Original Research

Effects of a high-salt diet on MAP and expression levels of renal ENaCs and aquaporins in SHR

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Impact Statement

Dietary salt intake is the most remarkably modifiable environmental risk factor for the etiology and progression of hypertension (HPN). Knowing the heterogeneity of HPN from abundant experimental, interventional, and epidemiological observations, it is likely to involve the intricate integration of multiple regulatory systems and the kidneys have long been implicated to play a central role in regulating blood pressure (BP). Defects in the kidney's sodium- and water-handling mechanisms have been mooted as one of the primary causes of HPN in high-salt (HS) intake. In addition, studies on the dysregulation of epithelial sodium channels (ENaCs) and aquaporins (AQPs) in spontaneously hypertensive rats (SHRs) as a consequence of the HS diet were far from complete. This study demonstrated suppression of mRNA expression levels of ENaCs and AQP subunits, thus suggesting that the high-salt-induced increase in BP of SHRs may not be solely due to renal sodium and water retention mechanism.

Abstract

An increase in blood pressure by a high-salt (HS) diet may change the expression levels of renal epithelial sodium channels (ENaCs) and aquaporins (AQPs). Spontaneously hypertensive rats (SHRs) and Wistar Kyoto (WKY) rats were exposed to HS and regular-salt (RS) diets for 6 weeks. Mean arterial pressure (MAP) and plasma atrial natriuretic peptide (ANP), angiotensin II (Ang II), aldosterone, and arginine vasopressin (AVP) levels were determined. Expression of mRNA levels of ENaCs and AQPs were quantified by real-time PCR. The MAP was higher in SHRs on the HS diet. Plasma Ang II and aldosterone levels were low while plasma ANP level was high in both strains of rats. Renal expression of mRNA levels of α-, β-, and γ-ENaCs was lowered in SHRs on the HS diet. Meanwhile, renal AQP1, AQP2, and AQP7 mRNA expression levels were lowered in both strains of rats on the HS diet. Suppression of mRNA expression levels of ENaC and AQP subunits suggests that the high-salt-induced increase in the MAP of SHR may not be solely due to renal sodium and water retention.

Keywords: High-salt diet, mean arterial pressure (MAP), epithelial sodium channels (ENaCs), aquaporins (AQPs), kidneys

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Introduction

Cardiovascular diseases, such as high blood pressure (BP), or hypertension (HPN), are chronic disorders that gradually decrease life quality and increase the need for medical and social assistance. Despite the prevalence and manace of essential HPN, little is known about the mechanisms of its etiology. Nevertheless, a number of unmodifiable that include heritability ranging from 30% to 60%,[1] and modifiable risk factors have been identified. The major modifiable risk factor for developing HPN is dietary salt intake.[2]

In the present study, we have compared Spontaneously Hypertensive rats (SHRs) with normotensive Wistar Kyoto (WKY) rats. The fully inbred SHR is an excellent hereditary model for human HPN,[3] and elevations in BP develop with age without the need for dietary or environmental stimuli. Different mechanisms have been associated with the development of HPN in SHRs such as high ventricular stiffness[4] and dysfunction of baroreflex sensitivity[5] prior to end-organ damage.[6,7] We recently reported that high-salt (HS) diet intake led to changes in various gene expressions at different brain regions regulating BP.[8] Elevated renin-angiotensin system (RAS) and sympathetic nervous system have also been reported to contribute to the pathogenesis of genetic and salt-sensitive HPN.[9]

Kidneys have long been implicated in playing a central role in regulating BP. Defects in the kidney's sodium- and water-handling mechanisms have been mooted as one of the primary causes of HPN in the HS intake.[10] The kidneys have the capacity to return altered BP to baseline level by

increasing or decreasing sodium and water excretion in response to elevated or reduced BP.[11] This is accomplished in the kidney by the presence of renal membrane-bound protein, that is, epithelial sodium channel (ENaC), sodium-potassium ATPase, sodium-hydrogen exchanger and sodium-potassium-2 chloride co-transporter in the loop of Henle, sodium-phosphate co-transporter in the proximal tubules, and sodium chloride co-transporter in the distal tubules that fine-tune sodium reabsorption[12] and aquaporins (AQPs) that facilitate the transport of water and in some cases, other small uncharged solutes.[13,14] Furthermore, there is no other sodium transporter beyond the kidney's cortical collecting duct (CD) except ENaC which further strengthens the importance of this transporter in BP regulation.[15]

In this study, we focus on ENaC and AQP- both of which play essential roles in sodium and water homeostasis. The ENaCs are composed of three homologous subunits, that is, α, β, and γ.[16] The alpha subunit is required for channel activity, and it is critical for the formation of ions in the permeating pore, whereas β and γ subunits are necessary for maximal channel expression and activity at the cell surface and may also play a regulatory role.[17] Nevertheless, all three subunits significantly affect multimeric ENaC sodium transport capacity. The ENaC subunits are regulated by a variety of hormones, especially aldosterone.[18] The aldosterone acts through mineralocorticoid receptor (MR), which in turn regulates ENaCs transcription.[19] Besides aldosterone, arginine vasopressin (AVP) also acts as an antidiuretic hormone (ADH) that increases sodium reabsorption.[20] Apart from these two hormones, angiotensin II (Ang II)[21] has also been implicated in sodium transport. In addition, atrial natriuretic peptide (ANP) has been reported to be an inhibitor of ENaC.[22] Malfunctions of ENaC subunits affect their responses to dietary salt and thus disturb sodium homeostasis. The functional role of ENaC in the development of salt-sensitive HPN has been widely studied and a variety of responses have been reported.[23,24] Thus, investigation on ENaC and its role in sodium handling in response to HS diet intake is continually expanding.

Meanwhile, the AQPs are essential to maintaining water balance which also affects BP. To date, 13 AQP isoforms (AQP0 to AQP12) have been identified in mammals. Among them, six isoforms have been reported in the kidney, that is, AQP1, AQP2, AQP3, AQP4, AQP6, and AQP7.[25,26] Renal AQPs are necessary for the osmotic equilibration,[27] and numerous studies have been documented the association between increased AQP levels and the pathogenesis of HPN.[28] A physiologically relevant role in water reabsorption has been demonstrated for AQP1 to AQP4. The majority of the water reabsorption in the kidney occurs via AQP1, localized in the apical and basolateral membranes of proximal tubule, descending loop of Henle, and descending vasa recta; and AQP2, expressed in the apical membrane of the CD.[27,29,30] Similarly, to ENaCs, the expressions of AQPs in the kidneys were also found to be influenced by hormones.

We hypothesized that chronic HS diet intake affects expressions of ENaC and AQP subunits in the kidney, leading to sodium and water retention, respectively, and the subsequent increase in BP.

Materials and methods

Ethical approval

All the experimental protocols involving animals and housing thereof were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of the Universiti Malaya (Reference: 2014-01-07/Physio/R/HSZ) which maintains a full Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accreditation.

Experimental design and diet treatment

Since sex differences play an important role in influencing the development of hypertension,[31,32] only male rats were used in this study as to minimize the confounding effect of sex hormones. The WKY rats and SHRs used in this study were bred at the Universiti Malaya Animal Experimental Unit from stock obtained from BioLASCO (Taiwan). After being weaned at 5 weeks of age, rats were housed in groups of four to five under controlled laboratory conditions (temperature 23°C ± 5°C, 12:12-h light/dark cycle and humidity 50% to 60%) with food and water provided *ad libitum* for at least 1 week prior to the onset of experimentation. Six-week-old WKY rats and SHRs were randomly assigned to receive food with either **an** RS content (0.2% w/v NaCl) or **an** HS content (4% w/v NaCl; Harlan Teklad, Germany) with free access to water. The potassium content in both diets was 0.6% (w/v). Four groups were thus studied:

Group 1: WKY receiving regular-salt diet (WRS).
Group 2: WKY receiving high-salt diet (WHS).
Group 3: SHR receiving regular-salt diet (SRS).
Group 4: SHR receiving high-salt diet (SHS).

Measurements of blood pressure, food, and water intakes and body weight

Eight rats at the age of 12 weeks from each group were anesthetized with sodium pentobarbital (60 mg/ kg; i.p.) (Sigma Aldrich, USA). A small incision (1.5–2 cm) was made in the neck for tracheostomy and carotid artery cannulation. The carotid artery was cannulated with a cannula pre-filled with heparinized normal saline (5IU/mL) which was connected to a pressure transducer (MLT0380, ADInstruments). The transducer output was amplified and recorded continuously by PowerLab Data Acquisition System (ADInstruments, Sydney, Australia). The whole setup was allowed to stabilize for 30 to 45 min with the baseline recording carried out for 10 to 15 min. Systolic (SBP), diastolic (DBP), and mean arterial pressures (MAP) were determined on the BP tracing. Meanwhile, weekly intake of feed and drinking fluids was estimated throughout the experimental period. The food and water intakes were measured by subtracting the measured amounts provided to the remaining amounts in the cage. Additionally, the body weight (BW) of the rats in both groups was recorded before and after treatment, that is, at weeks 6 and 12. The change in BW was calculated by subtracting the final weight from the initial weight, and the percentage was estimated.

Tissue collection

At the end of the diet treatment, that is, at week 12, rats were euthanized (between hours 08:00 and 11:00) by by stunning, followed immediately by decapitation with an animal guillotine, and whole kidneys were harvested and snap-frozen in dry ice. All tissues collected were stored at −80°C until further use.

Plasma analyses

Trunk blood was collected in a chilled, peptidase inhibitor (for ANP) and heparinized (for Ang II, aldosterone and AVP) coated vacutainers and plasma was obtained by centrifugation at 3000 r/min, 4°C for 20 min. The ANP level was quantified using the radioimmunoassay procedure as previously described by Gutkowska *et al*.[33] Meanwhile, plasma Ang II (catalog number: E-EL-R1430), aldosterone (catalog number: ADI-900-173), and AVP (catalog number: ADI-900-017A) levels were quantified by using a competitive enzyme-linked immunosorbent assay (ELISA) kits (Elabscience, China and Enzo Life Sciences, USA).

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analyses

The mRNA from the kidney was extracted using Qiazol lysis buffer followed by cDNA synthesis by Bio-Rad iScript Reverse Transcription Supermix for RT-qPCR (Biorad, Hercules, CA, USA) according to the manufacturer's instruction. The steady-state of ENaCs and AQPs expression level in the kidney's mRNA was measured using RT-qPCR. All primers for α-ENaC encoded by *Scnn1a* (5′-CCTAAGCCCAAGGGAGTTGA-3′ and 5′-ACACTACAAGGCTTCCGACA-3′), β-ENaC encoded by *Scnn1b* (5′-TGGACATTGGTCAGGAGGAC-3′ and 5′-AGCAGCACCCCAATAGAAGT-3′), γ-ENaC encoded by *Scnn1g* (5′-TGAGGCTTCCGAGAAATGGT-3′ and 5′-AATACTGTTGGCTGGGCTCT-3′), *AQP1* (5′-ACCCA CTGGAGAGAAACCAG-3′ and 5′-AGAGTAGC GATGCTCAGACC-3′), *AQP2* (5′-AACTACCTGCT GTTCCCCTC-3′ and 5′-ACTTCACGTTCCTCCCAGTC-3′), *AQP3* (5′-GAACCCTGCTGTGACCTTTG-3′ and 5′-AGTGTGTAGATGGGCAGCTT-3′), *AQP4* (5′-ACA CGAAAGATCAGCATCGC-3′ and 5′-TGACCAGGTA GAGGATCCCA-3′), *AQP6* (5′-GGATCTTCTGGGT AGGACCG-3′ and 5′-ACGGTCTTGGTGTCAGGAAA-3′), *AQP7* (5′-TATCTTCGCCATCACGGACA-3′ and 5′-CCC AAGAACGCAAACAAGGA-3′), and Gapdh (5′-GCT ACACTGAGGACCAGGTT-3′ and 5′-TCATTGAG AGCAATGCCAGC-3′) were designed from NCBI official website (http://www.ncbi.nlm.nih.gov). The qRT-PCR reactions were carried out using SYBR green master mix buffer (Roche).

Statistical analyses

Statistical analysis was performed using GraphPad Prism 9.1 (GraphPad Software, La Jolla, CA, USA). All data are expressed as the mean ± standard error of means (SEM) of four to eight rats. Comparisons between groups were performed by two-way analysis of variance (ANOVA) with Tukey's post hoc test. The differences were considered statistically significant at P values <0.05.

Results

HS diet increases MAP in SHR

The HS diet significantly increases MAP in SHR when compared with WKY rats and, in comparison, with their littermate on the RS diet (Figure 1). However, the MAP of WKY rats, on the other hand, does not show any significant difference between HS and RS groups. The two-way ANOVA analyses showed that the effect of diet and genotype are significant on SBP, DBP, and MAP.

HS diet increases water intake in both SHR and WKY rats

The HS diet has a significant effect on water intake. As shown in Figure 2(A), both SHR and WKY rats show similar water intake when compared with their respective controls on the RS diet.

HS diet increases food intake in SHR

Both SHS and WHS did not show any significant differences when compared either with their respective control groups or between strains in their food consumption during the 6 weeks of free access to HS and RS food intake (Figure 2(B)).

HS diet decreases BW in SHR

The BW of SHS was significantly lower compared with its respective control groups, SRS (Figure 2(C)). Meanwhile, there was no significant change in the BW of WHS compared with WRS.

HS diet induces a similar hormonal response in both SHR and WKY rats

Our findings show that both diet and genotype have a significant effect on the ANP level. The ANP level was higher in SHR when compared with WKY rats on the RS diet as well as their respective control groups (Figure 3(A)). In addition, our results also show that diet has a significant effect on Ang II and aldosterone levels. The HS diet possesses a reducing trend in both strains of rats; however, the result was not significant (Figure 3(B)). Meanwhile, the HS diet significantly reduces aldosterone levels in both strain of rats compared with their littermate on the RS diet (Figure 3(C)). However, neither genotype nor diet nor their interaction has a significant effect on the AVP level in SHR and WKY rats (Figure 3(D)).

HS diet lowers mRNA expression levels of ENaC subunits in the kidney

The HS diet was found to be able to lower the mRNA expression of the *Scnn1a* gene encoding α-EnaC in the kidneys of both SHRs and WKY rats when compared to their counterparts, that is, SHS versus SRS and WHS versus WRS ($P<0.01$), respectively, as evidenced in Figure 4(A). Meanwhile, *Scnn1g*, gene encoding γ ENaC, was found to be significantly ($P<0.01$) lower in WHS when compared with WRS (Figure 4(C)).

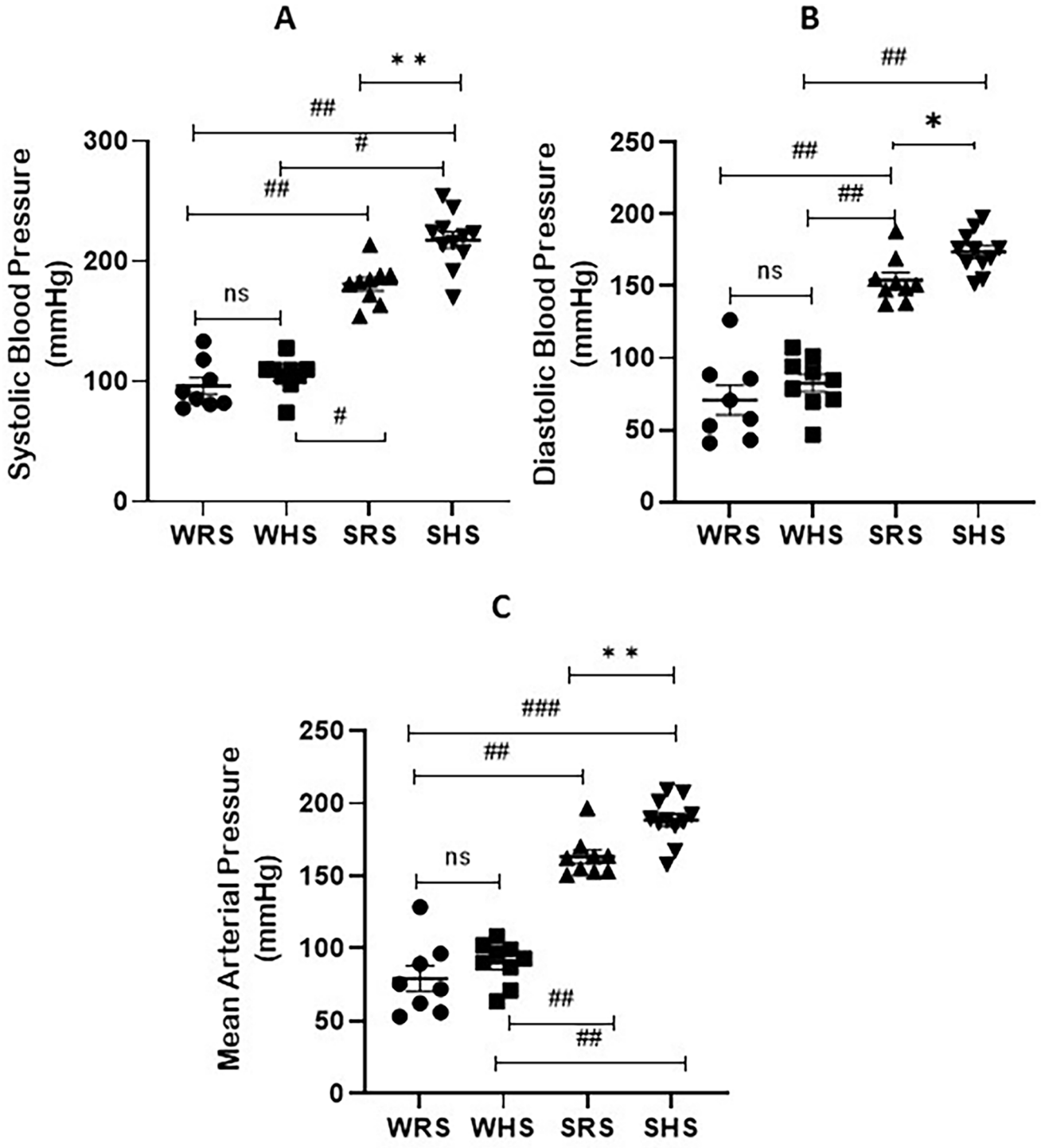

Figure 1. Changes in systolic blood pressure (Panel A), diastolic blood pressure (Panel B) and mean arterial pressure (Panel C) after HS diet. Data are presented as mean ± SEM; $n = 8$ rats. The $^{**}P < 0.01$, compared between SHS with SRS and $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared between either SRS or SHS with WRS or WHS using two-way ANOVA with Tukey's post hoc test.
WRS: WKY rats fed with regular-salt diet; WHS: WKY rats fed with high-salt diet; SRS: SHRs fed with regular-salt diet; SHS: SHRs fed with high-salt diet.

HS diet lowers mRNA expression levels of AQP subunits in the kidney

The expression levels of AQP1 (Figure 5(A)) and AQP7 (Figure 5(F)) were markedly lower with $P < 0.05$ and $P < 0.01$, respectively, in WKY rats being fed with HS diet when compared with WKY rats on the RS diet. Meanwhile, the SHRs did not show significant expression change of parallel comparison. However, the level of AQP2 was found to be significantly lower in SHS when compared with SRS ($P < 0.05$) (Figure 5(B)).

Discussion

This study demonstrated an increase in BP only in SHRs on HS diet, although water intake was elevated in both SHR and WKY rats on HS diet. In addition, the plasma hormonal

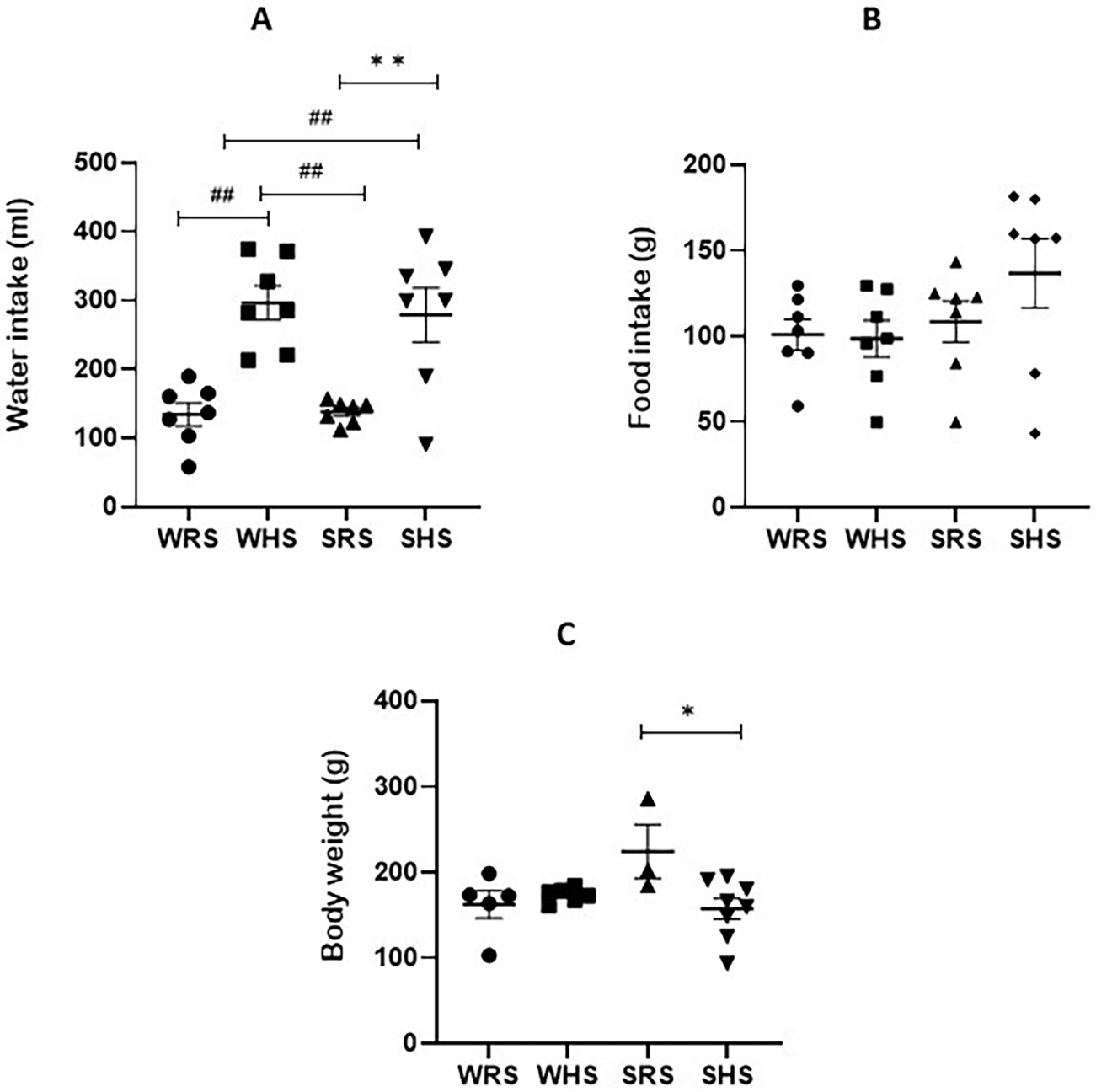

Figure 2. Changes in water intake (Panel A), food intake (Panel B), and body weight (Panel C) after HS diet. Data are presented as mean ± SEM; $n = 8$ rats. The $^{*}P < 0.05$ and $^{**}P < 0.01$, compared between SHS with SRS and $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ compared between either SRS or SHS with WRS or WHS using two-way ANOVA with Tukey's post hoc test.
WRS: WKY rats fed with regular-salt diet; WHS: WKY rats fed with high-salt diet; SRS: SHRs fed with regular-salt diet; SHS: SHRs fed with high-salt diet.

levels of Ang II, aldosterone, ANP, and AVP were similar in both strains of rats. The HS diet reduced the expression levels of ENaCs and AQP1, AQP2, and AQP7. All these changes observed in both strains of rats (SHRs and WKY rats) on the HS diet were in line with the physiological response, except for SHRs that developed HPN.

We found that both diet and genotype affect arterial pressure as SHRs had higher MAP than WKY rats on the HS diet as well as on RS (Figure 1). This is an expected outcome as SHRs are known to develop HPN following salt intake, unlike WKY rats.[34]

An HS diet is able to enhance water intake by stimulating the thirst center,[35,36] which is evidenced in the present study (Figure 2) as the water intake increased in both SHRs and WKY rats. The increase in water intake could potentially lead to volume expansion and therefore be one of the reasons for BP elevation in SHRs as these strains of rats have been reported to have low urine output, creatinine clearance, and urinary sodium excretion when compared with WKY rats.[37] Thus, the elevation in the MAP of SHR in the present study may as a result of volume expansion under the influence of raised salt intake as reported by Qi *et al*.[38]

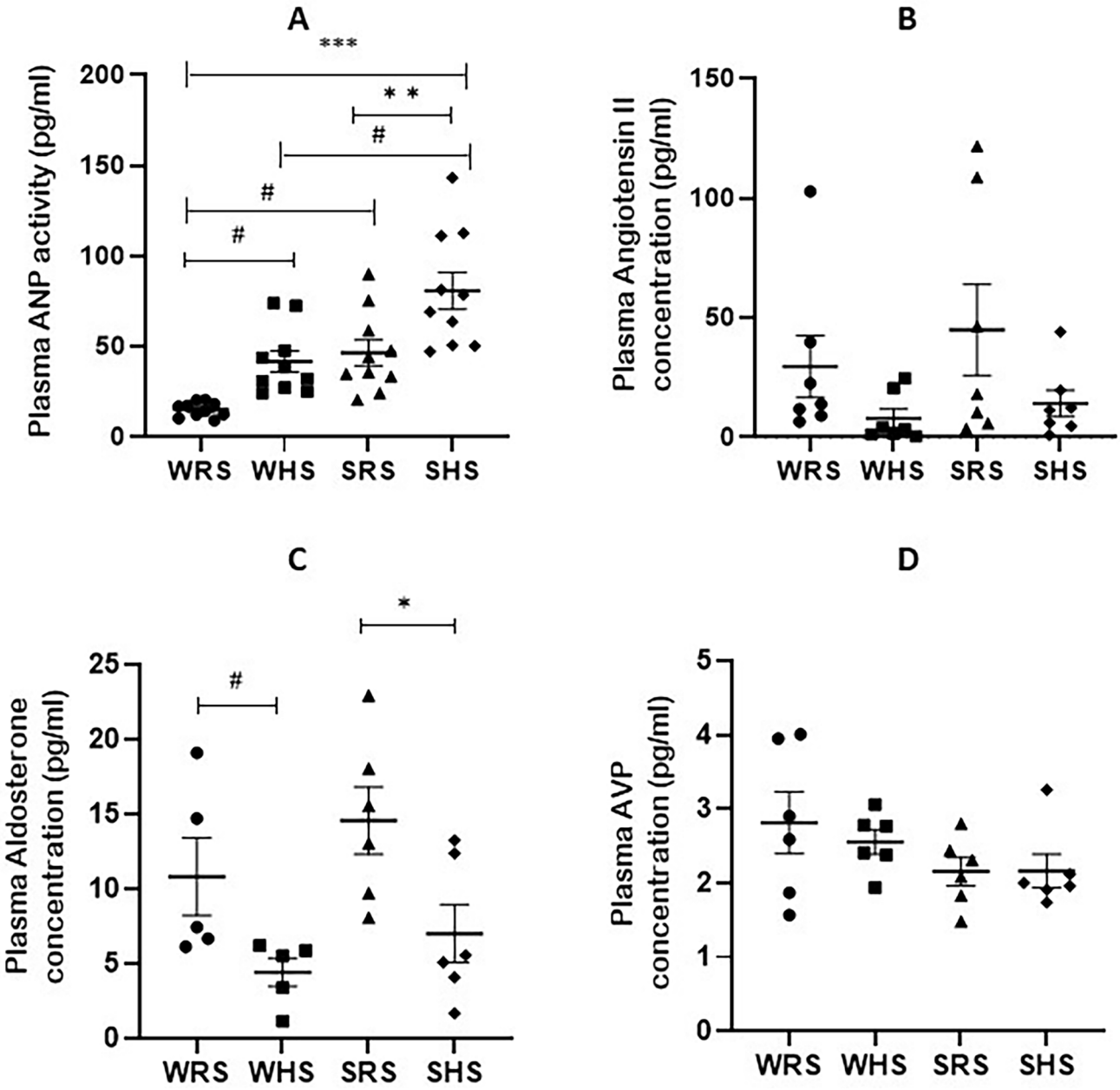

Figure 3. Changes in plasma hormonal levels of atrial natriuretic peptide (ANP) (Panel A), angiotensin II (Panel B), aldosterone (Panel C), and arginine vasopressin (AVP) (Panel D) levels in SHRs and WKY rats after HS diet. Data presented as mean ± SEM; $n = 6$ rats. The $^{*}P < 0.05$ and $^{**}P < 0.01$ SHS compared with SRS, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared between either SRS or SHS with WRS or WHS using two-way ANOVA with Tukey's post hoc test.
WRS: WKY rats fed with regular-salt diet; WHS: WKY rats fed with high-salt diet; SRS: SHRs fed with regular-salt diet; SHS: SHRs fed with high-salt diet.

However, this study also showed that the BW of SHR on HS diet significantly decreased compared with SHR on the RS diet though the food intake in SHR on HS was higher than that of SHS on RS diet. This could be that the high dietary sodium suppressed digestive efficiency via RAS as observed in the study conducted by Weidemann *et al*.,[39] on mice. A similar finding was reported by Mutchler *et al*.,[40] that associated reduced BW with higher metabolic demand and increased in fatty acid oxidation.

In order to study the mechanism behind these responses, we measured plasma Ang II and aldosterone levels, the two main components of the renin-angiotensin-aldosterone system (RAAS). Our results showed that the HS diet suppressed Ang II and aldosterone (Figures 3(B) and (C)) levels in both strains of rats, which indicates a normal physiological response. Furthermore, reduction in aldosterone level and renin activity following salt intake in SHRs has been reported before.[41] The authors also reported an increase in Ang II level in SHRs on the HS diet, which contradict the present finding. In addition, the higher BP in SHRs might result from higher MR activity and their downstream signaling pathway. The SHRs have been reported to show high MR expression compared with WKY rats[42,43] indicating that the changes in BP in SHRs might be MR dependent. This is

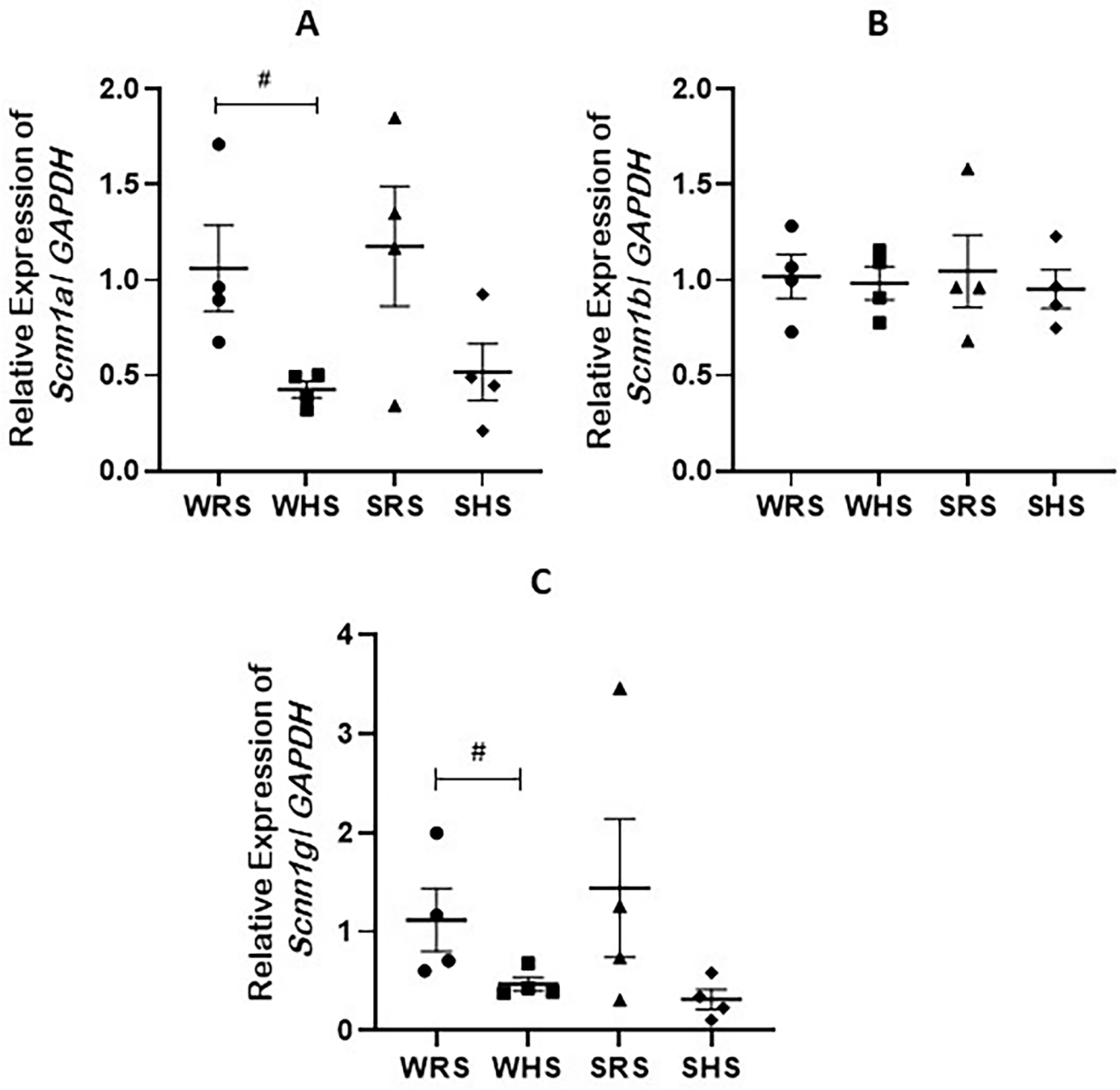

Figure 4. Relative mRNA expression levels of *Scnn1a* encoding α-ENaC (Panel A), *Scnn1b* encoding β-ENaC (Panel B), and *Scnn1g* encoding γ-ENaC (Panel C) in the kidneys under the influence of HS diet. Data are presented as mean ± SEM; $n = 4$ rats. The $^{*}P < 0.05$ SHS compared with SRS and $^{\#}P < 0.05$ WHS compared with WRS using two-way ANOVA with Tukey's post hoc test.
WRS: WKY rats fed with regular-salt diet; WHS: WKY rats fed with high-salt diet; SRS: SHRs fed with regular-salt diet; SHS: SHRs fed with high-salt diet.

further evidenced in the present study as there was a slight increase in aldosterone level (around 30%) in SHRs when compared with WKY rats.

We also measured two peptides that are involved in Na^+ and volume homeostasis, that is, ANP and AVP. Our results showed that the ANP level was altered in a similar manner in both SHRs and WKY rats on the HS diet whereby SHS and WHS had higher ANP levels when compared with their respective controls on the RS diet. An increase in ANP could inhibit aldosterone level, and this has been shown in this study, which also explains the reduced aldosterone level earlier. As ANP is an essential indicator of blood volume, the increase in plasma ANP in this study may corroborate our finding (Figure 2) that showed high water consumption of SHRs and WKY rats being fed with HS diet. A higher water consumption due to HS intake would have to increase extracellular fluid (ECF) volume and this would have increased the stretch of cardiac chambers thus surging the secretion of ANP. Teleologically, the response of ANP would be logical in being protective against excessive sodium and water retention. Nevertheless, plausibly for the increased of ANP and BP only in SHR on HS diet remains to be elucidated though our findings in accordance with results from studies conducted by Sagnella *et al*. and Kohno *et al*.[44] which

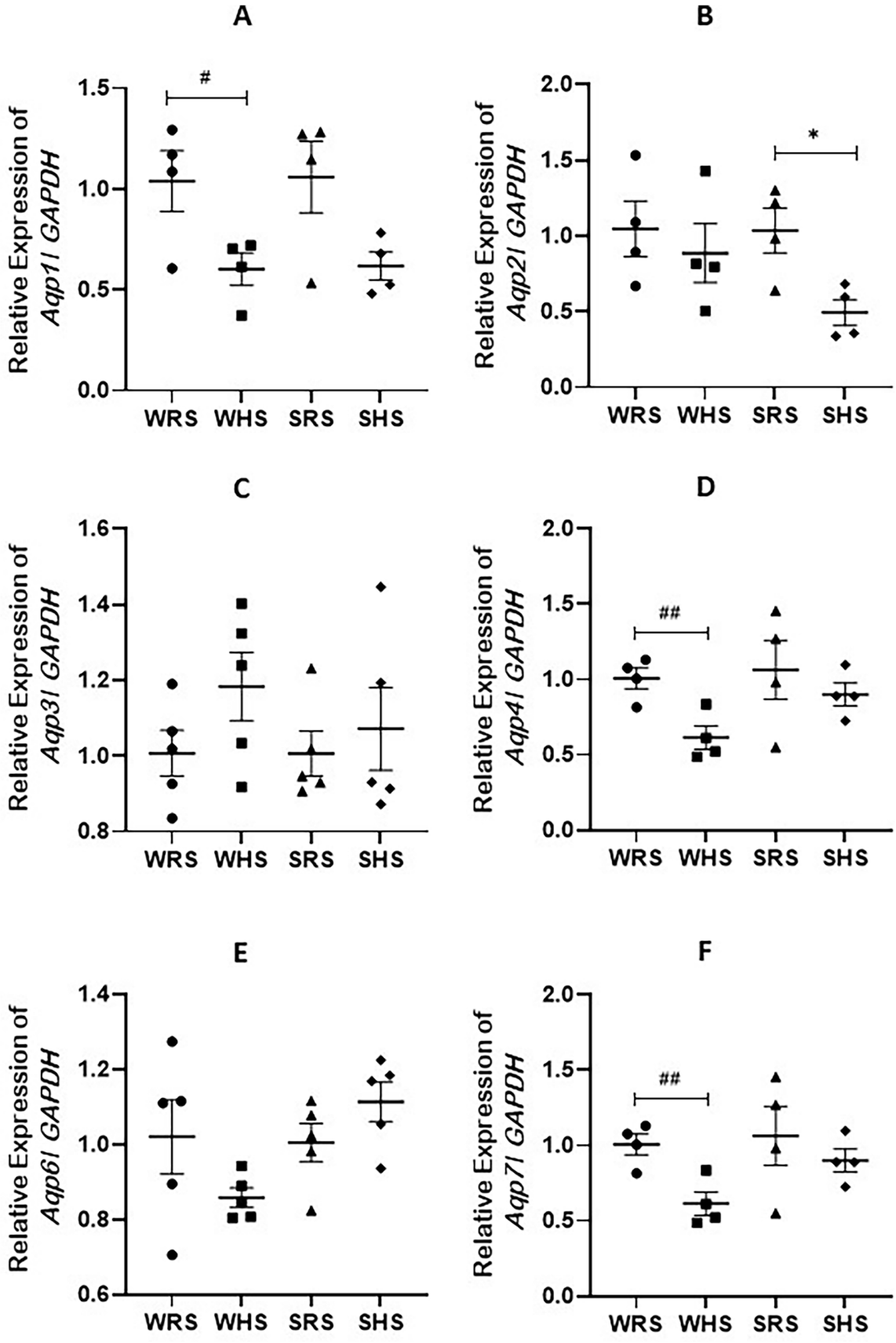

Figure 5. Relative mRNA expression levels of *AQP1* (Panel A), *AQP2* (Panel B), *AQP3* (Panel C), *AQP4* (Panel D), *AQP6* (Panel E), and *AQP7* (Panel F) in the kidneys under the influence of HS diet. Data are presented as mean ± SEM; $n = 4$ rats. The $^{*}P < 0.05$ SHS compared with SRS, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ WHS compared with WRS using two-way ANOVA with Tukey's post hoc test.
WRS: WKY rats fed with regular-salt diet; WHS: WKY rats fed with high-salt diet; SRS: SHRs fed with regular-salt diet; SHS: SHRs fed with high-salt diet.

demonstrated higher plasma ANP levels in patients with essential and salt-sensitive HPN.

In addition, we measured AVP level in order to assess the cause of increased water intake in rats. Our previous results showed that SHRs on the HS diet had higher AVP and oxytocin levels expressed in supraoptic nucleii and subfornical organ.[8] However, in this study, plasma AVP level did not change in response to the HS diet. It has been reported that enhanced thirst appeared to normalize plasma AVP concentrations in subjects on HS intake,[45] and this may serve as the possible explanation in the present results as SHRs and WKY rats fed with a high-salt diet showed higher water consumption compared to WKY rats of regular-salt diet. Moreover, the current and previous results[8] imply the essential role of the brain in the regulation of BP in SHRs.

As all the above findings remain to answer the increased BP only in SHRs on HS diet, we furthered our investigation on the Na^+ (ENaC) and water (AQP) transporters in the kidney. The mRNA expression level of both ENaC and AQP subunits were analyzed. mRNA expression of α-, β-, and γ-ENaC subunits was downregulated in SHRs fed with HS diet when compared with SHRs on RS diet (Figure 4). The SHRs on RS (0.2% Na^+ content) diet has been reported to be able to retain an excessive amount of sodium resulting from reduced glomerular filtration,[37,46] enhanced tubular reabsorption,[37,47] and increased protein abundance of ENaC subunits in various part of kidney segments.[37] This, in turn, contributes to the elevated BP in these rats. However, the 4% HS diet in this study did not enhance the mRNA level in SHRs suggesting that the HS diet induces compensatory natriuresis to maintain sodium homeostasis[23,48] in SHRs. One of the compensatory natriuretic mechanisms could be the low plasma Ang II as well as aldosterone levels and reduced in these two plasma proteins has been reported to lower α-ENaC mRNA levels.[48] Aldosterone secretion from the adrenal cortex is stimulated by Ang II upon activation of the RAS. It is known for its essential role in the transcription of gene encoding α-ENaC, thus activating its activity.[49,50] Therefore, our findings well correlate with reduced plasma Ang II and aldosterone levels with the low mRNA expression of the α-ENaC subunit and thus the lower protein content of α-ENaC.

Meanwhile, the mRNA expressions of β- and γ-ENaCs, which are known to be expressed independent of aldosterone,[51,52] were also found to be depressed in SHRs of being fed with HS diet. Activities of both β- and γ-ENaCs have been reported to be regulated by α-ENaC.[53] Hence, the low expression of β- and γ-ENaCs could be due to the low level of α-ENaC. Therefore, it is postulated that co-expression of all ENaC subunits would result in a fully operating channel as their co-existence was required for maximal ENaC channel function.[54] Furthermore, the low plasma aldosterone and high plasma ANP of SHRs fed with the HS diet may indicate that the high MAP in SHR caused by the HS diet (Figure 1) was not due to alteration in the activity of ENaC and may involve other mechanisms such as activation of sympathetic nervous activity,[12,55] enhancement of reactive oxygen species (ROS),[23,56] and stimulation of cardiovascular control center in the brain. However, the lower mRNA levels of α- and γ-ENaCs in WKY rats fed with a high-salt diet are in accordance with the claim that under physiological conditions, in normotensive rats (Dahl-salt-resistance/SD/WKY rats) there is neither no change in expression nor decreased expression of ENaC in the kidney in response to high-salt diet.[57–59]

This study showed various mRNA AQP expression patterns in SHRs and WKY rats in response to the HS diet. In both SHR and WKY rats on the HS diet, the mRNA levels of *AQP1*, *AQP2*, and *AQP7* were found to be lower when compared to their counterparts on the RS diet (Figure 5). AQP1 is the major water channel in the renal proximal tubule (PT) and loop of Henle that is responsible for reabsorbing 80% of glomerular filtrate.[60,61] It has been reported that renal and cardiac AQP1 expressions were downregulated in conditions such as renal fibrosis in mice[62] and HS-induced HPN.[63] The present result is in accordance with the finding by Penna *et al*.[64] who showed that 8% of HS downregulated APQ1. Hence, the downregulation of AQP1 in both SHRs and WKY rats could be interpreted as a compensatory mechanism to prevent large water reabsorption in the PT and the consequent expansion of ECF.[26] Furthermore, the downregulation of AQP1 also could be associated with low Ang II levels as Ang II has been reported to increase AQP1 expression in the PT cells.[65]

AQP2 is well recognized as an AVP-regulated water channel that is expressed in the principal cell of the CD. It plays a key role in urine concentration and body-water homeostasis through short- and long-term regulations of water permeability at the CD.[66,67] The low mRNA level of AQP2 in WKY rats in this study is in accordance with the study by Roxas *et al*.,[68] which showed low expression of AQP2 transcript in SD rats fed with an HS diet. In addition, stimulation of thirst by HS diet may also be a possible explanation for the suppressed AQP2 in both strains of rats which excessive water drinking keeps circulating AVP levels very low, resulting presumably in suppressed AQP2 levels in the kidneys.[69] However, the observed change in SHRs requires further studies.

Meanwhile, AQP7 localized at the brush border of PT where AQP1 is also located has been classified as aquaglyceroporins because of its credibility to transport water, glycerol, and urea just like AQP3. In this study, mRNA expression of AQP7 level (Figure 5(F)) was low in both strains of rats being fed with HS diet. This observed change in AQP7 is in a similar manner as that of AQP1, suggesting a substantial contribution of AQP7 in water reabsorption in the PT. This observation is in support with the study by Sohara *et al*.[70] that showed *AQP1/AQP7* double knockout mice showed reduced urinary concentrating ability compared with *AQP1* solo knockout mice. However, compared to AQP1, the contribution of AQP7 to water permeability in PT is small and remains to be further examined.

However, the mRNA expression levels of *AQP3* and *AQP4* (Figure 5(C) and (D)) were enhanced in both strains of rats fed with the HS diet. Both AQP3 and AQP4 are constitutively localized in the basolateral membrane in principal cells of CD. To be more precise, AQP3 is found in cortical and outer medullary CD, whereas AQP4 is located primarily in inner medullary CD. They both represent potential exit pathways, that is, the increased intracellular water absorbed by AQP2 is transported to blood by AQP3 and AQP4[30] according to an osmotic gradient. The upregulation of AQP3 and AQP4

mRNA expression as a consequence of the HS diet in this study indicates that the increased water reabsorption in CD may contribute to extracellular volume expansion, which is a typical characteristic of salt-sensitive HPN. This is further supported by our findings (Figure 1) that showed the higher MAP in SHRs and WKY rats consuming the HS diet. Furthermore, SHRs are known to have a high AQP3 level.[26,71]

Interestingly, AQP6 displayed upregulation in mRNA levels in SHRs and WKY rats. The AQP6 is known to have low water permeability, acting mainly as an anion transporter, is thought to be involved in urinary acid secretion.[13,14] Furthermore, AQP6 is co-localized with H^+ ATPase, suggesting that low pH could activate the protein. These indicate that AQP6 is most likely not involved in the transepithelial water transport;[72] therefore, the upregulation of its mRNA (Figure 5(E)) level as a consequence of the HS diet hugely remains unexplained.

This study showed that both SHRs and WKY rats possessed similar responses to HS intake though a higher BP is seen in SHRs. This could be explained based on other studies that reported SHRs to be relatively resistant or less susceptible to kidney damage until the age of 1 year[73] and other mechanisms such as changes in sodium transport at brain tissue, hyperactivity of the sympathetic nervous system,[74,75] differential regulation of tissue-specific RAS, structural changes in kidney that includes a low number of glomeruli.[76] Another possible reason for higher MAP in SHRs is SHRs have vascular smooth muscle cells that take up sodium excessively due to alteration of Na^+-K^+ pump;[77] consequently, increases intracellular sodium concentration $[Na^+]_i$ than induces a rise in calcium concentration via sodium–calcium exchanger that further causes vasoconstriction. Therefore, an augmentation in sodium load such as high dietary salt intake is predicted to elevate the $[Na^+]_i$ even more,[78] thus elevating the MAP unlike in WKY rats.[34]

Conclusions

In summary, HS intake markedly increased MAP in SHRs, which does not seem to be associated with renal expressions of ENaC and AQP subunits. The lower expression of ENaC and AQP subunits as a consequence of HS intake suggests stimulation of the BP regulatory system in SHRs in an attempt to maintain the MAP; and here it is likely via natriuresis activated by ANP. A significantly higher plasma ANP level and lower plasma aldosterone level seen in this study strongly correlate with the suppression of ENaC and AQP subunits. Furthermore, the present finding suggests that the kidney sodium- and water-handling channels may not be directly responsible for the increase in MAP by HS diet intake in SHRs. Thus, the role of ENaC and AQP subunits in salt-sensitive HPN is more toward the maintenance of BP rather than causing it to be elevated.

AUTHORS' CONTRIBUTIONS

CDR and SZH participated in the design and interpretation of the studies; CDR conducted the experiments and data analyses; CDR and KG wrote the original draft of manuscript; CDR, SZH, KG and SKL involved in review and editing of the manuscripts. All authors read and approved the final manuscript.

DECLARATION OF CONFLICTING INTERESTS

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