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ORIGINAL RESEARCH

Salt-Sensitive Hypertension, Renal Injury, and Renal Vasodysfunction Associated With Dahl Salt-Sensitive Rats Are Abolished in Consomic SS.BN1 Rats

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BACKGROUND: Abnormal renal hemodynamic responses to salt-loading are thought to contribute to salt-sensitive (SS) hypertension. However, this is based largely on studies in anesthetized animals, and little data are available in conscious SS and salt-resistant rats.

METHODS AND RESULTS: We assessed arterial blood pressure, renal function, and renal blood flow during administration of a 0.4% NaCl and a high-salt (4.0% NaCl) diet in conscious, chronically instrumented 10- to 14-week-old Dahl SS and consomic SS rats in which chromosome 1 from the salt-resistant Brown-Norway strain was introgressed into the genome of the SS strain (SS.BN1). Three weeks of high salt intake significantly increased blood pressure (20%) and exacerbated renal injury in SS rats. In contrast, the increase in blood pressure (5%) was similarly attenuated in Brown-Norway and SS.BN1 rats, and both strains were completely protected against renal injury. In SS.BN1 rats, 1 week of high salt intake was associated with a significant decrease in renal vascular resistance (–8%) and increase in renal blood flow (15%). In contrast, renal vascular resistance failed to decrease, and renal blood flow remained unchanged in SS rats during high salt intake. Finally, urinary sodium excretion and glomerular filtration rate were similar between SS and SS.BN1 rats during 0.4% NaCl and high salt intake.

CONCLUSIONS: Our data support the concept that renal vasodysfunction contributes to blood pressure salt sensitivity in Dahl SS rats, and that genes on rat chromosome 1 play a major role in modulating renal hemodynamic responses to salt loading and salt-induced hypertension.

Key Words: blood flow regulation ■ blood pressure ■ kidney ■ renal physiology ■ salt-sensitivity hypertension

The cause of salt-sensitive (SS) hypertension is complex and can involve dysfunction within many organ systems.[1] Nevertheless, there is wide recognition that renal dysfunction plays a major role in the pathogenesis of SS hypertension.[1] Recently, Kurtz and colleagues have emphasized the importance of renal vasodysfunction, or abnormal renal vascular responses to salt loading, in the initiation of SS hypertension.[2,3] In support of this concept, subjects with salt-induced hypertension exhibit subnormal decreases in total peripheral resistance (TPR) as compared with salt-resistant subjects when administered a high salt (HS) load, despite similar increases in sodium retention and cardiac output.[4] Importantly, the impaired TPR responses during salt loading involve subnormal decreases in renal vascular resistance (RVR).[2,3,5] Although it is recognized that renal hemodynamic responses to an HS diet may importantly modulate blood pressure

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CLINICAL PERSPECTIVE

What Is New?

- This study demonstrates that introgression of chromosome 1 from the salt-resistant Brown-Norway rat into the genetic background of the Dahl salt-sensitive rat (ie, consomic SS.BN1 rat) provides the same protection against salt-induced hypertension as compared with parental Brown-Norway rats.
- Salt-resistant SS.BN1 rats, but not salt-sensitive rats, exhibit early and sustained decreases in renal vascular resistance when administered a high salt diet for 1 week, despite similar levels of urinary sodium excretion and glomerular filtration rate.

What Are the Clinical Implications?

- Sustained decreases in renal vascular resistance may be important in preventing increases in blood pressure during the consumption of a high-salt diet.
- In developed countries, in which the daily intake of dietary salt is high, therapies that promote renal vasodilation may help reduce the prevalence of salt-induced hypertension.

Nonstandard Abbreviations and Acronyms

BN	Brown-Norway
C_{Cr}	creatinine clearance
HR	heart rate
HS	high salt
RBF	renal blood flow
RM	repeated measures
RVR	renal vascular resistance
SS	salt sensitive
TIF	tubulointerstitial fibrosis
TPR	total peripheral resistance

(BP) salt sensitivity, the underlying mechanisms remain poorly understood.

Dahl SS rats are commonly used to investigate the mechanisms contributing to SS hypertension.[1,6,7] They share many of the same phenotypes as at-risk clinical populations, such as marked increases in BP and renal disease, with increases in dietary salt consumption. Consistent with clinical studies, RVR responses to an HS diet are impaired in Dahl SS rats as compared with Dahl salt-resistant rats,[8–11] despite similar increases in sodium retention and cardiac output.[12,13] However, in previous investigations, renal blood flow (RBF) was assessed in anesthetized rats and/or at a single time point following several weeks of an HS (8% NaCl) diet. There are limitations to this approach, which include the dampening effects of anesthesia on absolute levels of BP and RBF, their spontaneous fluctuations,[14,15] and the assessment of BP and RBF at only a single time point during HS intake. The assessment of BP and RBF in conscious animals avoids the confounding effects of anesthesia and permits the longitudinal investigation of renal hemodynamics before and during HS intake. Thus, this experimental approach is critical with respect to understanding the role of renal hemodynamics in the development of SS hypertension.

The primary goal of the present study was to compare the renal hemodynamic responses to salt loading between conscious, chronically instrumented Dahl SS rats and a consomic rat strain developed by investigators at the Medical College of Wisconsin, in which chromosome 1 from the salt-resistant Brown-Norway (BN) rat was introgressed into the Dahl SS background (SS.BN1) via breeding strategies.[16] A previous study demonstrated that SS.BN1 rats exhibit significantly lower levels of BP and proteinuria after 3 weeks of HS intake as compared with parental SS rats as well as the majority of other consomic SS.BN strains.[16] However, the extent to which SS.BN1 rats were protected against salt-induced increases in BP as compared with parental BN and SS rats was not determined, because BP was not assessed before the HS challenge. Therefore, we performed an initial experiment to assess BP in SS, BN, and SS.BN1 rats at baseline during the administration of a 0.4% NaCl diet and over 3 weeks during the administration of an HS or 0.4% NaCl diet. In addition, because we observed significant differences in the RVR response to HS between SS.BN1 and SS rats, we also examined glomerular filtration rate (GFR) and urinary sodium excretion in SS.BN1 and SS rats during the acute (ie, 1–7 days) and chronic (ie, 3 weeks) periods after transitioning from a 0.4% NaCl to HS diet. The data show that SS.BN1 rats exhibit a similar salt-resistant BP phenotype as compared with BN rats, whereas SS rats develop more severe increases in BP during HS intake. Over 1 week of HS intake, significant and sustained decreases in RVR were observed in SS.BN1 but not SS rats. The differences in the RVR response to HS between SS.BN1 and SS rats were not attended by differences in GFR or urinary sodium excretion. Collectively, these data indicate that renal vascular responses to an acute HS challenge may importantly modulate BP salt sensitivity.

METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request.

Animals

Experiments were performed in 10- to 14-week-old inbred rats obtained from colonies maintained at East Tennessee State University. The rat colonies at East Tennessee State University were originally established with breeding pairs of inbred Dahl SS/JrHsdMcwi rats (hereafter referred to as SS rats), BN/NHsd/Mcwi rats (hereafter referred to as BN rats), and SS-Chr 1^{BN}/Mcwi rats (hereafter referred to as SS.BN1 rats) obtained from the Medical College of Wisconsin. All rats were housed in temperature-controlled environments (20–23 °C), maintained on a 0.4% NaCl diet (number 113755; Dyets), and provided water ad libitum. Some groups of rats were also administered an HS diet (4.0% NaCl, number 113756; Dyets) for 1 or 3 weeks and provided water ad libitum. A total of 139 rats were used in this study. Both male and female rats of each strain were studied; however, there were no significant sex differences observed for any variable in response to HS intake across strains. Therefore, male and female data were combined for each strain. We did not always investigate the effects of the 0.4% and 4.0% NaCl diets within or across strains at the same time. As such, it is possible that genetic variations among litters may have contributed to potential confounders within groups of rats. All animals were cared for in accordance with the *Guide for the Care and Use of Laboratory Animals* and the protocols approved by the Institutional Animal Care and Use Committee at East Tennessee State University.

Protocol 1: Assessment of BP and Renal Injury in BN, SS, and SS.BN1 Rats Administered a 0.4% NaCl or HS Diet for 3 Weeks

As described previously,[17,18] BN, SS, and SS.BN1 rats were surgically instrumented with a BP radiotelemeter catheter (HD-S10; Data Sciences International). Rats were anesthetized with isoflurane (5% induction/2.5% maintenance in 100% oxygen), and the femoral artery was isolated via an inguinal incision. Then a midline incision was made, the body of the transmitter was placed within the abdominal cavity, and the tip of the catheter was routed to the femoral region using an 18-gauge needle. The BP catheter was inserted into the femoral artery and advanced to the abdominal aorta, with the tip of the catheter positioned below the renal arteries. The abdominal incision was closed with 4-0 Vicryl suture and skin incisions closed with staples. Bupivacaine was applied to all incision sites, and rats were administered Tylenol via the drinking water (≈200 mg/kg per day) 1 day before and for 3 days following surgery.

At 7 days after surgery, BP was recorded continuously, 24 hours per day (10 s every 10 minutes at 500 Hz) for 3 consecutive days, during which rats were administered a 0.4% NaCl diet. Heart rate (HR), derived from the interbeat interval of the BP signal, and locomotor activity (1 Hz) were also recorded (Data Sciences International; Ponemah data acquisition software, version 6.41). Rats were then placed in metabolic cages, and 24-hour urine samples were collected for the assessment of proteinuria (Bradford method). Following urine collections, different groups of rats were either maintained on the 0.4% NaCl diet or switched to an HS diet for 3 weeks. Recordings of BP, HR, locomotor activity, and 24-hour urine collections were repeated every week in a similar fashion to baseline measurements. At the end of the 3-week protocol, kidneys were perfuse-fixed with a periodate-lysine-paraformaldehyde solution, weighed, and placed in periodate-lysine-paraformaldehyde overnight at 4 °C. The next day, kidneys were switched to a solution of 10 mM PBS + 0.2% sodium azide and stored at 4 °C before paraffin embedding.

As described previously,[17,18] renal injury, consisting of glomerulosclerosis, tubulointerstitial fibrosis (TIF), and vascular injury, was assessed in 4- to 5-μm-thick sections stained with periodic acid–Schiff in a blinded fashion by an experienced renal pathologist (M.M.P.). In each section, glomerulosclerosis was quantified as the percentage of 100 evaluated glomeruli exhibiting lesions of segmental, global, or ischemic glomerulosclerosis. TIF was assessed on a scale of 0 to 4, with 0=no TIF, 1=1% to 25%, 2=26% to 50%, 3=51% to 75%, and 4=76% to 100% of the section exhibiting TIF. Vascular injury was assessed as the number of vessels exhibiting lesions of necrosis, thrombosis, aneurysmal dilation, and/or onion skinning per 100 glomeruli.

Protocol 2: Renal Function in SS and SS.BN1 Rats During Administration of a 0.4% NaCl Diet and After Administration of an HS Diet for 3 Weeks

SS and SS.BN1 rats administered a 0.4% NaCl diet were placed in metabolic cages for baseline 24-hour urine collection. The next day, a blood sample was obtained via tail nick for the determination of plasma creatinine. Rats were then administered an HS diet for 3 weeks, following which urine and blood samples were collected in a similar fashion to baseline measurements. After 3 weeks of HS intake, rats were anesthetized, and a terminal blood sample was obtained via the vena cava for the determination of plasma sodium concentration. Kidneys were perfuse-fixed with periodate-lysine-paraformaldehyde and processed for the determination of renal injury, as described above. Urine and plasma samples were stored at –20 °C before analysis.

Protocol 3: Assessment of BP and RBF in Conscious SS and SS.BN1 Rats During Administration of a 0.4% NaCl or HS Diet for 1 Week

As described previously,[15,17–19] SS and SS.BN1 rats were anesthetized with isoflurane (5% induction/2.5% maintenance in 100% oxygen) and surgically instrumented with a BP radiotelemeter catheter (HD-S10; Data Sciences International) and an ultrasonic RBF probe (1PRB; Transonic), which was placed on the left renal artery. The RBF probe cable was secured to the posterior abdominal wall using Dacron mesh, routed to the midscapulae region, and secured to skin with a silicone button using 3-0 nylon suture. Bupivacaine was applied to all incision sites, and rats were administered Tylenol via the drinking water (≈200 mg/kg per day) 1 day before and for 3 days following surgery. Following 1 week of recovery, BP, RBF, and HR were then recorded at a sampling rate of 1000 Hz for 2 to 3 hours on 3 consecutive days while administered a 0.4% NaCl diet. After these baseline recordings, separate groups of SS and SS.BN1 rats were either maintained on a 0.4% NaCl diet or administered an HS diet for the next 7 days. Beginning on day 3 of 0.4% NaCl or HS intake, BP, RBF, and HR were assessed for 5 consecutive days (ie, days 3–7 of 0.4% NaCl or HS intake) in a similar fashion to baseline recordings. RVR was calculated for each recording by dividing the average BP by the average RBF over the entire recording.

Protocol 4: Assessment of Renal Function in SS and SS.BN1 Rats During Administration of a 0.4% NaCl Diet and 1 Week of an HS Diet

Before surgery, a 24-hour urine sample and blood sample were obtained in SS and SS.BN1 rats maintained on a 0.4% NaCl diet. As described previously,[15,17,20] rats were then anesthetized with isoflurane (5% induction/2.5% maintenance in 100% oxygen), and an osmotic minipump (ALZET; 2ML2) containing 20 mg/mL of fluorescein isothiocyanate (FITC)-inulin (Sigma) in 10 mM PBS (pH=7.4) was implanted subcutaneously between the scapulae. Bupivacaine was applied to the incision site, and rats were administered Tylenol via the drinking water (≈200 mg/kg per day) for 2 days following surgery. Two days following surgery, rats were placed in metabolic cages for 24-hour urine collections during 0.4% NaCl intake. The next day, a blood sample was obtained via tail nick for the determination of plasma FITC-inulin and plasma creatinine concentrations. All rats were then administered an HS diet for 7 days. Twenty-four-hour urine samples and plasma samples were obtained on days 1, 3, 5, and 7 of HS intake in a similar fashion to baseline measurements. After the final collection, rats were anesthetized, a terminal blood sample was obtained via the vena cava for the determination of plasma sodium concentration, and kidneys were perfuse-fixed with periodate-lysine-paraformaldehyde and processed for the determination of renal injury, as described above. Urine and plasma samples were stored at 4°C before analysis.

Urine and Plasma Analysis

Urine creatinine and plasma creatinine concentrations were determined via colorimetric analysis (BioAssay Systems, Hayward, CA; catalog number DICT-500). Urine sodium and plasma sodium concentrations were measured using a Beckman Coulter AU480 Chemistry Analyzer. The fractional excretion of sodium was calculated using the equation: fractional excretion of sodium=([urine sodium]×[plasma creatinine]) / ([plasma sodium]×[urine creatinine])×100.

Determination of Glomerular Filtration Rate via Chronic FITC-Inulin Administration

As described previously,[15,17,20] FITC-inulin concentrations in plasma and urine were determined via fluorescence (excitation: 485 nm, emission: 530 nm) using a microplate reader (Synergy H1; BioTek). Plasma and urine samples from rats before osmotic minipump implantation were included in the assay to correct for background fluorescence.

Statistical Analysis

We tested the null hypothesis that there is no difference in the RVR response to salt loading between SS and SS.BN1 rats. A 2-way repeated-measures (RM) ANOVA with Tukey post hoc analysis was used to test for differences in the primary outcome variable (ie, RVR) between groups of SS and SS.BN1 rats fed either a 0.4% NaCl or HS diet. Secondary outcome variables included BP, HR, proteinuria, urinary sodium excretion, locomotor activity, and renal injury during 3 weeks of 0.4% NaCl or HS intake in BN, SS, and SS.BN1 rats. A 1-way ANOVA with Tukey post hoc analysis or unpaired *t* test was used to test for differences in baseline data between groups. A 2-way RM ANOVA with Tukey post hoc analysis was used to test for differences in BP, HR, locomotor activity, and proteinuria between groups over time. A Kruskal-Wallis 1-way ANOVA with Dunn post hoc analysis was used to assess differences in indices of renal injury between groups of rats fed a 0.4% NaCl or HS diet. A Mann-Whitney rank sum test was used to assess differences in renal injury between rats fed a 0.4% NaCl and HS diet within each strain. A Bonferroni correction was used for the multiple Mann-Whitney tests (adjusted significance rate was $P<0.017$). Linear regression analysis was used to calculate the

slope of relationship between BP and indices of renal injury. Additional secondary outcome variables included the effects of 1 week and 3 weeks of HS intake on renal function (eg, GFR, urinary sodium excretion, and urine volume), proteinuria, and renal injury in SS and SS.BN1 rats. A 2-way RM ANOVA with Tukey post hoc analysis was used to test for differences in renal function and proteinuria between strains over time. A Mann-Whitney rank sum test was used to assess differences in renal injury between SS and SS.BN1 rats. Unless stated otherwise, percent change calculations are based on the average value over the 1- or 3-week protocol versus baseline values. Potential outliers within groups were determined using the Grubb test[21] and the ROUT method.[22] We did not detect any outliers within any group in all experiments using either method. All data are presented as mean±SE and, unless stated otherwise, $P<0.05$ was considered statistically significant. Statistical analyses were performed using SigmaPlot (version 12.5; Systat Software). Tests for outliers were performed using Prism (version 7.05; GraphPad).

RESULTS

Salt-Induced Hypertension and Renal Injury in BN, SS, and SS.BN1 Rats

Presurgical body weight was lower ($P<0.01$, 1-way ANOVA with Tukey post hoc comparison) in BN (202±15 g, n=16) versus SS.BN1 (257±13 g, n=16) and SS (269±12 g, n=23) rats maintained on a 0.4% NaCl diet. One week after the surgical implantation of BP radiotelemetry devices, during which all rats remained on a 0.4% NaCl diet, body weight was again lower ($P<0.01$, 1-way ANOVA with Tukey post hoc comparison) in BN (214±15 g, n=16) versus SS.BN1 (272±14 g, n=16) and SS (287±13 g, n=23) rats. As determined by a 2-way RM ANOVA with Tukey post hoc comparison, body weight increased ($P<0.001$) in all groups over the 3-week protocol. However, a statistically significant interaction between group and time was not detected ($P_{INTERACTION}=0.12$). This could be due to the large variability in body weight within groups because of the inclusion of both male and female rats. For this reason, we also assessed whether the percent change in body weight differed among groups as determined by a Kruskal-Wallis 1-way ANOVA with Dunn post hoc comparison. Despite a significant ANOVA ($P<0.005$), no significant differences were observed between HS versus 0.4% NaCl groups within BN (13±1% versus 8±1%, respectively), SS.BN1 (9±1% versus 5±1%, respectively), or SS (7±1% versus 7±1%, respectively) rats after post hoc analyses. Of note, 3 SS rats within the HS group exhibited a baseline mean arterial pressure (MAP) >150 mm Hg, more severe hypertension during HS, and weight loss during the third week of HS. We have observed similar weight loss in rats that develop more severe BP elevations in other experimental models of hypertension.[23] If these 3 SS rats are excluded from statistical analyses, significant differences in the percent change in body weight are observed between HS versus 0.4% NaCl–fed BN ($P<0.01$) rats, but not SS.BN1 ($P=0.07$) or SS rats (9±1% versus 7±1%, respectively, $P=0.60$) as determined by a 1-way ANOVA with Tukey comparison.

Baseline MAP during 0.4% NaCl intake was different ($P<0.001$, 1-way ANOVA with Tukey comparison) across all groups with SS (133±2 mm Hg, n=23) > SS.BN1 (116±1 mm Hg, n=16) > BN (94±1 mm Hg, n=16). As shown in Figure 1A, the increase in MAP over the 3-week protocol in rats fed an HS diet was greater ($P<0.001$) in SS (20±1%) versus SS.BN1 (5±1%) and BN (5±1%) rats, which were not statistically different from each other. In rats administered a 0.4% NaCl diet, there was a modest, but significant ($P<0.001$), increase in MAP in SS rats (5±1%), but not SS.BN1 (1±1%) or BN (–2±1%) rats.

Baseline systolic and diastolic BP during 0.4% NaCl intake were different ($P<0.001$, 1-way ANOVA with Tukey post hoc comparison) across all groups with SS>SS.BN1>BN. Baseline pulse pressure was higher ($P<0.001$, 1-way ANOVA with Tukey post hoc comparison) in SS versus BN and SS.BN1 rats, which were not significantly different from each other. As shown in Figure S1, an HS diet led to significant increases in systolic (18±1%, $P<0.001$), diastolic (21±2%, $P<0.001$), and pulse (8±4%, $P<0.05$) pressures in SS rats over the 3-week protocol. In BN and SS.BN1 rats, HS led to modest increases ($P<0.01$) in systolic BP; however, neither diastolic BP nor pulse pressure significantly increased above baseline levels in either group.

Baseline HR during 0.4% NaCl intake was lower ($P<0.001$, 1-way ANOVA with Tukey post hoc comparison) in BN (374±7 beats/min, n=16) versus SS (419±4 beats/min, n=23) and SS.BN1 (430±5 beats/min, n=16) rats, which were not significantly different from each other. As shown in Figure 1B, such differences in HR persisted over the entire protocol in both 0.4% NaCl– and HS-fed BN versus respective SS and SS.BN1 rats ($P<0.05$). A significant difference in HR was also observed between groups of BN rats administered a 0.4% NaCl versus HS diet at baseline ($P<0.05$, 1-way ANOVA with Tukey post hoc comparison) and over the entire protocol ($P<0.005$). The significantly higher HR in the BN group fed an HS diet was largely because of 3 rats with HRs >400 beats/min. If these 3 rats are excluded from statistical analysis, no significant differences in HR are observed between BN groups at baseline ($P=0.625$) or over the entire protocol ($P=0.103$; Figure S2). In addition, the pattern of HR change in the BN HS group over the 3-week protocol is similar to

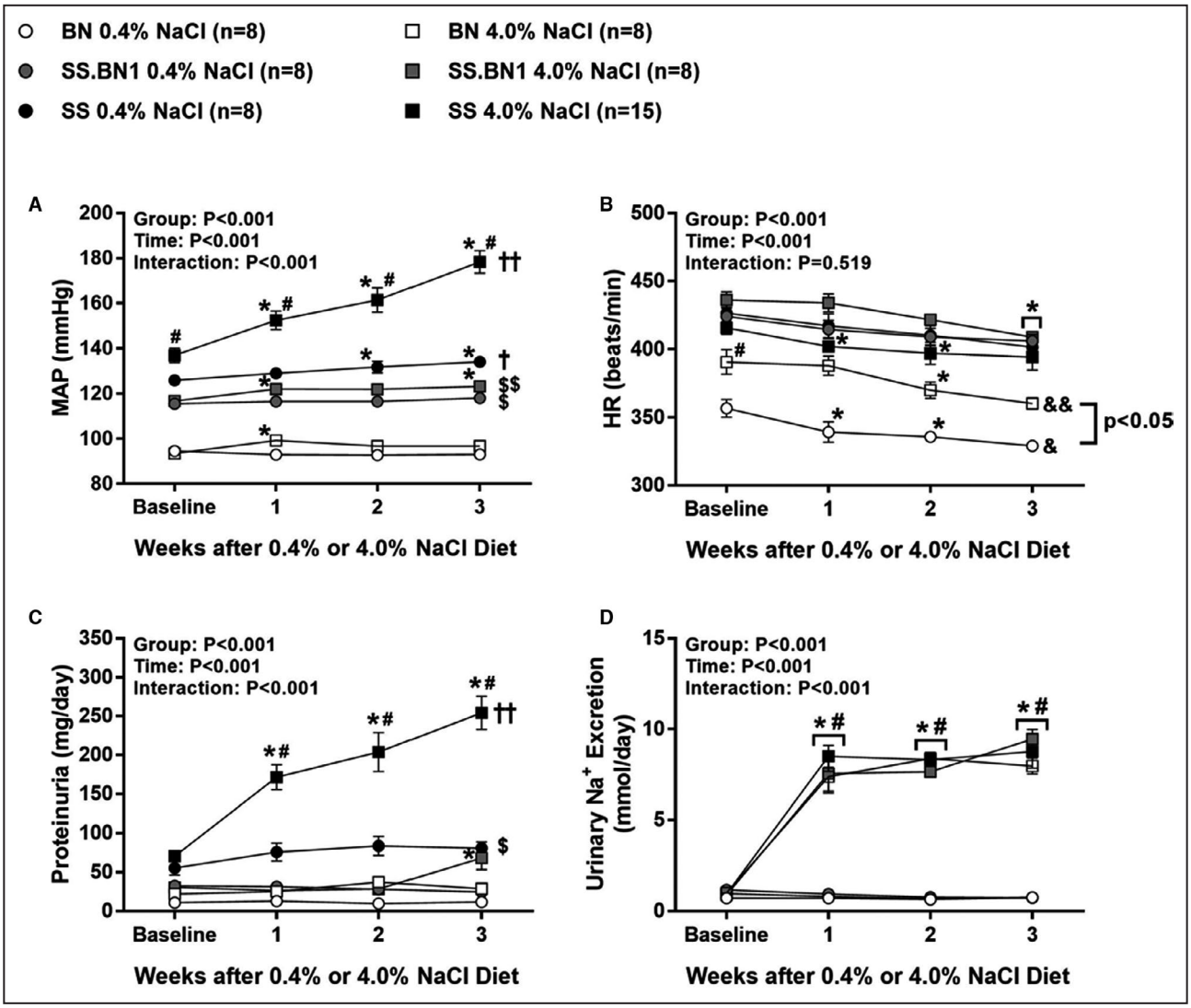

Figure 1. Blood pressure, heart rate (HR), proteinuria, and urinary sodium excretion in rats fed a 0.4% NaCl (n=8 BN, n=8 SS.BN1, n=8 SS) or high-salt (4.0% NaCl; n=8 BN, n=8 SS.BN1, n=15 SS) diet for 3 weeks.
A 1-way ANOVA was used to assess differences in (**A**) mean arterial pressure (MAP), (**B**) HR, (**C**) proteinuria, and (**D**) urinary sodium excretion between groups at baseline during 0.4% NaCl intake. A 2-way repeated measures ANOVA with Tukey comparison was used to assess differences between groups over time. Data are mean±SE, and $P<0.05$ was considered statistically significant. *$P<0.05$ vs respective baseline value. #$P<0.05$ vs respective 0.4% NaCl group. †$P<0.05$ vs BN and SS.BN1 rats fed a 0.4% NaCl diet. ††$P<0.05$ vs BN and SS.BN1 rats fed a high-salt diet. \$$P<0.05$ vs BN rats fed a 0.4% NaCl diet. \$\$ $P<0.05$ vs BN rats fed a high-salt diet. &$P<0.05$ vs SS.BN1 and SS rats fed a 0.4% NaCl diet. &&$P<0.05$ vs SS.BN1 and SS rats fed a high-salt diet. The bracket over the asterisk (*) indicates all groups significantly different ($P<0.05$) from respective baseline values. The bracket over the number symbol (#) indicates all high-salt groups significantly different ($P<0.05$) from respective 0.4% NaCl groups. BN indicates Brown-Norway; SS, salt sensitive; and SS.BN1, chromosome 1 from the salt-resistant BN rat introgressed into the Dahl SS background.

that observed when these 3 BN rats are included in the analysis (Figure 1B). In general, HR decreased over time in all groups. In rats administered a 0.4% NaCl diet, there was a −6±1%, −3±1%, and −4±1% decrease in HR in BN, SS.BN1, and SS rats, respectively. In rats administered an HS diet, there was a −4±1%, −3±1%, and −4±1% decrease in HR in BN, SS.BN1, and SS rats, respectively.

Baseline proteinuria during 0.4% NaCl intake was elevated ($P<0.001$, 1-way ANOVA with Tukey post hoc comparison) in SS (65±5 mg/d, n=23) versus BN (17±2 mg/d, n=16) and SS.BN1 (32±4 mg/d, n=16) rats, which were not significantly different from each other. As shown in Figure 1C, HS intake exacerbated ($P<0.01$) proteinuria in SS rats, whereas no significant changes were observed in either BN or SS.BN1 rats except for a modest increase ($P<0.05$) in proteinuria in SS.BN1 rats during the third week of HS intake.

No significant differences in urinary sodium excretion were observed between BN (0.9±0.1 mmol/d,

n=16), SS.BN1 (1.0±0.1 mmol/d, n=16), and SS (1.0±0.1 mmol/d, n=23) rats at baseline during 0.4% NaCl intake. As shown in Figure 1D, HS intake significantly increased urinary sodium excretion in all groups. No significant differences in urinary sodium excretion were observed among BN, SS.BN1, and SS rats within the 0.4% NaCl or HS diet groups. Similar results were observed when urinary sodium excretion was normalized to body weight (data not shown). There were no significant differences in locomotor activity among BN, SS.BN1, and SS rats fed either a 0.4% NaCl or HS diet, and HS intake did not significantly alter locomotor activity in any strain (Figure S3).

In rats administered a 0.4% NaCl diet, the level of glomerulosclerosis was significantly greater in SS versus SS.BN1 and BN rats ($P<0.05$). Although TIF was only observed in SS rats fed a 0.4% NaCl diet, there was not a statistically significant difference in TIF as compared with SS.BN1 and BN rats. HS intake exacerbated ($P<0.01$) both indices of renal injury in SS rats but not SS.BN1 or BN rats (Figures 2A and 2B). Representative images of glomeruli and tubulointerstitium from BN, SS.BN1, and SS rats are shown in Figure 2C. Injury to renal arterioles was only observed in 3 of 15 SS kidneys and none of the BN or SS.BN1 kidneys. Of note, a small number of glomeruli of BN rats exhibited thrombotic microangiopathy (Figure S4), which were excluded from analysis. Thrombotic microangiopathy has previously been observed in BN rats from various distributors (personal communication with Dr Will Cupples and Dr Anil Bidani). The quantitative relationships between the average systolic BP and percent glomerulosclerosis (Figure 3A) and TIF (Figure 3B) show that both indices of renal injury were strongly correlated ($P<0.0001$) with systolic BP.

Right kidney weight normalized to body weight (grams per kilogram) was different ($P<0.05$, 1-way ANOVA with Tukey comparison) across all groups fed a 0.4% NaCl diet: BN (3.5±0.1), SS.BN1 (5.1±0.2), and SS (6.5±0.5). In rats fed an HS diet, normalized kidney weight was greater ($P<0.01$, 1-way ANOVA with Tukey post hoc comparison) in SS (7.0±0.3 g/kg) versus SS.BN1 (5.3±0.3 g/kg) and BN (4.6±0.3 g/kg) rats, which were not significantly different from each other. In BN, but not SS.BN1 and SS rats, normalized kidney weight was greater ($P<0.01$) in rats fed an HS versus 0.4% NaCl diet (unpaired t test with Bonferonni correction for multiple comparisons; adjusted significance of $P<0.02$).

Of note, baseline MAP was 11 mm Hg higher in SS rats subsequently fed the HS versus 0.4% NaCl diet ($P<0.05$, 1-way ANOVA with Tukey post hoc comparison), and this was associated with a 15-mg/d higher level of proteinuria, although the difference in baseline proteinuria was not significant ($P=0.440$, 1-way ANOVA with Tukey post hoc comparison). As mentioned previously, such differences in baseline MAP were largely the result of 3 SS rats in the HS group with baseline MAPs >150 mm Hg. Although none of the data from these 3 rats were identified as outliers, it is possible their inclusion may have predisposed the SS HS group to develop greater increases in salt-induced hypertension and renal injury than would be expected for SS rats. For this reason, we repeated the statistical analyses for the data shown in Figures 1 through 3 after removing these 3 SS rats from the HS group. As shown in Figure S5, this attenuated the difference in baseline MAP (6 mm Hg) and proteinuria (8 mg/d), and no significant differences in MAP ($P=0.237$) or proteinuria ($P=0.915$) were observed between groups (1-way ANOVA with Tukey post hoc comparison). Moreover, the percent increase in MAP and proteinuria in SS rats fed an HS diet was similar whether these 3 SS rats were included (20±1% and 213±27%, respectively) or excluded (19±1% and 225±32%, respectively) from analysis. As shown in Figure S6, the exclusion of these 3 SS rats did not significantly alter the average level of glomerulosclerosis (17±2%) and TIF (1.3±0.1) as compared with the average level of glomerulosclerosis (19±2%) and TIF (1.6±0.2) observed when they were included in the analysis (Figure 2). Finally, as shown in Figure S7, the exclusion of these 3 SS rats did not alter the robust and significant relationship between BP and glomerulosclerosis and TIF as compared with when they were included in analysis (Figure 3).

Renal Function in SS and SS.BN1 Rats Administered a 0.4% NaCl Diet and After Administration of an HS Diet for 3 Weeks

In SS and SS.BN1 rats, 24-hour urine samples and blood samples were obtained during 0.4% NaCl intake and after 3 weeks of HS intake. On a 0.4% NaCl diet, no significant differences in body weight, water intake, urine output, creatinine clearance (C_{Cr}), or sodium excretion were observed between SS and SS.BN1 rats (Table). Three weeks following administration of an HS diet, the percent increase in body weight was not significantly different between SS.BN1 (16±1%) and SS (13±2%) rats. In both strains, 24-hour water intake and urine output significantly increased with HS intake; however, the increases were greater ($P<0.01$) in SS versus SS.BN1 rats (Table). Although a significant interaction was observed in the 2-way RM ANOVA on the change in C_{Cr} from 0.4% NaCl to HS intake between SS.BN1 (14±7%) and SS (–9±12%) rats, no significant differences were observed following post hoc analysis. No significant differences were observed in urinary sodium excretion (Table), plasma sodium concentration (140±1 versus 140±2 mmol/L, respectively), and fractional excretion of sodium (2.6±0.2% versus 2.9±0.4%) between SS.BN1 versus SS rats, respectively, after 3 weeks of HS intake.

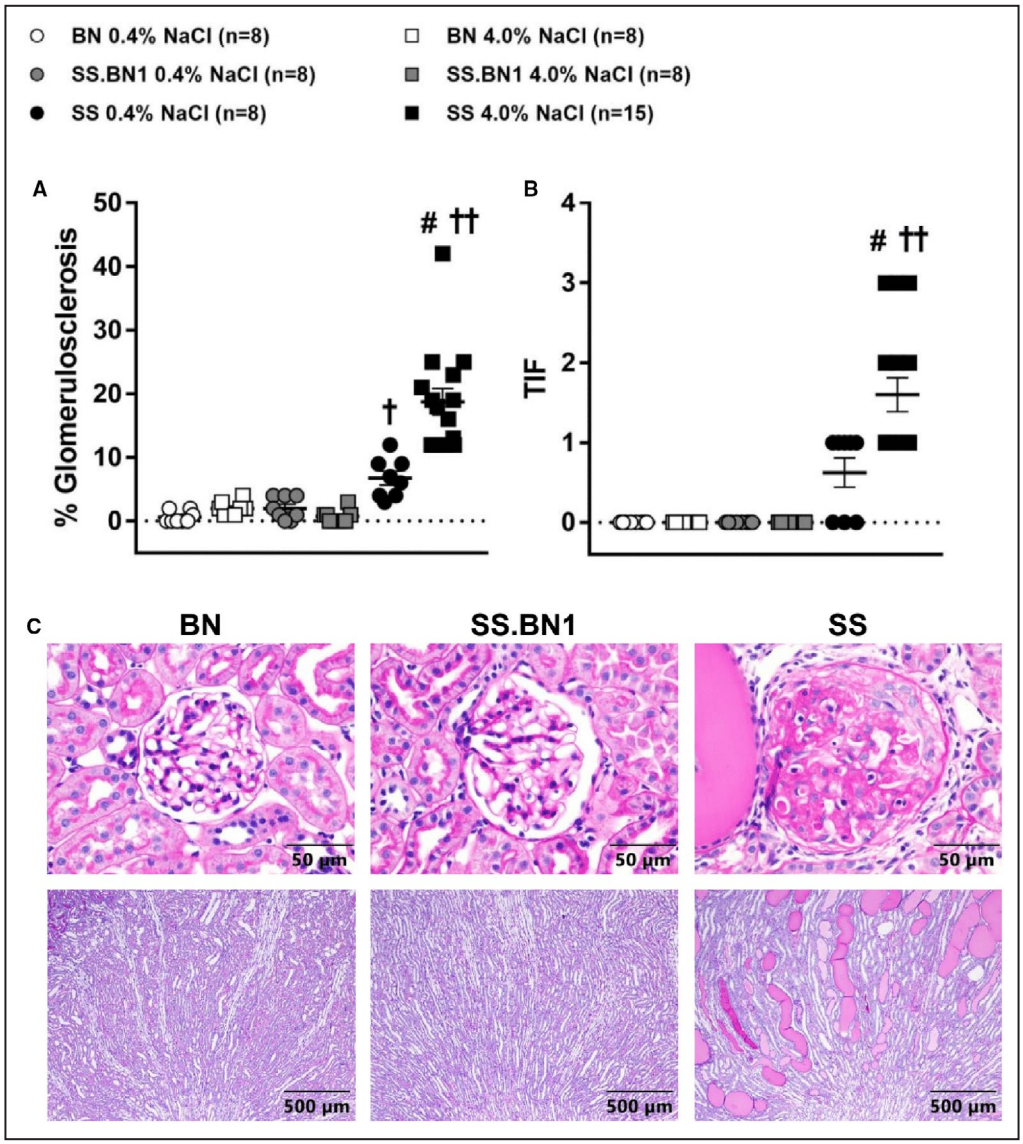

Figure 2. Renal injury in rats fed a 0.4% NaCl (n=8 BN, n=8 SS.BN1, n=8 SS) or a high-salt (4.0% NaCl; n=8 BN, n=8 SS.BN1, n=15 SS) diet for 3 weeks.
A Kruskal-Wallis test with Dunn analysis was used to assess differences in the semiquantitative analysis of (**A**) glomerulosclerosis and (**B**) tubulointerstitial fibrosis (TIF) across groups of rats fed a 0.4% NaCl or high-salt diet. A Mann-Whitney rank sum test was used to assess differences in renal injury between rats fed a 0.4% NaCl and high-salt diet within each strain. A Bonferroni correction was used for the multiple Mann-Whitney tests (adjusted significance rate was $P<0.017$). Representative images of glomeruli and tubulointerstitium stained with Periodic acid-Schiff from rats fed a high-salt diet are shown (**C**). Data are mean±SE unless noted otherwise. $P<0.05$ was considered statistically significant. #$P<0.017$ vs respective 0.4% NaCl group. †$P<0.05$ vs BN and SS.BN1 rats fed a 0.4% NaCl diet. †† $P<0.05$ vs BN and SS.BN1 rats fed a high-salt diet. BN indicates Brown-Norway; SS, salt sensitive; and SS.BN1, chromosome 1 from the salt-resistant BN rat introgressed into the Dahl SS background.

Normalized kidney weight was greater ($P<0.0001$, unpaired *t* test) in SS versus SS.BN1 rats (7.4±0.3 versus 6.0±0.3 g/kg, respectively), and HS intake led to robust renal injury in SS but not SS.BN1 rats ($P<0.001$), evident by both glomerulosclerosis (20±4 versus 2±1%, respectively) and TIF (1.3±0.2 versus 0.1±0.1 score, respectively). Vascular injury was only observed in 1 of 10 SS kidneys.

Renal Hemodynamic Responses in Conscious SS and SS.BN1 Rats Administered a 0.4% NaCl or HS Diet for 1 Week

During the first 30 minutes of the 2- to 3-hour recordings, BP, HR, and RVR were significantly higher, and RBF was significantly lower, as compared with

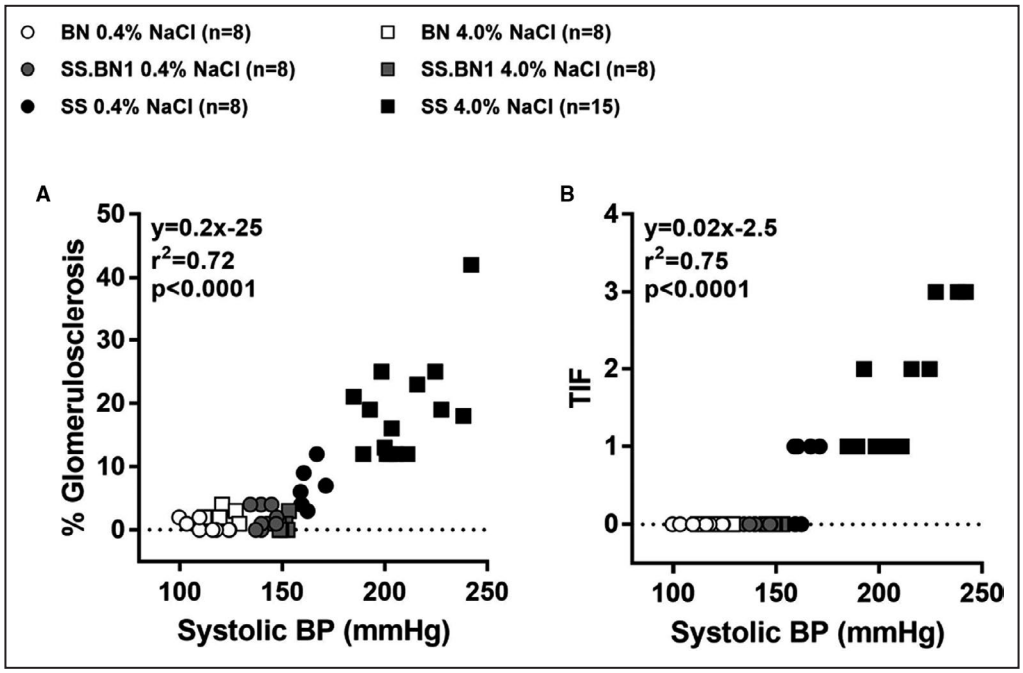

Figure 3. Quantitative relationships between blood pressure (BP) and indices of renal injury in rats fed a 0.4% NaCl (n=8 BN, n=8 SS.BN1, n=8 SS) or a high-salt (4.0% NaCl; n=8 BN, n=8 SS.BN1, n=15 SS) diet for 3 weeks.

The quantitative relationships between systolic BP and (**A**) percent glomerulosclerosis and (**B**) tubulointerstitial fibrosis (TIF) were assessed using linear regression analysis. BN indicates Brown-Norway; SS, salt sensitive; and SS.BN1, chromosome 1 from the salt-resistant BN rat introgressed into the Dahl SS background.

the remainder of the recordings in both strains. This was likely because of stress associated with connection of the flow meter extension cable to the flow probe connector. Therefore, the first 30 minutes of all recordings were not included in the analysis. For statistical analysis, the average of the 3 baseline recordings during 0.4% NaCl intake was compared with the average of 5 recordings during 1 week of a 0.4% NaCl or HS diet. At baseline during 0.4% NaCl intake in SS (n=28) versus SS.BN1 (n=23) rats, MAP (136±2 versus 116±1 mm Hg, respectively, $P<0.001$), HR (386±5 versus 436±6 beats/min, respectively, $P<0.001$), and RVR (21±1 versus 17±1 mm Hg/mL per minute, respectively, $P<0.05$) were significantly

Table. Body Weight, Water Consumption, and Renal Function During 0.4% NaCl Intake and After 3 Weeks of High-Salt (4.0% NaCl) Intake in SS.BN1 (n=10) and SS (n=10) Rats

	Body weight, g	H_2O intake, mL/d	Urine output, mL/d	C_{Cr}, mL/min per kilogram BW	Na^+ excretion, mmol/d
SS.BN1, n=10					
0.4% NaCl	238±14	18±1	11±1	6.4±0.4	0.9±0.1
4.0% NaCl	276±15*	27±2*	24±2*	7.5±0.5	10.4±1.0*
SS, n=10					
0.4% NaCl	271±17	16±1	10±1	7.6±0.9	1.0±0.1
4.0% NaCl	305±18*	38±4*†	34±3*†	6.4±0.6	11.0±1.5*
2-way RM ANOVA					
Strain	P=0.196	P<0.05	P<0.05	P=0.916	P=0.709
Time	P<0.001	P<0.001	P<0.001	P=0.663	P<0.001
Interaction	P=0.570	P<0.05	P<0.01	P<0.05	P=0.770

A 2-way RM ANOVA with Tukey post hoc comparison was used to assess the effects of strain and salt on the measured parameters. Data are mean±SE, and $P<0.05$ was considered statistically significant. BW indicates body weight, C_{Cr}, creatinine clearance, RM, repeated measures; and SS.BN1, chromosome 1 from the salt-resistant Brown-Norway rat introgressed into the Dahl salt-sensitive background.

*$P<0.05$ vs respective 0.4% NaCl salt value.

†$P<0.05$ vs respective SS.BN1 group.

different between groups (unpaired *t* tests). In contrast, RBF was not significantly different in SS (6.9±0.4 mL/min) versus SS.BN1 (7.0±0.3 mL/min) rats (unpaired *t* test). After these baseline measurements, different groups of SS and SS.BN1 rats were either maintained on the 0.4% NaCl diet or administered an HS diet, and hemodynamic assessments were repeated on days 3 to 7 of the 0.4% NaCl or HS diet. We chose this time frame to investigate the relatively early renal hemodynamic responses to HS intake. As shown in Figure 4A, HS intake increased MAP in both SS (10±2%, $P<0.001$) and SS.BN1 rats (4±1%, $P<0.01$). In SS.BN1 rats, HS intake was associated with a significant decrease in RVR (–8±3%, $P<0.05$) and increase in RBF (15±4%, $P<0.001$). In contrast, RVR (10±5%, $P=0.06$) and RBF (2±4%, $P=0.85$) were not significantly different during HS versus respective baseline levels in SS rats. During HS intake, HR decreased in SS (–6±1%, $P<0.001$) but not SS.BN1 (–2±2%, $P=0.08$) rats as compared with their respective baseline values. The significant decrease in HR in SS rats was possibly mediated by an arterial baroreflex response to the greater increase in BP. In both SS and SS.BN1 rats maintained on a 0.4% NaCl diet, no significant changes were observed in MAP, RVR, or RBF as compared with their respective baseline levels. In contrast, HR significantly decreased from baseline levels in both SS and SS.BN1 rats maintained on a 0.4% NaCl diet. The daily average hemodynamic data over the 3 baseline recordings and over days 3 to 7 of 0.4% NaCl or HS intake are shown in Figure S8. A representative 3-s sample of BP and RBF from a conscious, chronically instrumented rat is provided in Figure S9 to illustrate the quality of the individual BP and RBF waveforms.

Renal Function in SS and SS.BN1 Rats Administered a 0.4% NaCl Diet and During 1 Week of an HS Diet

In SS and SS.BN1 rats with chronically implanted osmotic minipumps containing FITC-inulin, 24-hour urine samples and blood samples were obtained during 0.4% NaCl intake and at 1, 3, 5, and 7 days of HS intake. Two days following the implantation of minipumps, body weight was not significantly different between SS.BN1 (320±6 g) versus SS (339±7 g) rats administered a 0.4% NaCl diet. No significant differences were found with respect to the change in body weight over the 7-day protocol between groups. In both strains, there was a modest increase ($P<0.005$) in body weight at day 1 of HS intake. However, over the remainder of the protocol, body weight decreased slightly such that there were no significant differences at day 7 of HS intake versus values during 0.4% NaCl intake. As shown in Figure 5A, GFR, as determined via the chronic administration of FITC-inulin, did not significantly differ between SS.BN1 and SS rats over the entire protocol. In both strains, GFR was lower ($P<0.05$) on day 3 of HS intake versus 0.4% NaCl values, but GFR returned to baseline levels on days 5 and 7 of HS intake. The same pattern was observed in both groups when GFR was normalized to body weight (data not shown). We also assessed C_{Cr} to estimate GFR. As shown in Figure S10, no significant differences in C_{Cr} (absolute and normalized values) were observed between groups over the entire protocol. As shown in Figure 5B, urinary sodium excretion significantly increased ($P<0.001$) in both groups on day 1 of HS intake as compared with 0.4% NaCl values and remained significantly elevated in both groups over the 7 days of HS intake. No significant differences in urinary sodium excretion were observed between groups over the entire protocol. The same pattern was observed in both groups when urinary sodium excretion was normalized to body weight (data not shown). No significant differences ($P=0.08$) in plasma sodium concentration were observed between SS.BN1 and SS rats (141±2 versus 148±3 mmol/L, respectively). Fractional excretion of sodium at day 7 of HS intake was also not significantly different between SS.BN1 and SS rats when calculated using GFR values based on FITC-inulin (1.9±0.2% versus 1.7±0.2%, respectively) or C_{Cr} (2.5±0.5% versus 3.0±0.6%, respectively) methods.

In SS, but not SS.BN1 rats, proteinuria significantly increased ($P<0.001$) on day 1 of HS intake as compared with 0.4% NaCl values and remained significantly elevated for the remainder of the protocol (Figure 5C). Similar to the 3-week HS study, 24-hour urine output and water intake increased ($P<0.001$) during HS versus 0.4% NaCl intake in both groups (Figure S11). The increase in urine and drinking volumes was greater in SS versus SS.BN1 rats on day 3 ($P<0.05$) and day 7 ($P<0.05$) of HS intake.

Normalized kidney weight was greater ($P<0.01$, unpaired *t* test) in SS versus SS.BN1 rats (6.1±0.3 versus 4.8±0.2 g/kg, respectively), and renal injury was more severe in SS versus SS.BN1 rats ($P<0.005$), evident by both glomerulosclerosis (16±2 versus 0.5±0.2%, respectively) and TIF (1.3±0.3 versus 0.1±0.1 score, respectively). Vascular injury was not observed in SS or SS.BN1 rats.

DISCUSSION

The major goal of the present study was to determine the BP and renal hemodynamic responses to an HS diet in conscious, chronically instrumented Dahl SS and consomic SS.BN1 rats. The novel findings of the study are: (1) consomic SS.BN1 rats exhibit the same protection against salt-induced hypertension and renal

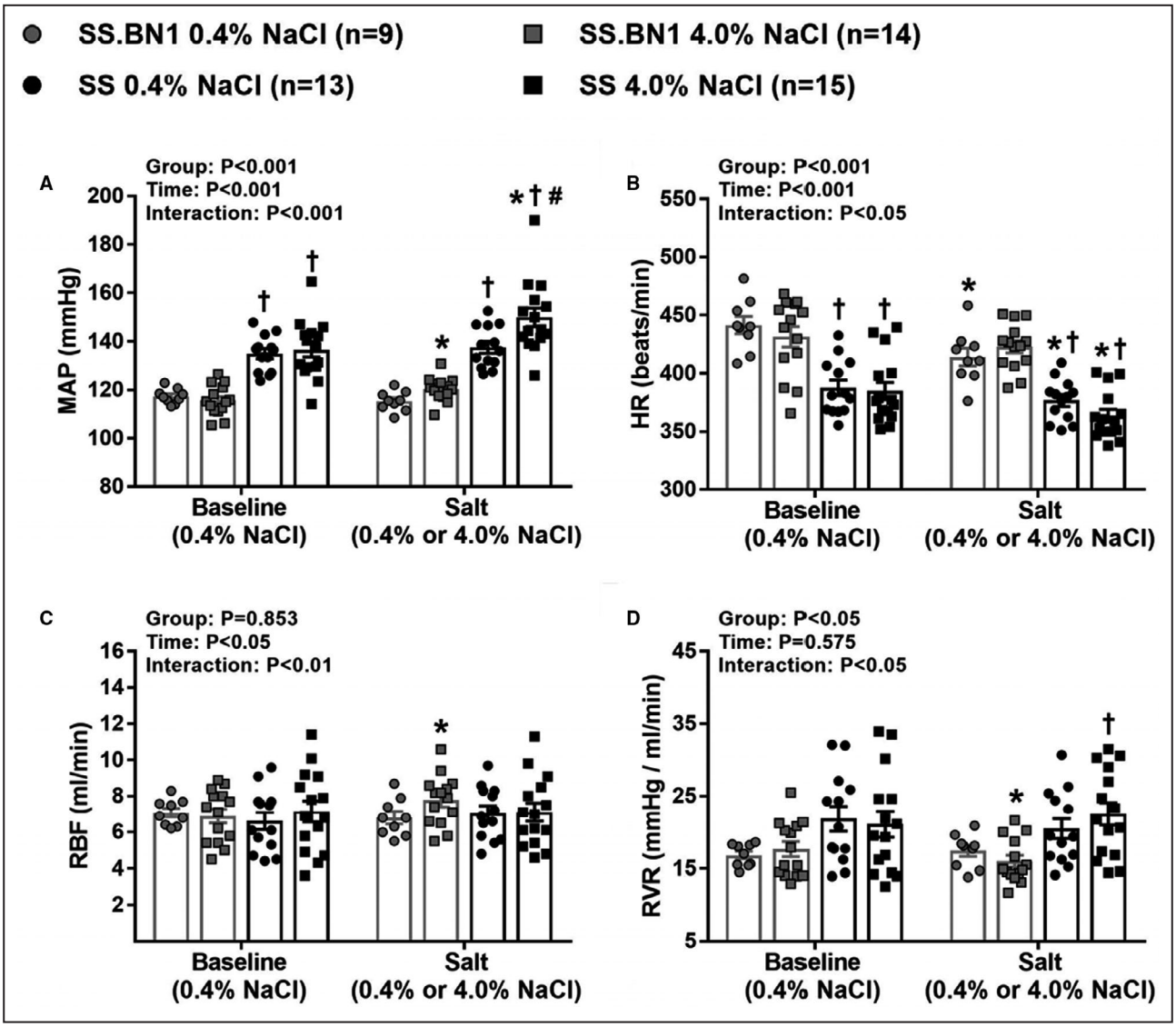

Figure 4. Systemic and renal hemodynamics in conscious, chronically instrumented rats during the administration of a 0.4% NaCl diet (ie, baseline) and during days 3 to 7 after the transition to a high-salt (4.0% NaCl) diet (n=14 SS.BN1, n=15 SS) or the continued administration of a 0.4% NaCl diet (n=9 SS.BN1, n=13 SS).

A 1-way ANOVA with Tukey comparison was used to assess differences in (**A**) mean arterial pressure (MAP), (**B**) heart rate (HR), (**C**) renal blood flow (RBF), and (**D**) renal vascular resistance (RVR) between groups at baseline during 0.4% NaCl intake. A 2-way repeated measures ANOVA with Tukey comparison was used to assess differences between groups over time. For statistical analysis, the average value of days 3 to 7 of high-salt or 0.4% NaCl intake was compared with the average of the 3 baseline measurements during 0.4% NaCl intake. Data are mean±SE, and $P<0.05$ was considered statistically significant. *$P<0.05$ vs respective baseline value. †$P<0.05$ vs respective SS.BN1 group. #$P<0.05$ vs respective 0.4% NaCl group. SS indicates salt sensitive; and SS.BN1, chromosome 1 from the salt-resistant BN rat introgressed into the Dahl SS background.

injury as compared with salt-resistant BN rats, (2) salt-resistant SS.BN1 rats exhibit significant and sustained renal vasodilation during the first week of HS intake, and (3) renal vasodilation is not observed in Dahl SS rats during HS intake (ie, renal vasodysfunction). Such findings are consistent with observations in SS and salt-resistant clinical populations[2,3] and thus support the concept that renal hemodynamic responses to an increase in dietary salt consumption may importantly modulate BP salt sensitivity.

Protection Against Salt-Induced Hypertension and Renal Injury Is Similar in SS.BN1 and BN Rats

Initial experiments on the entire panel of SS.BN rats demonstrated that SS.BN1 rats have substantially reduced BP and renal injury after 3 weeks of an HS (8% NaCl) diet as compared with parental SS rats.[16] However, the extent to which SS.BN1 rats were protected from salt-induced hypertension as compared

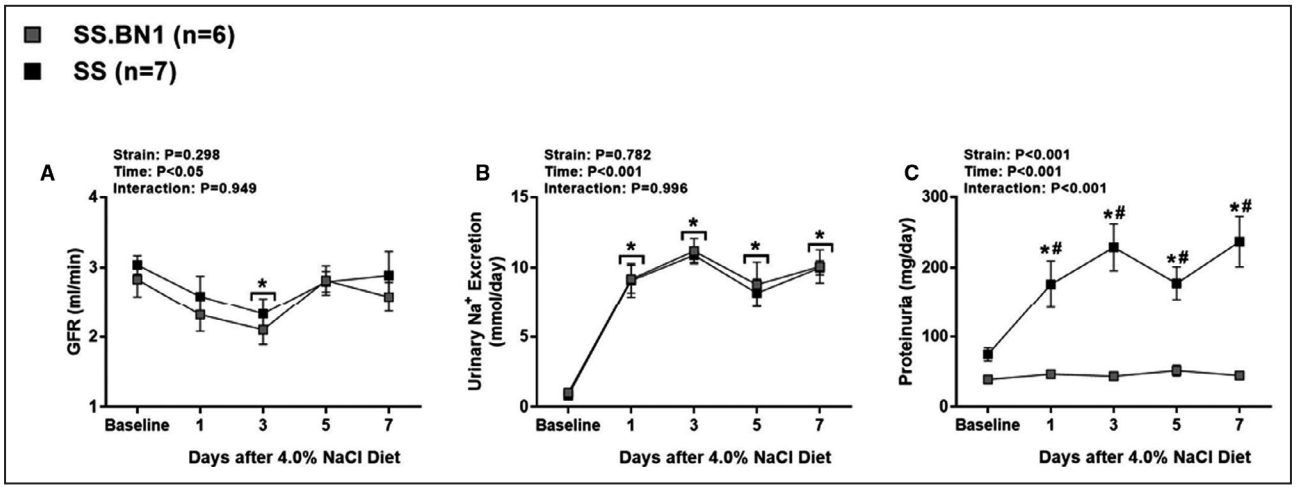

Figure 5. Renal function in SS.BN1 (n=6) and SS (n=7) rats during 0.4% NaCl intake and during days 1, 3, 5, and 7 of high-salt (4.0% NaCl) intake.

A 2-way repeated measures ANOVA with Tukey comparison was used to assess differences in (**A**) glomerular filtration rate (GFR), (**B**) urinary Na^+ excretion, and (**C**) proteinuria between groups over time. Data are mean±SE, and $P<0.05$ was considered statistically significant. *$P<0.05$ vs respective baseline value. #$P<0.05$ vs respective SS.BN1 group. The bracket under the asterisk (*) indicates all groups significantly different ($P<0.05$) from respective baseline values. SS indicates salt sensitive; and SS.BN1, chromosome 1 from the salt-resistant BN rat introgressed into the Dahl SS background.

with parental SS and BN rats (ie, the change in BP from 0.4% NaCl to HS) was not examined. Our data show that despite a genetic background that is approximately 90% homologous to parental SS rats and a baseline BP that is approximately 25% higher than parental BN rats, SS.BN1 rats exhibit a strikingly similar protection against salt-induced hypertension as compared with BN rats. These findings are consistent with the identification of several quantitative trait loci and specific genes on rat chromosome 1 that contribute to salt-induced hypertension.[24–27] Although BP was significantly lower in SS.BN1 versus SS rats during 0.4% NaCl intake, which is consistent with several known BP quantitative trait loci on rat chromosome 1,[28] it was still significantly higher than BN rats. Such data indicate that genes on other chromosomes in addition to chromosome 1 can affect BP in the absence of an HS diet. In any event, these data highlight the major importance of genetic variants on chromosome 1 in modulating physiologic pathways that regulate BP salt sensitivity.

As expected, HS intake significantly worsened glomerulosclerosis and TIF in SS rats. The strong correlation between BP and renal injury in SS rats is consistent with a previous study showing that elevated BP, per se, accelerates renal injury in HS-fed SS rats.[29] The quantitative relationships between BP and indices of renal injury in SS rats revealed a systolic BP threshold for injury of approximately 150 mm Hg. This value is much lower than that observed in other genetically hypertensive rat strains (eg, spontaneously hypertensive rat and stroke-prone spontaneously hypertensive rat)[30] and pharmacologically induced models of hypertension in rats with intact kidneys.[18] Impaired RBF autoregulation, which enhances the susceptibility to hypertensive renal injury,[30] has been documented in Dahl SS rats.[9,31–33] Moreover, defects in glomerular structure/function, which could theoretically increase the susceptibility to any given level of renal BP transmission, have also been reported in Dahl SS rats.[34,35] In contrast to SS rats, renal injury was minimal in SS.BN1 rats after 3 weeks of an HS diet, comparable to that of the parental BN strain. The protection against both glomerulosclerosis and TIF in SS.BN1 rats is superior to that observed in other consomic strains (eg, SS.BN5 and SS.BN13), which exhibit less protection against glomerular injury.[36,37] Because SS hypertension was largely blunted in SS.BN1 rats, it remains to be determined whether the susceptibility to hypertensive renal injury is altered in SS.BN1 versus SS rats. Of note, genetic variants on chromosome 1 of BN rats have been shown to both decrease[38,39] and increase[40] the susceptibility to hypertensive renal injury. Future studies in which more severe hypertension is produced in SS.BN1 rats are required to determine whether they are also protected against hypertensive renal injury.

Renal Hemodynamic Responses to HS Intake in SS Versus SS.BN1 Rats

Kurtz and colleagues have recently proposed a vasodysfunction theory of salt-induced hypertension.[2,3] The theory holds that BP salt sensitivity is caused by a failure of TPR, including RVR, to decrease in response to sodium and water retention and the ensuing increase

in plasma volume and cardiac output during an HS load. Notably, they emphasize that salt-induced hypertension is not caused by a greater degree of sodium retention, plasma volume expansion, and increase in cardiac output as compared with salt-resistant subjects, but rather by an abnormal vascular response to such changes. This concept is supported by previous studies that have reported similar degrees of sodium retention and increases in cardiac output in response to an HS load in SS versus salt-resistant humans[4,5] and rats.[12,13]

Using gold-standard BP and RBF measurements in conscious, chronically instrumented rats, we found that SS.BN1, but not SS, rats exhibit significant and sustained renal vasodilation during 1 week of an HS diet. The persistent renal vasodilation during HS intake in salt-resistant SS.BN1 rats and the failure of the renal vasculature to dilate in SS rats is similar to the TPR and RVR responses observed between salt-resistant and SS clinical populations during an HS challenge.[2–5,41] Although we did not assess plasma volume in the present study, the different RVR response to HS intake between SS and SS.BN1 rats occurred despite similar increases in urinary sodium excretion as well as similar levels of GFR during 1 week of an HS challenge. Thus, the renal vasodilation observed in SS.BN1 rats is unlikely to protect against salt-induced hypertension by causing greater amounts of sodium excretion as compared with SS rats. Alternatively, the renal vasodilation accompanied by modest elevations in systemic BP in SS.BN1 rats fed an HS diet would be expected to enhance renal BP transmission and sodium excretion.[42–44] Although it is possible that such renal vasodilation permitted SS.BN1 rats to achieve sodium balance with minimal increases in BP during 1 week of HS intake, this concept is controversial,[3,45–49] and we did not directly assess the effects of renal perfusion pressure on sodium excretion in the present study. Thus, the importance of decreases in RVR versus decreases in vascular resistance in other organ beds on mitigating increases in BP during the early stages of an HS challenge in SS.BN1 rats will require additional studies. Nevertheless, these data support the idea that renal vasodysfunction during HS intake may importantly contribute to BP salt sensitivity.

The mechanisms responsible for the impaired renal vasodilatory response to an HS diet in Dahl SS rats require further investigations. One likely possibility includes endothelial dysfunction and reduced NO bioavailability. As recently described in several review articles,[2,3,41] normally functioning endothelium increases NO production in response to salt-induced increases in extracellular fluid volume and cardiac output, which promotes a decrease in RVR and TPR. In states of endothelial dysfunction, NO bioavailability is reduced, which can lead to impaired vasodilatory responses to salt-induced increases in cardiac output. In this regard, endothelial dysfunction and reduced NO bioavailability are common features of Dahl SS rats.[50–53] In addition, Dahl SS rats exhibit increased levels of reactive oxygen species,[54,55] which can directly reduce NO bioavailability. Interestingly, a recent study demonstrated that mutation of *Plekha7*, which is located on chromosome 1, in Dahl SS rats increased NO bioavailability, improved both flow-mediated and endothelium-dependent vasodilation in isolated arteries, reduced TPR, and attenuated SS hypertension.[25]

Other potential causes of renal vasodysfunction in SS rats during HS intake include the presence of TIF. Previous studies have demonstrated that TIF, per se, alters renal hemodynamics[56] and increases the susceptibility to salt-induced hypertension.[56,57] In addition, the significantly higher BP in SS versus SS.BN1 rats during 0.4% NaCl intake may have directly contributed to endothelial dysfunction, thus impairing the ability of the renal vasculature to appropriately respond to physiological changes induced by HS intake. However, the similar protection against salt-induced hypertension in SS.BN1 versus BN rats, despite significantly higher levels of baseline BP in SS.BN1 rats, suggests other factors are also important. On the other hand, it is possible that a BP threshold higher than that observed in SS.BN1 rats during 0.4% NaCl intake may be required to cause endothelial dysfunction and renal vasodysfunction during an HS intake. Finally, abnormalities within other pathways (eg, neural, humoral) may also contribute to renal vasodysfunction during HS intake in Dahl SS rats.

The sustained renal vasodilation in SS.BN1 rats and the lack thereof in SS rats during the first week of HS intake could be interpreted as an impaired versus intact chronic RBF autoregulatory response, respectively. However, it is important to consider the mechanisms contributing to the acute versus chronic regulation of RBF when interpreting such data. Traditionally, the term RBF autoregulation has been used to describe and evaluate the role of the myogenic and tubuloglomerular feedback mechanisms in responding to acute (ie, over seconds to minutes) changes in BP.[30,58,59] Importantly, the primary function of the myogenic response is to protect the glomerular capillaries against pressure-induced injury.[14,59] Although these acute autoregulatory mechanisms are also thought to act as chronic stabilizers of RBF and GFR, there are other mechanisms that are clearly capable of regulating RBF and GFR over longer time periods in response to metabolic, excretory, or physiological stimuli.[14,59] For example, uninephrectomy and pregnancy both result in chronic increases in RBF with minimal changes in BP, yet the acute RBF autoregulatory response remains intact.[59,60] Moreover, RBF and GFR can be well regulated over extended periods of time (eg, days to

weeks) even when the 2 known mechanisms of RBF autoregulation, the myogenic and tubuloglomerular feedback response, are completely abolished (eg, rats with 5/6 renal ablation treated with calcium channel blockers).[59,60] Notably, such rats are susceptible to hypertensive injury caused by impaired myogenic autoregulation.

We speculate that the decrease in RVR observed in SS.BN1 rats during 1 week of HS intake was an appropriate chronic response, and that acute RBF autoregulatory responses remained intact, as evidenced by their minimal levels of renal injury. In contrast, we speculate that the stability of RBF in Dahl SS rats over 1 week of HS intake, which would appear as an appropriate chronic autoregulatory response to increases in BP, may be inappropriate and even accompanied by impaired myogenic autoregulation that increases the susceptibility to hypertensive renal injury. As noted earlier, future studies will be required to characterize the myogenic and tubuloglomerular feedback autoregulatory responses in SS versus SS.BN1 rats as well as the degree to which SS.BN1 rats are protected against hypertension-induced renal injury.

Limitations

We did not investigate the renal hemodynamic responses to HS in BN rats because it was beyond the scope of the present study. Nevertheless, it will be important to determine whether BN rats, as well as other salt-resistant strains (eg, Sprague-Dawley), also exhibit significant decreases in RVR during HS intake, as observed in SS.BN1 rats in the present study. It is also important to emphasize that the present investigation was conducted in a colony of SS rats obtained from the Medical College of Wisconsin (ie, Dahl SS/JrHsdMcwi) and maintained at East Tennessee State University, fed an AIN-76A diet (Dyets), in which casein is the primary source of protein, and in which renal hemodynamics were assessed for 2 to 3 hours per day. It is now recognized that there are differences in the various colonies of Dahl SS rats maintained at different institutions/facilities.[7] In addition, dietary factors, especially the source of protein,[61,62] have been shown to play a major role in determining the severity of hypertension and renal injury in SS rats. Thus, the extent to which Dahl SS rats from other colonies/facilities exhibit impaired renal hemodynamic responses to HS intake and the potential role of dietary factors in modulating this response merit future investigations. Finally, it will be important to confirm whether the renal hemodynamic responses to HS observed in the present study are also observed when RBF is measured 24 hours a day for several weeks during HS intake.

During the development of the SS.BN consomic panel of rats at the Medical College of Wisconsin, marker-assisted selection was used to confirm that individual chromosomes from the BN rat were introgressed into the SS genetic background.[63] However, almost 2 decades have passed since the creation of consomic SS.BN rats, and we did not genotype SS and SS.BN1 in the present study. Thus, we cannot exclude the possibility that SS and SS.BN1 rats differ in other regions of the genome besides chromosome 1.

We used a colorimetric method to measure creatinine in SS versus SS.BN1 rats. Because the colorimetric method is sensitive to noncreatinine chromogens in rat plasma,[64] this makes estimating of GFR based on C_{Cr} less reliable. This may have contributed to the lack of a significant difference in C_{Cr} after 3 weeks of HS intake in SS versus SS.BN1 rats. Cowley and colleagues found that GFR significantly decreased after 2 weeks of HS (4.0% NaCl) intake in Dahl SS rats when GFR was assessed with a more accurate method (ie, FITC-sinistrin).[65]

Lastly, it is important to emphasize that baseline measurements were assessed in rats fed a 0.4% NaCl diet, which is in excess of that required (≈0.1% NaCl) for normal growth and reproduction in laboratory rats.[66,67] Based on an estimated consumption of 15 to 20 g of rat chow per day by a 300-g rat at our animal facility, the baseline sodium consumption during 0.4% NaCl intake in the present study was approximately 30 to 40 mg/d (ie, 1.3–1.7 mmol/d) or 100 to 135 mg/kg per day (ie, 4.3–5.7 mmol/kg per day). This level of sodium consumption in rats is approximately 2.0 to 2.7 times higher than the average daily sodium intake by adults in the United States when normalized to body mass.[68] Thus, the BP and renal hemodynamic data obtained in the present study and its translation to clinical populations should be considered with this issue in mind.

Perspectives

The early and sustained renal vasodilation observed in SS.BN1 rats upon an increase in dietary salt intake suggests that it may importantly contribute to their salt-resistant BP phenotype. The extent to which decreases in RVR in conscious, chronically instrumented SS.BN1 rats extend beyond 1 week of HS intake and whether this response is caused by increases in plasma volume are important questions to address in the future. In contrast to SS.BN1 rats, RVR failed to decrease in Dahl SS rats during HS intake (ie, renal vasodysfunction). Future studies examining whether the impaired vascular responses to an HS diet in conscious, chronically instrumented SS versus SS.BN1 rats are specific for the renal vasculature, or involve nonrenal vascular beds, would be of great interest. In any event, SS.BN1 rats will undoubtedly be useful in future studies investigating potential regions of or genes on chromosome 1 that modulate endothelial function and renal vascular

responses to an HS diet. Such data may improve our understanding of the genetic and pathophysiological mechanisms contributing to salt-induced hypertension in clinical populations.

ARTICLE INFORMATION

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Disclosures

None.

Supplementary Material

Figures S1–S11

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SUPPLEMENTAL MATERIAL

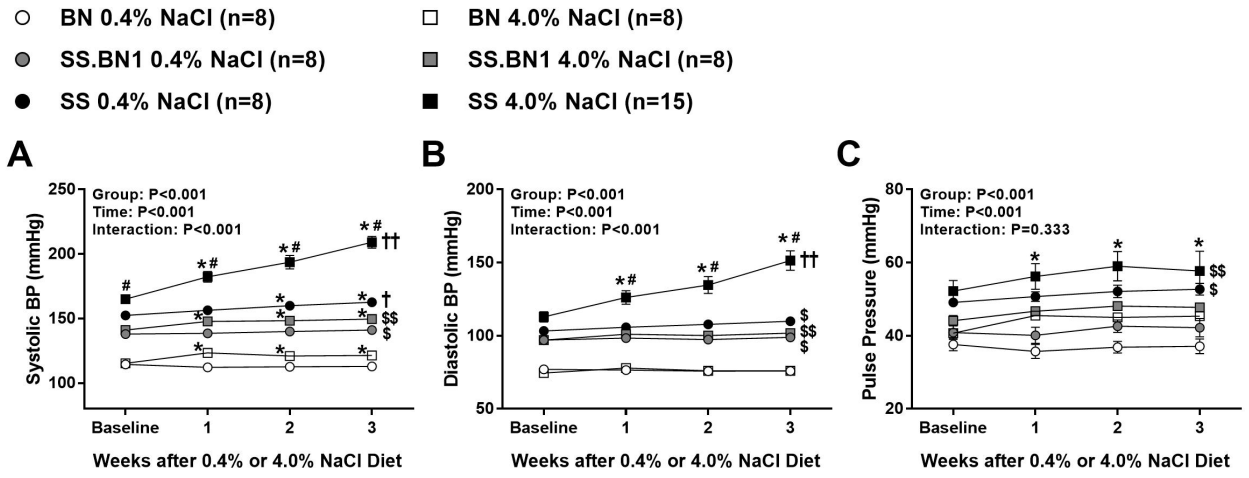

Figure S1. Blood pressure (BP) components in rats fed a 0.4% NaCl (n=8 BN, n=8 SS.BN1, n=8 SS) or high salt (4.0% NaCl; n=8 BN, n=8 SS.BN1, n=15 SS) diet for 3 weeks. A 1-way ANOVA with Tukey *post hoc* comparison was used to assess differences in A) systolic BP, B) diastolic BP, and C) pulse pressure between groups at baseline during 0.4% NaCl intake. A 2-way repeated measures ANOVA with Tukey's *post hoc* comparison was used to assess differences between groups over time. Data are mean±SE and P<0.05 was considered statistically significant. * P<0.05 vs. respective baseline value; # P<0.05 vs. respective 0.4% NaCl group; † P<0.05 vs. BN and SS.BN1 rats fed a 0.4% NaCl diet; †† P<0.05 vs. BN and SS.BN1 rats fed a high salt diet; $ P<0.05 vs. BN rats fed a 0.4% NaCl diet; $$ P<0.05 vs. BN rats fed a high salt diet.

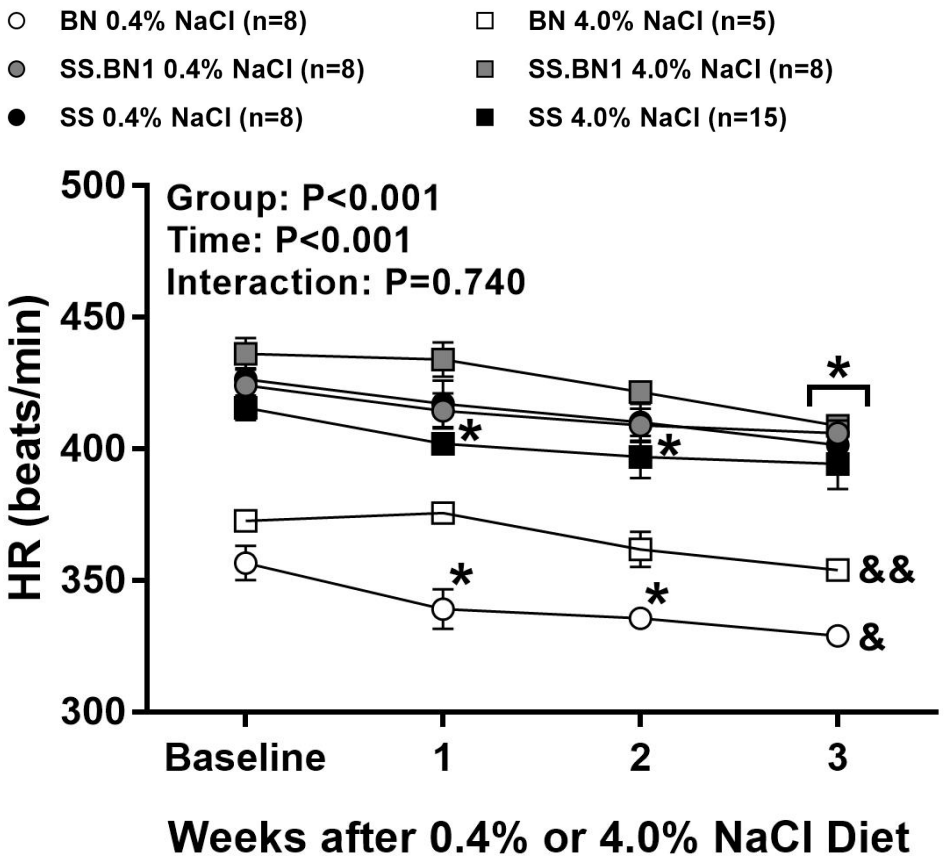

Figure S2. Analysis of heart rate (HR) when 3 BN rats within the high salt group with baseline HR's > 400 beats/min were excluded from analysis. Similar to Figure 1B, the average HR values are shown for rats fed a 0.4% NaCl (n=8 BN, n=8 SS.BN1, n=8 SS) or high salt (4.0% NaCl; n=5 BN, n=8 SS.BN1, n=15 SS) diet for 3 weeks. A 1-way ANOVA with Tukey *post hoc* comparison was used to assess differences in HR between groups at baseline during 0.4% NaCl intake. A 2-way repeated measures ANOVA with Tukey's *post hoc* comparison was used to assess differences in HR between groups over time. Data are mean±SE and $P<0.05$ was considered statistically significant. * $P<0.05$ vs. respective baseline value; * over bracket indicates all groups significantly different ($P<0.05$) from their respective baseline values; & $P<0.05$ vs. SS.BN1 and SS rats fed a 0.4% NaCl diet; && $P<0.05$ vs. SS.BN1 and SS rats fed a high salt diet.

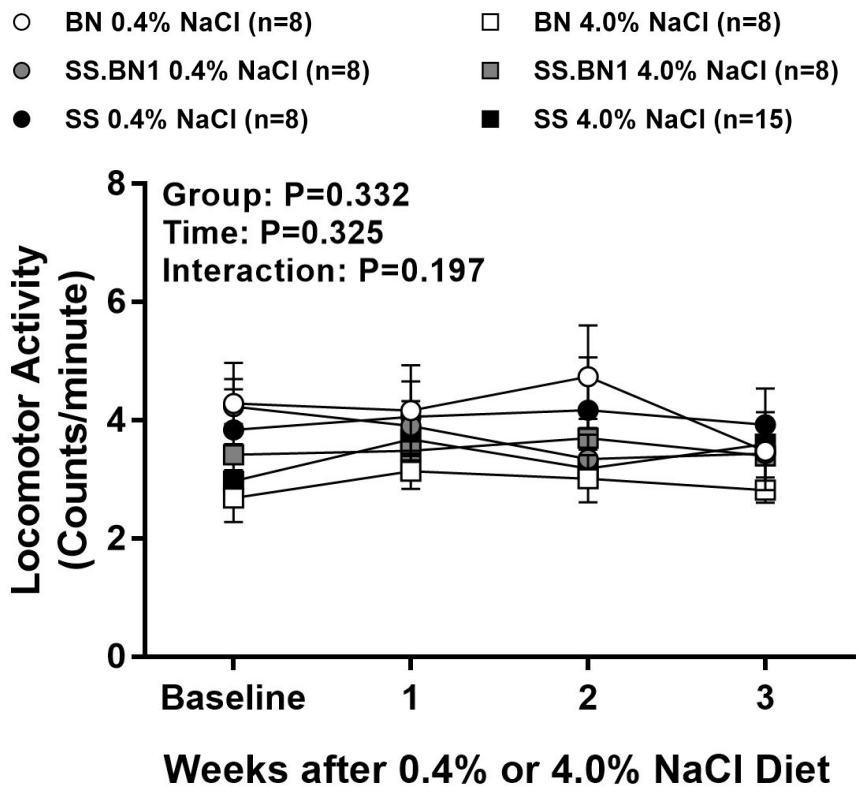

Figure S3. Locomotor activity (daily average) in rats fed a 0.4% NaCl (n=8 BN, n=8 SS.BN1, n=8 SS) or high salt (4.0% NaCl; n=8 BN, n=8 SS.BN1, n=15 SS) diet for 3 weeks. A 2-way repeated measures ANOVA with Tukey's *post hoc* comparison was used to assess differences in locomotor activity between groups over time. Data are mean±SE and $P<0.05$ was considered statistically significant. No significant differences were observed.

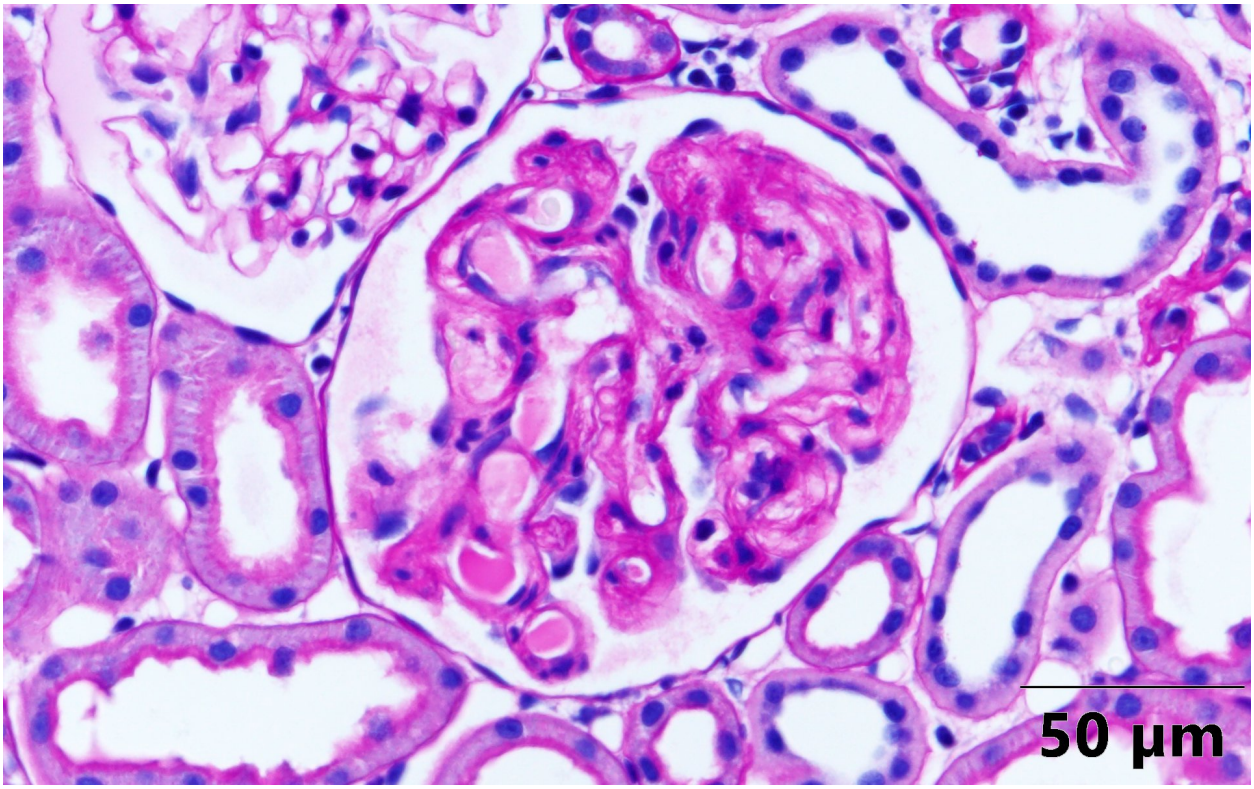

Figure S4. Illustration of thrombotic microangiopathy (TMA) pathology observed in BN rats. The prevalence of TMA was very low in BN rats. Glomeruli with TMA were excluded from the analysis of glomerulosclerosis.

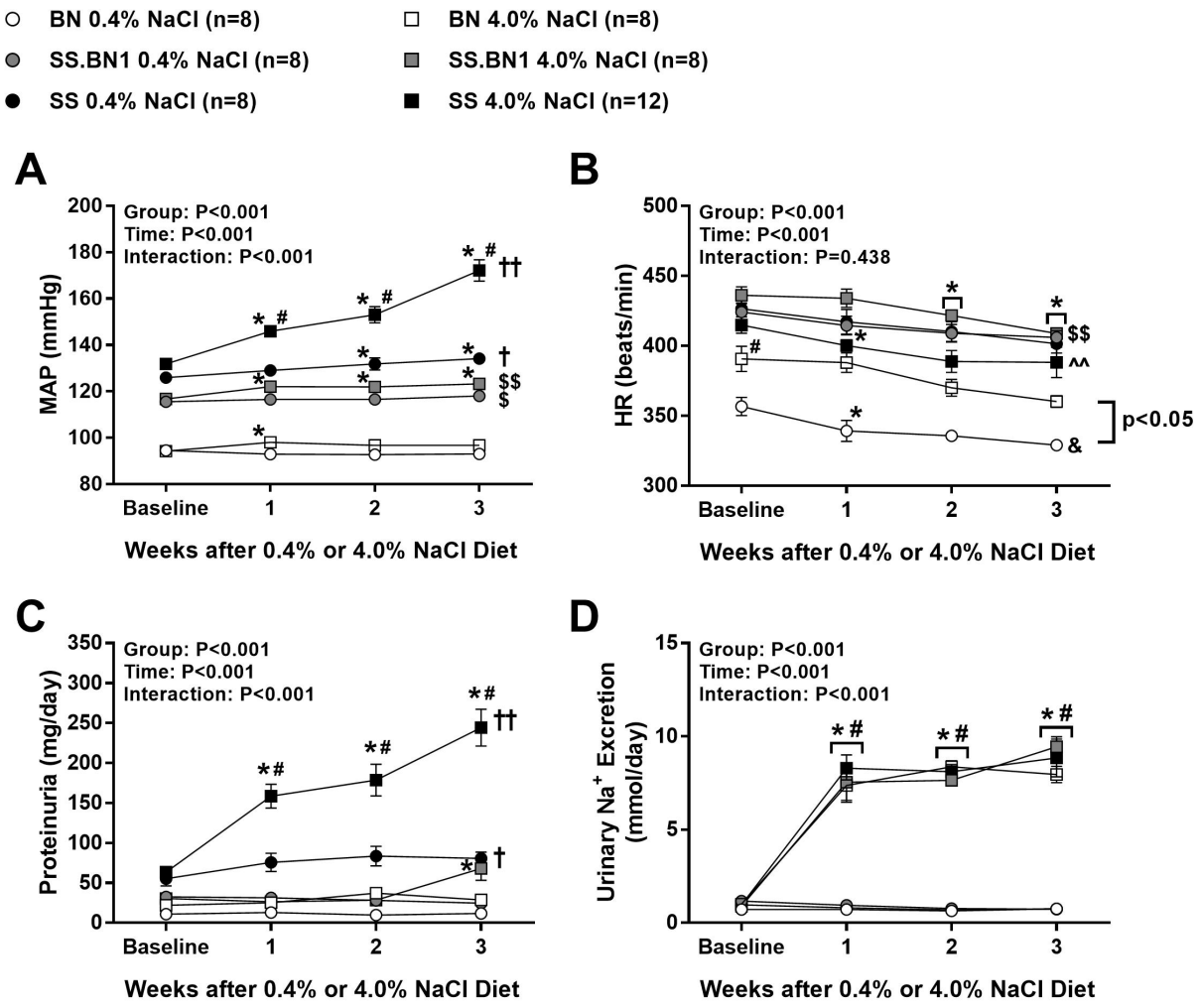

Figure S5. Analysis of mean arterial pressure (MAP), heart rate (HR), proteinuria, and urinary sodium excretion when 3 SS rats within the high salt group with a baseline MAP > 150 mmHg were excluded from analysis. Similar to Figure 1A, the average values for A) MAP, B) HR, C) proteinuria, and D) urinary sodium excretion are shown for rats fed a 0.4% NaCl (n=8 BN, n=8 SS.BN1, n=8 SS) or high salt (4.0% NaCl; n=8 BN, n=8 SS.BN1, n=12 SS) diet for 3 weeks. A 1-way ANOVA with Tukey *post hoc* comparison was used to assess differences between groups at baseline during 0.4% NaCl intake. A 2-way repeated measures ANOVA with Tukey's *post hoc* comparison was used to assess differences between groups over time. Data are mean±SE and P<0.05 was considered statistically significant. * P<0.05 vs. respective baseline value; # P<0.05 vs. respective 0.4% NaCl group; † P<0.05 vs. BN and SS.BN1 rats fed a 0.4% NaCl diet; †† P<0.05 vs. BN and SS.BN1 rats fed a high salt diet; $ P<0.05 vs. BN rats fed a 0.4% NaCl diet; $$ P<0.05 vs. BN rats fed a high salt diet; ^^ P<0.05 vs. SS.BN1 rats fed a high salt diet; & P<0.05 vs. SS.BN1 and SS rats fed a 0.4% NaCl diet; * over bracket indicates all groups significantly different (P<0.05) from their respective baseline values; # over bracket indicates all high salt groups significantly different (P<0.05) from their respective 0.4% NaCl counterparts.

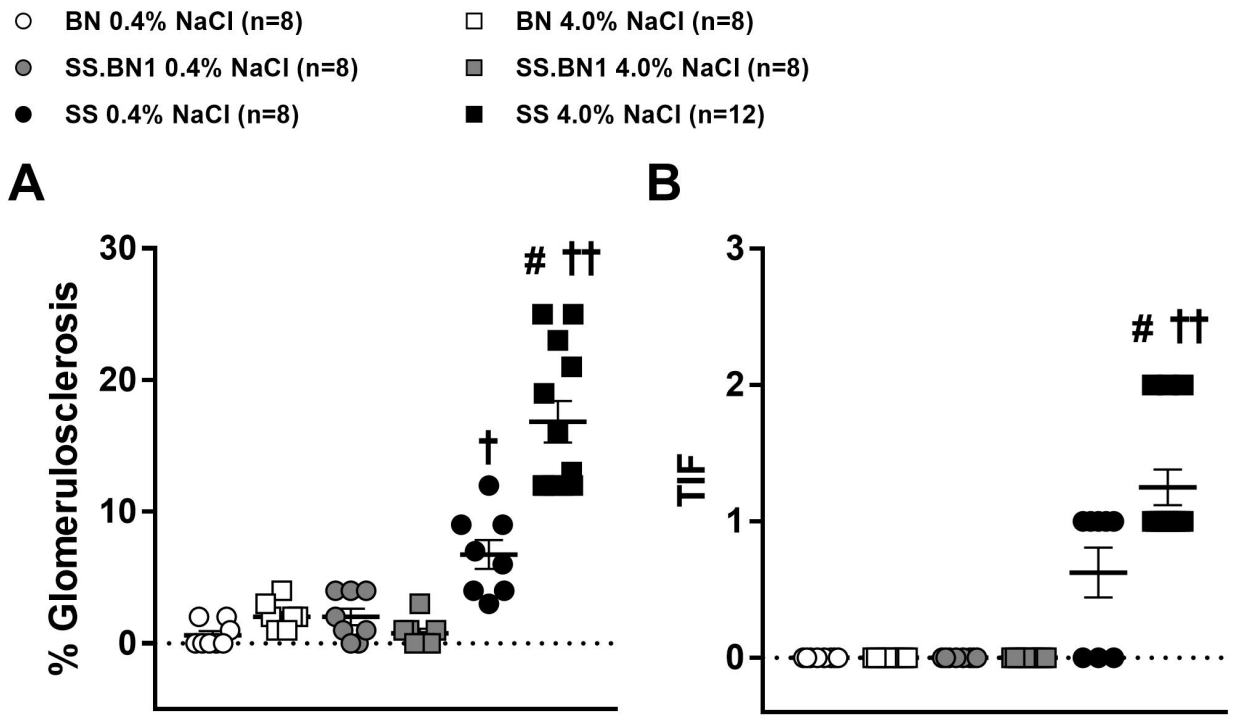

Figure S6. Analysis of renal injury when 3 SS rats within the high salt group with a baseline MAP > 150 mmHg were excluded from analysis. Similar to Figure 2, the average values for A) glomerulosclerosis and B) tubulointerstitial fibrosis (TIF) are shown for rats fed a 0.4% NaCl (n=8 BN, n=8 SS.BN1, n=8 SS) or high salt (4.0% NaCl; n=8 BN, n=8 SS.BN1, n=12 SS) diet for 3 weeks. A Kruskal-Wallis test with Dunn's *post hoc* analysis was used to assess differences in glomerulosclerosis and TIF across groups of rats fed a 0.4% NaCl or high salt diet. A Mann-Whitney rank sum test was used to assess differences in renal injury between rats fed a 0.4% NaCl and high salt diet with each strain. A Bonferroni correction was used for the multiple Mann-Whitney tests (adjusted significance rate was $P<0.017$). Data are mean±SE and unless noted otherwise, $P<0.05$ was considered statistically significant. # $P<0.05$ vs. respective 0.4% NaCl group; † $P<0.05$ vs. BN and SS.BN1 rats fed a 0.4% NaCl diet; †† $P<0.05$ vs. BN and SS.BN1 rats fed a high salt diet.

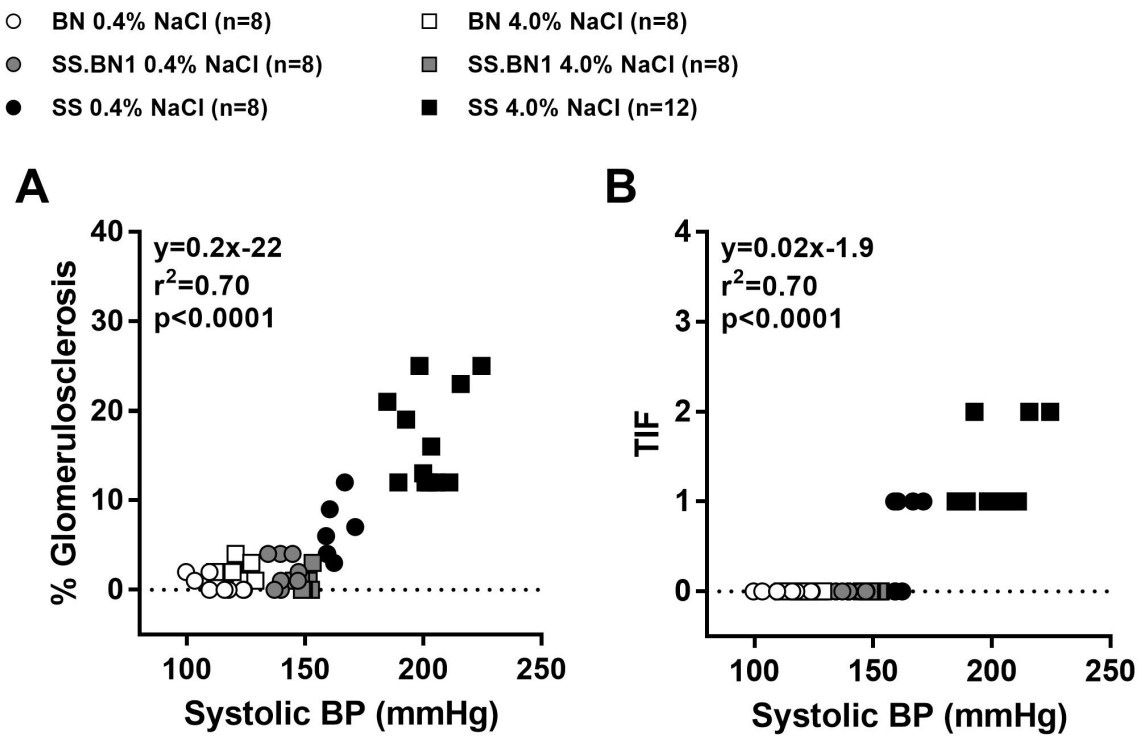

Figure S7. Quantitative relationships between systolic blood pressure (BP) and indices of renal injury when 3 SS rats within the high salt group with a baseline MAP > 150 mmHg were excluded from analysis. Similar to Figure 3, the figure shows the quantitative relationships between systolic BP and A) % glomerulosclerosis and B) tubulointerstitial fibrosis (TIF) as assessed by linear regression analysis in rats fed a 0.4% NaCl (n=8 BN, n=8 SS.BN1, n=8 SS) or high salt (4.0% NaCl; n=8 BN, n=8 SS.BN1, n=12 SS) diet for 3 weeks.

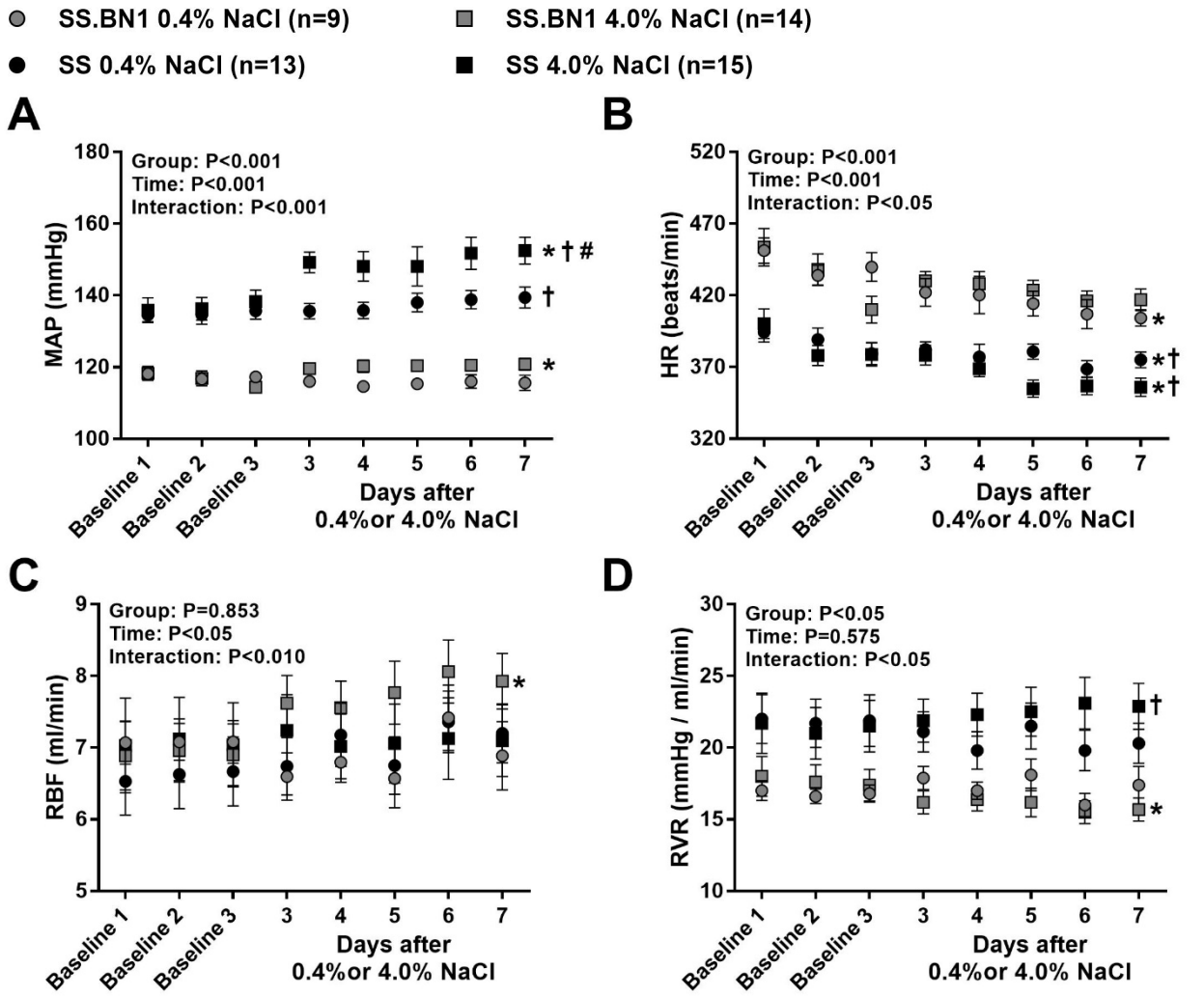

Figure S8. Daily average of systemic and renal hemodynamics in conscious, chronically instrumented rats at baseline while administered a 0.4% NaCl diet and over days 3-7 during the administration of a high salt (4.0% NaCl) diet (n=14 SS.BN1; n=15 SS) or continued administration of a 0.4% NaCl diet (n=9 SS.BN1; n=13 SS). A 2-way repeated measures ANOVA with Tukey's *post hoc* comparison was used to assess differences in A) mean arterial pressure (MAP), B) heart rate (HR), C) renal blood flow (RBF), and D) renal vascular resistance (RVR) between groups over time. For statistical analysis, the average values of days 3-7 of high salt or 0.4% NaCl intake were compared to the average of the 3 baseline measurements. Data are mean±SE and P<0.05 was considered statistically significant. * P<0.05 vs. respective baseline value; † P<0.05 vs. respective SS.BN1 group; # P<0.05 vs. respective 0.4% NaCl group.

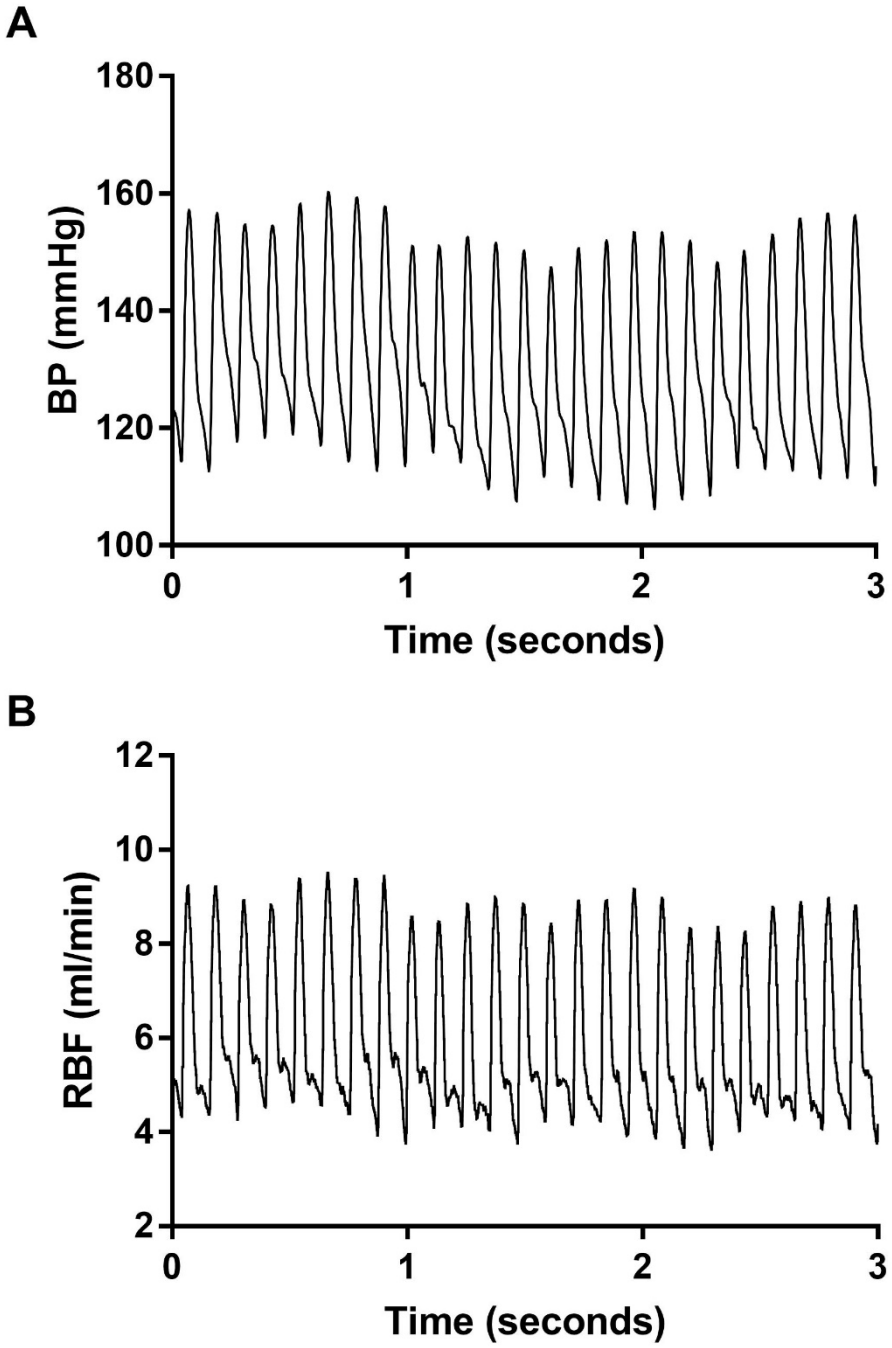

Figure S9. Representative 3-second waveform of A) blood pressure (BP) and B) renal blood flow (RBF) recorded from a conscious, chronically instrumented rat.

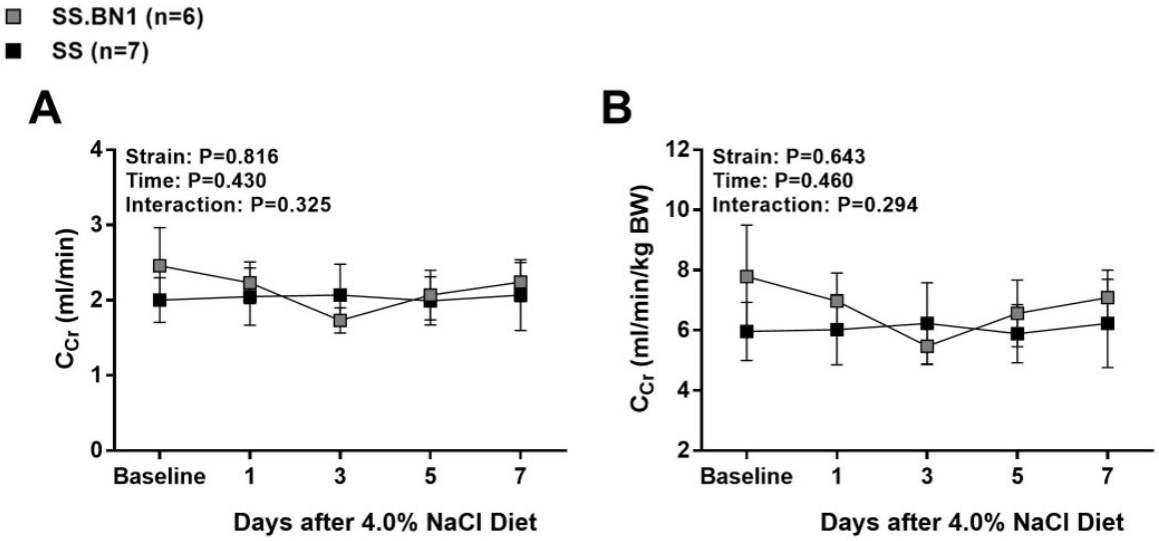

Figure S10. Creatinine clearance (C_{Cr}) in 10-14-week-old SS.BN1 (n=6) and SS (n=7) rats during 0.4% NaCl intake and days 1, 3, 5, and 7 of high salt (4.0% NaCl) intake. A 2-way RM ANOVA with Tukey's *post hoc* comparison was used to assess differences in A) C_{Cr}, and B) C_{Cr} normalized to body weight (BW) between groups over time. Data are mean±SE and $P<0.05$ was considered statistically significant.

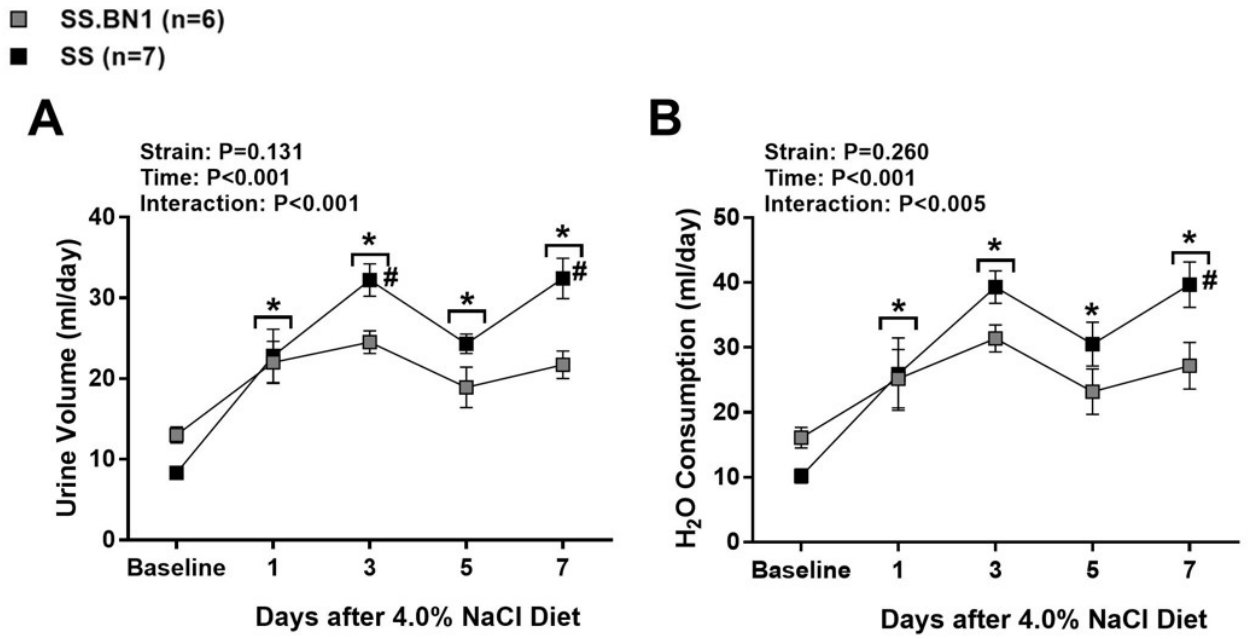

Figure S11. 24-hour urine volume and water consumption in 10-14-week-old SS.BN1 (n=6) and SS (n=7) rats during 0.4% NaCl intake and during days 1, 3, 5, and 7 of high salt (4.0% NaCl) intake. A 2-way RM ANOVA with Tukey's *post hoc* comparison was used to assess differences in A) urine volume and B) water consumption between groups over time. Data are mean±SE and $P<0.05$ was considered statistically significant. * over bracket indicates all groups significantly different ($P<0.05$) from respective baseline values; # $P<0.05$ vs. respective SS.BN1 group.