reducing the protein load within the ER (de Almeida et al., 2007; R. C. Rubenstein, Egan, & Zeitlin, 1997). It has been shown that 4-PBA reduces ER stress-induced apoptosis in the liver (Vilatoba et al., 2005); as well, it has been shown to protect against cerebral ischemic injury (X. Qi, Hosoi, Okuma, Kaneko, & Nomura, 2004). Proteinuria is a characteristic of diabetic nephropathy both in humans and animals and 4-PBA treatment of diabetic nephropathy in rats resulted in a significant reduction of ER stress markers, inflammatory cytokines and fibrosis factors (W. Qi et al., 2011). Further, 4-PBA treatment of a type I diabetic model in which diabetes was induced by with streptozotocin, resulted in a significant decrease of urinary protein excretion.

1.6.3 ER stress and vascular dysfunction

ER stress has been found to be associated with angiotensin (Ang) II-induced cardiac hypertrophy and fibrosis (Dickhout, Carlisle, et al., 2011; Kassan et al., 2012).

This damage was attenuated with ER stress inhibition by tauroursodeoxycholate (TUDCA) and 4-PBA. Ang II- induced hypertension in mice is associated with reduced vascular function; with vessels demonstrating reduced endothelial-dependent relaxation and epithelial nitric oxide synthase (eNOS) phosphorylation. ER stress inhibition resulted in improved vascular relaxation in these animals and reduced blood pressures (Kassan et al., 2012). Recently, we have shown that 4-PBA treatment in the SHR resulted in an attenuation of hypertension and an improvement of endothelial dependent relaxation (Werner, Yum, Lu, & Dickhout, 2013).

1.7 The myogenic response

To protect the kidneys from changes in systemic blood pressure and autoregulate renal blood flow, two mechanisms are known: tubuloglomerular feedback (TGF) and the myogenic response (Khavandi et al., 2009). These two mechanisms work in concert to allow a constant pressure to enter the kidneys; if these mechanisms become dysfunctional, the kidney is no longer protected from high arterial pressure (Takenaka, Forster, De Micheli, & Epstein, 1992). Damage to the GFB, particularly the podocytes are strongly linked to proteinuria. Therefore, dysfunctional autoregulation can result in hyperfiltration of the kidney and eventually lead to proteinuria. In TGF, specialized epithelial cells in the distal tubule called macula densa cells respond to changes in NaCl concentration in the lumen by signaling the afferent arterioles in an adenosine-dependent manner (Sun et al., 2001) while the myogenic response involves the constriction of blood vessels in response to increases in luminal pressure. TGF involves maintaining NaCl load

within the tubules, while the myogenic response involves protecting the GFB from incoming pressures.

Although high salt fed Dahl S rats showed a loss of renal blood flow autoregulation (Karlsen et al., 1997), this effect was not due to a reduction in TGF, as TGF was not affected by high salt feeding (Karlsen, Leyssac, & Holstein-Rathlou, 1998). Fawn-hooded hypertensive rats also demonstrate impaired renal blood flow autoregulation, however TGF was maintained, while the myogenic response was impaired (Cupples & Braam, 2007; Van Dokkum, Alonso-Galicia, et al., 1999; van Dokkum, Sun, et al., 1999; Verseput, Braam, Provoost, & Koomans, 1998). This suggests that the myogenic response plays an important role in autoregulation of renal blood flow and is interrupted in salt sensitive hypertension.

Hyperfiltration at the glomerulus can lead to renal pathology (Brenner et al., 1996; Brenner et al., 1982; Hostetter et al., 1981). This may be the case since increased filtration and pressure entering the glomerulus can lead to barotrauma of the filtration barrier and result in proteinuria. In this study, we hypothesized that inhibition of ER stress with 4-PBA, a known ER stress inhibitor, would reduce proteinuria by preserving myogenic constriction in the Dahl S rat thereby protecting the glomerular filtration barrier.

1.8 Fibrosis

High levels of protein in proximal tubule cells induce the expression of monocyte chemoattractant protein (MCP-1), a potent chemoattractant of monocytes/macrophages and T lymphocytes (Wang et al., 1997). This was also found in the protein-overload

model of proteinuria, in which rats develop significant levels of proteinuria with injections of bovine serum albumin. In these animals, genes associated with fibrosis such as the ones encoding for collagen I, III, fibronectin, MCP-1 and Transforming growth factor-β1 (TGF-β1) were upregulated after 2 weeks (A. A. Eddy & Giachelli, 1995). This suggests that high levels of protein in the proximal tubules lead to fibrosis. Interstitial monocytes, attracted to MCP-1, promote fibrosis by further producing and secreting cytokines and vasoactive molecules that attract myofibroblasts and activate resident fibroblasts (A. Eddy, 2001).

The myofibroblast is a key cell type involved in fibrosis of many tissues. Myofibroblasts continually remodel the extracellular matrix (ECM) by synthesizing components like collagen type I. After tissue injury, myofibroblasts form to take part in wound healing. Myofibroblasts will undergo apoptosis once damaged cells are replaced (Tomasek, Gabbiani, Hinz, Chaponnier, & Brown, 2002). However, chronic injury may result in large amounts of myofibroblasts and lead to fibrosis. Several studies have shown that fibrosis that occurs in idiopathic pulmonary fibrosis is correlated with dysregulated apoptosis of myofibroblasts (Jinta et al., 2010; Kis, Liu, & Hagood, 2011; Maher et al., 2010). Myofibroblasts can be identified by *de novo* synthesis of α-smooth muscle actin (α-SMA), a contractile component that is also present in smooth muscle cells (Hinz et al., 2007). The appearance α-SMA allows at least a two-fold increase of contractile strength compared to fibroblasts, which are negative for α-SMA (Hinz, Celetta, Tomasek, Gabbiani, & Chaponnier, 2001; Hinz et al., 2007).

Myofibroblasts may be derived from resident fibroblasts or even epithelial cells during the process of epithelial to mesenchymal transition (EMT). A study showed that 50% of the myofibroblasts found in the unilateral urethral obstruction (UUO) model of renal fibrosis are derived from resident fibroblasts (Spitler, Matsumoto, & Webb, 2013). Myofibroblasts can sustain contractile forces, which are stabilized by the synthesis of ECM components (Gabbiani, 2003; Tomasek et al., 2002). TGFβ1, a fibrogenic cytokine, induces synthesis of α-SMA and production of collagen type I in the presence of mechanical stretch (Gabbiani, 2003; Gabbiani, Le Lous, Bailey, Bazin, & Delaunay, 1976).

1.9 Hypothesis

We hypothesize that treatment of the Dahl S rat, a model of salt sensitive hypertension, with an ER stress inhibitor, 4-PBA, will preserve the myogenic constriction in renal blood vessels, preventing proteinuria and resultant CKD. In order to test this hypothesis, we will treat Dahl S rats with 4-PBA and determine if it preserves myogenic constriction, prevents proteinuria and reduces renal pathology associated with CKD. In the course of this work we will be guided by the following objectives:

1. Determine what the differences are in renal function and pathology between salt sensitive and salt insensitive models of hypertension.
2. Determine if treatment with 4-PBA preserves myogenic constriction in the Dahl S rat and if this reduces proteinuria or renal pathology.
3. Determine the effect of 4-PBA treatment on ER stress in the kidney.

Our findings demonstrate that the ER stress inhibitor, 4-PBA, prevents proteinuria in a salt sensitive hypertensive animal model of CKD, the Dahl S rat. It appears that this drug acts to preserve myogenic constriction in the arcuate artery of the kidneys during salt sensitive hypertension thereby preventing the development of ER stress within the podocytes of the glomerulus and preserving the glomerular filtration barrier. The studies described in this thesis will detail the basis for these conclusions and layout in greater detail the role of ER stress inhibition through the use of low molecular weight chemical chaperones in prevention of the progression of proteinuric CKD.

2.0 MATERIALS AND METHODS

2.1 Animal Study

At 12 weeks of age, SHR and WKY rats (bred at McMaster University Central Animal Facility) were placed on either normal salt (NS; AIN-76A; 0.4% NaCl) or a high salt diet (HS; AIN-76A; 8% NaCl, Research Diets, New Brunswick, NJ) for 4 weeks. N=5-8. Age matched Dahl S, and BN13 rats (Charles River) were also placed on either NS or HS diets for 4 weeks. N=10 in each Dahl S group and N=6 in each BN13 group. Throughout the 4-week salt experiment, animals were placed in metabolic cages at 0, 1, and 3 weeks. 24 hour urine samples were collected and analyzed for total protein and albumin. Blood pressure was measured at 0, 1, 3, and 4 weeks on salt diets using tail cuff plethysmography in the BN13 and Dahl S rats (Kent Scientific, CODA system).

In order to determine the role of ER stress in the progression of CKD in the Dahl S rat, a subset of the Dahl S and BN13 animals were placed on HS diet and treated with a protein folding chaperone, 4-PBA in the drinking water (1g/kg/day); 4-PBA treatment started 1 week prior to diet change.

To determine the effect of 4-PBA on blood pressure in another model of hypertension that is independent of salt, 4-PBA (1g/kg/day) was given to NS fed SHR rats. 4-PBA treatment started one week prior to the switch from chow diet to the control NS diet. Blood pressure was measured every week from baseline (before 4-PBA treatment) to the end of the study (5 weeks).

2.2 Tissue collection

At the end of the study period, animals were sacrificed and tissue collected. To assess renal function, blood was collected by left ventricular puncture in heparinized tubes and plasma prepared for creatinine and blood urea nitrogen (BUN) measurements. Kidneys were perfused with Hank's basic salt solution (HBSS) to clear blood. A segment of the arcuate artery of the left kidney was removed to perform vascular studies. Samples of the right kidney were removed and either fixed in 4% buffered paraformaldehyde and processed for paraffin embedding or frozen at -80°C for biochemical analysis.

2.3 Urine Analysis

Urine protein concentration was analyzed by Core lab at St Joseph's Hamilton Healthcare and 24 hour protein was then calculated using 24 hour urine volume collected in metabolic cages. Albumin was analyzed using a rat albumin enzyme-linked immunosorbent assay (ELISA) kit and following manufacturer's directions (Bethyl Laboratory, Texas, USA). 96 well plates were coated with primary antibody diluted in coating buffer overnight and blocked for 1 hour. Diluted samples (1:1 000- 1:100 000) were incubated in wells for one hour and then incubated with HRP detection antibody. After washing the wells, TMB substrate solution (Sigma) was placed in each well and the reaction was stopped with 0.18 M H_2SO_4. Plates were then read at 450 nm with a plate reader (Molecular Devices Spectra Max Plus384 Absorbance Microplate Reader).

2.4 Vascular Studies

To determine the effect of high salt feeding and ER stress inhibition through 4-PBA treatment on the myogenic response, vascular studies were performed. Renal arcuate

arteries were dissected from surrounding kidney tissue. Vessels were then transferred to a dual vessel chamber containing 37°C Hank's buffered salt solution (HBSS), either with or without Ca (HBSS, composition 0.137 M NaCl, 5.4 mM KCl, 0.25 mM Na_2HPO_4, 0.44 mM KH_2PO_4, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$, 4.2 mM $NaHCO_3$) and mounted in blind-sac method where the proximal end of the artery was cannulated on a glass pipette and the distal end was ligated by a 10-0 surgical suture to maintain intraluminal pressure as described previously (Dickhout & Lee, 1997). The chamber was connected to the PS-200 system which pressurized the vessel with a servo controller and a peristaltic pump. Vessel lumen diameter was recorded continuously with a video dimension analyzer (living systems instrumentation, Burlington, VT, USA). Leica WILD M3C microscope with HITACHI KP-113 CCD camera was used to image the vessel and a TOSHIBA video recorder was used to record the experiment.

After equilibrating the vessels for 60min, at 40 mmHg of pressure lumen-diameter curves were generated by exposing the arcuate arteries to step-wise increases in intraluminal pressure from 80 to 180 mmHg (20 mmHg steps, 5 min each). Changes in lumen diameter were calculated as the difference between the lumen diameter at each pressure point and the lumen diameter at 80 mmHg.

To determine whether the myogenic response was L-type calcium channel dependent, vessels were treated with nifedipine (10 μm). To determine the effect of ER stress on myogenic constriction, vessels were incubated with ER stress inducer, tunicamycin (1μg/ml), for 6 hours with or without 4-PBA (1 mM) before mounting.

2.5 Tissue Analysis

Paraffin embedded kidney samples were sectioned into 4µm slices using a Leica microtome and placed on Superfrost microscope slides (Fisherbrand). Periodic acid-Schiff (PAS) staining was performed to observe intraluminal protein casts as a marker of proteinuria and renal injury. To observe fibrotic regions in the kidney sections, tissues were stained using the picro-sirius red (PSR) method and were with imaged polarized light microscopy. Sections were also immunostained for α-smooth muscle actin (α-SMA) by the Core lab at McMaster University. α-SMA is a marker for myofibroblasts, a key cell type involved in renal interstitial fibrosis. Cubilin (sc-20609; Santa Cruz) expression was also observed in kidney sections through immunohistochemical staining. Immunohistochemical staining was preformed as described previously (Dickhout, Lhotak, et al., 2011). After passing paraffin embedded kidney sections through a series of xylene and decreasing percentages of ethanol, sections were rinsed with water and 1x Tris-buffered saline (TBS). Sections were blocked for 15 minutes in goat serum and incubated in primary cubilin antibody (1:50 dilution) for 1 hour. After washes with 1x TBS, sections were incubated in goat anti-rabbit biotinylated secondary (1:500 dilution; Vector, BA-1000) for 30 minutes. Sections were then incubated with streptavidin horseradish peroxidase (1:200 dilution; Vector, SA-5004) for 10 minutes. Staining was developed by incubation in NovaRED peroxidase substrate kit (Vector) following manufacturer's directions.

Protein casts were quantified as previously, (Cowley et al., 2001) from PAS stained kidney sections using light microscope images taken with an BX41 Olympus

microscope. 10 images were taken each of the renal cortex and medulla at 20x magnification. Protein casts were identified as bright pink regions within enlarged tubules of the kidney. To quantify α-SMA staining, Metamorph software (Molecular Devices) was used. Images of the cortex or medulla from each animal were converted into binary form using Metamorph software by colour thresholding. The area density of α-SMA positive regions was determined by Metamorph software. We measured newly deposited collagen with PSR staining imaged through polarized light microscopy as shown previously (Sadoun & Reed, 2003). Images were thresholded for intensity of collagen birefringence and area density of newly deposited collagen was determined. To assess proximal tubule reabsorption of proteins on proteinuria and the effects of high salt feeding or ER stress inhibition through 4-PBA treatment, the molecular mechanism of protein reabsorption was assessed through cubilin immunohistochemistry. Images were separated into an RGB stack in ImageJ. Using the blue channel, a consistent threshold level was used to determine the area density of positive cubilin staining. Electron microscopy was performed on glomeruli to assess the integrity of the filtration barrier. Kidneys were processed through standard sequential fixation in 2% gluteraldehyde, 1% OsO4 in 0.1 M cacodylate buffer, then dehydrated through a graded series of ethanol and embedded in Spurr's resin. Toluidine blue sections were cut with an ultramicrotome and regions were selected to cut ultrathin sections. Ultrathin sections, 100 nm in thickness, were cut, stained in lead citrate and uranyl acetate, and visualized on a Joel 1200 Exm TEM scan electron microscope.

2.6 Western Blotting

Renal tissue was homogenized using 600uL of lysis buffer (ddH_2O, 0.5M Tris pH6.8, glycerol, 10% SDS, NaF, Na_3VO_4, protease inhibitor cocktail) a standard Bio-Rad DC assay was performed with a plate reader (Molecular Devices Spectra Max Plus384 Absorbance Microplate Reader). After the addition of DTT and Bromothymol blue, samples were run on a 10% SDS-PAGE gel and transferred to a polyvinylidene difluoride (PVDF) membrane (Milipore) using the Trans-Blot Semi-Dry Electrophoretic Transfer Cell (Bio Rad). Blots were blocked for one hour with 5% milk in TBST. Blots were probed with the following antibodies: KDEL mouse antibody (1:1000; SPA-827; Enzo Life Science), CHOP rabbit antibody (1:200; SC-793; Santa Cruz), β-actin mouse antibody (Dako) Goat anti-mouse IgG- HRP conjugate (1:10000, Bio Rad), Goat anti-rabbit IgG- HRP conjugate (1:5000, Bio Rad). Washes were performed with TBST for 5 minutes 3 times. Bands were visualized using Amersham Enhanced chemiluminescence (ECL, GE Healthcare) and Amersham Hyperfilm Chemiluminescence film (GE Healthcare). ChemiDoc XRS+ System (Bio Rad, California) was used to image film, as previously described (Carlisle et al., 2012).

2.7 Statistical Analysis

Quantitative results are expressed as the mean ± standard error of the mean and were analyzed using the Student's t-test or ANOVA with Bonferroni post-test correction for multiple comparisons. Linear regression analysis was performed with GraphPad Prism

to determine the predictive value of systolic blood pressure for 24hr protein or albumin excretion in the Dahl S rat. Significant differences were recognized at the 95% level.

3.0 RESULTS

3.1 Objective 1: Determine what the differences are in renal function and pathology between salt sensitive and salt insensitive models of hypertension.

3.1.1 Effect of high salt feeding on blood pressure in SHR and Dahl S rats

To determine salt sensitivity of blood pressure in the SHR and Dahl S rats, both strains and their control animal models were placed on either a normal salt (NS; 0.4% NaCl) or high salt diet (HS; 8% NaCl). The SHR and Dahl S rats are two established models of hypertension. Blood pressure was measured after 4 weeks of HS feeding. Along with the two models of hypertension (SHR and Dahl S) blood pressure was measured in their controls (WKY and BN13, respectively). The Dahl S and BN13 both showed a significant increase in SBP while the SHR and WKY animals did not have an elevation of SBP after 4 weeks of HS feeding (Figure 2A). The SBP of the Dahl S increased from 150.8 ±4.0 mmHg to 174.7 ±5.5 mmHg with HS feeding ($p < 0.05$); the SBP of the BN13 increased from 131.5 ±3.1 mmHg to 149.4 ±2.6 mmHg ($p < 0.05$) after 4 weeks. Measurements of DBP resulted in similar trends as SBP. The Dahl S and BN13 showed significantly increased DBP after 4 weeks of HS diet compared to NS fed animals, while SHR and WKY did not show a change in DBP (Figure 2B). The DBP of the Dahl S increased from 108.8 ±3.9 mmHg to 128.0 ±6.6 mmHg with HS diet compared to NS diet ($p < 0.05$), while the BN13 had a DBP of 92.0 ±1.8 mmHg with NS feeding and 101.6 ±3.2 mmHg with HS feeding ($p < 0.05$). Although BN13 animals displayed significantly lower blood pressure than the Dahl S, as shown previously (Cowley et al., 2001), it is interesting to note that both SBP and DBP were significantly

increased in these animals with HS feeding. The SHR rats had a blood pressure of 197.4/142.8 ± 7.1/8.3 mmHg on a NS diet. On a HS diet, the SHR rats had a blood pressure of 195.1/135.0 ± 8.4/5.0 mmHg. The WKY rats had a blood pressure 142.6/85.5 ± 4.9/5.7 mmHg on the NS diet; on a HS diet, the WKY rats had a 135.2/82.2 ± 6.5/6.5 mmHg.

3.1.2 Progression of blood pressure over 4 weeks of HS feeding

SBP and DBP were measured at weeks 0, 1, 3, and 4 weeks of HS feeding in the WKY, SHR, BN13, and Dahl S rats to determine the whether progression of salt sensitive hypertension occurred after a certain number of weeks after HS feeding. WKY and SHR did not develop a significant increase of either SBP (Figure 3A) or DBP (Figure 3C) at any point compared to baseline blood pressure levels with NS or HS feeding. This was also true in the BN13 (Figure 3B and Figure 3D). The Dahl S rats developed a significant elevation of SBP and DBP after 3 weeks of HS feeding compared to baseline levels. Dahl S rats fed the NS diet maintained baseline levels of SBP and DBP throughout the course of the study.

3.1.3 BUN and serum creatinine in salt sensitive and insensitive models of hypertension

To determine whether HS feeding for 4 weeks had an effect on renal function compared to NS feeding in the animal models, BUN and serum creatinine levels were measured. SHR, BN13, and Dahl S rats did not have increased BUN levels after 4 weeks of HS feeding compared to NS feeding (Figure 4A). HS fed Dahl S had significantly

increased BUN levels compared to HS fed SHR. WKY rats had a reduced BUN level after HS feeding compared to NS. Serum creatinine levels in all 4 strains of rats showed no elevation with HS feeding (Figure 4B). However, the Dahl S on the NS diet did have significantly increased serum creatinine levels compared to SHR on the same diet.

3.1.4 The effect of HS feeding on protein excretion of 4 strains of rats

As a measure of renal function and pathology, protein and albumin levels in urine were measured. Urine was collected over 24 hours after 3 weeks of salt diets and analyzed for protein and albumin. Total protein excretion was significantly increased in Dahl S rats, from 65.8 ±9.6 mg/24 hrs to 150.5 ±17.4 mg/24 hrs with HS feeding compared to NS ($p < 0.05$) (Figure 5A). HS feeding did not significantly increase total protein excretion in WKY, SHR, and BN13 rats. However, there was a trend in the BN13 ($p=0.09$). The HS fed Dahl S rat demonstrated increased total protein compared to SHR on the same diet. This relationship was also found in with total albumin excretion (Figure 5B). After 3 weeks of HS feeding, the Dahl S excreted a significantly greater amount of albumin compared to NS fed animals (Figure 5B). NS fed Dahl S excreted 32.7 ±6.9 mg/24 hrs, while HS fed Dahl S excreted 101.9 ±23.9 mg/24 hrs ($p < 0.05$).

3.1.5 Protein cast formation

Using PAS-stained kidney sections, protein casts were identified in both the renal medulla (Figure 6A) and cortex (Figure 7A). Protein casts were identified as intratubular PAS-positive regions but differed structurally from that of the proximal tubular brush

border due to the lack of villi; the majority of these casts were located in enlarged tubules within the medulla. Quantification showed that the WKY, SHR, and BN13 rats did not have significantly increased area density of protein casts after 4 weeks of HS feeding in the medulla (Figure 6B). The Dahl S rats showed a significantly increased area density of protein casts in the medulla when fed a HS diet compared to NS diet. On a NS diet, the Dahl S rats had 4.7 ± 0.85% area density of protein casts in the medulla, while Dahl S rats on a HS diet had 12.0 ±2.2% after 4 weeks. A similar trend was observed in the renal cortex (Figure 7B). Dahl S rats on a HS diet had 2.8 ± 0.71% area density of protein casts in the cortex, this was a significant increase compared to NS fed Dahl S (1.1 ±0.25%). The WKY, SHR, and BN13 rats did not show a significant difference between HS and NS fed groups. However, the SHR demonstrated a protection of renal pathology on HS diet compared to the Dahl S, as a significant reduction of area density of protein casts was found in both the cortex and medulla.

3.1.6 Myogenic Constriction

To define myogenic constriction in the study, the arcuate artery of the normotensive WKY rat was used. Vessels incubated in Ca-free HBSS exhibited no constriction and passive dilation was shown (Figure 8A). When vessels were placed in a normal HBSS solution, constriction occurred with increasing pressures and was significantly different compared to Ca-free HBSS ($p < 0.05$). In vessels treated with nifedipine, an L-type Ca channel blocker, the myogenic constriction was abolished (Figure 8B), demonstrating an L-type Ca channel dependent mechanism of the myogenic

constriction. Myogenic constriction was studied in SHR and WKY fed either a NS diet or a HS diet (Figure 9). Arcuate arteries from HS fed SHR and WKY showed a significant reduction of the myogenic response compared to the NS fed groups. However, in both groups SHR vessels showed significantly greater myogenic constriction then the WKY vessels.

3.1.7 The effect of hypertension on proteinuria in salt sensitive and salt insensitive models.

Using a definition of hypertension in which the SBP is greater than 140 mmHg, the rate of protein excreted per mmHg above this level was determined. This allowed us to establish whether there was a difference between the salt sensitive, Dahl S, and the salt insensitive, SHR, in the response of proteinuria to blood pressure. There was a significantly greater increase in 24-hour protein excretion for every mmHg increase in blood pressure above 140 mmHg in the Dahl S than in the SHR rats on HS diet (Figure 10). This suggests a difference in the ability of blood pressure to cause protein excretion between a salt sensitive and salt insensitive model of hypertension.

3.1.8 Effect of 4-PBA treatment on blood pressure in a salt insensitive model of hypertension, SHR

To determine whether 4-PBA treatment had a blood pressure lowering effect independent of salt sensitivity, NS fed SHR were treated with 4-PBA (1g/kg/day) for 5 weeks. After 5 weeks of 4-PBA treatment, blood pressure was measured (Figure 11).

There was a significant reduction of SBP and DBP in SHR with 4-PBA treatment compared to untreated controls. The blood pressure of untreated SHR was 206.1/143.6 ±4.3/4.4 mmHg. 5 weeks of 4-PBA treatment reduced blood pressure to 179.0/121.2 ±2.1/4.3 mmHg.

3.2 Objective 2: Determine if treatment with 4-PBA preserves myogenic constriction in the Dahl S rat and if this reduces proteinuria or renal pathology.

3.2.1 4-PBA and Blood Pressure

After determining the effect of HS feeding in the Dahl S and BN13 rats, 4-PBA treatment was given to HS fed animals to determine the effect of treatment on salt sensitive animals. 4-PBA treatment of HS Dahl S significantly reduced SBP from 174.7 ±5.5 mmHg to 159.1 ±4.1 mmHg (#, $p < 0.05$) (Figure 12A). 4-PBA treatment of BN13 rats on HS diet also significantly lowered SBP from 149.4 ±2.6 mmHg to 130.7 ±4.1 mmHg (#, $p < 0.05$). 4-PBA treatment significantly lowered DBP in the BN13 animals (101.6 ±3.2 mmHg to 89 ±4.4 mmHg; #, $p < 0.05$) (Figure 12B). 4-PBA treatment prevented a significant salt-induced increase in DBP in the Dahl S rats but did not significantly lower DBP ($p = 0.21$).

3.2.2 4-PBA and Proteinuria

After determining that the Dahl S excrete significantly high levels of protein and albumin in the urine with 4 weeks of HS feeding compared to NS feeding (Figure 5A and 5B), the effect of 4-PBA treatment on these parameters was measured in the animals. 4-

PBA treatment of HS fed Dahl S significantly reduced the amount of protein and albumin excreted compared to untreated animals (Figure 13A). Although 4-PBA treatment reduced protein and albumin excretion in BN13 animals, this was not significant ($p = 0.21$ and 0.25, respectively). When observing the development of proteinuria over the course of the study, HS feeding resulted in significantly increased protein excretion after the first week in the Dahl S compared to protein levels at week 0 (&, $p < 0.05$) (Figure 13B). After 3 weeks, HS feeding resulted in significantly increased protein and albumin excretion compared to levels at Week 0 (&, $p < 0.05$). 4-PBA treated animals maintained baseline levels of protein and albumin excretion over the course of the study. Protein excretion in HS fed BN13 remained constant throughout the 3 weeks of observation, however albumin excretion significantly increased after 3 weeks compared to albumin levels at Week 0 (Figure 13C).

3.2.3 Myogenic constriction in response to high salt feeding and 4-PBA treatment

After establishing that the renal arcuate artery is capable of a myogenic response at intralumenal pressures of (100, 120, 140, 160, and 180 mmHg) (Figure 8A). The arcuate artery from NS fed Dahl S animals was tested and demonstrated a reduction in lumen diameter (negative change in diameter) when intralumenal pressure was increased (Figure 14A). HS feeding for 4 weeks attenuated myogenic constriction in the Dahl S. 4-PBA treatment in HS fed Dahl S rats resulted in the preservation of the myogenic response in the arcuate arteries ($p < 0.05$). Unlike the Dahl S rat, salt sensitivity of myogenic constriction was not observed with BN13 animals (Figure 14B).

To determine the importance of blood pressure in the level of protein and albumin excretion, linear regression analysis was performed using SBP as a predictor of 24-hour total protein or albumin excretion. SBP was found to be a weak predictor (R^2= 0.21) of total protein excretion in the Dahl S rats (Figure 15A). SBP showed little predictive value for albumin excretion (R^2 = 0.017) (Figure 15B). This suggests that the effect of 4-PBA to significantly reduce proteinuria may be due to effects in addition to blood pressure.

3.2.4 4-PBA and intratubular protein casts

To determine the effect of 4-PBA treatment on renal pathology in a salt sensitive hypertensive model, kidney sections were PAS stained to observe for intratubular protein casts in the cortex and medulla (Figure 16A). 4-PBA treatment in HS fed Dahl S significantly reduced area density of protein casts in both the cortex and medulla compared to untreated HS fed animals (Figure 16B). 4-PBA treatment reduced the area density of protein casts in the medulla from 12.0 ±2.2% to 3.9 ±0.6% ($p < 0.05$); in the cortex, the area density was reduced from 2.8 ± 0.71% to 0.78 ±0.2% ($p < 0.05$). 4-PBA treatment lowered the protein cast area density in the BN13 of the cortex and medulla, however this was not statistically significant ($p = 0.72$ and 0.4, respectively).

3.2.5 Reabsorption of proteins in the proximal tubule

To determine whether 4-PBA, a low molecular weight chaperone, prevented proteinuria by helping to fold endocytic receptors in the proximal tubules and aid in their trafficking to the cellular membrane, kidney tissues were stained for cubilin expression

(Figure 17A). Although not significant, there was a trend for 4-PBA treatment to increase cubilin expression compared to untreated HS fed animals. Area density of cubilin expression in Dahl S HS animals was 2.42 ±0.4%, the area density with 4-PBA treatment was increased to 3.34 ±0.7% ($p = 0.28$) (Figure 17B). Higher levels of cubilin may indicate a larger reabsorptive capacity in the proximal tubules, thereby reducing the amount of total protein or albuin appearing in the final urine. Increased cubilin levels with 4-PBA treatment may partially explain reduced levels of proteinuria observed in this group.

3.2.6 4-PBA and the integrity of the glomerular filtration barrier

To determine whether 4-PBA treatment preserved the integrity of the GFB in HS fed Dahl S, transmission electron microscopy (TEM) images were taken. The GFB of the Dahl S animals on a HS diet showed podocyte effacement and GBM expansion (Figure 18 B1-4). 4-PBA treatment protected the GFB from these injuries in HS fed Dahl S (Figure18C1-4). It is interesting to note that the rough ER of the podocytes showed dilation with HS feeding in the Dahl S (Figure 18 B-4) and this was prevented by 4-PBA treatment (Figure 18 C-4). HS fed BN13 animals did not show damage in the GFB, as podocytes, the GBM, and the fenestrated endothelial cells remained intact and normal (Figure 19).

To determine whether the rough ER showed dilation in the proximal tubules, where protein reabsorption occurs and may produce a high demand for reabsorptive protein synthesis with proteinuria, TEM images were taken. There was no visible

difference in the amount of rough ER or dilation of the rough ER with HS feeding compared to NS in the Dahl S (Figure 20) and BN13 (Figure 21). This suggests that HS feeding for 4 weeks may not act in the proximal tubules.

3.2.7 4-PBA and interstitial fibrosis

To determine the effect of 4-PBA treatment on renal interstitial fibrosis, the expression of α-SMA, a marker for myofibroblasts, was observed with immunohistochemistry staining (Figure 22A). α-SMA -positive cells were concentrated mainly in the renal medulla and found around protein casts. The Dahl S developed significantly increased α-SMA area density from 2.08 ±0.27% to 3.93 ±0.41% with HS feeding ($p < 0.05$) (Figure 22B). 4-PBA treatment significantly reduced α-SMA positive area density of HS fed Dahl S to 2.54 ±0.4% in the medulla. Although the area density of α-SMA staining was lower in the Dahl S renal cortex than in the medulla, the same trends were observed. There was no significant difference in area density between NS and HS diets in the BN13 either in the cortex or medulla.

Collagen deposition was observed with PSR staining. Similar to protein casts and α-SMA staining, regions of collagen deposition were largely located in the medulla (Figure 23A). Collagen deposition was located in the areas surrounding protein casts in both renal cortex and medulla. HS feeding in Dahl S resulted in significantly increased collagen deposition compared to NS fed animals; showing an increase in area density from 1.31 ±0.31 to 2.23 ±0.25% in the medulla ($p < 0.05$)(Figure 23B). Compared to untreated HS fed Dahl S, 4-PBA treatment resulted in significantly reduced collagen

deposition in the medulla (1.13 ±0.32%; $p < 0.05$). BN13 animals did not show and increase in renal interstitial fibrosis in response to HS feeding.

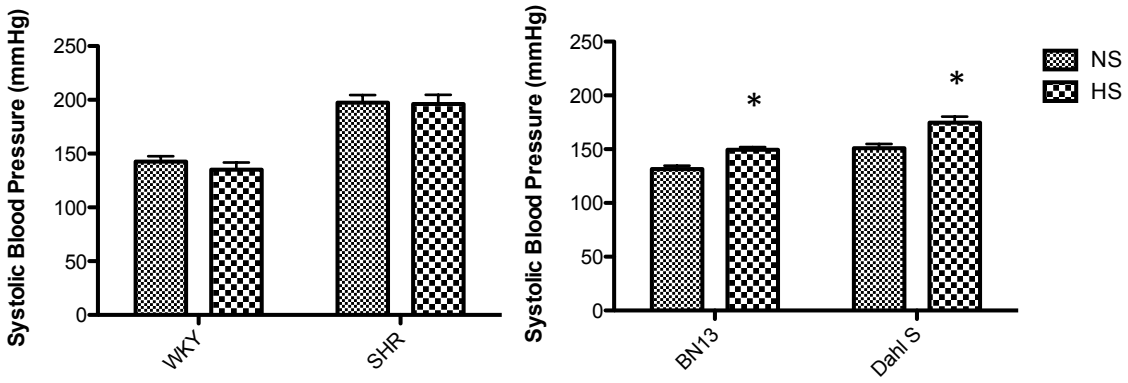
3.3 Objective 3: Determine the effect of 4-PBA treatment on ER stress in the kidney.

3.3.1 4-PBA and ER stress in the kidney

To study the effect of ER stress on myogenic constriction in the arcuate artery, renal vessels were micro-dissected from normotensive Sprague Dawley rats and subject to ER stress-inducer tunicamycin (TM, 1 µg/mL) treatment or drug vehicle. Additionally, TM and 4-PBA co-treatment were performed to determine if ER stress inhibition could effect myogenic constriction. TM treatment resulted in an attenuation of the myogenic response, while 4-PBA co-treatment resulted in some protection (Figure 24).

To study the effect of 4-PBA on ER stress in broad regions of the kidney Western blotting was performed for the ER stress marker GRP78 in the renal cortex and medulla (Figure 25A). Tissue homogenates of the renal cortex demonstrated a significant increase in GRP78 expression with HS feeding compared to NS ($p < 0.05$) (Figure 25B). 4-PBA treatment did not significantly reduce GRP78 expression in the renal cortex, but did in the renal medulla ($p < 0.05$). CHOP expression was not found in tissue homogenates due to large amounts of background signal.

A


Systolic Blood Pressure (mmHg)
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WKY
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Systolic Blood Pressure (mmHg)
250
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BN13
Dahl S
NS
HS

B

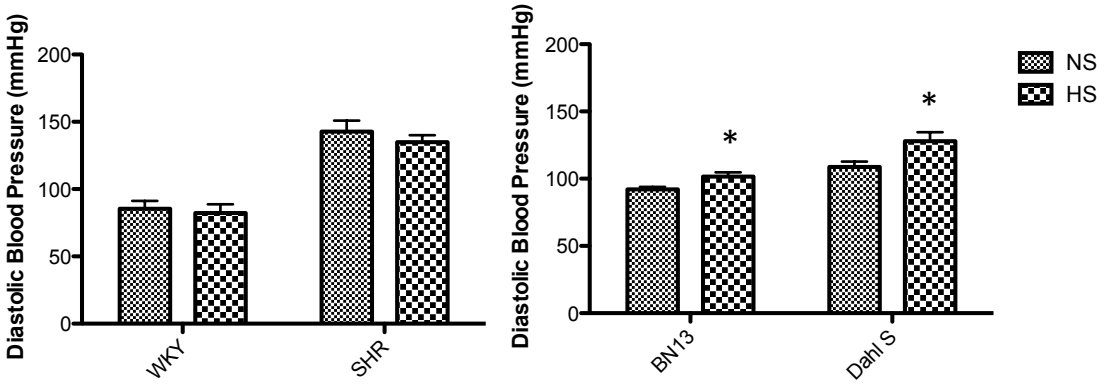

Diastolic Blood Pressure (mmHg)
200
150
100
50
0
WKY
SHR
Diastolic Blood Pressure (mmHg)
200
150
100
50
0
*
*
BN13
Dahl S
NS
HS

Figure 2. Blood pressure after 4 weeks of salt diet in four strains of rat demonstrate varying salt sensitivities. (A) SBP measured after 4 weeks of salt diets in WKY, SHR, BN13, and Dahl S rats demonstrate an elevated blood pressure in the SHR regardless of salt diet. The WKY animals did not exhibit hypertension with either NS or HS diet. The BN13 and Dahl S animals demonstrated significantly elevated SBP with HS feeding. The Dahl S had a greater SBP compared to the BN13 rats. (B) DBP demonstrated similar trends as SBP; BN13 and Dahl S animals developed significantly elevated DBP with HS feeding. N=5 in SHR and WKY groups; N=10 in Dahl S; N=6 BN13 groups. *, $p < 0.05$ vs same strain on NS.

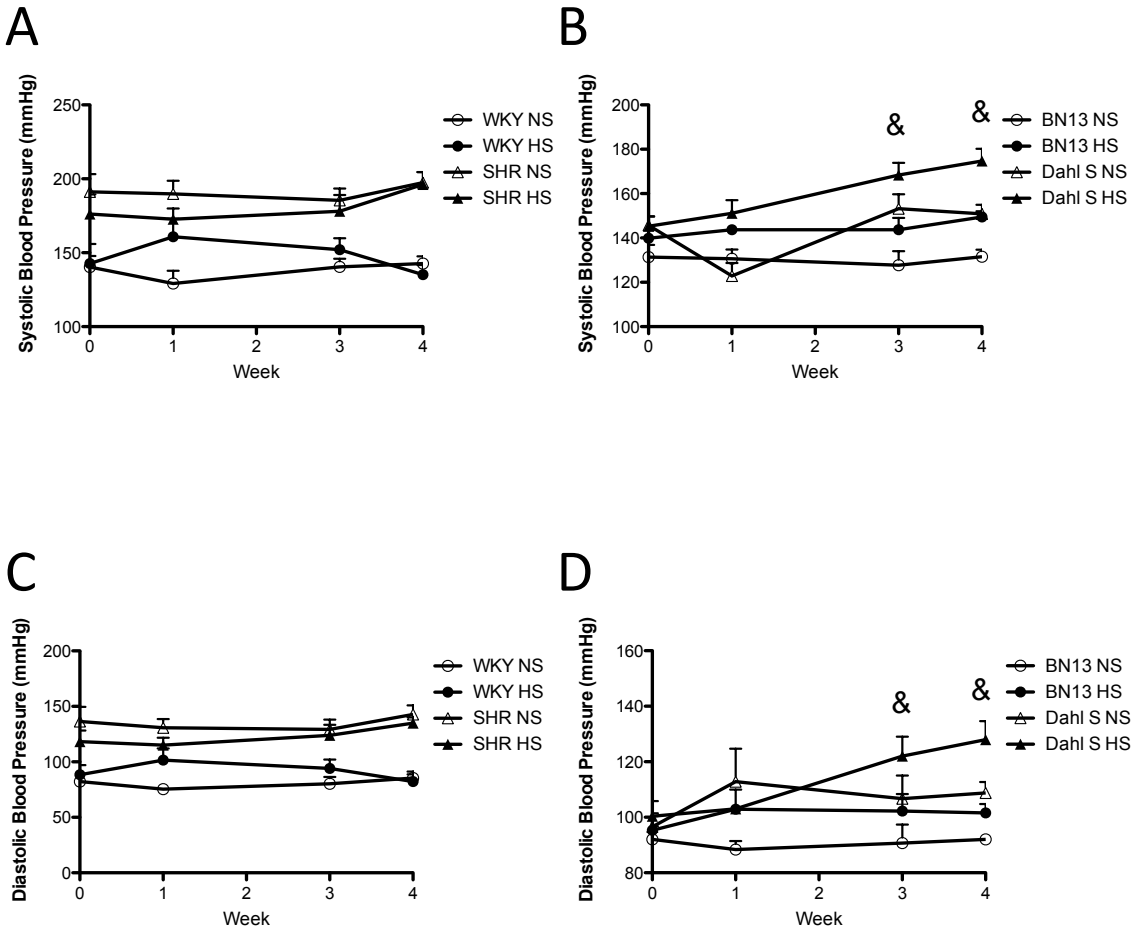
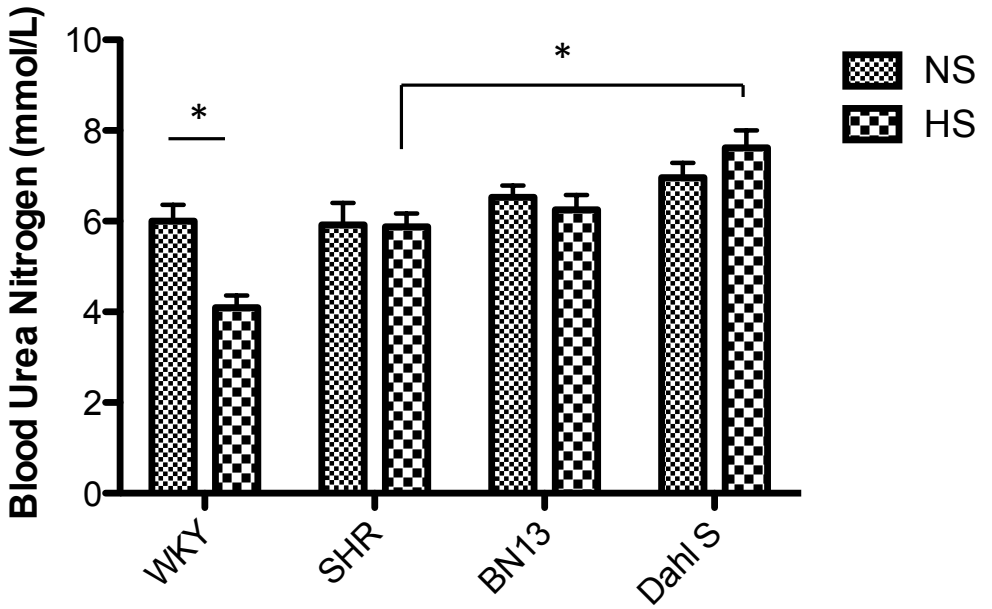

A
Systolic Blood Pressure (mmHg)
250
200
150
100
0
1
2
3
4
Week
WKY NS
WKY HS
SHR NS
SHR HS
B
Systolic Blood Pressure (mmHg)
200
180
160
140
120
100
&
&
Week
BN13 NS
BN13 HS
Dahl S NS
Dahl S HS
C
Diastolic Blood Pressure (mmHg)
200
150
100
50
0
Week
WKY NS
WKY HS
SHR NS
SHR HS
D
Diastolic Blood Pressure (mmHg)
160
140
120
100
80
&
&
Week
BN13 NS
BN13 HS
Dahl S NS
Dahl S HS

Figure 3. Development of blood pressure over 4 weeks of high salt (HS) feeding in four strains of rats. (A, C) SBP and DBP of WKY and SHR at weeks 0, 1, 3, and 4 of salt diet starting at 12 weeks of age. (B, D) SBP and DBP measured at weeks 0, 1, 3, and 4 of salt diets in Dahl S and BN13 . Data represented as mean ± SEM. &, $p < 0.05$ vs week 0. N=5 in SHR and WKY groups; N=10 in Dahl S; N=6 BN13 groups.

A


Blood Urea Nitrogen (mmol/L)
10
8
6
4
2
0
*
*
NS
HS
WKY
SHR
BN13
Dahl S

B

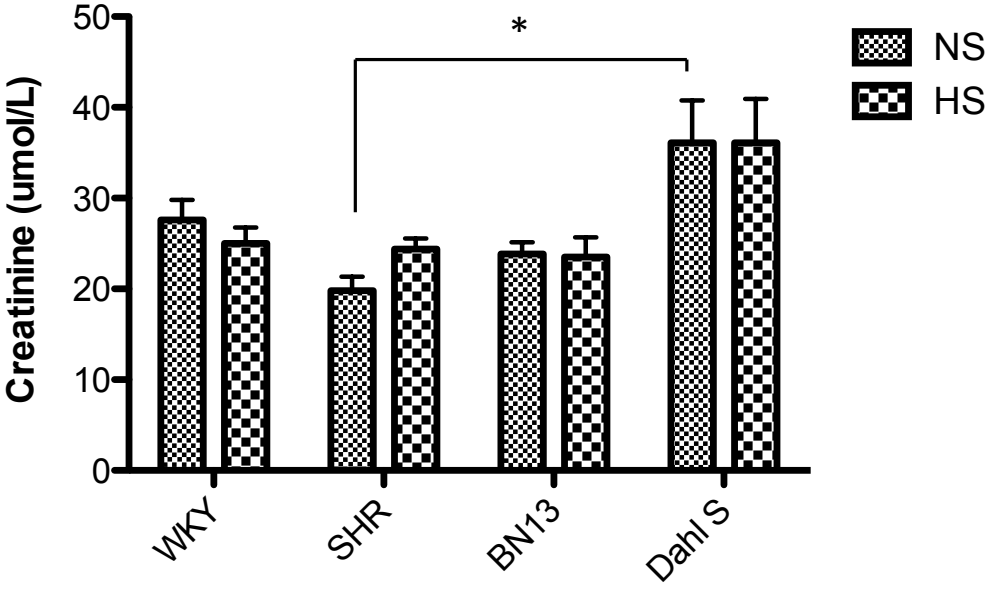

Creatinine (umol/L)
50
40
30
20
10
0
*
NS
HS
WKY
SHR
BN13
Dahl S

Figure 4. Analysis of blood urea nitrogen (BUN) and plasma creatinine in four strains of rat. Plasma collected at time of sacrifice after 4 weeks of salt diets was analyzed for BUN (A) and plasma creatinine (B) as measures of renal function. Data represented as mean ± SEM. *, $p<0.05$. N=5 in SHR and WKY groups; N=10 in Dahl S; N=6 BN13 groups.

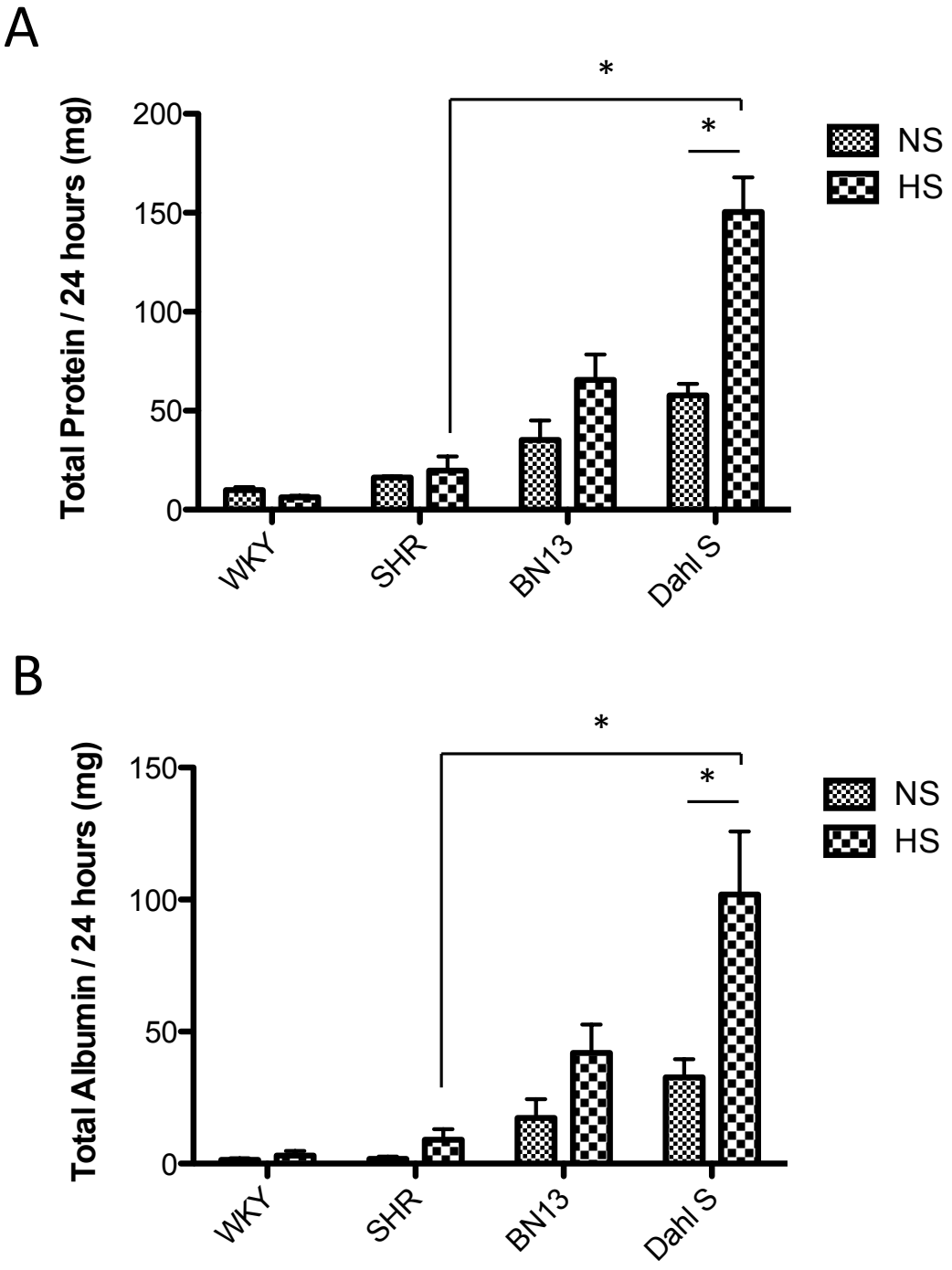
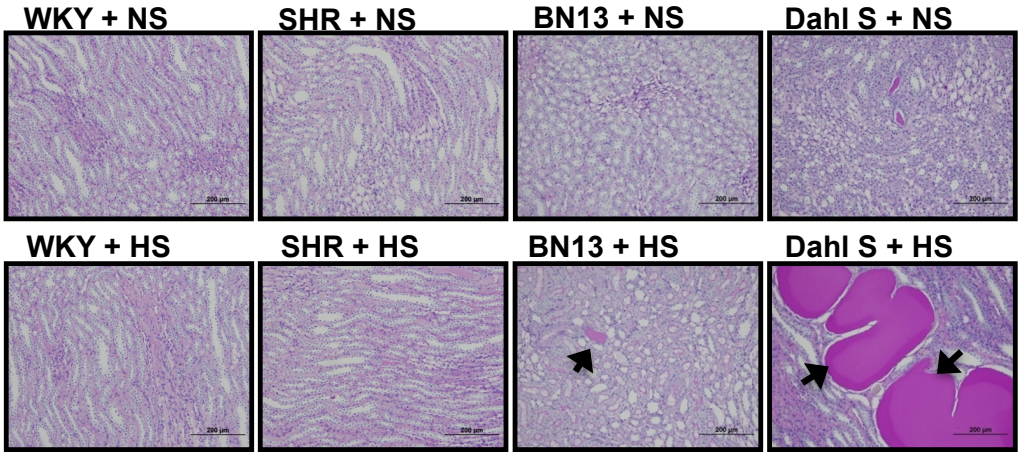

A
Total Protein / 24 hours (mg)
200
150
100
50
0
*
*
NS
HS
WKY
SHR
BN13
Dahl S
B
Total Albumin / 24 hours (mg)
150
100
50
0
*
*
NS
HS
WKY
SHR
BN13
Dahl S

Figure 5. The effect of high salt feeding on total protein and albumin excretion in four strains of rat. (A) After 3 weeks of salt feeding, 24 hour urine samples were collected from WKY, SHR, BN13, and Dahl S and analyzed for protein excretion. (B) Urinary albumin excretion over 24 hours was measured from urine samples. Data represented as mean ± SEM. *, $p<0.05$. N=5 in SHR and WKY groups; N=10 in Dahl S; N=6 BN13 groups.

A


WKY + NS
SHR + NS
BN13 + NS
Dahl S + NS
WKY + HS
SHR + HS
BN13 + HS
Dahl S + HS

B

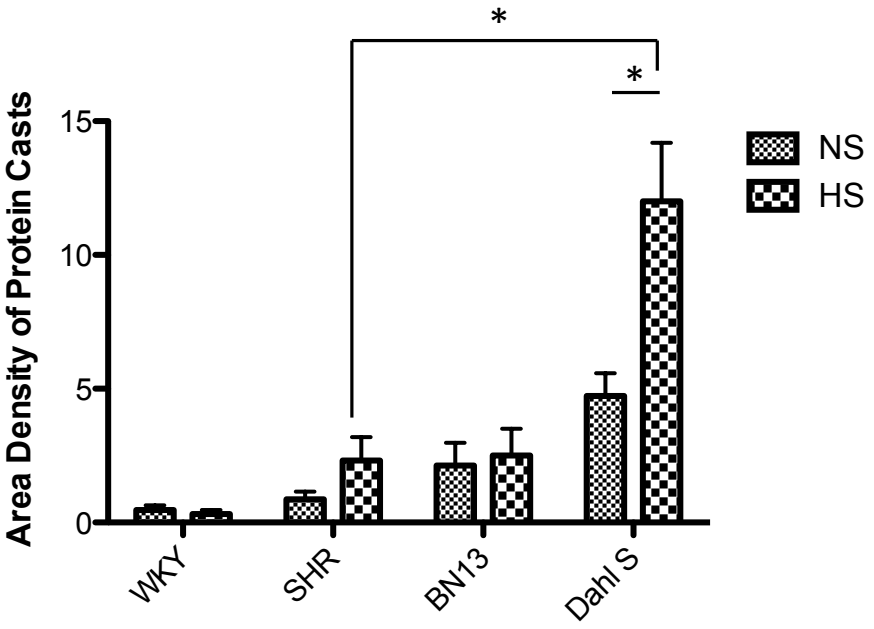
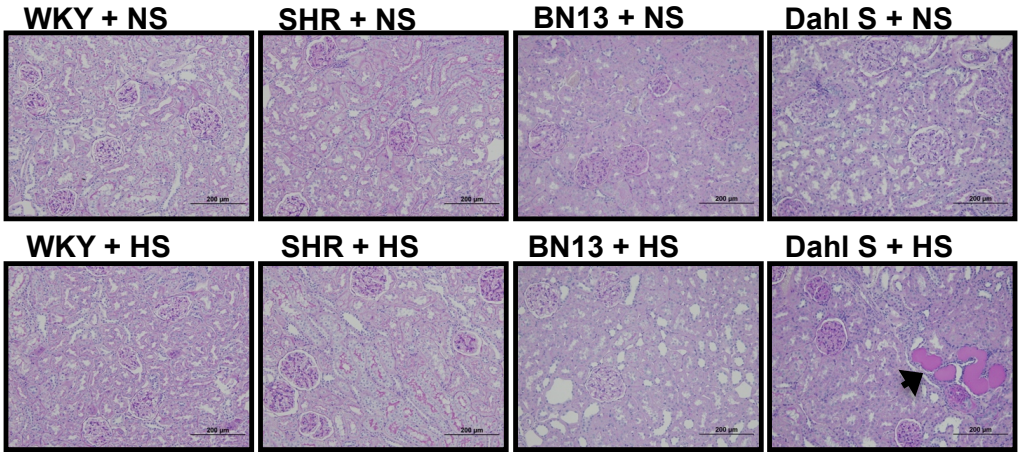

*
*
Area Density of Protein Casts
15
10
5
0
WKY
SHR
BN13
Dahl S
NS
HS

Figure 6. The effect of high salt feeding on the development of intratubular protein casts in the medulla of four strains of rat. (A) Paraffin-embedded kidneys were sectioned and PAS stained; images of renal medulla were taken at 20x. (B) Area density was quantified from 10 images per animal and represented as mean ± SEM. *, $p<0.05$ vs same strain on NS. N=5 in SHR and WKY groups; N=10 in Dahl S; N=6 BN13 groups.

A


WKY + NS
SHR + NS
BN13 + NS
Dahl S + NS
WKY + HS
SHR + HS
BN13 + HS
Dahl S + HS

B

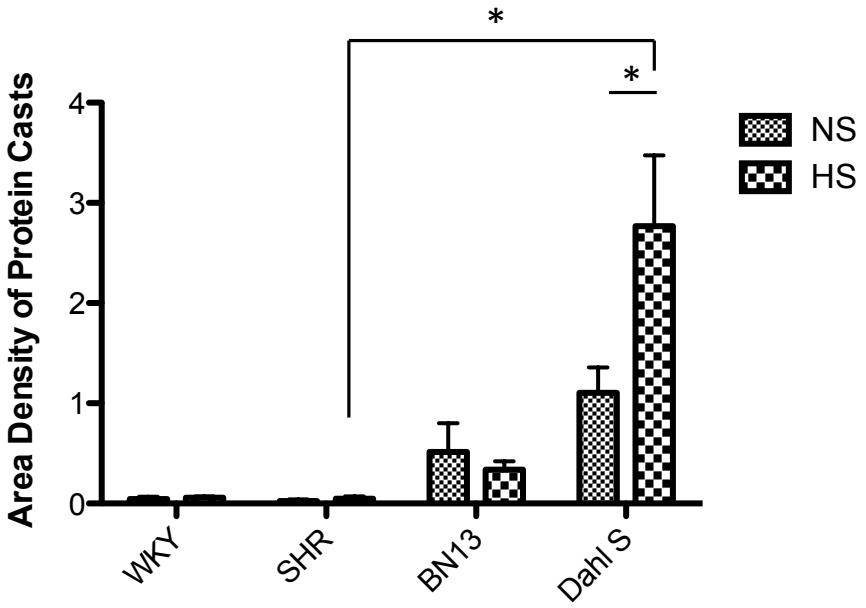
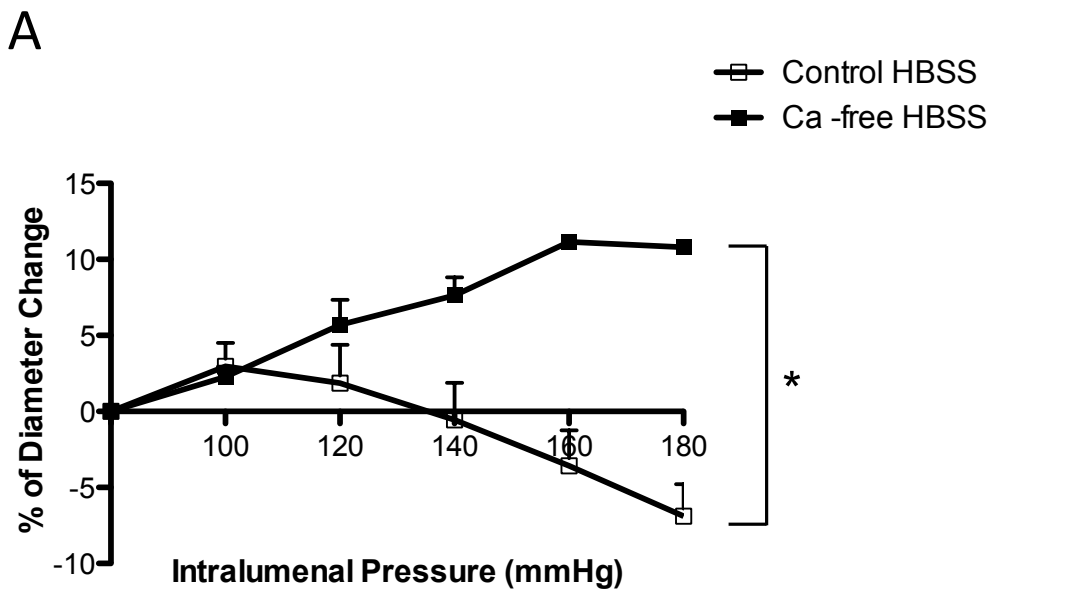

Area Density of Protein Casts
0
1
2
3
4
*
*
WKY
SHR
BN13
Dahl S
NS
HS

Figure 7. The effect of high salt feeding on the development of intratubular protein casts in the cortex of four strains of rat. (A) Paraffin-embedded kidneys were sectioned and PAS stained; images of renal cortex were taken at 20x. (B) Area density was quantified from 10 images per animal and represented as mean ± SEM. *, $p<0.05$. N=5 in SHR and WKY groups; N=10 in Dahl S; N=6 BN13 groups.


A
Control HBSS
Ca -free HBSS
15
10
5
0
-5
-10
% of Diameter Change
100
120
140
160
180
Intralumenal Pressure (mmHg)
*

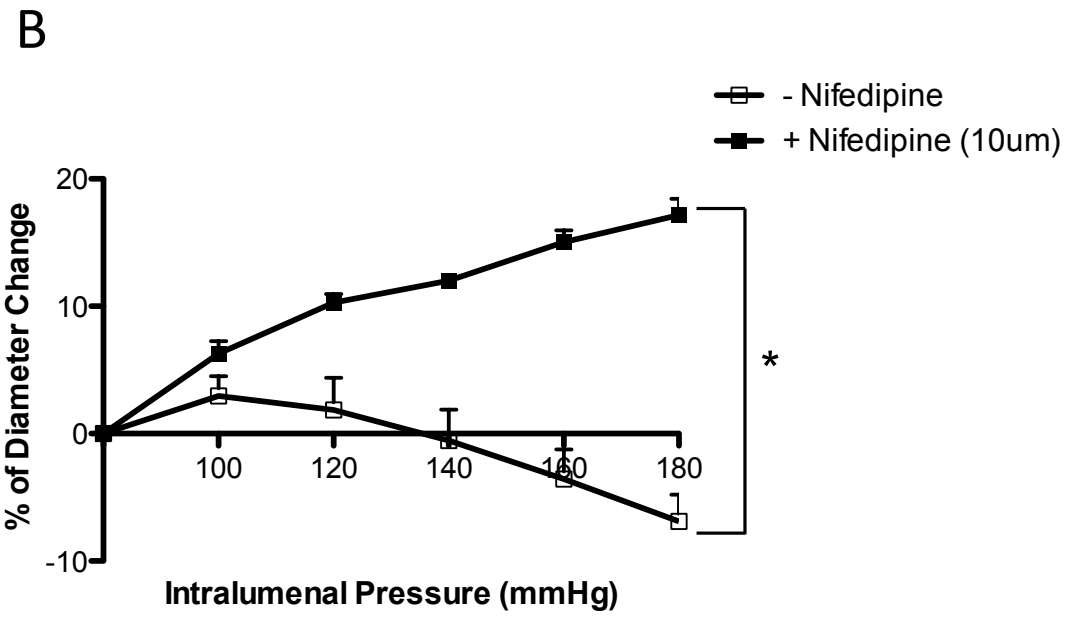
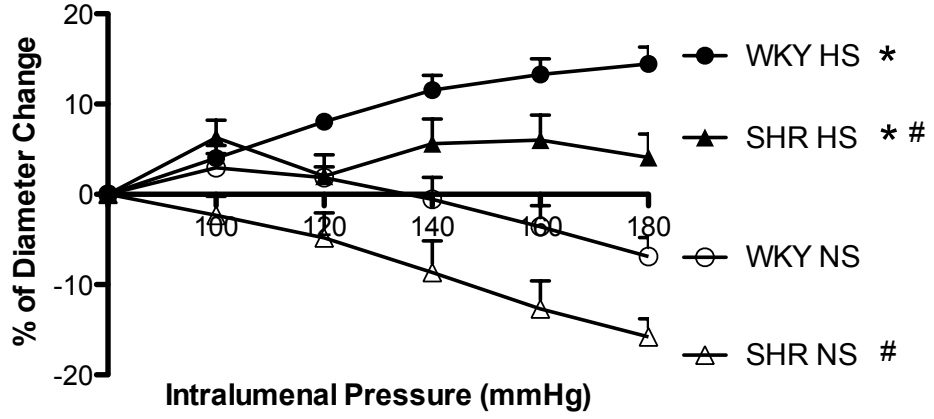

B
- Nifedipine
+ Nifedipine (10um)
20
10
0
-10
% of Diameter Change
100
120
140
160
180
Intralumenal Pressure (mmHg)
*

Figure 8. Myogenic constriction in the arcuate artery of the normotensive WKY rat is L-type calcium channel dependent. (A) Arcuate arteries from NS fed WKY rats were placed in normal HBSS (N = 7) or calcium free HBSS (N=4) and the percent of lumen diameter change was measured at pressures between 80 – 180 mmHg in 20 mmHg increments. The space between the two lines represents the tension generated by myogenic constriction. (B) Using an L-type calcium channel blocker, nifedipine (10um) myogenic constriction was tested at the above pressures (N=5).

A


20
10
0
-10
-20
% of Diameter Change
100
120
140
160
180
Intralumenal Pressure (mmHg)
WKY HS *
SHR HS * #
WKY NS
SHR NS #

B

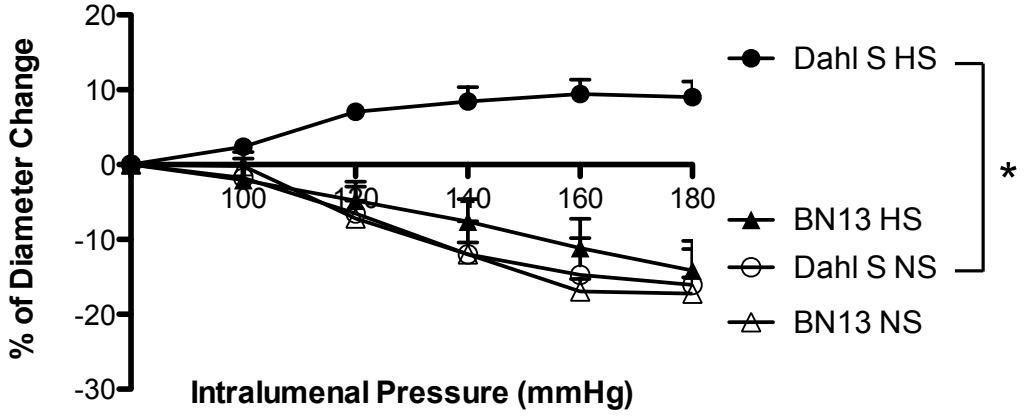

20
10
0
-10
-20
-30
% of Diameter Change
100
120
140
160
180
Intralumenal Pressure (mmHg)
Dahl S HS
BN13 HS
Dahl S NS
BN13 NS
*

Figure 9. The effect of salt feeding on the myogenic response in the arcuate artery of 4 models of rats. The percent diameter change of the lumen of the arcuate artery was measured at 80, 100, 120, 140, 160, and 180 mmHg of lumen pressure in HBSS. (A) The arcuate artery from HS fed animals had an attenuated myogenic response compared to NS in both WKY and SHR strains. *, $p < 0.05$: NS vs HS on same strain; #, $p < 0.05$: WKY vs SHR on same diet. N=7 in WKY groups; N=7 in SHR NS; N=8 in SHR HS. (B) Vessels from HS fed Dahl S resulted in a significant reduction of myogenic constriction. *, $p < 0.05$. N= 6 in BN13 groups. N= 8 in Dahl S NS; N=10 in Dahl S HS.

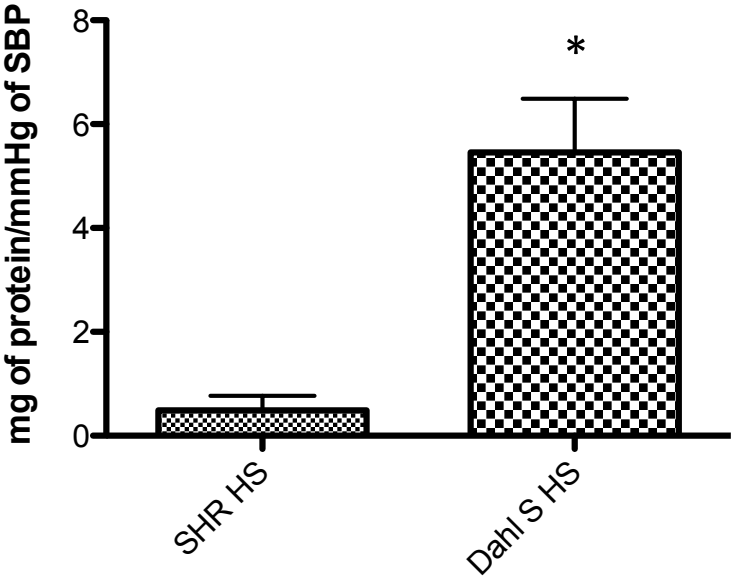

mg of protein/mmHg of SBP
8
6
4
2
0
*
SHR HS
Dahl S HS

Figure 10. Salt sensitivity and the effect of blood pressure on protein excretion. The SBPs of HS fed Dahl S (N=10) and HS fed SHR (N=5) was subtracted from 140 mmHg (> 140 mmHg was used to define hypertension). The protein excreted per mmHg demonstrates that for each mmHg of SBP over 140 mmHg, the Dahl S excretes a significantly greater amount of protein compared to the SHR. (*, $p < 0.05$).

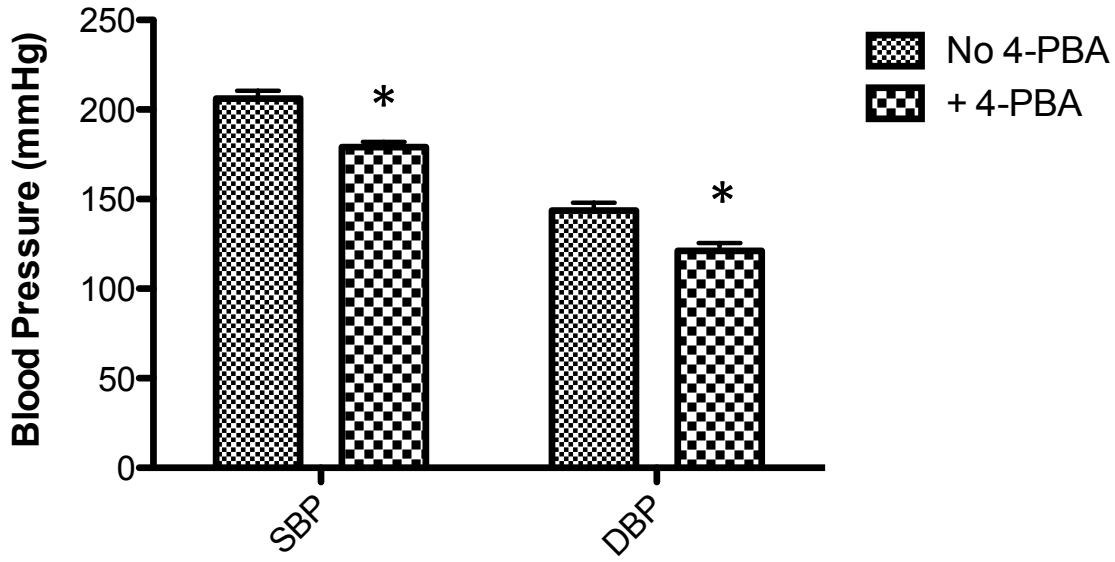
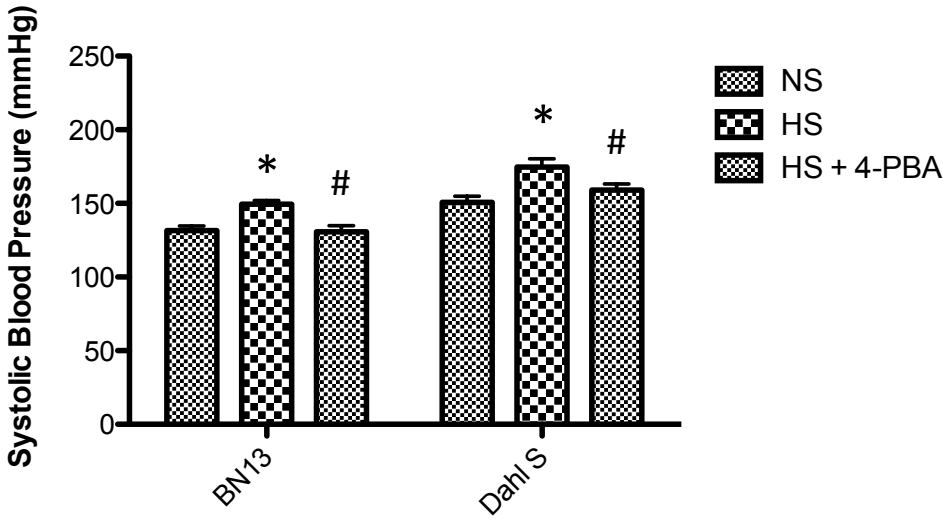

Blood Pressure (mmHg)
250
200
150
100
50
0
*
*
SBP
DBP
No 4-PBA
+ 4-PBA

Figure 11. The effect of 4-PBA treatment on blood pressure on SHR. SHR fed a normal salt diet were treated with 4-PBA (1g/kg/day) for 5 weeks. Blood pressure was measured using tail cuff plethysmography. Data represented as mean ± SEM. *, $p < 0.05$ vs NS. N= 7-8.

A


Systolic Blood Pressure (mmHg)
250
200
150
100
50
0
*
#
*
#
BN13
Dahl S
NS
HS
HS + 4-PBA

B

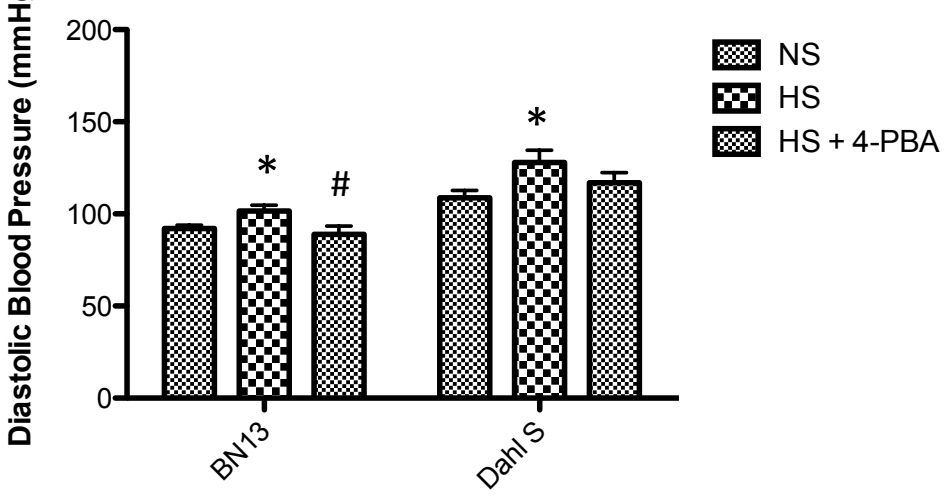
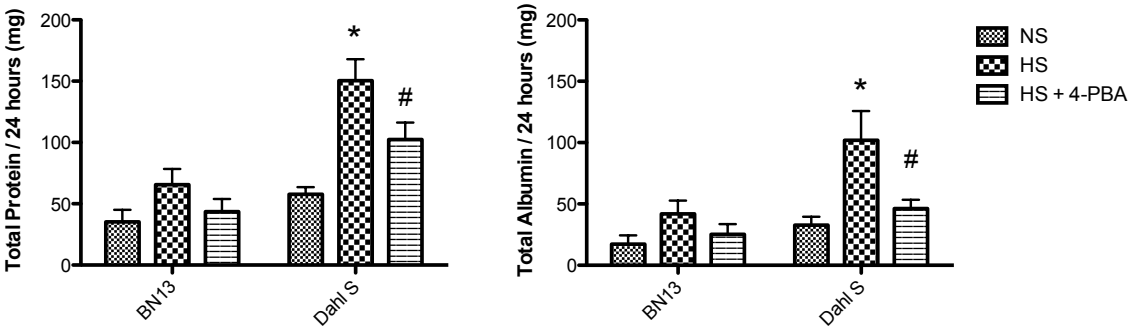
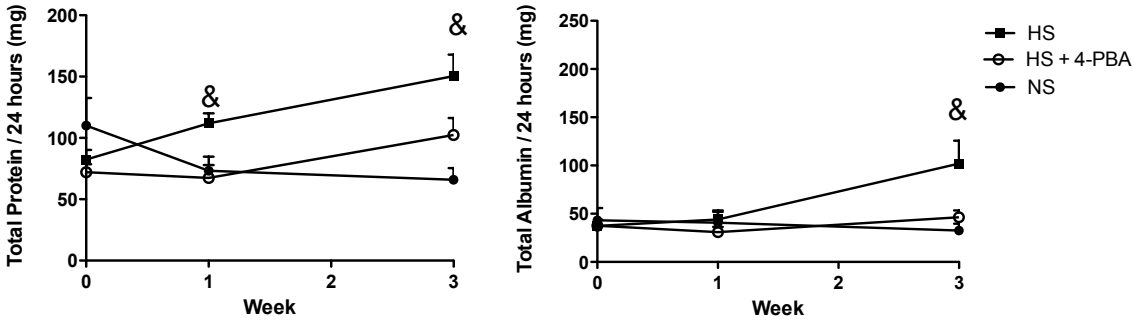

Diastolic Blood Pressure (mmHg)
200
150
100
50
0
*
#
*
BN13
Dahl S
NS
HS
HS + 4-PBA

Figure 12. The effect of 4-PBA treatment on blood pressure on Dahl S animals fed a HS diet for 4 weeks. 4-PBA treatment (1g/kg/day) was given one week prior to HS feeding in BN13 and Dahl S. Blood pressure was measured using tail cuff plethysmography after 4 weeks of salt diet. NS fed animals acted as the control group. Data represented as mean ± SEM. *, $p < 0.05$ vs NS; #, $p < 0.05$ vs HS of same strain. N= 10.

A



B Dahl S



C BN13

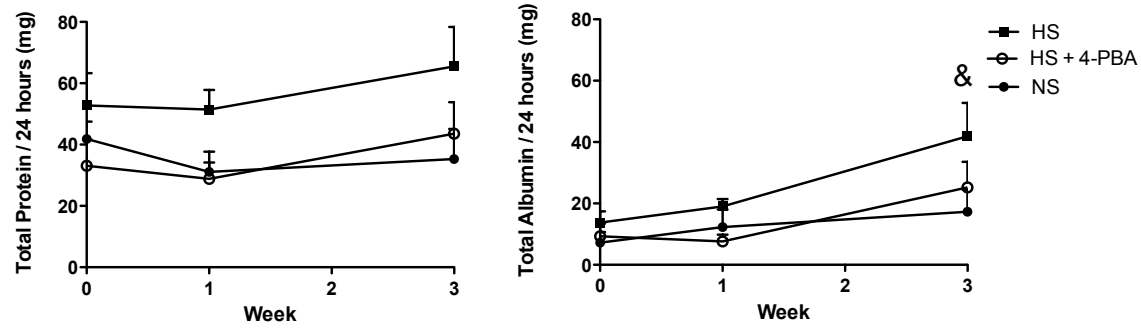
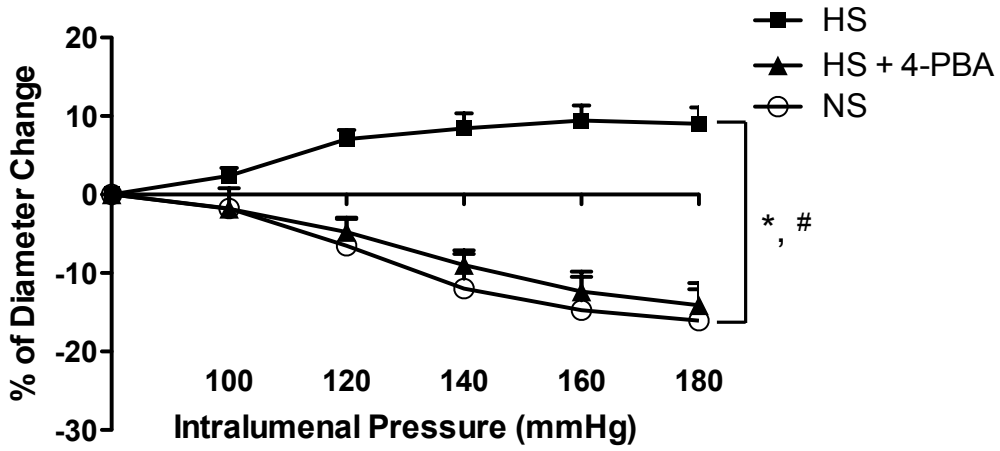


Figure 13. The effect of 4-PBA treatment on proteinuria in Dahl S animals fed a HS diet for 3 weeks. 4-PBA treatment (1g/kg/day) was given one week prior to HS feeding in BN13 and Dahl S. (A) Urine was collected and analyzed for protein and albumin excretion after 3 weeks of salt feeding. *, $p < 0.05$ vs NS; #, $p < 0.05$ vs HS of same strain. (B) Urine was collected at weeks 0, 1, and 3 to determine progression of proteinuria and albuminuria in Dahl S rats. (C) Progression of proteinuria and albuminuria in BN13 rats. NS fed animals acted as the control group in both Dahl S and BN13. Data represented as mean ± SEM. N= 6 in BN13 groups; N= 10 in Dahl S groups.

A


HS
HS + 4-PBA
NS
20
10
0
-10
-20
-30
% of Diameter Change
100
120
140
160
180
Intralumenal Pressure (mmHg)
*, #

B

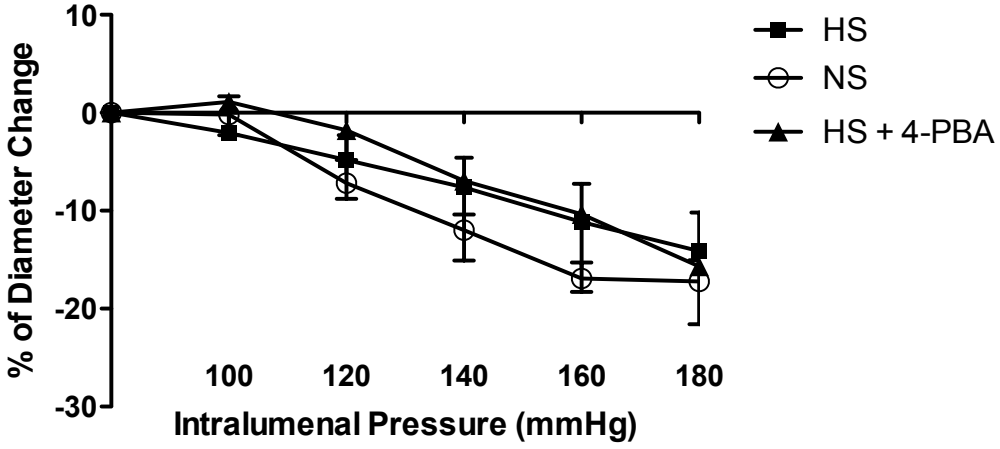
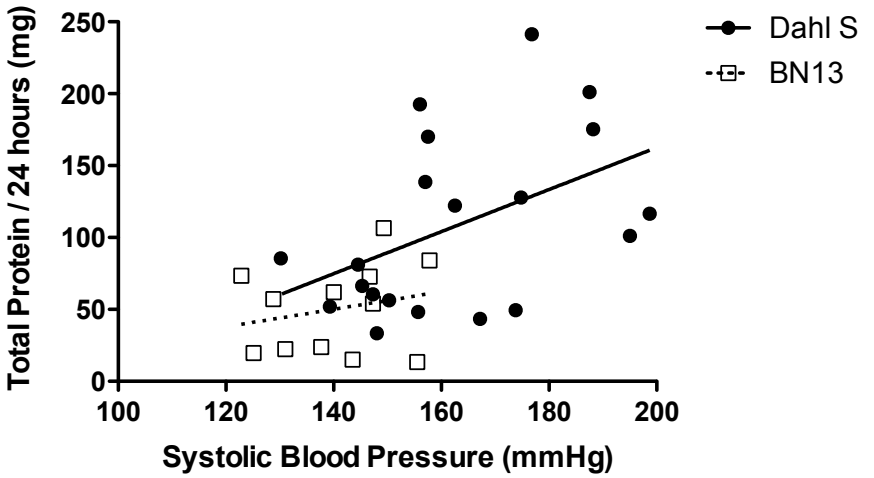

HS
NS
HS + 4-PBA
10
0
-10
-20
-30
% of Diameter Change
100
120
140
160
180
Intralumenal Pressure (mmHg)

Figure 14. Myogenic response in in the Dahl S and BN13 rats, the effect of salt and 4-PBA treatment. 4-PBA treatment (1g/kg/day) was given one week prior to HS feeding in BN13 and Dahl S. Myogenic constriction was measured at intralumenal pressures of 80, 100, 120, 140, 160, and 180 mmHg. (A) The arcuate artery from the Dahl S show an attenuation of myogenic constriction with HS feeding for 4 weeks, this was protected with 4-PBA treatment. N= 4-6. *, $p < 0.05$ vs NS; #, $p < 0.05$ vs HS. (B) The arcuate artery from BN13 rats did not show a change of myogenic constriction with HS feeding compared to NS. N= 6. Data represented as mean ± SEM.

A


Total Protein / 24 hours (mg)
250
200
150
100
50
0
100
120
140
160
180
200
Systolic Blood Pressure (mmHg)
Dahl S
BN13

B

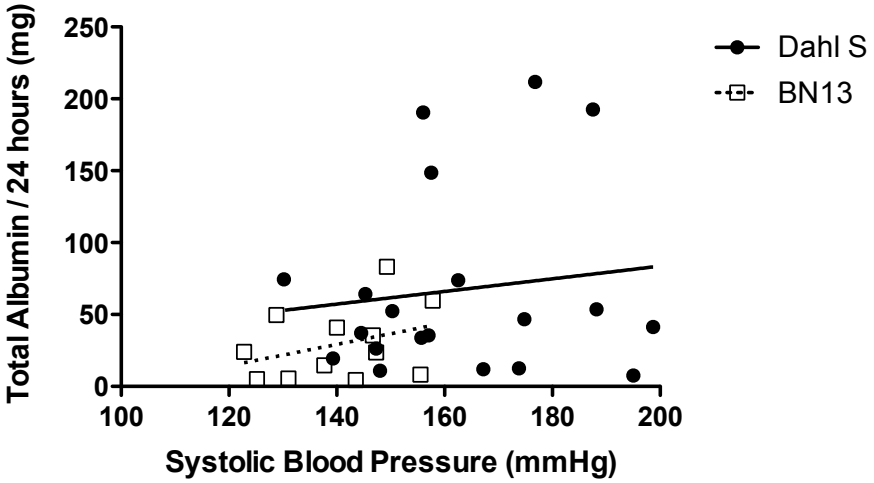

Total Albumin / 24 hours (mg)
250
200
150
100
50
0
100
120
140
160
180
200
Systolic Blood Pressure (mmHg)
Dahl S
BN13

Figure 15. The role of SBP on total protein and albumin excretion in Dahl S and BN13 rats. (A) Total urinary protein excretion over 24 hours is represented per animal with SBP at 4 weeks of salt feeding as a predictor. Data represent HS and NS fed Dahl S and BN13 rats. (B) Total albumin excretion over 24 hours vs SBP at 4 weeks of salt diet is represented. Albumin excretion of both HS and NS fed Dahl S and BN13 rats are presented. N=20 in Dahl S; N=12 in BN13.

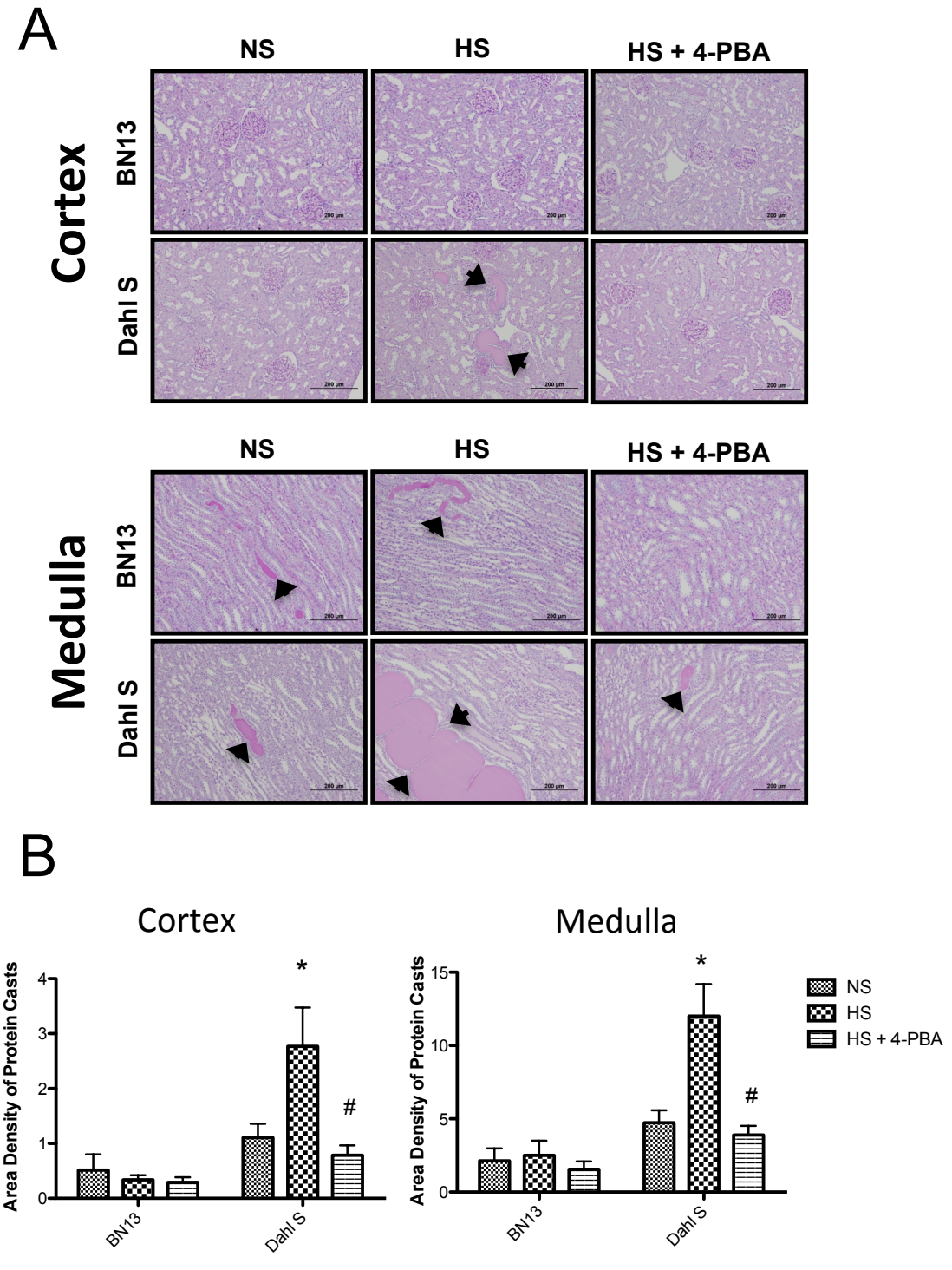
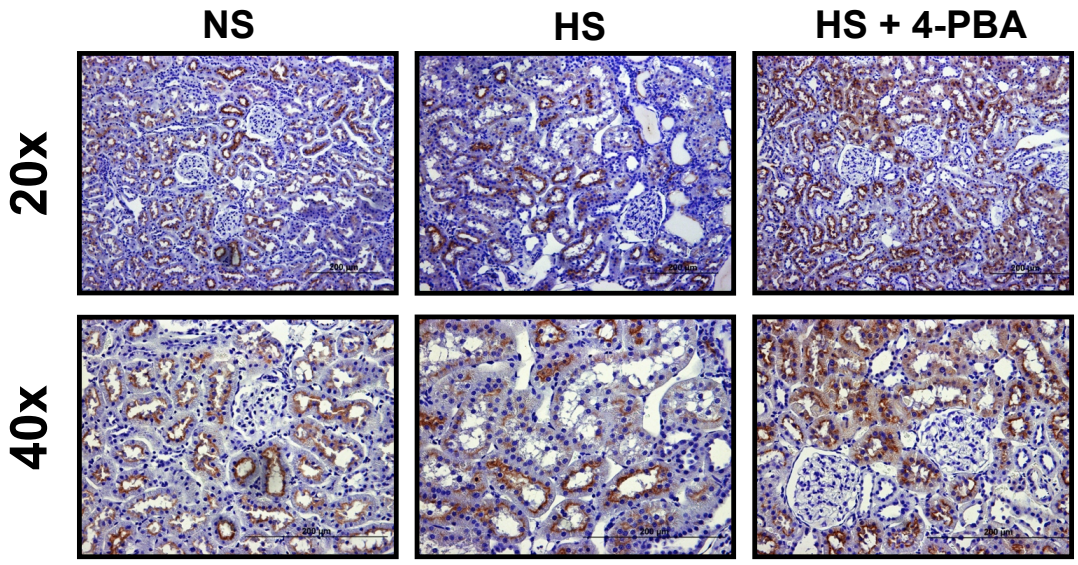

A
NS
HS
HS + 4-PBA
Cortex
BN13
Dahl S
NS
HS
HS + 4-PBA
Medulla
BN13
Dahl S
B
Cortex
Medulla
Area Density of Protein Casts
4
3
2
1
0
*
#
BN13
Dahl S
Area Density of Protein Casts
15
10
5
0
*
#
BN13
Dahl S
NS
HS
HS + 4-PBA

Figure 16. Treatment with 4-PBA, prevents renal pathology in HS fed Dahl S. PAS staining of the renal cortex and medulla of Dahl S and BN13 rats taken at 20x. (A) shows the accumulation of protein casts (arrows). HS fed Dahl S rats demonstrated large protein casts that were reduced with 4-PBA treatment. (B) Quantification of area density of protein casts in cortex and medulla shows a significant reduction with 4-PBA treatment. Chromosome 13 substituted Dahl S animals (BN13) did not demonstrate increases in protein cast area with HS feeding compared to NS feeding. *, $p < 0.05$ vs NS of same strain; #, $p < 0.05$ vs HS of same strain. N=6 in BN13 groups. N=10 in Dahl S groups. Data represented as mean ± SEM.

A


NS
HS
HS + 4-PBA
20x
40x

B

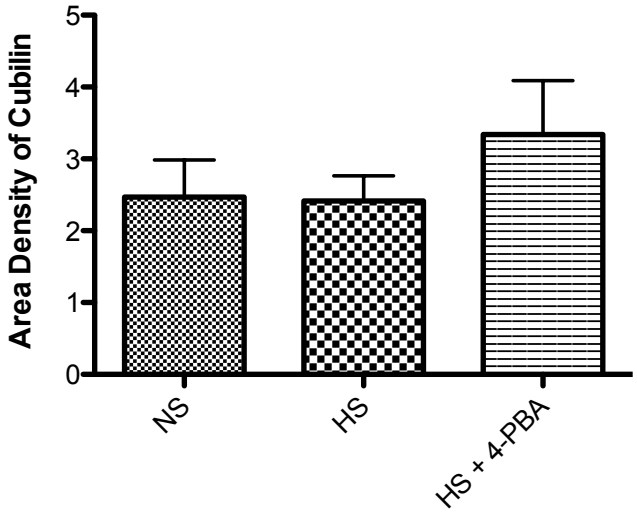

Area Density of Cubilin
0
1
2
3
4
5
NS
HS
HS + 4-PBA

Figure 17. The effect of 4-PBA on cubilin expression in the proximal tubules of the renal cortex. (A) Immunohistochemistry staining of paraffin-embedded kidney tissues demonstrate cubilin expression in the brush border of the proximal tubules of the kidney imaged at 20x. (B) Animals fed HS diet for 4 weeks demonstrated similar cubilin expression compared to the NS fed group (2.41 ±0.35% vs 2.47 ±0.51% cubilin area density). With 4-PBA treatment, HS fed Dahl S demonstrated increased cubilin expression (3.34 ±0.74% cubilin positive area). N= 10. Data represented as mean ± SEM.

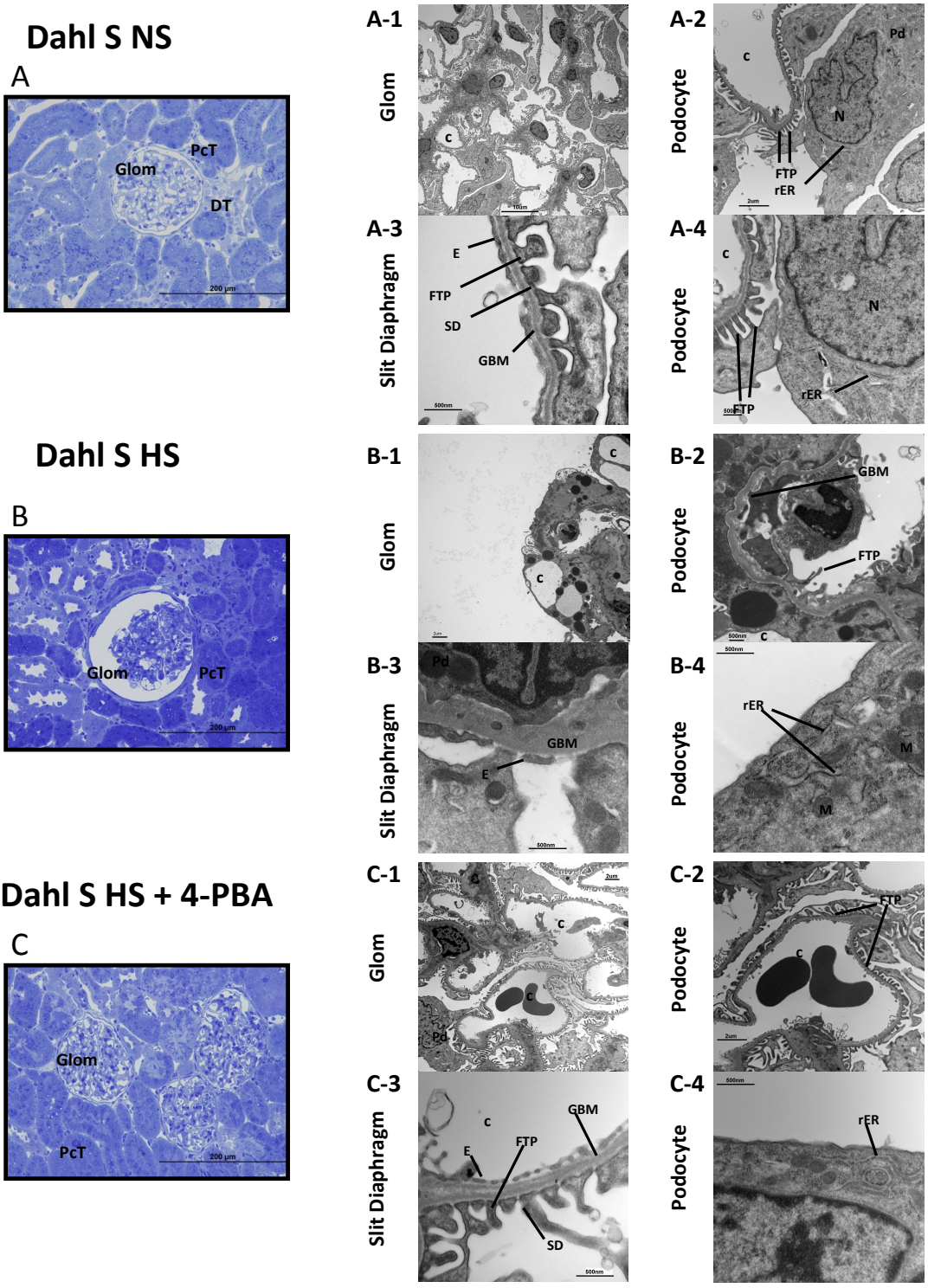

Dahl S NS
A
PcT
Glom
DT
200 µm
A-1
Glom
C
A-2
Podocyte
Pd
C
N
FTP
rER
A-3
Slit Diaphragm
E
FTP
SD
GBM
A-4
Podocyte
C
N
rER
FTP
Dahl S HS
B
Glom
PcT
200 µm
B-1
Glom
C
B-2
Podocyte
GBM
FTP
C
B-3
Slit Diaphragm
Pd
GBM
E
B-4
Podocyte
rER
M
M
Dahl S HS + 4-PBA
C
Glom
PcT
200 µm
C-1
Glom
C
C
Pd
C-2
Podocyte
FTP
C
C-3
Slit Diaphragm
C
GBM
E
FTP
SD
C-4
Podocyte
rER

Figure 18. The effect of 4-PBA on glomerular filtration barrier and endoplasmic reticulum of the foot processes in Dahl S rats. Blues taken at 20x with light microscopy. (A-1, B1, and C-1) Low magnification of the glomerulus in NS, HS, and HS + 4-PBA Dahl S depicting strucutres like the capillary (c). (A-2, B-2, C-2) Middle magnification of the glomerular filtration barrier (GFB), including the, glomerular basement membrane (GBM) and podocyte foot processes (FTP). (A-3, B-3, C-3) High magnification of the GFB, showing the slit diaphragm (SD) and fenestrated endothelium (E). (A-4, B-4, C-4) High magnification of the podocyte depicting intracellular organelles like the mitochondria (M), nucleus (N), rough ER (rER).

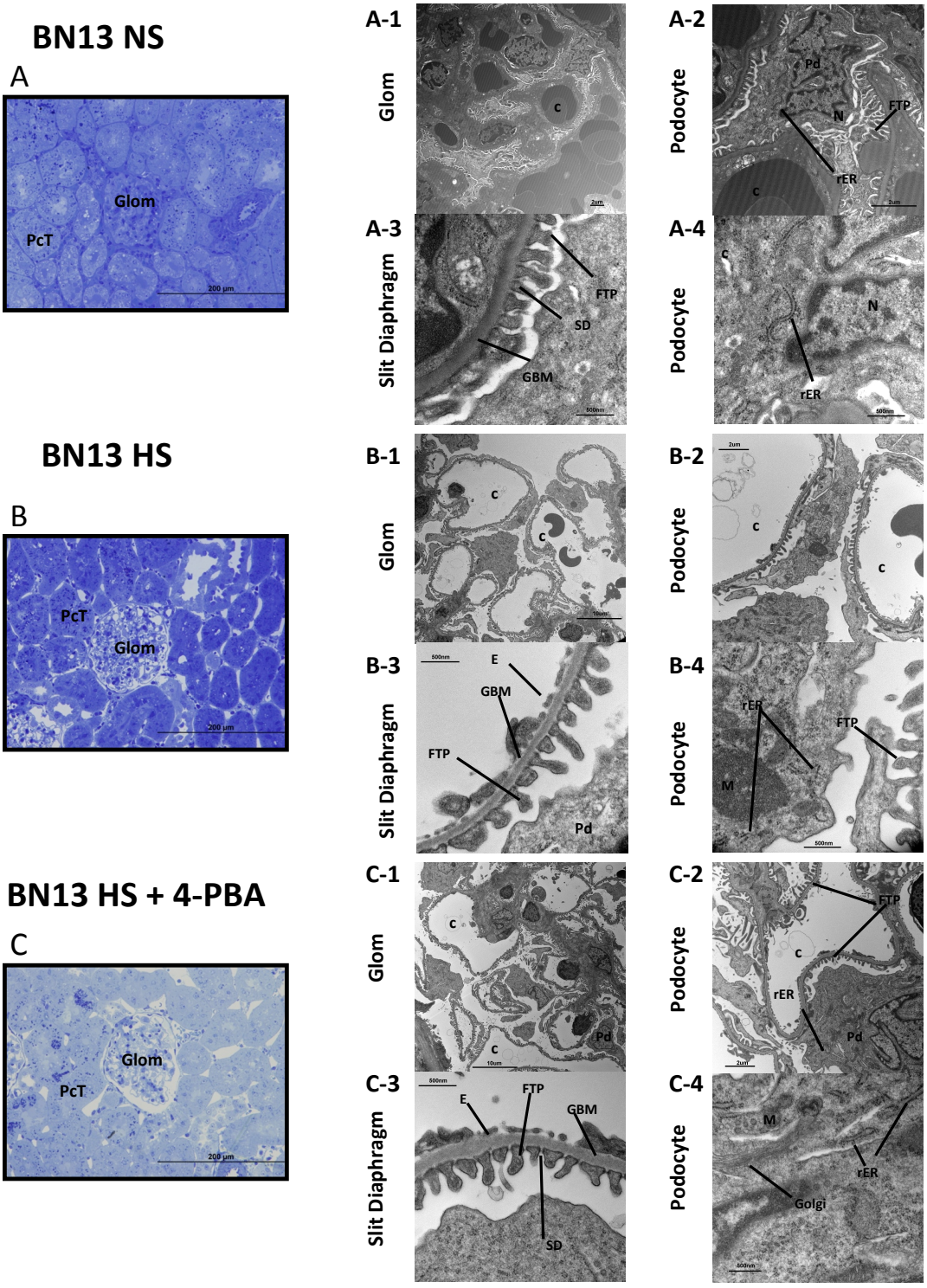
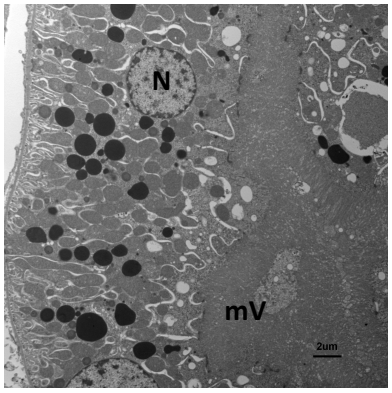

BN13 NS
A
Glom
PcT
200 µm
A-1
Glom
c
A-2
Podocyte
Pd
FTP
N
rER
c
A-3
Slit Diaphragm
FTP
SD
GBM
A-4
Podocyte
c
N
rER
BN13 HS
B
PcT
Glom
200 µm
B-1
Glom
c
c
B-2
Podocyte
c
c
B-3
Slit Diaphragm
E
GBM
FTP
Pd
B-4
Podocyte
rER
FTP
M
BN13 HS + 4-PBA
C
Glom
PcT
200 µm
C-1
Glom
c
c
Pd
C-2
Podocyte
FTP
c
rER
Pd
C-3
Slit Diaphragm
E
FTP
GBM
SD
C-4
Podocyte
M
rER
Golgi

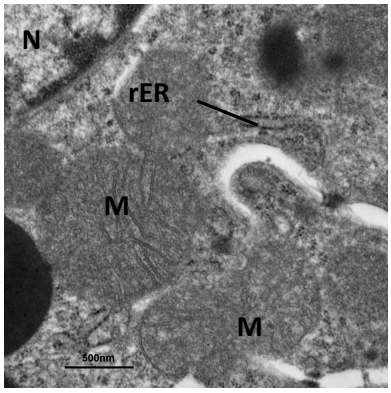
Figure 19. The effect of HS feeding on glomerular filtration barrier and endoplasmic reticulum of the foot processes in BN13 rats. (A-1, B1, and C-1) Low magnification of the glomerulus in NS, HS, and HS + 4-PBA BN13 depicting structures like the capillary (c). (A-2, B-2, C-2) Middle magnification showing the glomerular basement membrane (GBM) and podocyte foot processes (FTP). (A-3, B-3, C-3) High magnification of the GFB, showing the slit diaphragm (SD) and fenestrated endothelium (E). (A-4, B-4, C-4) High magnification of the podocyte depicting intracellular organelles like the mitochondria (M), nucleus (N), rough ER (rER).

Dahl S NS

A

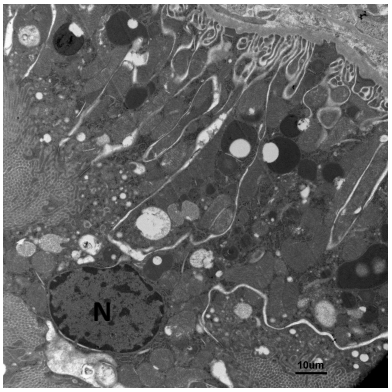


B

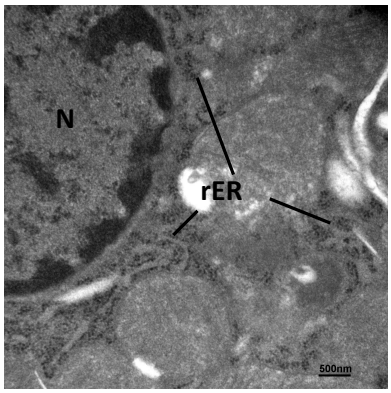


Dahl S HS

C

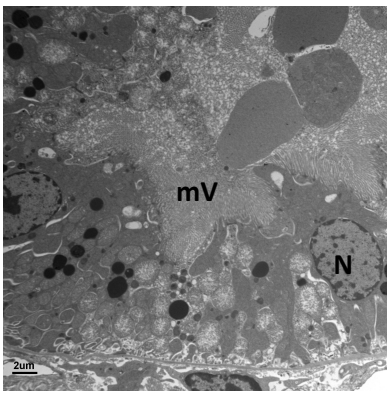


D



Dahl S HS + 4-PBA

E



F

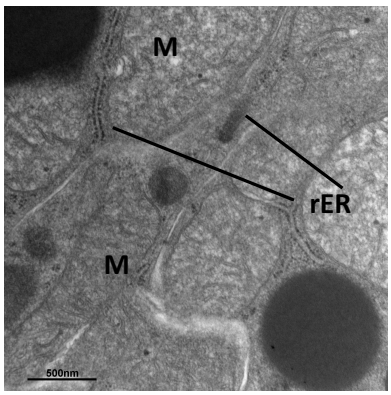


Figure 20. The effect of 4-PBA on endoplasmic reticulum of proximal tubules cells in the Dahl S rat. (A and B) TEM images of proximal tubules of the Dahl S rat on NS diet. (C and D) Images from Dahl S on HS diet. (E-F) Images from Dahl S on HS diet with 4-PBA treatment. mV- microvilli; M-mitochondria; N- nucleus rER- rough ER.

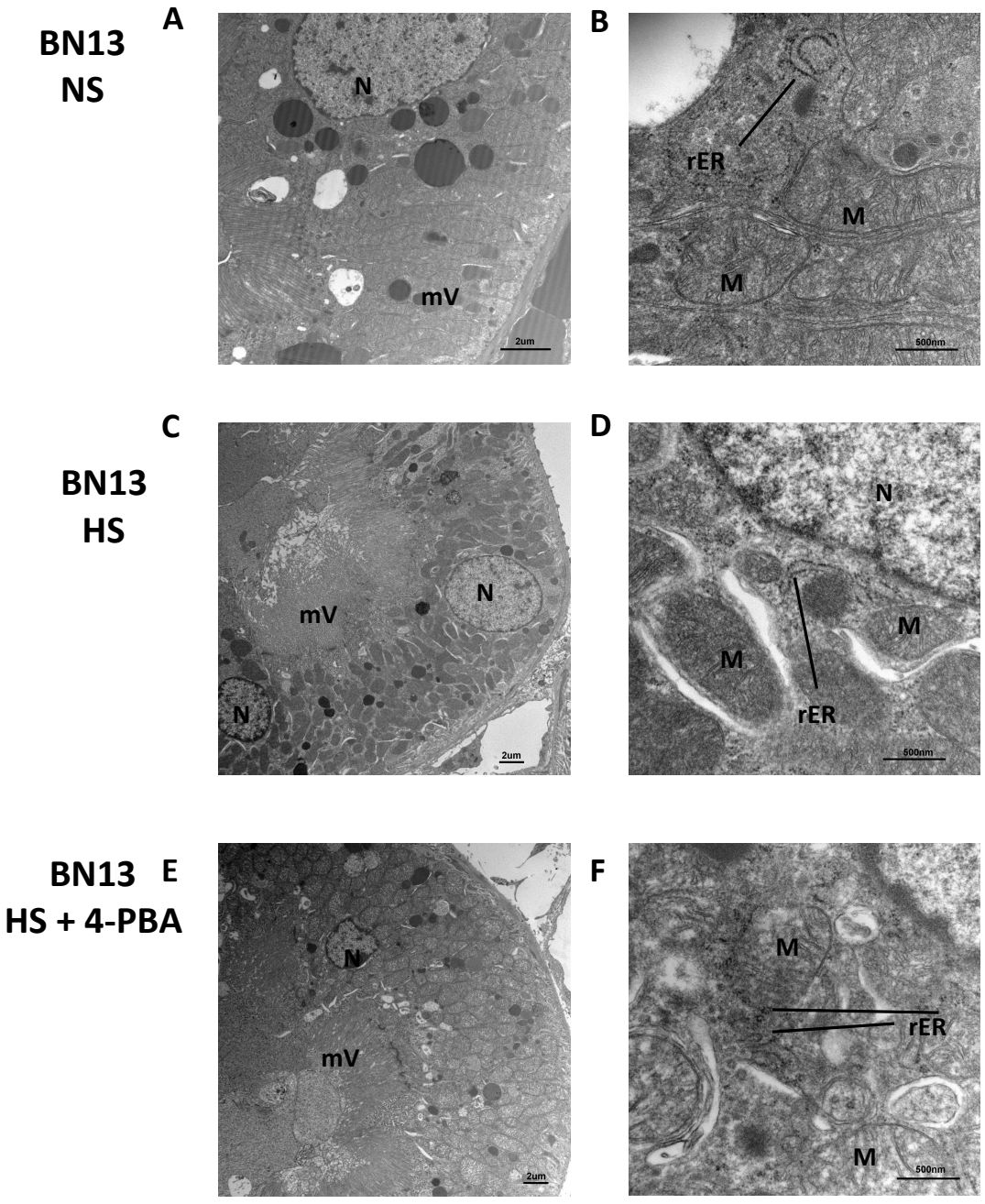

BN13
NS
A
N
mV
2um
B
rER
M
M
500nm
C
BN13
HS
mV
N
N
2um
D
N
M
M
rER
500nm
BN13
HS + 4-PBA
E
N
mV
2um
F
M
rER
M
500nm

Figure 21. The effect of 4-PBA on endoplasmic reticulum of proximal tubules cells in the BN13 rat. (A and B) TEM images of proximal tubules of the BN13 rat on NS diet. (C and D) Images from BN13 rat on HS diet. (E-F) Images from BN13 rat on HS diet with 4-PBA treatment. mV- microvilli; M-mitochondria; N- nucleus rER- rough ER.

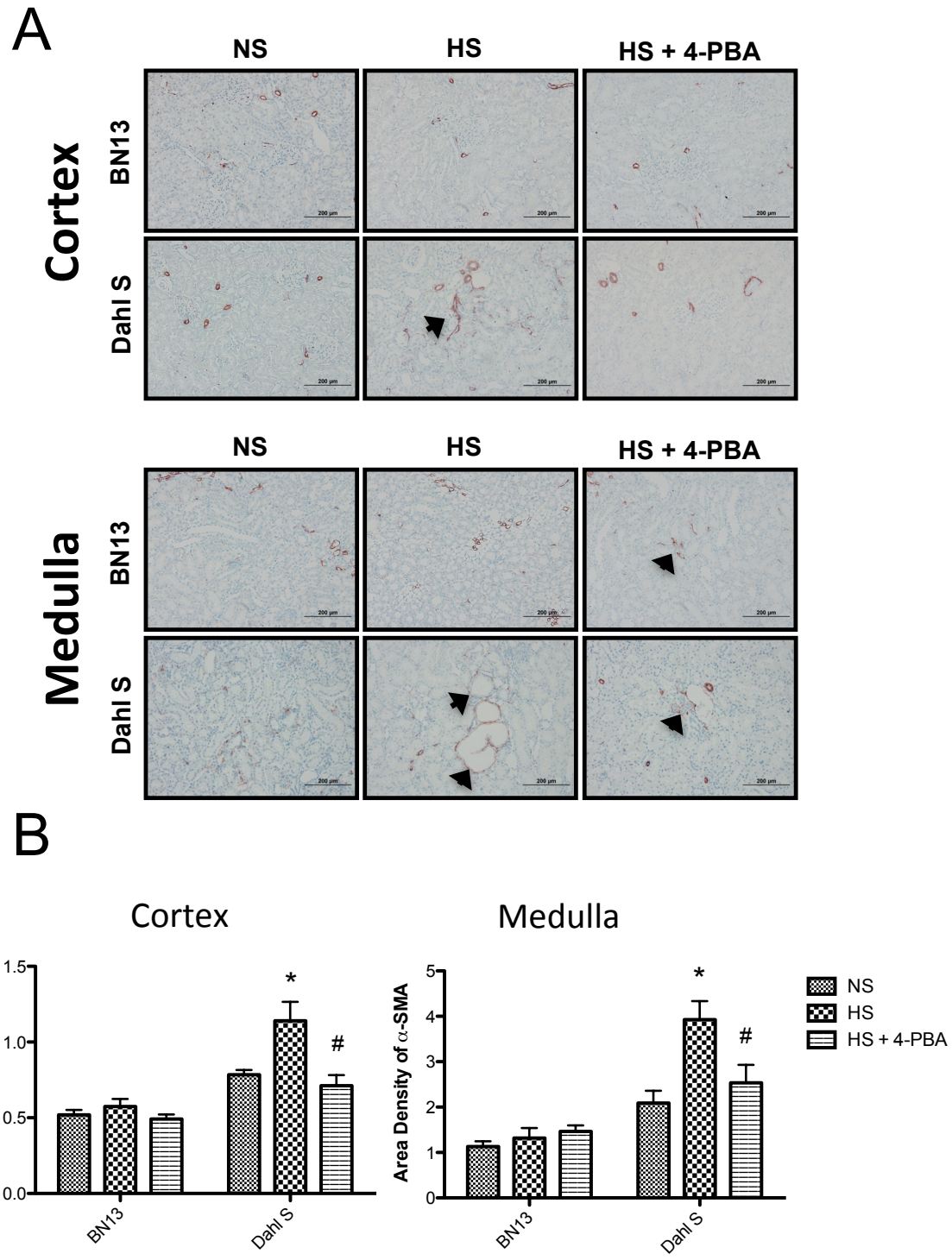

A
NS
HS
HS + 4-PBA
Cortex
BN13
Dahl S
Medulla
B
Cortex
Medulla
Area Density of α-SMA
BN13
Dahl S
NS
HS
HS + 4-PBA

Figure 22. Effect of 4-PBA treatment and chromosome 13 on α-smooth muscle actin expression in rat kidneys. (A) Immunohistochemistry staining for α-smooth muscle actin (α-SMA) (arrows) in HS fed Dahl S and BN13, either treated with 4-PBA or untreated. (B) Quantification of α-SMA staining demonstrated significant increases in α-SMA staining in the renal cortex and medulla with HS feeding compared to NS (*, $p<0.05$, N=10). 4-PBA treatment of HS fed Dahl S resulted in a significant reduction of α-SMA staining in the renal cortex and medulla (#, $p<0.05$, N=10). Data represented as mean ± SEM.

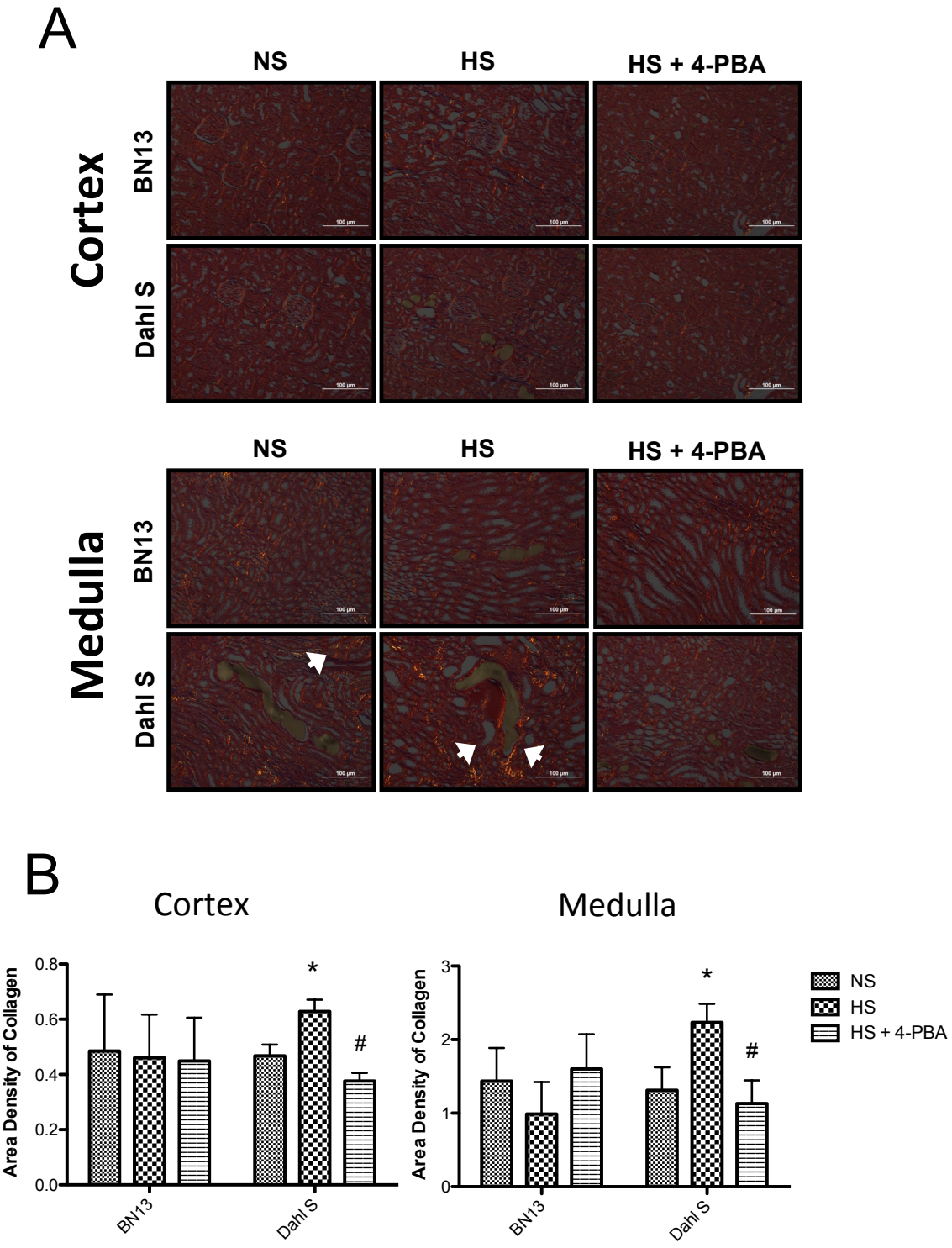

A
NS
HS
HS + 4-PBA
Cortex
BN13
Dahl S
NS
HS
HS + 4-PBA
Medulla
BN13
Dahl S
B
Cortex
Medulla
Area Density of Collagen
0.8
0.6
0.4
0.2
0.0
*
#
BN13
Dahl S
Area Density of Collagen
3
2
1
0
*
#
BN13
Dahl S
NS
HS
HS + 4-PBA

Figure 23. The effect of 4-PBA on collagen deposition in Dahl S rats. (A) Picro-sirius red staining (PSR) demonstrates regions of collagen deposition in renal cortex and medulla (arrows) in Dahl S and BN13 rats. (B) Quantification of collagen deposition showed similar trends in between the cortex and medulla of the Dahl S. HS feeding increased area density of collagen compared to NS Dahl S (*, $p<0.05$, N=10). 4-PBA treatment protected the Dahl S from increased deposits (#, $p<0.05$, N=10). Chromosome 13 substitution prevented an increase in collagen deposition with HS feeding compared to NS feeding. N=6. Data represented as mean ± SEM.

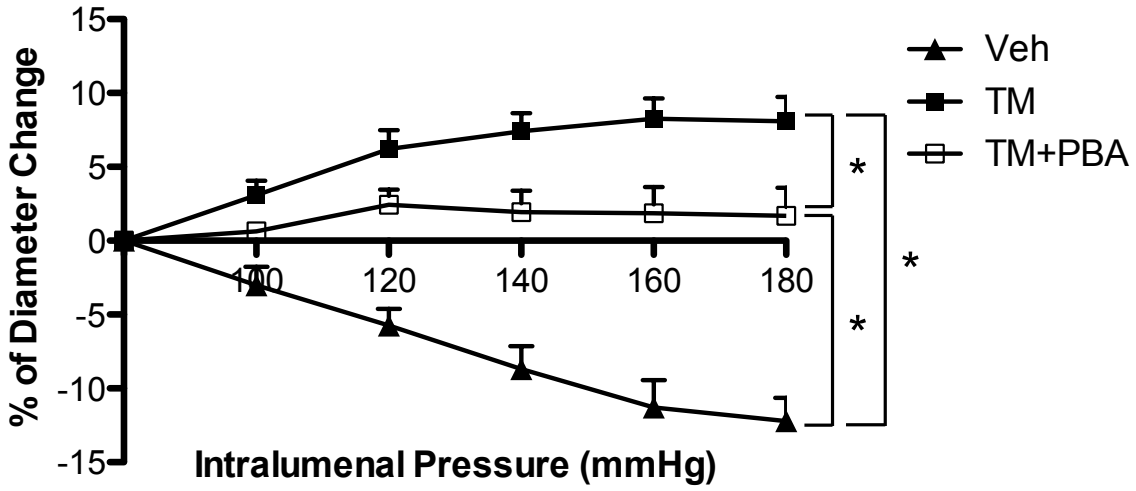
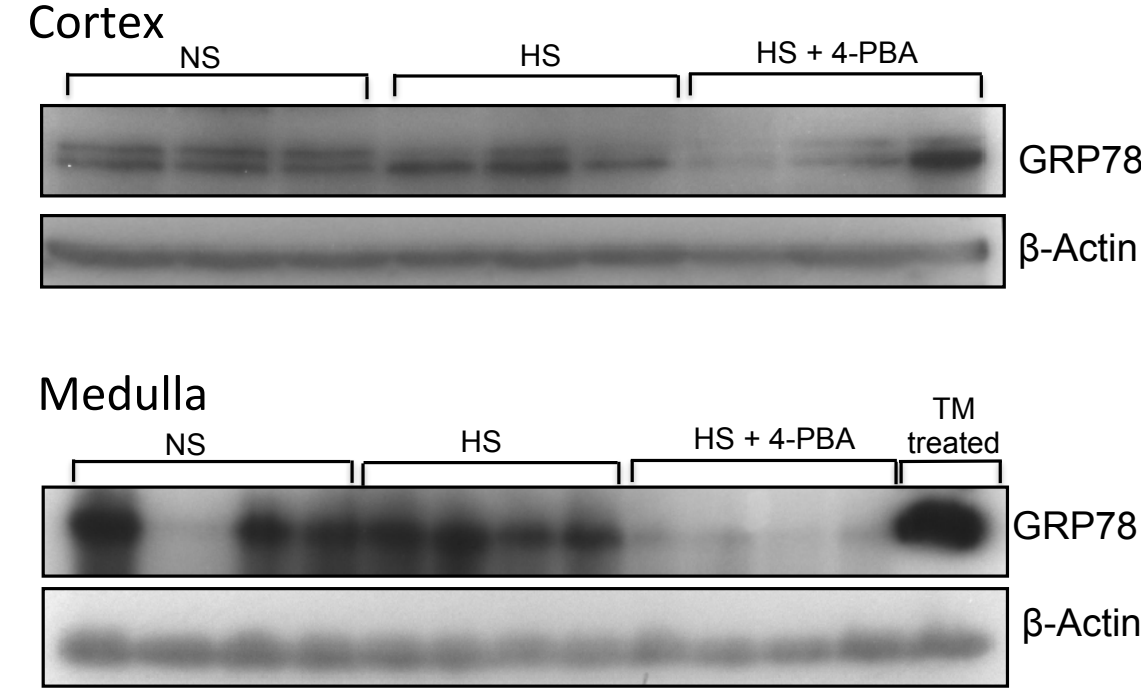

15
10
5
0
-5
-10
-15
% of Diameter Change
100
120
140
160
180
Intralumenal Pressure (mmHg)
Veh
TM
TM+PBA
*
*
*

Figure 24. The effect of ER stress induced with tunicamycin (TM) treatment on the myogenic response of the arcuate artery. Arcuate arteries from Sprague-Dawley rats were treated for TM (1ug/ml) for 6 hours with or without 4-PBA (1mM). TM treatment resulted in attenuation of the myogenic response. 4-PBA treatment of TM treated vessels protected the loss of myogenic constriction. N=9. Data represented as mean ± SEM. *, $p < 0.05$.

A


Cortex
NS
HS
HS + 4-PBA
GRP78
β-Actin
Medulla
NS
HS
HS + 4-PBA
TM treated
GRP78
β-Actin

B

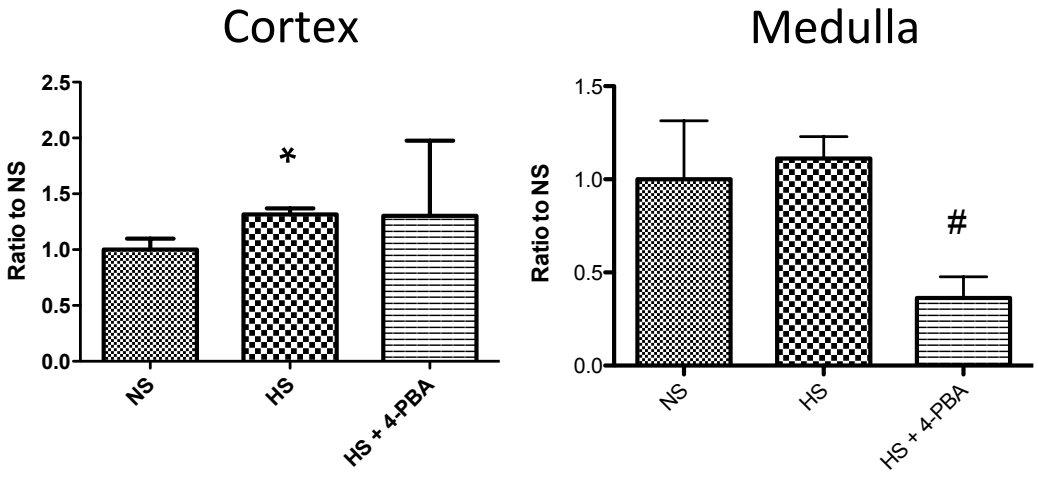

Cortex
Ratio to NS
2.5
2.0
1.5
1.0
0.5
0.0
*
NS
HS
HS + 4-PBA
Medulla
Ratio to NS
1.5
1.0
0.5
0.0
#
NS
HS
HS + 4-PBA

Figure 25. The effect of 4-PBA on ER stress in the kidney of HS fed Dahl S. (A) Homogenates of renal cortex and medulla were examined for ER stress marker, GRP78 using Western blot. (B) Bands were analyzed with densitometry and data is represented as mean of ratio to NS groups ± SEM. The renal cortex demonstrated a significant increase in GRP78 expression with HS feeding compared to NS fed controls (*, $p < 0.05$, N= 3). HS fed Dahl S treated with 4-PBA demonstrated a significant reduction of GRP78 expression in the renal medulla compared to untreated (#, $p < 0.05$, N= 4).

4.0 DISCUSSION

4.1 Objective 1: Determine what the differences are in renal function and pathology between salt sensitive and salt insensitive models of hypertension.

4.1.1 Hypertension

Two models of hypertension were used in this study, the salt sensitive Dahl S rat and the salt insensitive SHR rat. The Dahl S rat developed significantly increased blood pressure after 4 weeks of HS feeding; the increased blood pressure after salt loading is defined as salt sensitivity. The SHR did not develop elevated blood pressure with HS feeding compared to NS fed animals. However, the SHR had elevated blood pressure compared to the Dahl S animal regardless of diet. At 3-4 weeks of age, the SHR develop structural changes in small resistant arteries compared to the normotensive WKY rats (Dickhout & Lee, 1997). These structural changes include increased medial volume and smooth muscle cell hypertrophy. These vessel changes in the resistant vasculature may contribute to increased total peripheral resistance, resulting in the early development of hypertension in these animals (Dickhout & Lee, 1997). It is thought that volume expansion due to retention of sodium, as a result of inhibited sodium excretion, plays a role in salt sensitive hypertension in the Dahl S rat (Liu et al., 2011; Nishida, Hiruma, Kemuriyama, & Hagisawa, 2013). Increased sodium retention is thought to increase sympathetic nerve activity, this in turn results in increased blood pressure (Nishida et al., 2013).

In a study of Dahl S rats, severe hypertension, proteinuria, and glomerulosclerosis developed after 6 weeks of HS feeding beginning at 4 weeks of age (Nagase et al., 2006).

Treatment with hydralazine, an antihypertensive drug acting as a vasodilator, in these Dahl S rats resulted in a significant reduction of blood pressure, but proteinuria and glomerulosclerosis was not ameliorated. However, treatment with eplerenone, an aldosterone antagonist, resulted in attenuation of both proteinuria and glomerulosclerosis.

Aldosterone is a hormone that stimulates sodium reabsorption and water retention within the kidney, thereby increasing blood pressure. Aldosterone is also found to increase production of reactive oxygen species (ROS) in podocytes in vivo and in vitro (Shibata, Nagase, Yoshida, Kawachi, & Fujita, 2007). This suggests that the protective effects of eplerenone are due to inhibition of aldosterone induced podocyte injury (Zhu et al., 2011). Diabetic nephropathy, a kidney disease characterized by the appearance of proteinuria, appears to be driven by podocyte injury (Pagtalunan et al., 1997). Therefore, protection of podocytes from injury may be essential in slowing the progression of proteinuria and CKD. We tested this theory by examining the ability for vessels to protect the filtration barrier of the glomerulus through myogenic constriction and the effect of salt loading on myogenic constriction.

4.1.2 Salt sensitivity and renal damage

We found that salt sensitive hypertensive animals were prone to renal damage compared to salt insensitive hypertensive animals when fed a HS diet. After establishing that the SHR develops high blood pressure independent of salt intake, and the Dahl S develops hypertension after 4 weeks of HS feeding, we found that the Dahl S rats on a HS diet had significantly reduced renal function and increased pathology compared to SHR

on a HS diet. Proteinuria and albuminuria levels were also significantly greater in the Dahl S rats compared to the salt insensitive, SHR rats.

The African American population is at a greater risk for salt sensitive hypertension. Several studies have found a significant elevation of urinary protein excretion in blacks than whites with hypertension (Summerson, Bell, & Konen, 1995). This difference of protein excretion was also found in blacks and whites with diabetes (Young et al., 2005). It is known that the black population is at a greater risk for the progression of CKD to ESRD. Taken together, this suggests the importance of proteinuria in CKD progression of salt sensitive individuals. Similar to this human population, the Dahl S rat model of salt sensitive hypertension is prone to proteinuria and significant renal damage in comparison to the SHR despite the presence of significant blood pressure elevation in the SHR.

Based on quantitative trait loci (QTL) mapping study, it was found that the Dahl S allele of a QTL region on rat chromosome 19 was linked to increased albumin excretion (Siegel et al., 2004). When chromosome 19 from the SHR was substituted into the Dahl S background, there was a significant reduction of urinary protein and albumin excretion and atrial natriuretic peptide expression, although there was still an effect of salt loading (Wendt et al., 2007). Although there was an effect with high salt feeding, SBP was significantly reduced with chromosome 19 substitution. This suggests that chromosome 19 from the salt insensitive SHR rat offers protection of renal function and reduces blood pressure in conditions of high salt feeding.

4.1.3 Salt sensitivity and myogenic constriction

To determine how salt played a role in proteinuria in HS fed Dahl S, myogenic constriction of renal blood vessels was observed. A previous study has shown that inhibition of high systemic blood pressure reaching the kidney in the Dahl S rat protected the kidney from renal intratubular protein casts formation and interstitial fibrosis (Mori et al., 2008). An additional study determined that high dietary salt impaired the myogenic response in the renal arcuate artery (Takenaka et al., 1992). This finding is similar to a previous study in which high dietary salt impaired the myogenic response of large arterioles from spinotrapezius muscle (Nurkiewicz & Boegehold, 1998), while tubuloglomerular feedback (TGF), the other mechanism responsible for renal blood flow autoregulation remains intact with HS feeding (Karlsen et al., 1998).

In this study, we found that the arcuate artery from the SHR demonstrated some retention of the myogenic response after 4 weeks of HS feeding. This was not found to be true in the Dahl S rats, which demonstrated a dilation of the vessel. Therefore, the myogenic constriction may play a role in protecting the kidneys of the SHR rat with HS feeding and this protection is lost in the Dahl S due its salt sensitivity. This data suggests that salt sensitivity may affect vessel function. The Afro-Caribbean population, prone to salt sensitive hypertension and ESRD, was also found to have decreased small artery function compared to Caucasians (Kalra et al., 2005). This suggests that salt sensitive individuals are susceptible to end organ damage due to impaired function of small arteries.

The salt sensitivity of myogenic constriction found in the Dahl S may explain the observation that for every mmHg greater than 140 mmHg, the Dahl S excreted significantly more urinary protein compared to the SHR. Blood pressure played a greater role in the level of proteinuria in the Dahl S rat than it did in the SHR, this may be due to the greater effect of salt on myogenic constriction in the Dahl S. This study has shown that high blood pressure alone is insufficient to cause renal injury and that salt sensitivity may play a role in hypertension-induced proteinuria and CKD through the effect on myogenic constriction.

4.2 Objective 2: Determine if treatment with 4-PBA preserves myogenic constriction in the Dahl S rat and if this reduces proteinuria or renal pathology.

4.2.1 Reduction of blood pressure with 4-PBA

Recently, it was shown that 4-PBA treatment in mice infused with tunicamycin or angiotensin II resulted in a reduction of SBP and DBP (Liang et al., 2013). A significant reduction in blood pressure was also found when SHR rats were treated with ER stress inhibitors, TUDCA or 4-PBA (Spitler et al., 2013). These results are consistent with our findings that demonstrate the blood pressure lowering effects of 4-PBA in SHR and HS fed Dahl S, two models of essential hypertension. Here, we show that 4-PBA significantly reduced SBP in HS fed Dahl S rats; there was a trend for 4-PBA to reduce DBP, but this did not reach statistical significance ($p= 0.22$). In BN13 rats, which also experienced salt sensitive elevations of blood pressure, although at a lower level than the Dahl S rat, 4-PBA also significantly reduced SBP and DBP. This result suggests that the blood pressure

lowering effect of 4-PBA is not linked to the genes on chromosome 13 that drive salt sensitive hypertension in the Dahl S rat since this effect was independent of chromosome 13 substitution. Further, this suggests that the blood pressure lowering effect is not limited to hypertension that is secondary to salt sensitivity or CKD, since 4-PBA was able to lower blood pressure in the SHR with NS feeding.

4.2.2 4-PBA and chronic renal damage

ER stress inhibition with either 4-PBA or TUDCA was found to significantly reduced the activity of TGFβ1, a fibrogenic cytokine, in a model of Ang-II induced hypertension in mice, leading to a reduction of cardiac hypertrophy and fibrosis (Kassan et al., 2012). This suggests that ER stress plays a role in fibrosis by inducing TGFβ1 activity. Therefore, ER stress may be implicated in renal fibrosis, as was found in the unilateral ureteral obstruction (UUO)- model of renal fibrosis (Chiang et al., 2011).

This study has demonstrated the ability of 4-PBA, an ER stress inhibitor, to reduce chronic renal damage by reducing pathological features similar to common abnormalities in patients with CKD, including fibrotic protein deposition and myofibroblast accumulation. 4-PBA treatment of HS fed Dahl S rats was able to significantly lower proteinuria and albuminuria, markers of CKD as well as risk factors for the progression of CKD. Possibly due to the role 4-PBA plays in reducing proteinuria, the amount renal fibrosis was also reduced in the Dahl S. Renal fibrosis was determined using PSR staining as well as α-SMA expression as a marker of myofibroblasts.

4-PBA may protect against chronic renal damage in the Dahl S model of salt sensitive hypertension by influencing blood pressure (as discussed above), myogenic constriction of the vessels upstream of the glomerulus, changes in the GFB and proximal tubular mediated reabsorption. We analyzed these factors to determine how 4-PBA reduced proteinuria and albuminuria levels in the Dahl S rat.

4.2.3 4-PBA treatment and the myogenic response

The GFB may become damaged in conditions of hyperfiltration, due to the loss of renal blood flow autoregulation. The glomerulus is protected from high systemic blood pressure by two mechanisms, TGF and myogenic constriction of vessels upstream of the glomerulus. ER stress has been associated with blood vessel dysfunction, particularly endothelial-dependent relaxation (Kassan et al., 2012) and agonist mediated constriction (Spitler et al., 2013). Myogenic constriction was studied to determine if its dysfunction, with high salt feeding, was related to ER stress and if treatment with an ER stress inhibitor, 4-PBA, could prevent myogenic dysfunction.

We found that 4-PBA treatment prevented the loss of the myogenic response found with HS feeding in the Dahl S. The myogenic response was completely protected in vessels from 4-PBA treated Dahl S rats, as myogenic constriction was comparable to the NS fed Dahl S rats. This may provide an understanding of how 4-PBA is able to reduce the severity of proteinuria in this model of salt-sensitive hypertension. The inhibition of the myogenic response allows unregulated hydrostatic pressure to enter the glomerulus, allowing a larger number of serum proteins to pass through the filtration

barrier. Vessels dissected from the normotensive Sprague-Dawley rat treated with the ER stress inducer, tunicamycin, resulted in an attenuation of myogenic constriction similar to the response found in the HS fed Dahl S rat. We were able to show that co-treatment of arcuate arteries with tunicamycin and 4-PBA resulted in a protection from the loss of the myogenic response. This suggests that ER stress plays a role in vessel dysfunction leading to the loss of the myogenic response, and this can be rescued with ER stress inhibition.

4.2.4 Glomerular damage

The GFB consists of three components: the fenestrated endothelium, the glomerular basement membrane (GBM), and the podocyte foot processes. The podocyte has been implicated as the major component of the GFB that has been linked to proteinuria and CKD (Kriz, 2002). Dysfunction or damage in any of these components has been shown to lead to proteinuria (Kalluri, 2006).

In the remnant kidney model in rats, where 5/6 of renal mass was ablated, protein was found to accumulate in podocytes and lead to increased TGFβ1 activity, suggesting that protein uptake within the podocytes can lead to injury (Abbate et al., 2002). Further, protein overload in rats was linked to podocyte injury and increased urinary protein excretion (Davies, Messina, Thumwood, & Ryan, 1985). Cultured podocytes in conditions of albumin-overload was associated with ER stress (Chen et al., 2011). Taken together, high passage of proteins through the GFB may result in ER stress of the podocyte, resulting in higher levels of proteinuria and renal injury.

We found that 67% of protein that was excreted in the urine of HS fed Dahl S rats, was albumin; this provides evidence that large amounts of plasma proteins are entering the nephrons. Transmission electron microscopy (TEM) of the GFB from HS fed Dahl S rats showed podocyte effacement and detachment, as well as an expansion of the GBM. With 4-PBA treatment, the Dahl S rats demonstrated protection of the GFB, particularly protection from podocyte effacement. This suggests that 4-PBA protects podocytes from injury in HS fed Dahl S by protecting the myogenic response, thereby preventing high systemic blood pressure from reaching the glomerulus. 4-PBA may be protecting the kidney from proteinuria by protecting the myogenic response from HS feeding, thereby preventing barotrauma to the GFB. 4-PBA may also protect the GFB directly by inhibiting ER stress in the podocytes of the GFB.

4.2.5 4-PBA and protein reabsorption

Another possible mechanism of 4-PBA to reduce protein excretion in the urine of the HS fed Dahl S rats is through its properties to augment protein folding and protein trafficking, particularly in the proximal tubules. Imerslund-Gräsbeck syndrome (IGS) is found in both humans and dogs in which there is a mutation preventing the proper processing of cubilin. Individuals with IGS develop proteinuria, suggesting an important role of cubilin-mediated uptake of proteins to prevent proteins from reaching the distal nephron. The gene encoding for cubilin, CUBN, has been linked to albuminuria in a genome-wide association study (Böger et al., 2011). An increased demand for reabsorptive proteins, like cubilin, may result in a large demand for proteins to fold in the

ER. The large demand to fold membrane proteins in the ER may result in ER stress and UPR activation. 4-PBA, through its protein folding properties, may allow proper folding of cubilin, alleviating ER stress and preventing the UPR. In this study, staining for cubilin in the brush border of the proximal tubules resulted in an increase of cubilin with 4-PBA treatment. However, this trend did not reach statistical significance. TEM images of the renal proximal tubules did not show a noticeable difference between the Dahl S groups: NS diet, HS diet, and HS diet with 4-PBA treatment in terms of dilation of the rough ER, a direct measure of ER stress. This suggests that the main action of 4-PBA to prevent renal injury in salt sensitive hypertension may not be inhibition of ER stress within the proximal tubules. Further studies into whether there is an effect on cubilin folding with 4-PBA treatment are necessary. Future studies may also look into whether megalin, a mediator of cubilin uptake of proteins, is also affected with 4-PBA treatment.

4.3 Objective 3: Determine the effect of 4-PBA treatment on ER stress in the kidney.

4.3.1 High salt feeding and ER stress

High salt feeding in the Dahl S results in a significant increase of the renin-angiotensin-aldosterone system (RAAS) locally in the kidney (Bayorh et al., 2005). Aldosterone, a steroid hormone, acts on the distal tubules to increase sodium reabsorption and water retention. In addition to the effects of aldosterone on podocytes, aldosterone has also been linked to vascular inflammation (Blasi et al., 2003). Aldosterone acts on mineralocorticoid receptors, which are found both in vessels and renal epithelial cells (Blasi et al., 2003). This provides support that high salt feeding can result in inflammation

in both the vessel and within the tubules of the kidney. Further, cells suffering from chronic inflammation have been shown to increase GRP78 expression and induce ER stress (Kitamura, 2011; Shkoda et al., 2007). Under conditions of inflammation, ROS are produced by activated macrophages (Drath & Karnovsky, 1975), which in turn is a known factor inducing ER stress (Zhang & Kaufman, 2008). Another possible connection between inflammation and ER stress is the effect of inflammatory cytokines. Several studies have found ER stress induction in mice with IL-6 or IL-1β injections (Kitamura, 2011). Tumor necrosis factor-α (TNF-α) has also been shown to activate the three known pathways of ER stress, PERK, ATF6, and IRE1 (Xue et al., 2005).

4.3.1 4-PBA and ER stress in renal tissues of the Dahl S

4-PBA treatment of rats with diabetic nephropathy, a proteinuric kidney disease, resulted in reduced cortical ER stress, oxidative stress, and urinary protein excretion (Z. F. Luo et al., 2010). Our proposed mechanism of the role of high salt diet in proteinuria involves the affect of high salt feeding on ER stress of the renal vasculature. Dysfunctional vascular function as a result of high salt feeding may be linked to an impaired myogenic response, which in turn results in barotrauma to the glomerular filtration barrier. As discussed above, 4-PBA was found to protect the myogenic response by inhibiting ER stress. Co-treatment of vessels with tunicamycin and 4-PBA resulted in a protection of the myogenic response. Further, the effects of 4-PBA treatment on the expression of ER stress marker, GRP78, in the renal cortex and medulla were also assessed.

Excessive serum proteins within the nephron may signal increased expression of reabsorptive proteins such as cubilin and megalin. A large demand for reabsorptive proteins in the proximal tubules may cause ER stress. When large amounts of serum proteins are not reabsorbed in the proximal tubules, protein casts can form, causing blockage and loss of the nephron. Further, when ER stress persists, damage to the cells may occur resulting in apoptosis (Dickhout, Carlisle, et al., 2011) or EMT (Carlisle et al., 2012) and thereby contribute to nephron loss. As nephron loss proceeds, the remaining nephrons must carry a greater proportion of the filtration load leading to a vicious circle of hyperfiltration, proteinuria, ER stress and nephron loss.

The renal cortex showed a significantly increased GRP78 expression in HS fed Dahl S, suggesting the cells of the renal cortex, possibly the proximal tubules and podocytes, may be undergoing ER stress. 4-PBA treatment did not significantly reduce GRP78 in the cortex. Variability within groups was seen, suggesting that sampling of renal tissue played an important role in the amount of GRP78 protein expression found. 4-PBA treatment demonstrated a reduction of GRP78 in the renal medulla, suggesting that 4-PBA inhibited ER stress in the renal medulla. Interestingly, there was a greater amount of renal pathology in the renal medulla compared to the cortex. CHOP expression was difficult to elucidate, due to background signal of the Western blot.

Ultrastructural observations of podocytes, a crucial cell type that forms the slit diaphragm with its foot processes, demonstrated an expansion of the lumen of the rough ER. ER stress is associated with an dilation of the rough ER (Hartley et al., 2010; Schönthal, 2012). The dilation of the rough ER in the podocytes suggest that these cells

may be undergoing ER stress. This was prevented with ER stress inhibition with 4-PBA. The same expansion of the ER was not found in the proximal tubule cells, suggesting the effect of HS feeding on proteinuria may be due to its effects on the glomerular filtration barrier rather than the proximal tubules.

5.0 CONCLUSIONS

In conclusion, 4-PBA treatment of a salt sensitive rat model of essential hypertension reduced proteinuria and prevented high salt feeding induced albuminuria, and renal fibrosis. 4-PBA was found to reduce blood pressure in both salt sensitive and insensitive rat models of hypertension. 4-PBA preserved myogenic constriction, which was lost with high salt feeding in the salt sensitive rat model, the Dahl S rat. Further, 4-PBA protected the glomerular filtration barrier of the Dahl S rat from podocyte foot process effacement and detachment. As well, 4-PBA appears to protect the podocyte from ER stress since HS fed Dahl S rat podocytes display dilated rough ER which was prevented by 4-PBA treatment. 4-PBA treatment did not significantly reduce ER stress marker, GRP78, in the cortex. However, it did result in significant reduction of GRP78 in the medulla as seen by Western blotting. The expression of the reabsorptive protein, cubilin, did not display a large difference in expression with 4-PBA treatment. Therefore, 4-PBA appears to have had renoprotective effects by a combination of lowering blood pressure and preserving myogenic constriction preventing damage to the GFB of the Dahl S rat.

REFERENCES

(2007). "KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease." Am J Kidney Dis **49**(2): S12-S154.

Abbate, M., C. Zoja, et al. (2002). "Transforming growth factor-beta1 is up-regulated by podocytes in response to excess intraglomerular passage of proteins: a central pathway in progressive glomerulosclerosis." Am J Pathol **161**(6): 2179-2193.

Al-Solaiman, Y., A. Jesri, et al. (2009). "Low-Sodium DASH reduces oxidative stress and improves vascular function in salt-sensitive humans." J Hum Hypertens **23**(12): 826-835.

Arora, P., P. Vasa, et al. (2013). "Prevalence estimates of chronic kidney disease in Canada: results of a nationally representative survey." CMAJ **185**(9): E417-423.

Bakris, G. L., M. R. Weir, et al. (2003). "Effects of blood pressure level on progression of diabetic nephropathy: results from the RENAAL study." Arch Intern Med **163**(13): 1555-1565.

Bayorh, M. A., A. A. Ganafa, et al. (2005). "Alterations in aldosterone and angiotensin II levels in salt-induced hypertension." Clin Exp Hypertens **27**(4): 355-367.

Bertani, T., F. Cutillo, et al. (1986). "Tubulo-interstitial lesions mediate renal damage in adriamycin glomerulopathy." Kidney Int **30**(4): 488-496.

Bertani, T., G. Rocchi, et al. (1986). "Adriamycin-induced glomerulosclerosis in the rat." Am J Kidney Dis **7**(1): 12-19.

Bianchi, S., R. Bigazzi, et al. (1999). "Microalbuminuria in essential hypertension: significance, pathophysiology, and therapeutic implications." Am J Kidney Dis **34**(6): 973-995.

Blasi, E. R., R. Rocha, et al. (2003). "Aldosterone/salt induces renal inflammation and fibrosis in hypertensive rats." Kidney Int **63**(5): 1791-1800.

Böger, C. A., M.-H. Chen, et al. (2011). "CUBN is a gene locus for albuminuria." Journal of the American Society of Nephrology **22**(3): 555-570.

Brater, D. C. (1999). "Effects of nonsteroidal anti-inflammatory drugs on renal function: focus on cyclooxygenase -2–selective inhibition." Am J Med **107**(6, Supplement 1): 65-70.

Brenner, B. M., E. V. Lawler, et al. (1996). "The hyperfiltration theory: a paradigm shift in nephrology." Kidney Int **49**(6): 1774-1777.

Brenner, B. M., T. W. Meyer, et al. (1982). "Dietary protein intake and the progressive nature of kidney disease: the role of hemodynamically mediated glomerular injury in the pathogenesis of progressive glomerular sclerosis in aging, renal ablation, and intrinsic renal disease." N Engl J Med **307**(11): 652-659.

Carlisle, R. E., A. Heffernan, et al. (2012). "TDAG51 mediates epithelial-to-mesenchymal transition in human proximal tubular epithelium." Am J Physiol Renal Physiol **303**(3): F467-481.

Carretero, O. A. and S. Oparil (2000). "Essential hypertension part I: definition and etiology." Circulation **101**(3): 329-335.

Chen, S., F. F. He, et al. (2011). "Calcium entry via TRPC6 mediates albumin overload-induced endoplasmic reticulum stress and apoptosis in podocytes." Cell Calcium **50**(6): 523-529.

Chiang, C.-K., S.-P. Hsu, et al. (2011). "Endoplasmic reticulum stress implicated in the development of renal fibrosis." Molecular Medicine **17**(11-12): 1295.

Cianciaruso, B., V. Bellizzi, et al. (1998). "Salt intake and renal outcome in patients with progressive renal disease." Miner Electrolyte Metab **24**(4): 296-301.

Coresh, J., B. C. Astor, et al. (2003). "Prevalence of chronic kidney disease and decreased kidney function in the adult US population: Third national health and nutrition examination survey." American Journal of Kidney Diseases **41**(1): 1-12.

Cowley, A. W., Jr., R. J. Roman, et al. (2001). "Brown Norway chromosome 13 confers protection from high salt to consomic Dahl S rat." Hypertension **37**(2 Part 2): 456-461.

Cupples, W. A. and B. Braam (2007). "Assessment of renal autoregulation." Am J Physiol Renal Physiol **292**(4): F1105-1123.

Cybulsky, A. V. (2010). "Endoplasmic reticulum stress in proteinuric kidney disease." Kidney Int **77**(3): 187-193.

Davies, D. J., A. Messina, et al. (1985). "Glomerular podocytic injury in protein overload proteinuria." Pathology **17**(3): 412-419.

de Almeida, S. F., G. Picarote, et al. (2007). "Chemical chaperones reduce endoplasmic reticulum stress and prevent mutant HFE aggregate formation." J Biol Chem **282**(38): 27905-27912.

Dickhout, J. G., R. E. Carlisle, et al. (2011). "Interrelationship between cardiac hypertrophy, heart failure, and chronic kidney disease: endoplasmic reticulum stress as a mediator of pathogenesis." Circ Res **108**(5): 629-642.

Dickhout, J. G., G. S. Hossain, et al. (2005). "Peroxynitrite causes endoplasmic reticulum stress and apoptosis in human vascular endothelium: implications in atherogenesis." Arterioscler Thromb Vasc Biol **25**(12): 2623-2629.

Dickhout, J. G. and J. C. Krepinsky (2009). "Endoplasmic reticulum stress and renal disease." Antioxid Redox Signal **11**(9): 2341-2352.

Dickhout, J. G. and R. M. Lee (1997). "Structural and functional analysis of small arteries from young spontaneously hypertensive rats." Hypertension **29**(3): 781-789.

Dickhout, J. G. and R. M. Lee (2000). "Increased medial smooth muscle cell length is responsible for vascular hypertrophy in young hypertensive rats." Am J Physiol Heart Circ Physiol **279**(5): H2085-2094.

Dickhout, J. G., S. Lhotak, et al. (2011). "Induction of the unfolded protein response after monocyte to macrophage differentiation augments cell survival in early atherosclerotic lesions." FASEB J **25**(2): 576-589.

Drath, D. B. and M. L. Karnovsky (1975). "Superoxide production by phagocytic leukocytes." The Journal of experimental medicine **141**(1): 257-262.

Eddy, A. (2001). "Role of cellular infiltrates in response to proteinuria." Am J Kidney Dis **37**(1 Suppl 2): S25-29.

Eddy, A. A. and C. M. Giachelli (1995). "Renal expression of genes that promote interstitial inflammation and fibrosis in rats with protein-overload proteinuria." Kidney Int **47**(6): 1546-1557.

Fox, C. S., M. G. Larson, et al. (2004). "Predictors of new-onset kidney disease in a community-based population." JAMA: the journal of the American Medical Association **291**(7): 844-850.

Gabbiani, G. (2003). "The myofibroblast in wound healing and fibrocontractive diseases." J Pathol **200**(4): 500-503.

Gabbiani, G., M. Le Lous, et al. (1976). "Collagen and myofibroblasts of granulation tissue. A chemical, ultrastructural and immunologic study." Virchows Arch B Cell Pathol **21**(2): 133-145.

Ge, Y., S. R. Murphy, et al. (2013). "Endogenously produced 20-HETE modulates myogenic and TGF response in microperfused afferent arterioles." Prostaglandins Other Lipid Mediat **102-103**: 42-48.

Grantham, J. J. and S. Slusher (2003). "Polycystic kidney disease." Science and Medicine **9**(3): 128-139.

Harder, D. R., D. Gebremedhin, et al. (1994). "Formation and action of a P-450 4A metabolite of arachidonic acid in cat cerebral microvessels." Am J Physiol **266**(5 Pt 2): H2098-2107.

Hartley, T., M. Siva, et al. (2010). "Endoplasmic reticulum stress response in an INS-1 pancreatic beta-cell line with inducible expression of a folding-deficient proinsulin." BMC Cell Biol **11**: 59.

Hemmelgarn, B. R., B. J. Manns, et al. (2010). "Relation between kidney function, proteinuria, and adverse outcomes." JAMA **303**(5): 423-429.

Hinz, B., G. Celetta, et al. (2001). "Alpha-smooth muscle actin expression upregulates fibroblast contractile activity." Mol Biol Cell **12**(9): 2730-2741.

Hinz, B., S. H. Phan, et al. (2007). "The myofibroblast: one function, multiple origins." Am J Pathol **170**(6): 1807-1816.

Hoagland, K. M., A. K. Flasch, et al. (2004). "Elevated BSC-1 and ROMK Expression in Dahl Salt-Sensitive Rat Kidneys." Hypertension **43**(4): 860-865.

Hostetter, T. H., J. L. Olson, et al. (1981). "Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation." Am J Physiol **241**(1): F85-93.

Hostetter, T. H., J. L. Olson, et al. (2001). "Hyperfiltration in remnant nephrons: a potentially adverse response to renal ablation." J Am Soc Nephrol **12**(6): 1315-1325.

Hoy, W. E., M. D. Hughson, et al. (2006). "Reduced nephron number and glomerulomegaly in Australian Aborigines: a group at high risk for renal disease and hypertension." Kidney Int **70**(1): 104-110.

Hoy, W. E., Z. Wang, et al. (2001). "The natural history of renal disease in Australian Aborigines. Part 1. Changes in albuminuria and glomerular filtration rate over time." Kidney Int **60**(1): 243-248.

Hsu, C. Y., C. Iribarren, et al. (2009). "Risk factors for end-stage renal disease: 25-year follow-up." Arch Intern Med **169**(4): 342-350.

Hsu, C. Y., F. Lin, et al. (2003). "Racial differences in the progression from chronic renal insufficiency to end-stage renal disease in the United States." J Am Soc Nephrol **14**(11): 2902-2907.

Igarashi, P. and S. Somlo (2007). "Polycystic kidney disease." J Am Soc Nephrol **18**(5): 1371-1373.

Imai, E., M. Horio, et al. (2007). "Estimation of glomerular filtration rate by the MDRD study equation modified for Japanese patients with chronic kidney disease." Clin Exp Nephrol **11**(1): 41-50.

Imig, J. D., A. P. Zou, et al. (1996). "Formation and actions of 20-hydroxyeicosatetraenoic acid in rat renal arterioles." Am J Physiol **270**(1 Pt 2): R217-227.

Inagi, R., M. Nangaku, et al. (2005). "Involvement of endoplasmic reticulum (ER) stress in podocyte injury induced by excessive protein accumulation." Kidney Int **68**(6): 2639-2650.

Inagi, R., M. Nangaku, et al. (2005). "Novel serpinopathy in rat kidney and pancreas induced by overexpression of megsin." J Am Soc Nephrol **16**(5): 1339-1349.

Ishimitsu, T., Y. Uehara, et al. (1992). "Interferon gamma attenuates hypertensive renal injury in salt-sensitive Dahl rats." Hypertension **19**(6 Pt 2): 804-808.

Jinta, T., Y. Miyazaki, et al. (2010). "The pathogenesis of chronic hypersensitivity pneumonitis in common with idiopathic pulmonary fibrosis: expression of apoptotic markers." Am J Clin Pathol **134**(4): 613-620.

Johnson, R. J., J. Feehally, et al. (2003). Comprehensive clinical nephrology, Mosby London.

Kalluri, R. (2006). "Proteinuria with and without renal glomerular podocyte effacement." Journal of the American Society of Nephrology **17**(9): 2383-2389.

Kalra, L., C. Rambaran, et al. (2005). "Ethnic differences in arterial responses and inflammatory markers in Afro-Caribbean and Caucasian subjects." Arterioscler Thromb Vasc Biol **25**(11): 2362-2367.

Karlsen, F. M., C. B. Andersen, et al. (1997). "Dynamic autoregulation and renal injury in Dahl rats." Hypertension **30**(4): 975-983.

Karlsen, F. M., P. P. Leyssac, et al. (1998). "Tubuloglomerular feedback in Dahl rats." Am J Physiol **274**(6 Pt 2): R1561-1569.

Kassan, M., M. Galan, et al. (2012). "Endoplasmic reticulum stress is involved in cardiac damage and vascular endothelial dysfunction in hypertensive mice." Arterioscler Thromb Vasc Biol **32**(7): 1652-1661.

Kauser, K., J. E. Clark, et al. (1991). "Inhibitors of cytochrome P-450 attenuate the myogenic response of dog renal arcuate arteries." Circ Res **68**(4): 1154-1163.

Khavandi, K., A. S. Greenstein, et al. (2009). "Myogenic tone and small artery remodelling: insight into diabetic nephropathy." Nephrol Dial Transplant **24**(2): 361-369.

Kimura, K., H. Jin, et al. (2008). "Dysfunction of the ER chaperone BiP accelerates the renal tubular injury." Biochem Biophys Res Commun **366**(4): 1048-1053.

Kis, K., X. Liu, et al. (2011). "Myofibroblast differentiation and survival in fibrotic disease." Expert Rev Mol Med **13**: e27.

Kitamura, M. (2011). "Control of NF-kappaB and inflammation by the unfolded protein response." Int Rev Immunol **30**(1): 4-15.

Klag, M. J., P. K. Whelton, et al. (1996). "Blood pressure and end-stage renal disease in men." N Engl J Med **334**(1): 13-18.

Kriz, W. (2002). "Podocyte is the major culprit accounting for the progression of chronic renal disease." Microscopy research and technique **57**(4): 189-195.

Lais, L. T., L. L. Rios, et al. (1977). "Arterial pressure development in neonatal and young spontaneously hypertensive rats." Blood Vessels **14**(5): 277-284.

Lea, J., T. Greene, et al. (2005). "The relationship between magnitude of proteinuria reduction and risk of end-stage renal disease: Results of the african american study of kidney disease and hypertension." Archives of Internal Medicine **165**(8): 947-953.

Lee, C. S., R. H. Cha, et al. (2010). "Ethnic coefficients for glomerular filtration rate estimation by the Modification of Diet in Renal Disease study equations in the Korean population." J Korean Med Sci **25**(11): 1616-1625.

Levey, A. S., J. P. Bosch, et al. (1999). "A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group." Ann Intern Med **130**(6): 461-470.

Levey, A. S., J. Coresh, et al. (2003). "National Kidney Foundation practice guidelines for chronic kidney disease: evaluation, classification, and stratification." Ann Intern Med **139**(2): 137-147.

Liang, B., S. Wang, et al. (2013). "Aberrant endoplasmic reticulum stress in vascular smooth muscle increases vascular contractility and blood pressure in mice deficient of AMP-activated protein kinase-alpha2 in vivo." Arterioscler Thromb Vasc Biol **33**(3): 595-604.

Lindenmeyer, M. T., M. P. Rastaldi, et al. (2008). "Proteinuria and hyperglycemia induce endoplasmic reticulum stress." J Am Soc Nephrol **19**(11): 2225-2236.

Liu, J., Y. Yan, et al. (2011). "Impairment of Na/K-ATPase signaling in renal proximal tubule contributes to Dahl salt-sensitive hypertension." Journal of Biological Chemistry **286**(26): 22806-22813.

Luft, F. C., J. Z. Miller, et al. (1991). "Salt sensitivity and resistance of blood pressure. Age and race as factors in physiological responses." Hypertension **17**(1 Suppl): I102-108.

Luo, S., C. Mao, et al. (2006). "GRP78/BiP is required for cell proliferation and protecting the inner cell mass from apoptosis during early mouse embryonic development." Mol Cell Biol **26**(15): 5688-5697.

Luo, Z. F., B. Feng, et al. (2010). "Effects of 4-phenylbutyric acid on the process and development of diabetic nephropathy induced in rats by streptozotocin: regulation of endoplasmic reticulum stress-oxidative activation." Toxicol Appl Pharmacol **246**(1-2): 49-57.

Luyckx, V. A. and B. M. Brenner (2010). "The clinical importance of nephron mass." Journal of the American Society of Nephrology **21**(6): 898-910.

Ma, Y. C., L. Zuo, et al. (2006). "Modified glomerular filtration rate estimating equation for Chinese patients with chronic kidney disease." J Am Soc Nephrol **17**(10): 2937-2944.

Ma, Y. H., D. Gebremedhin, et al. (1993). "20-Hydroxyeicosatetraenoic acid is an endogenous vasoconstrictor of canine renal arcuate arteries." Circ Res **72**(1): 126-136.

Maestri, N. E., S. W. Brusilow, et al. (1996). "Long-Term Treatment of Girls with Ornithine Transcarbamylase Deficiency." New England Journal of Medicine **335**(12): 855-860.

Maher, T. M., I. C. Evans, et al. (2010). "Diminished prostaglandin E2 contributes to the apoptosis paradox in idiopathic pulmonary fibrosis." Am J Respir Crit Care Med **182**(1): 73-82.

Malhi, H. and R. J. Kaufman (2011). "Endoplasmic reticulum stress in liver disease." J Hepatol **54**(4): 795-809.

McMahon, E. J., J. D. Bauer, et al. (2013). "A Randomized Trial of Dietary Sodium Restriction in CKD." J Am Soc Nephrol.

Mederos y Schnitzler, M., U. Storch, et al. (2008). "Gq-coupled receptors as mechanosensors mediating myogenic vasoconstriction." EMBO J **27**(23): 3092-3103.

Mogensen, C. (1986). "Early glomerular hyperfiltration in insulin-dependent diabetics and late nephropathy." Scandinavian journal of clinical & laboratory investigation **46**(3): 201-206.

Molsted, S., L. Prescott, et al. (2007). "Assessment and clinical aspects of health-related quality of life in dialysis patients and patients with chronic kidney disease." Nephron Clin Pract **106**(1): c24-33.

Moreno, C., J. M. Williams, et al. (2011). "Narrowing a region on rat chromosome 13 that protects against hypertension in Dahl SS-13BN congenic strains." American Journal of Physiology - Heart and Circulatory Physiology **300**(4): H1530-H1535.

Mori, T., A. Polichnowski, et al. (2008). "High perfusion pressure accelerates renal injury in salt-sensitive hypertension." J Am Soc Nephrol **19**(8): 1472-1482.

Muntner, P., D. Gu, et al. (2004). "Factors associated with hypertension awareness, treatment, and control in a representative sample of the chinese population." Hypertension **43**(3): 578-585.

Nagase, M., S. Shibata, et al. (2006). "Podocyte injury underlies the glomerulopathy of Dahl salt-hypertensive rats and is reversed by aldosterone blocker." Hypertension **47**(6): 1084-1093.

Nishida, Y., M. Hiruma, et al. (2013). "Sympathetic Activity in Dahl Salt-Sensitive Hypertension-Why is a Nitric Oxide-Induced Inhibitory System Up-Regulated in the Sympathetic Center." J Nephrol Ther **3**(131): 2161-0959.1000.

Norris, K. C., T. Greene, et al. (2006). "Baseline predictors of renal disease progression in the African American Study of Hypertension and Kidney Disease." J Am Soc Nephrol **17**(10): 2928-2936.

Nurkiewicz, T. R. and M. A. Boegehold (1998). "High dietary salt alters arteriolar myogenic responsiveness in normotensive and hypertensive rats." Am J Physiol **275**(6 Pt 2): H2095-2104.

Ohse, T., R. Inagi, et al. (2006). "Albumin induces endoplasmic reticulum stress and apoptosis in renal proximal tubular cells." Kidney Int **70**(8): 1447-1455.

Pagtalunan, M. E., P. L. Miller, et al. (1997). "Podocyte loss and progressive glomerular injury in type II diabetes." J Clin Invest **99**(2): 342-348.

Painter, R. C., T. J. Roseboom, et al. (2005). "Microalbuminuria in adults after prenatal exposure to the Dutch famine." J Am Soc Nephrol **16**(1): 189-194.

Perlmutter, D. H. (2002). "Chemical chaperones: a pharmacological strategy for disorders of protein folding and trafficking." Pediatr Res **52**(6): 832-836.

Peterson, J. C., S. Adler, et al. (1995). "Blood pressure control, proteinuria, and the progression of renal disease. The Modification of Diet in Renal Disease Study." Ann Intern Med **123**(10): 754-762.

Pincus, D., M. W. Chevalier, et al. (2010). "BiP binding to the ER-stress sensor Ire1 tunes the homeostatic behavior of the unfolded protein response." PLoS Biol **8**(7): e1000415.

Qi, W., J. Mu, et al. (2011). "Attenuation of diabetic nephropathy in diabetes rats induced by streptozotocin by regulating the endoplasmic reticulum stress inflammatory response." Metabolism **60**(5): 594-603.

Qi, X., T. Hosoi, et al. (2004). "Sodium 4-phenylbutyrate protects against cerebral ischemic injury." Mol Pharmacol **66**(4): 899-908.

Raij, L., S. Azar, et al. (1985). "Role of hypertension in progressive glomerular immune injury." Hypertension **7**(3 Pt 1): 398-404.

Rosner, M. H. and W. K. Bolton (2006). "Renal function testing." Am J Kidney Dis **47**(1): 174-183.

Rubenstein, R. C., M. E. Egan, et al. (1997). "In vitro pharmacologic restoration of CFTR-mediated chloride transport with sodium 4-phenylbutyrate in cystic fibrosis epithelial cells containing delta F508-CFTR." J Clin Invest **100**(10): 2457-2465.

Rubenstein, R. C. and P. L. Zeitlin (2000). "Sodium 4-phenylbutyrate downregulates Hsc70: implications for intracellular trafficking of ΔF508-CFTR." American Journal of Physiology - Cell Physiology **278**(2): C259-C267.

Rugale, C., S. Delbosc, et al. (2003). "Sodium restriction prevents cardiac hypertrophy and oxidative stress in angiotensin II hypertension." Am J Physiol Heart Circ Physiol **284**(5): H1744-1750.

Sacks, F. M., L. P. Svetkey, et al. (2001). "Effects on blood pressure of reduced dietary sodium and the Dietary Approaches to Stop Hypertension (DASH) diet. DASH-Sodium Collaborative Research Group." N Engl J Med **344**(1): 3-10.

Sadoun, E. and M. J. Reed (2003). "Impaired angiogenesis in aging is associated with alterations in vessel density, matrix composition, inflammatory response, and growth factor expression." J Histochem Cytochem **51**(9): 1119-1130.

Schönthal, A. H. (2012). "Endoplasmic Reticulum Stress: Its Role in Disease and Novel Prospects for Therapy." Scientifica **2012**.

Shibata, S., M. Nagase, et al. (2007). "Podocyte as the target for aldosterone roles of oxidative stress and Sgk1." Hypertension **49**(2): 355-364.

Shkoda, A., P. A. Ruiz, et al. (2007). "Interleukin-10 blocked endoplasmic reticulum stress in intestinal epithelial cells: impact on chronic inflammation." Gastroenterology **132**(1): 190-207.

Siegel, A. K., P. Kossmehl, et al. (2004). "Genetic linkage of albuminuria and renal injury in Dahl salt-sensitive rats on a high-salt diet: comparison with spontaneously hypertensive rats." Physiol Genomics **18**(2): 218-225.

Spitler, K. M., T. Matsumoto, et al. (2013). "Suppression of endoplasmic reticulum stress improves endothelium-dependent contractile responses in aorta of the spontaneously hypertensive rat." Am J Physiol Heart Circ Physiol **305**(3): H344-353.

Summerson, J. H., R. A. Bell, et al. (1995). "Racial differences in the prevalence of microalbuminuria in hypertension." Am J Kidney Dis **26**(4): 577-579.

Sun, D., L. C. Samuelson, et al. (2001). "Mediation of tubuloglomerular feedback by adenosine: evidence from mice lacking adenosine 1 receptors." Proc Natl Acad Sci U S A **98**(17): 9983-9988.

Tabas, I. and D. Ron (2011). "Integrating the mechanisms of apoptosis induced by endoplasmic reticulum stress." Nat Cell Biol **13**(3): 184-190.

Takenaka, T., H. Forster, et al. (1992). "Impaired myogenic responsiveness of renal microvessels in Dahl salt-sensitive rats." Circ Res **71**(2): 471-480.

Thomas, M. C., J. Moran, et al. (2011). "The association between dietary sodium intake, ESRD, and all-cause mortality in patients with type 1 diabetes." Diabetes Care **34**(4): 861-866.

Tomasek, J. J., G. Gabbiani, et al. (2002). "Myofibroblasts and mechano-regulation of connective tissue remodelling." Nat Rev Mol Cell Biol **3**(5): 349-363.

Tonelli, M., N. Wiebe, et al. (2011). "Systematic review: kidney transplantation compared with dialysis in clinically relevant outcomes." Am J Transplant **11**(10): 2093-2109.

Tryggvason, K. and E. Pettersson (2003). "Causes and consequences of proteinuria: the kidney filtration barrier and progressive renal failure." J Intern Med **254**(3): 216-224.

Van Dokkum, R. P., M. Alonso-Galicia, et al. (1999). "Impaired autoregulation of renal blood flow in the fawn-hooded rat." Am J Physiol **276**(1 Pt 2): R189-196.

van Dokkum, R. P., C. W. Sun, et al. (1999). "Altered renal hemodynamics and impaired myogenic responses in the fawn-hooded rat." Am J Physiol **276**(3 Pt 2): R855-863.

Vegter, S., A. Perna, et al. (2012). "Sodium intake, ACE inhibition, and progression to ESRD." J Am Soc Nephrol **23**(1): 165-173.

Verseput, G. H., B. Braam, et al. (1998). "Tubuloglomerular feedback and prolonged ACE-inhibitor treatment in the hypertensive fawn-hooded rat." Nephrol Dial Transplant **13**(4): 893-899.

Vilatoba, M., C. Eckstein, et al. (2005). "Sodium 4-phenylbutyrate protects against liver ischemia reperfusion injury by inhibition of endoplasmic reticulum-stress mediated apoptosis." Surgery **138**(2): 342-351.

Wang, Y., J. Chen, et al. (1997). "Induction of monocyte chemoattractant protein-1 in proximal tubule cells by urinary protein." J Am Soc Nephrol **8**(10): 1537-1545.

Weiner, D. E., H. Tighiouart, et al. (2004). "Chronic kidney disease as a risk factor for cardiovascular disease and all-cause mortality: a pooled analysis of community-based studies." Journal of the American Society of Nephrology **15**(5): 1307-1315.

Wendt, N., A. Schulz, et al. (2007). "Rat chromosome 19 transfer from SHR ameliorates hypertension, salt-sensitivity, cardiovascular and renal organ damage in salt-sensitive Dahl rats." J Hypertens **25**(1): 95-102.

Werner, K., V. Yum, et al. (2013). "The endoplasmic reticulum stress inhibitor, 4-phenylbutyrate, reduces hypertension in the spntaneously hypertensive rat model of essential hypertension." J Am Soc Nephrol. Abstract. In Press.

Whaley-Connell, A., J. R. Sowers, et al. (2009). "Diabetes Mellitus and CKD Awareness: The Kidney Early Evaluation Program (KEEP) and National Health and Nutrition Examination Survey (NHANES)." Am J Kidney Dis **53**(4): S11-S21.

Woitas, R. P., B. Stoffel-Wagner, et al. (2000). "Correlation of serum concentrations of cystatin C and creatinine to inulin clearance in liver cirrhosis." Clinical chemistry **46**(5): 712-715.

Wu, J. and R. J. Kaufman (2006). "From acute ER stress to physiological roles of the Unfolded Protein Response." Cell Death Differ **13**(3): 374-384.

Wuhl, E. and F. Schaefer (2011). "Managing kidney disease with blood-pressure control." Nat Rev Nephrol **7**(8): 434-444.

Wyss, M. and R. Kaddurah-Daouk (2000). "Creatine and creatinine metabolism." Physiol Rev **80**(3): 1107-1213.

Xue, X., J.-H. Piao, et al. (2005). "Tumor necrosis factor α (TNFα) induces the unfolded protein response (UPR) in a reactive oxygen species (ROS)-dependent fashion, and the UPR counteracts ROS accumulation by TNFα." Journal of Biological Chemistry **280**(40): 33917-33925.

Young, B. A., W. J. Katon, et al. (2005). "Racial and ethnic differences in microalbuminuria prevalence in a diabetes population: the pathways study." J Am Soc Nephrol **16**(1): 219-228.

Zandi-Nejad, K., A. A. Eddy, et al. (2004). "Why is proteinuria an ominous biomarker of progressive kidney disease?" Kidney Int Suppl(92): S76-89.

Zatz, R. and B. M. Brenner (1986). "Pathogenesis of diabetic microangiopathy. The hemodynamic view." Am J Med **80**(3): 443-453.

Zhang, K. and R. J. Kaufman (2008). "From endoplasmic-reticulum stress to the inflammatory response." Nature **454**(7203): 455-462.

Zhu, C., S. Huang, et al. (2011). "Mitochondrial dysfunction mediates aldosterone-induced podocyte damage: a therapeutic target of PPARgamma." Am J Pathol **178**(5): 2020-2031.