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NULL MUTATION OF THE NADPH OXIDASE SUBUNIT p67^{PHOX}PROTECTS THE DAHL-S RAT FROMSALT-INDUCED REDUCTIONS IN MEDULLARY BLOOD FLOW AND GFR

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Abstract

Null mutations in the *p67^{phox}* subunit of NADPH-oxidase confer protection from salt-sensitivity on Dahl salt-sensitive (SS) rats. Here we track the sequential changes in medullary blood flow, glomerular filtration rate, urinary protein and mean arterial pressure in SS*p67^{phox}* null rats and wild-type littermates during 21-days of 4.0% NaCl (high-salt [HS]) diet. Optical fibers were implanted in the renal medulla and medullary blood flow measured in conscious rats by laser-Doppler flowmetry. Separate groups of rats were prepared with femoral venous catheters and glomerular filtration rate measured by the transcutaneous assessment of fluorescein isothiocyanate-sinistrin disappearance curves. Mean arterial blood pressure was measured by telemetry. In wild-type rats HS caused a rapid reduction in medullary blood flow which was significantly lower than control values by HS day-6. Reduced medullary blood flow was associated with a progressive increase in mean arterial pressure, averaging 170 ± 5 mmHg by HS salt day-21. A significant reduction in glomerular filtration rate was evident at day-14 HS, after the onset of hypertension and reduced medullary blood flow. In contrast, HS had no significant effect on medullary blood flow in SS*p67^{phox}* null rats and the pressor response to sodium was blunted, averaging 150 ± 3 mmHg at day-21 HS. Glomerular filtration rate was maintained throughout the study and proteinuria was reduced. In summary, when p67^{phox} is not functional in the SS rat HS does not cause reduced medullary blood flow and salt-sensitive hypertension is attenuated, consequently renal injury is reduced and glomerular filtration rate is maintained.

Keywords

Salt-Sensitive; NADPH-Oxidase; Hypertension; Dahl-S Rat; Kidney

Introduction

Essential hypertension is driven by a complex interplay of genetic and environmental factors. Consumption of a high salt diet is a major environmental factor, with blood pressure

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Disclosures
None

elevation in response to salt (salt-sensitivity) occurring in ~50% of essential hypertension patients[1]. In African-American populations this figure increases dramatically, with 75% of hypertensive patients being salt-sensitive[1–3] along with a higher incidence of end stage renal disease[4–6]. Despite this, the mechanisms underlying the progression of salt-sensitivity are poorly understood, which may explain why current treatments are effective in only ~50% of patients[7]. An area of particular controversy is the relationship between hypertension and chronic kidney disease, as recently reviewed by Pirkle and Freedman[8], with clinical trials reporting disappointing results when evaluating the effect of blood pressure control on the rate of decline of glomerular filtration rate and the progression of chronic kidney disease[9–12]. Therefore, characterizing the interplay between increased blood pressure, renal injury and reduced glomerular filtration rate is of interest.

In the Dahl salt-sensitive SS/JrHsdMcwi (SS) rat, as in the clinical condition, hypertension is greatly accelerated by a high salt (NaCl) diet, a trait that is genetically determined[13]. Many of the clinical characteristics of the disease are mimicked in these rats which present with progressive, low-renin hypertension, end stage renal disease, hyperinsulinemia and increased reactive oxygen species (ROS) production[14–19]. Defining the causes and consequences of salt-induced hypertension through the evaluation of its temporal progression in the SS rat will provide valuable insights into the pathology of the clinical condition.

Increased ROS production has been implicated in the development of salt-sensitive hypertension. Increased oxidative stress in the kidneys of SS rats has profound effects resulting in anti-natriuresis, reduced blood flow in the cortex and medulla, reduced glomerular filtration rate, chronic kidney disease and hypertension[19–24]. Notably, reduction of intramedullary ROS alone greatly attenuates the pressor response to a high salt diet in SS rats[19, 21].

ROS production is mediated by NADPH-oxidase, a complex enzyme consisting of six subunits: two membrane-bound ($gp91^{phox}$ and $p22^{phox}$) and three cytosolic components ($p67^{phox}$, $p47^{phox}$ and $p40^{phox}$) plus one G-protein (rac1/2)[25]. Chromosome substitution studies performed in our laboratory revealed that a 16 Mbp genomic region of chromosome 13 from Brown Norway salt-resistant rats conferred protection from salt-sensitivity onto SS rats[26]. One of the genes identified in this introgressed region of the congenic strain was *p67^{phox}*, which plays a crucial role in the activation of NADPH-oxidase[27]. We have recently demonstrated that SS rats exhibit higher NADPH-oxidase activity in the outer medulla compared to salt-resistant control rats[21] and uniquely overexpress the $p67^{phox}$ subunit of the enzyme[28]. Based on these data, a rodent model was developed in which the *p67^{phox}* gene was mutated in SS rats (SS*p67^{phox}* null rat). Study of the SS*p67^{phox}* null rat provided the first direct evidence of the physiological relevance of *p67^{phox}* in the etiology of blood pressure salt-sensitivity in the SS rat as the SS*p67^{phox}* null rats exhibited a substantial attenuation of both the hypertensive response and renal injury[28].

In the current study, the SS*p67^{phox}* null rat was used to determine the physiological role of increased ROS production in the initiation and maintenance of salt-sensitive hypertension in SS rats. Changes of medullary blood flow (MBF), glomerular filtration rate (GFR) and mean

arterial blood pressure (MAP) were determined throughout three weeks of the study to track the temporal progression of salt-sensitive hypertension in conscious SS*p67*^{phox} null rats and their salt-sensitive wild-type (WT) littermates. Importantly, this provided the sequential order of the pathological changes associated with the development of the salt-induced hypertension, with a focus on the role of increased ROS production.

Materials and Methods

Experimental Animals

Male SS*p67*^{phox} null rats and WT littermates, generated from heterozygous crosses, were obtained at weaning from colonies developed and maintained at the Medical College of Wisconsin. Breeders and offspring at weaning were fed a purified AIN-76A rodent food diet (Dyets, Bethleham, PA) containing 0.4% NaCl with *ad libitium* water. The high salt diet (HS) contained 4.0% NaCl. All experimental protocols were approved by the Medical College of Wisconsin Institutional Animal Care and Use Committee. Experimental methods describing the phenotyping protocols and statistics are detailed in the online-only supplemental data.

Results

MBF and MAP

Figure 1 summarizes the MBF and MAP in SS*p67*^{phox} null rats and their WT littermates over the course of the study. In the salt-sensitive WT rats, HS caused a rapid and sustained reduction in MBF, which fell by 20% over the first week of the salt challenge: from 0.59 ± 0.03 volts to 0.47 ± 0.05 volts. By day-6 HS MBF was significantly lower than the average control value. After this initial reduction, attenuated medullary perfusion persisted over the course of the study, with day-7 HS values being comparable to those recorded on day-14 HS (0.46 ± 0.02 volts) and day-21 HS (0.51 ± 0.03 volts) (Figure 1A). In the WT rats reduced MBF was associated with a progressive rise in MAP which increased from 128 ± 2 mmHg during the control period to 143 ± 3 mmHg at day-7 HS. Blood pressure continued to increase throughout the study, reaching 165 ± 6 mmHg at day-14 and 170 ± 5 mmHg at day-21 HS (Figure 1C). In contrast, null mutation of the *p67*^{phox} gene protected the kidneys of SS rats from salt-induced reductions of MBF. Notably, MBF was stable during the first week of HS, day-7 values (0.59 ± 0.04 volts) were equivalent to those recorded during the control period (0.58 ± 0.02 volts). Protection from reduced MBF persisted throughout the 3-week salt challenge and day-21 HS values were not significantly different from those recorded when the rats were maintained on 0.4% NaCl. Indeed at day-21 HS MBF had trended upward by ~20% from 0.58 ± 0.02 volts to 0.73 ± 0.04 volts suggesting an increased rather than decreased, medullary perfusion (Figure 1B). In parallel with the sustained MBF, the hypertensive response to HS was blunted in the SS*p67*^{phox} null rats throughout the study, increasing from 120 ± 3 mmHg during the control period to 150 ± 3 mmHg at day-21 HS (Figure 1D).

GFR and MAP

Figure 2 shows the MAP and GFR recorded in unrestrained SS*p67*^{phox} null and WT rats over 21-days HS diet. As in the MBF study, HS caused a progressive increase in MAP in the WT rats, from 126 ± 2 mmHg in the control period to 156 ± 8 mmHg on day-21 HS (Figure 2C). Although a small initial reduction in GFR was observed on day-2 HS, significant changes from the control period were not observed until day-14 HS in the WT rats, at which point GFR had reduced from an average of 1.48 ± 0.04 ml/min/100g bw to 1.28 ± 0.03 ml/min/100g bw. By day-21 GFR had reduced further, to 1.24 ± 0.03 ml/min/100g bw (Figure 2A). Notably, a significant increase in MAP was observed at day-7 HS in the WT rats, which preceded the reduction in GFR (Figure 2C). In contrast, the pressor response to the HS diet was blunted in the SS*p67*^{phox} null rats with MAP averaging only 132 ± 5 mmHg at day-21 with a significant increase not observed until day-14 HS (Figure 2D). There was no significant change in GFR over the 21-days HS in the SS*p67*^{phox} null rats, control values of 1.54 ± 0.05 ml/min/100g bw were comparable to day-21 HS values of 1.43 ± 0.03 ml/min/100g bw (Figure 2B).

Creatinine, Urinary Protein and Nitrate and Histological Assessment of Glomerular Damage

Figure 3 summarizes the urinary protein data over the course of the study. HS caused a significant increase in urinary protein excretion in the WT rats. In contrast to an absence of change of GFR, significant increases in urinary protein were evident at HS day-7 in the WT rats; levels of urinary protein were 2-fold higher than control values in all WT rats at this point. Urinary protein increased progressively throughout the study and was 4-fold higher than control values at HS-21 (Figure 3). In contrast, the protein measured in the urine of SS*p67*^{phox} null rats was substantially lower than WT rats throughout the study, and by day-14 HS this had reached significance (Figure 3). In correlation with the increased MBF, urinary nitrate excretion was significantly higher in the SS*p67*^{phox} null rats than the WT rats at day-21 HS (Figure S1).

Glomeruli injury was quantified in both the cortex and juxtamedullary boundary of WT and SS*p67*^{phox} null rats after 21-days HS. The data presented in Figure 4 show that glomeruli injury was significantly higher in the cortex of WT rats compared to the SS*p67*^{phox} null rats. In contrast there was no difference in the extent of injury observed in the juxtamedullay border region (Figure 4C). Trichrome staining was used for the assessment of tubular protein casts in the outer medulla in both groups of rats. At day-21 HS tubular injury was significantly attenuated in the SS*p67*^{phox} null rats (Figure 4F).

Finally, Table 1 summarizes the urinary creatinine clearance over the 21-day HS challenge. Plasma and urinary collections were made on a control day and days 7, 14 and 21 HS. Creatinine concentrations were measured by mass spectrometry with the aim of comparing creatinine clearance and the transcutaneous method as assessments of GFR. No significant reduction in creatinine clearance was observed over the 21-days HS in the WT rats, with day-21 HS values (1.00 ± 0.06 ml/min/100g bw) being comparable to control values (0.84 ± 0.04 ml/min/100g bw). Despite the significant reductions in GFR detected by the FITC-sinistrin excretion kinetics at HS-14, creatinine clearance was significantly higher than

control values at this time point in the WT rats. Throughout the study we found no correlation between creatinine clearance and GFR as determined by FITC-sinistrin clearance, further highlighting the limitation of this technique (Figure S2).

Discussion

The present study characterized the temporal changes in MBF, MAP and GFR in the development of hypertension in Dahl salt-sensitive (WT) and salt-resistant (SS*p67*^{phox} null) rats to examine the role of increased oxidative stress in the initiation and maintenance of salt-sensitive hypertension.

The data presented show that during the initiation of salt-sensitivity the hypertensive response to a HS diet is directly paralleled by a reduction in MBF in WT salt-sensitive rats. Sustained elevations in blood pressure were associated with the development of glomeruli injury, increased urinary protein, and subsequently reduced GFR. Null mutations in the *p67*^{phox} gene, which significantly reduces the production of ROS in the renal interstitium[28], protected the renal medulla from the ischemic effects of a HS diet. During the first week of salt-loading MBF was stable in the null rats, with HS day-7 values being comparable to those recorded during the control period. Moreover, protection from reductions of MBF persisted over the three weeks, and by HS-21 there was a tendency towards increased rather than decreased perfusion. Sustained MBF was associated with an attenuated pressor response to HS, reduced pressure-induced renal injury and the maintenance of GFR. Glomeruli and tubular injury were exacerbated in the kidneys of WT rats compared to SS*p67*^{phox} null rats at HS day-21. These data support the concept that reduced MBF, driven by increased ROS production in the renal medulla, plays a central role in the initiation of salt-sensitive hypertension and its resultant pathologies.

Previous studies from our laboratory have highlighted the key role that increased oxidative stress in the renal medulla plays in the development of salt-sensitive hypertension. We have demonstrated that DETC (superoxide dismutase inhibitor) infusion into the renal medulla of anaesthetised rats resulted in an immediate reduction in MBF and sodium excretion; MAP however, did not change during the 2-hour acute study[29]. In contrast, in comparable chronic studies 24-hours of DETC-infusion into the renal medulla did cause a significant increase in MAP which was paralleled by reduced MBF[30]. These data suggest that increased oxidative stress in the renal medulla induces reduced MBF and sodium retention which leads to the development of hypertension[29]. Accordingly, direct infusion of H_2O_2 into the renal medulla of normotensive rats caused a rapid and sustained increase in blood pressure[31]. Together these data indicate that endogenous ROS in the renal medulla is vasoconstrictive and the resultant reductions in MBF play a pathological role in the development of salt-sensitive hypertension. The observation that SS*p67*^{phox} null rats are protected from reduced MBF is therefore consistent with the demonstration that H_2O_2 and superoxide (O_2^-) production are significantly reduced in the renal medulla of SS*p67*^{phox} null rats compared to their WT littermates[28].

The balance between ROS and nitric oxide (NO) in the renal medulla is finely regulated, with NO acting as a buffer to the vasoconstrictive actions of ROS[32]. The medullary thick

ascending limb (mTAL) is a major site for both ROS and NO production, mediated by NADPH oxidase and NOS respectively[33–35]. Studies in isolated mTAL have demonstrated the importance of the balance between ROS and NO and coined the concept of tubulovascular crosstalk. We have demonstrated that NO and O_2^- produced by the mTAL can interact with surrounding outer medullary descending vasa recta promoting vasodilation and vasoconstriction respectively[34, 36]. Under normal physiological conditions NO is capable of buffering the vasoconstrictive effects of O_2^-[36]. In situations of increased ROS production, as during the consumption of a HS diet in SS rats, the balance is skewed towards excessive ROS production and MBF is compromised[30, 37]. In contrast, the results in this study show a tendency towards increased medullary perfusion from day-10 HS onwards in the SS*p67*^{phox} null rats. This may reflect a relative increase in NO in the renal medulla, occurring as a secondary consequence of the attenuated ROS production in the kidneys of SS*p67*^{phox} null rats. In support of this hypothesis we found that urinary nitrate levels were augmented in the SS*p67*^{phox} null rats relative to the WT rats at day-21 HS.

In the current study MBF was lower than the average control value from HS day-2 onwards in the WT rats and was significantly reduced by HS day-6. It may be of note that despite comparable pressor responses to 4.0% NaCl during week one and week two of salt loading in the WT rats, maximal reductions in MBF were observed in the first week of HS, after which medullary perfusion appeared to plateau. This observation is consistent with the concept that reductions in MBF are involved in the initiation of the hypertensive response to a HS diet and are not merely the consequence of increased blood pressure.

Pressure-induced renal injury is reduced in SSp67^{phox} null rats

In the current study we have used the transcutaneous assessment of FITC-sinistrin clearance to determine GFR in conscious, unrestrained rats. Sinistrin is a biologically inert fructose polymer which is freely filtered at the glomerulus and neither secreted nor reabsorbed by the renal tubules, making its clearance an ideal assessment of GFR[38]. The power of this novel technique is that the results are not confounded by the effects of repeated blood sampling or anaesthesia as is required in more traditional renal clearance studies. Here we have demonstrated that reductions in GFR occur after significant elevations in blood pressure in the WT rats. Blood pressure was significantly higher than control values at day-7 HS, at which point urinary protein excretion had also increased significantly suggestive of glomeruli injury. However, significant reductions in GFR were not observed until day-14 HS. Given these results we hypothesize that reduced GFR is the consequence of pressure-induced glomerular injury rather than a cause of hypertension. Similar observations were recently made in SS rats, in which reductions in GFR were observed 9-days after significant increases in blood pressure[39].

Increased salt consumption has been shown to increase *p67*^{phox} abundance and augment NADPH-oxidase activity in the renal cortex of SS rats[24]. In addition to increased cortical O_2^-, maintaining SS rats on 8.0% NaCl for 5-weeks was associated with increased MAP, reduced GFR and cortical glomerular sclerosis. Treatment with the anti-oxidant apocynin reduced NADPH-oxidase activity and consequently O_2^- production in the cortex. In correlation with the reduced cortical ROS, GFR was improved and MAP reduced[24]. The

current study shows that in SS*p67*^{phox} null rats, in which NADPH-oxidase activity is reduced[28], GFR is maintained throughout the salt challenge and glomeruli injury is reduced. These results implicate a role for increased ROS production in cortical injury and reduced GFR. Whether the improved glomeruli function is the direct result of reduced ROS or secondary to the reduced MAP cannot be conclusively determined. However, given that reductions in GFR occurred after the onset in hypertension in WT rats it is likely that attenuated pressure, as a result of reduced ROS production, conferred protection from cortical injury to the SS*p67*^{phox} null rats.

The concept of pressure-induced glomerular injury is consistent with results from studies in which one kidney of SS rats was protected from the hypertensive effects of a 14-day HS diet. Specifically, when renal perfusion pressure to the left kidney was servo-controlled with an aortic balloon implanted between the renal arteries, glomeruli injury was significantly reduced compared to the non-controlled, hypertensive right kidney. These results highlight the contribution of elevated renal perfusion pressure to glomeruli injury in salt-sensitive rats[40]. Whether extending the HS protocol would lead to the surpassing of a “pressure threshold” and a reduction in GFR in the SS*p67*^{phox} null rats is an interesting area of future research.

In the current study we have shown that reductions in GFR, which are secondary to increased blood pressure, are preceded by two fold increases in urinary protein. These data suggest that clinically there could be a period in the initial stages of hypertension during which the identification of proteinuria and the initiation of blood pressure treatments may reduce the progression of renal injury and the decline of GFR. The concept that anti-hypertensive therapies have a more beneficial effect on GFR in individuals with increased proteinuria may be reflective of this[41, 42].

In addition to the assessment of FITC-sinistrin clearance, creatinine clearance was measured in the rats to compare the sensitivity of this novel technique to that of one of the most commonly used experimental and clinical estimates of GFR. The results of the current study are in agreement with others which have shown the limitations of using creatinine clearance as a surrogate marker of GFR as we have previously discussed[39]. Specifically, at day-14 HS creatinine clearance increased in the WT rats relative to the control values and was comparable to those recorded in the SS*p67*^{phox} null rats at the same time point; this is despite the demonstration that GFR had significantly reduced in the WT rats at day-14 HS when using the FITC-sinistrin clearance method. Moreover, we found no correlation between creatinine clearance and GFR as determined by the elimination of FITC-sinistrin. Given that creatinine clearance is considered an overestimation of GFR it is surprising that these values were consistently lower than the GFR values obtained using FITC-sinistrin disappearance, an observation also made in our previous characterization of GFR in SS rats over a 21-d HS challenge[39]. The cause of this discrepancy is unclear, however it possible that factors such as skin perfusion and thickness affect light penetration and lead to a further over-estimation of GFR when using the transcutaneous method; the investigation of these factors is an important area of future research.

It is a technical limitation of these studies that GFR, MBF and MAP cannot be simultaneously measured in the same rat. The requirement of restraint in the MBF studies prevents concurrent assessment of GFR, and may account for the consistently higher blood pressures recorded over the 21-days of HS in the MBF study compared to the GFR study. Nevertheless, in both studies null mutation of the *p67^{phox}* gene conferred protection from salt-sensitivity to the SS rat, with day-21 HS MAP being at least 20 mmHg lower in the SS*p67^{phox}* null rats than the WT rats in both experiments.

Perspectives

In these studies we have made repeated measurements of MBF, GFR, MAP and urinary protein in conscious SS*p67^{phox}* null and WT rats over 21-days consumption of a HS diet. This is the first study to simultaneously track these parameters over a three week chronic salt challenge. The results presented have allowed us to determine both the sequential order of events involved in the initiation and maintenance of salt-sensitive hypertension, and the role of increased ROS production in its development. We have shown that reduced MBF, occurring as a result of increased NADPH oxidase-mediated ROS production, is involved in the initiation of salt-sensitive hypertension in the SS rat. In the SS*p67^{phox}* null rat, medullary perfusion was sustained throughout the study and the hypertensive response to HS was blunted. In the later stages of the condition we hypothesize that pressure-induced renal injury results in a reduction in GFR; indeed renal injury was reduced in the SS*p67^{phox}* null and GFR was maintained. Two fold increases in proteinuria occurred prior to significant reductions in GFR in WT rats. Clinically, increased proteinuria can be used to predict the rate of decline of GFR[41, 42]. Our data suggest that in hypertensive renal injury, early initiation of therapies to reduce the progression of salt-sensitive hypertension may improve clinical outcomes by preventing/delaying reductions in GFR. Screening for elevated proteinuria may help identify patients during the initiation of salt-sensitive hypertension, who could be at an increased risk of developing hypertensive renal injury.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Novelty and Significance

1) What is new

These studies are the first to measure MBF in conscious rats over 3 weeks. The combination of these studies with the chronic assessment of GFR has allowed the delineation of events involved in the initiation of salt-sensitive hypertension in the SS rat, with a focus on the role of oxidative stress generated by NADPH-oxidase

2) What is relevant

The SS rat is frequently used as a model of clinical salt-sensitive hypertension. The mechanisms involved in the initiation and progression of salt-sensitivity have not been fully defined. The data presented suggest that increased oxidative stress in the renal medulla results in medullary ischemia which is involved in the initiation of hypertension. Increased blood pressure leads to proteinuria and subsequently reduced GFR. Identification of these time points clinically may improve therapeutic management.

3) Summary

Reducing oxidative stress in the renal medulla, through null mutations in $p67^{phox}$, improves medullary blood flow during the initial stages of salt-sensitive hypertension, which promotes a blunted pressor response to salt and improved renal function.

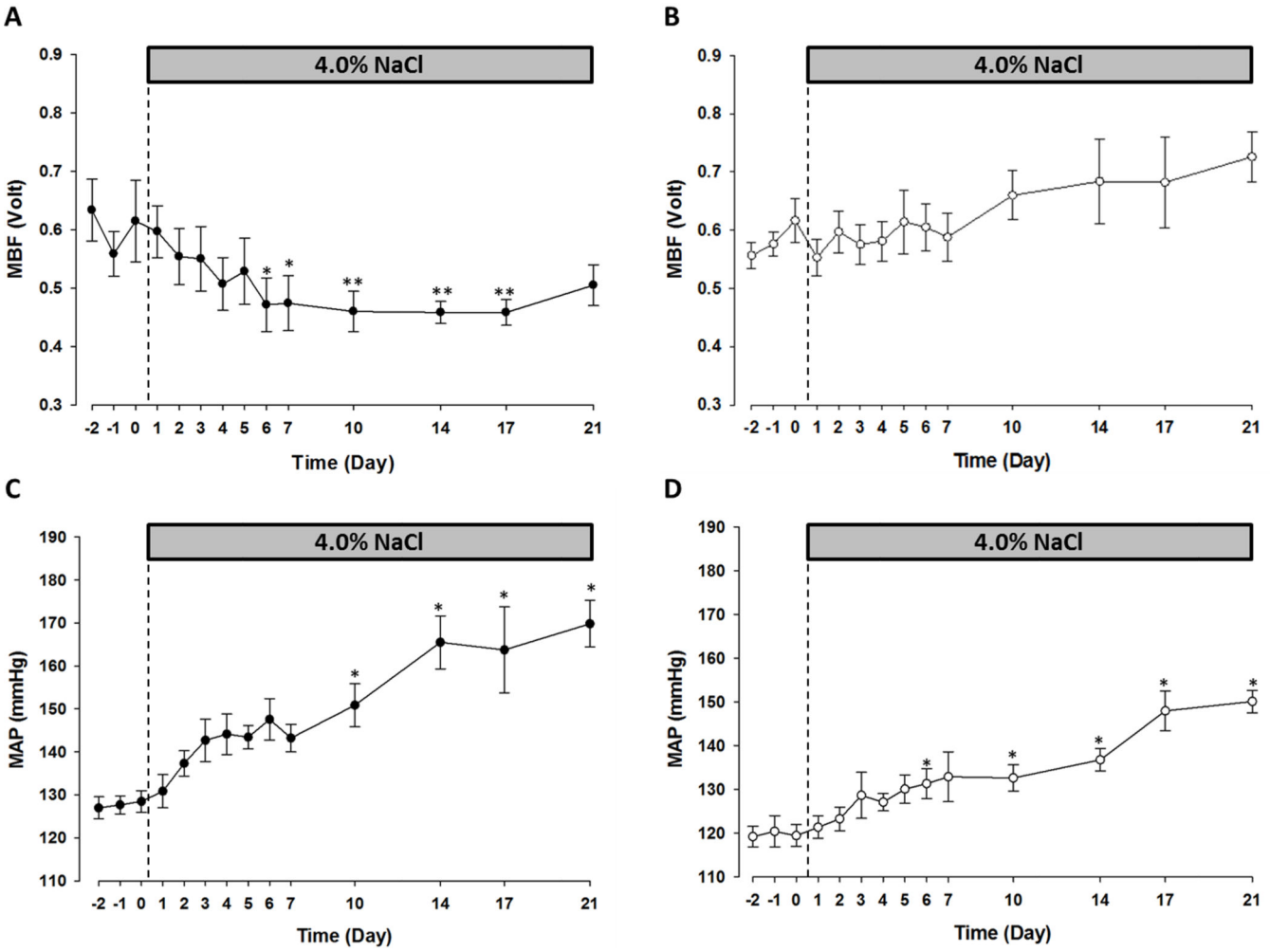

Figure 1.

SS*p67*^{phox} null rats are protected from salt-sensitive medullary ischemia and hypertension compared to wild-type (WT) littermates. Medullary blood flow (MBF) in WT (A) and SS*p67*^{phox} null (B) rats on 0.4% NaCl and 4.0% NaCl diets (n=6/7 rats per strain). Mean arterial pressure (MAP) in WT (C) and SS*p67*^{phox} null (D). n=5/7 per strain, *p<0.05, **p<0.01 compared to the average 0.4% NaCl measurement. Data are presented as mean values ± SE.

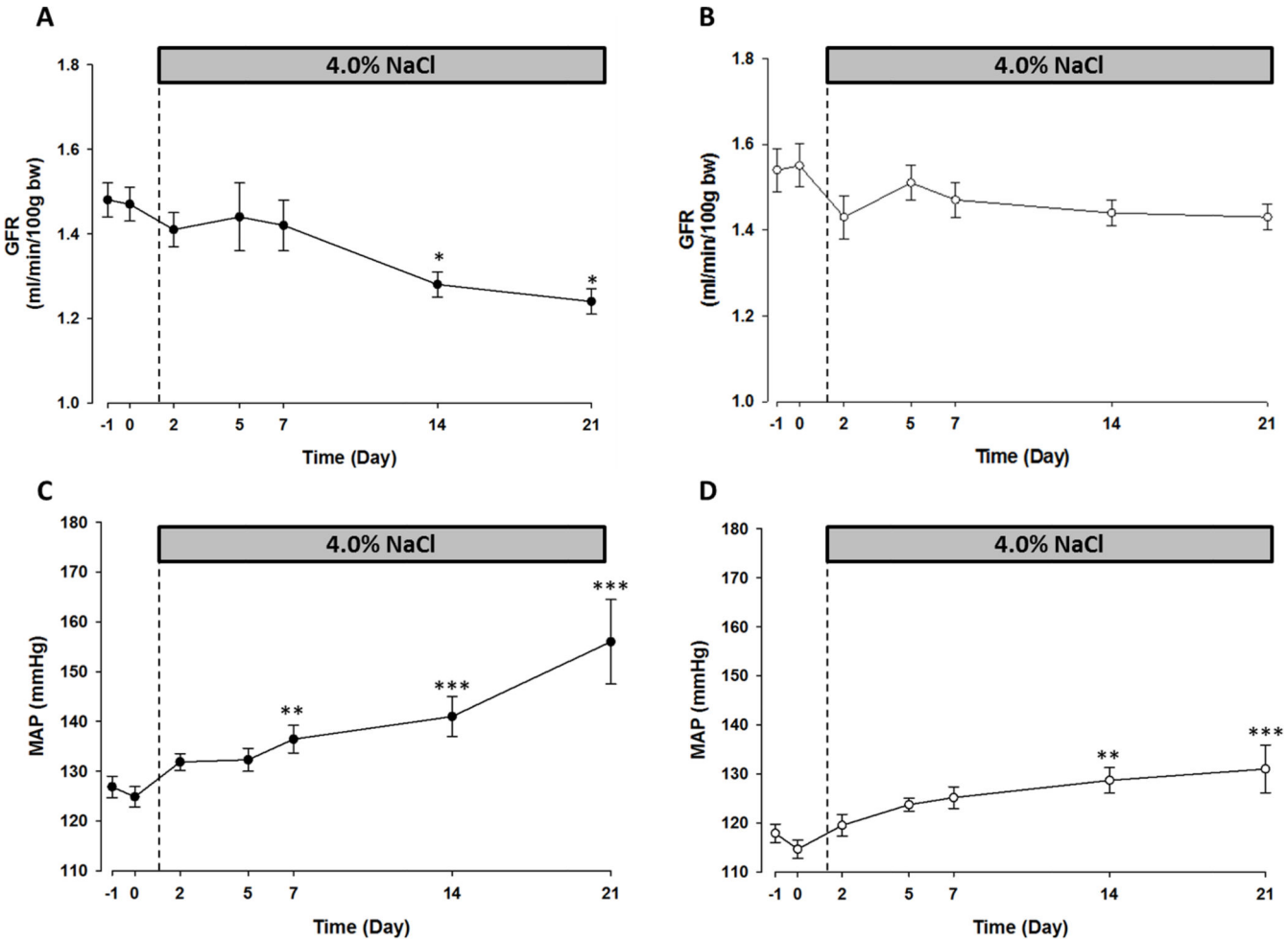

Figure 2.

Salt-sensitive hypertension is attenuated and glomerular filtration rate maintained in SS*p67*^{phox} null rats over 21-days 4.0% NaCl diet compared to wild-type (WT) littermates. Glomerular filtration rate (GFR) in WT (A) and SS*p67*^{phox} null (B) rats on 0.4% and 4.0% NaCl diets. Mean arterial blood pressure (MAP) in WT (C) and SS*p67*^{phox} null (D) rats. n=7/8 per strain, *=p<0.05, **p<0.01, ***p<0.001 compared to average 0.4% NaCl measurement. Data are mean values ± SE.

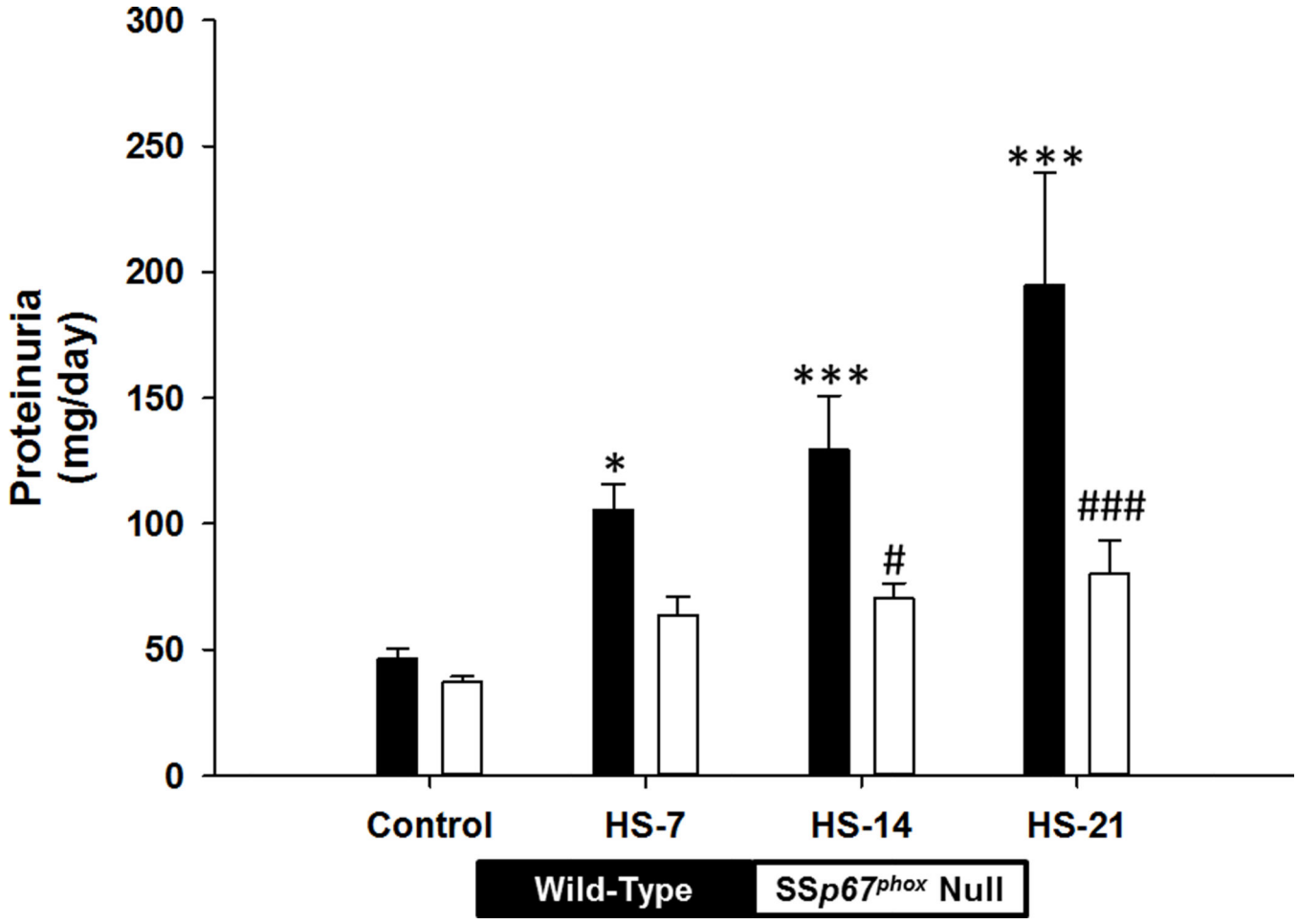

Figure 3.

Urinary protein is attenuated in SS*p67*^{phox} null rats compared to wild-type littermates. Proteinuria in wild-type (black bars) and SS*p67*^{phox} null (white bars) rats on a 0.4% NaCl control day, HS-7, HS-14, HS-21. *p<0.05, ***p<0.001 compared to 0.4% NaCl control measurement. #p<0.05 compared to wild-type rats at the same time point. Data are presented as mean values ± SE.

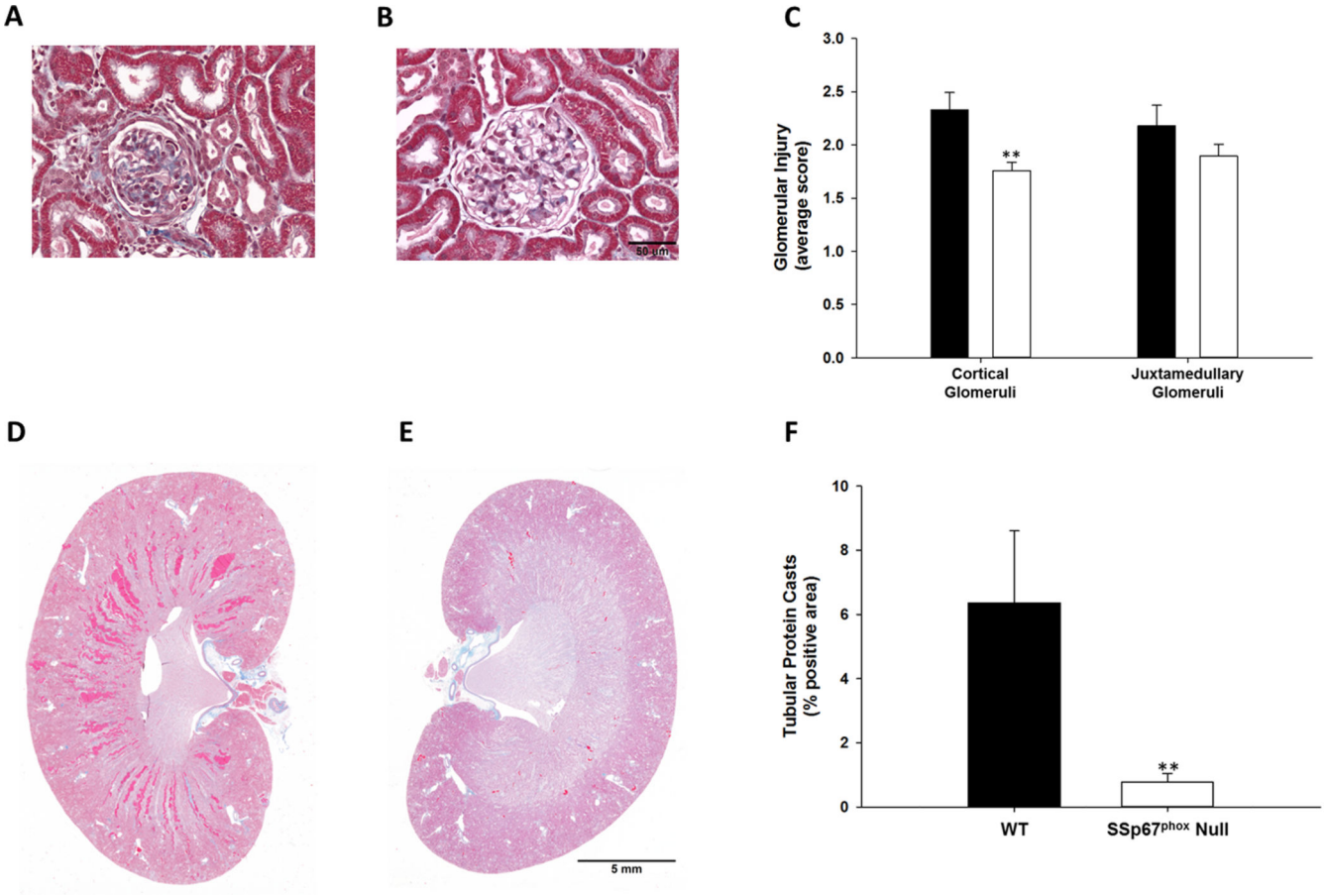

Figure 4.

Histological assessment indicates reduced renal injury in SS*p67*^{phox} null rats compared to wild-type (WT) littermates. Cortical glomerular injury in WT (A) and SS*p67*^{phox} null (B) rats, quantification of glomeruli injury in cortical and juxtamedullary glomeruli (C) in WT (black bars) and SS*p67*^{phox} null (white bars) rats. Trichrome staining of protein casts in WT (D) and SS*p67*^{phox} null (E) rats, quantification of tubular casts (F).

Table 1

Day	0.4% NaCl Control		HS-7		HS-14		HS-21	
Group	WT	SS*p67*^{phox}Null	WT	SS*p67*^{phox}Null	WT	SS*p67*^{phox} Null	WT	SS*p67*^{phox} Null
Creatinine Clearance (ml/min/100g bw)	0.84±0.04	0.89±0.06	1.13±0.05‡	1.07±0.05*	1.08±0.02†	1.08±0.04	1.00±0.06	0.96±0.08

Creatinine Clearance is not reflective of GFR in either wild-type (WT) or SS*p67*^{phox} null rats. Creatinine clearance was compared on a 0.4% NaCl control day and 4.0% NaCl day-7 (HS-7), day-14 (HS-14) and day-21 (HS-21). bw indicates body weight.

*p<0.05,

†p<0.01,

‡p<0.001 compared to control measurements.