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Age-related hypertension and salt sensitivity are associated with unique cortico-medullary distribution of D1R, AT1R, and NADPH-oxidase in FBN rats

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Abstract

We examined effects of normal (NS) and high salt (HS) on blood pressure (BP) and cortico-medullary distribution of dopamine D1 receptor (D1R), angiotensin AT1 receptor (AT1R), NADPH oxidase-*gp^{91phox}*, and sodium transporters (NHE-3, Na, K ATPase) in adult and aged rats. Aged rats fed with NS diet had higher BP, which further increased with HS. HS increased D1R mRNA and protein levels in cortex and medulla of adult rats. NS or HS fed-aged rats had higher AT1R and *gp^{91phox}* mRNA levels in cortex and medulla. Aged rats fed with NS diet had higher *gp^{91phox}* protein levels in cortex. HS diet increased AT1R and *gp^{91phox}* protein levels in medulla of aged rats. Aged rats fed with NS or HS diet had higher NHE-3 protein levels in medulla. HS increased Na, K ATPase protein levels in medulla of aged rats. HS increased urinary kidney injury molecule-1 (KIM-1) but not protein or albumin levels in aged rats. These results suggest that cortical *gp^{91phox}* and medullary NHE-3 contribute to age-related hypertension. Whereas D1R (cortical and medullary) together with medullary AT1R, *gp^{91phox}* and Na, K-ATPase contribute to salt sensitivity in aged rats. And, KIM-1 may be a better marker for kidney damage.

Keywords

Angiotensin II; aging; dopamine; GPCR; kidney oxidative stress

Introduction

Renal dopamine receptors belong to G-protein-coupled receptor (GPCR) family that play a pivotal role in sodium homeostasis and long-term regulation of blood pressure (BP) (1,2). These receptors are divided into two classes namely D1-like receptors and D2-like receptors. The D1-like receptors are further classified into D1 and D5 receptors subtypes, while D2-

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Declaration of interest

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like receptors into D2, D3, and D4 receptors subtype (3–5). Out of these dopamine receptor subtypes, D1 receptor plays a key role in BP regulation during sodium replete condition (1–3,6–8). Kidney sodium transporters such as Na, H-exchanger, and Na/K ATPase have also been implicated in the regulation of BP (5–7,9,10).

Renal angiotensin II receptors are another class of receptors belonging to the same GPCR family as of dopamine receptors and also play a critical role in BP regulation (11,12). There are mainly two types of angiotensin II receptors: type 1 (AT1) and type 2 (AT2) (13). Gene-targeting studies have shown that it is the AT1 receptor that plays a major role in producing angiotensin II response (14). Renal AT1 receptors act differently and their mechanisms contributing to BP regulation is different from D1 receptors (15,16). For example, unlike D1 receptors, AT₁ receptors stimulate renal Na, H-exchanger and Na, K-ATPase (17,18). Our recent report demonstrates that age-related increase in BP is associated with diminished D1 receptor and exaggerated AT1 receptor functions in the aging kidneys (19). Given that these receptors are distributed in cortico-medullary zones of the kidney and these zones perform specific functions (20–22), we tested whether there exists any relationship between cortico-medullary distribution of these receptors and BP during normal (NS) and high-salt (HS) feeding in the aging FBNs. In order to test this, adult and old rats were treated with NS and HS, their blood pressure was measured and kidneys were isolated to determine different parameters in cortex and medulla. *gp^{91phox}*-NADPH-oxidase is one of the parameters that by producing superoxide radical (23,24) increases cellular oxidative stress burden, which is directly linked to dysfunctions of these receptors and hypertension (25–28). We also determined sodium transporters NHE-3 and Na, K-ATPase as they play critical roles in the regulation of BP (29). Since renin–angiotensin–system (RAS) contributes to regulation of BP, we determined systemic and tissue levels of renin as an index of RAS function. Finally, proteinuria, albuminuria, and urinary kidney injury molecule-1 (KIM-1) levels were determined to assess kidney damage, if any, by high-salt feeding in FBNs.

Methods

Animals

Adult (2-month) and aged (20-month) male Fischer Brown F1 hybrid (FBN) rats raised by Harlan Laboratories (Indianapolis, IN) were purchased from National Institute on Aging (Bethesda, MA). The rats were housed in plastic cages in the University of Houston animal care facility and were used as per NIH guidelines and approved protocols by Institutional Animal Care and Use Committee. Animals had free access to NS and HS rodent chow and drinking water.

Animal surgery

Survival surgery in rats was performed as reported (30) to implant telemetry probe for measuring conscious blood pressure. The rats were given 1 week to recover from surgical procedure and no experimental manipulation was carried during this period.

Salt treatment

Iso-caloric NS (0.4% NaCl) and high-salt (HS) (8% NaCl) rat chow were purchased from Dyets, Inc. (Bethlehem, PA). Rats were fed with NS and HS diet for a period of 4 weeks. Conscious BP of rats was determined with computer-assisted software program (DSI, St. Paul, MN). Thereafter, rats were sacrificed, bladder urine and aortic blood were obtained, and kidneys were harvested. Longitudinal section of kidneys was carried out and cortical and medullary tissues were separated with a razor blade.

Real-time PCR

Total RNA from cortical and medullary tissues was purified using a kit-based method (Qiagen Inc, Valencia, CA) and used (2 µg RNA) to synthesize cDNA using Advantage RT for PCR kit (Clontech Inc, Mountain View, CA). The cDNA obtained was further diluted five times and used (10 µl) in the real-time PCR reaction with TaqMan rat-specific primers for angiotensin AT1 receptor (AT1R), dopamine D1 receptor (D1R) (D1R primers used do not give D5R product), and *gp91^{phox}* from Applied Biosystems (Life Technologies, Grand Island, NY). 18S rRNA was run in parallel as an internal control and used to normalize the data. Data were compared with adult NS-treated samples using the Delta-Delta Ct method.

Western immunoblotting

Western immunoblotting on tissue homogenates and membranes was performed by standard methods as described previously (31). Briefly, tissue homogenates were made in lysis buffer (20mM Tris-HCl (pH 7.5), 150mM NaCl, 1mM Na₂EDTA, 1mM EGTA, 1% Triton, 2.5mM sodium pyrophosphate, 1mM beta-glycerophosphate, 1mM Na₃VO₄, 1 µg/ml leupeptin, protease inhibitor cocktail) as described. Crude plasma membranes from tissues were also made in lysis buffer using our published method (31). Protein concentrations in the homogenate and membrane samples as well as in bladder urine were determined by the BCA protein assay kit (Pierce BCA Protein Assay Kit, Thermo Scientific, Rockford, IL). Equal amount of protein samples (15 µg) was used for Western immunoblotting using specific antibodies for D1R (D1R antibody used does not cross-react with D5R), NHE-3, NA, K ATPase (EMD Millipore, Billerica, MA), AT1R (Abcam®, Cambridge, MA), and *gp91^{phox}* NADPH-oxidase (BD Biosciences, San Jose, CA). Protein band density was quantified with the Flourchem image software (Proteinsimple, Santa Clara, CA). The protein band density for homogenate samples was normalized with protein-loading control, β-actin.

Assays for renin, KIM-1, and microalbumin

Renin levels in plasma and tissues were determined using Sensolyte® 520 Rat Renin Assay kit (AnaSpec, Fremont, CA) according to the instructions of the manufacturer. Rat-specific KIM-1 (Oxiselect Rat KIM-1 ELISA kit, Cell Biolabs Inc., San Diego, CA) and microalbumin (Rat Albumin EIA kit, Cayman Chemical Company, Ann Arbor, MI) kits were used to measure these proteins in the urine.

Data analysis

Data are presented as mean $\pm$ SEM. One-way ANOVA followed by Newman–Keul’s post-hoc test was applied to achieve significance using statistical analysis software (Graphpad Prism, San Diego, CA). The minimum level of significance was considered at $p < 0.05$.

Results

Blood pressure phenotypes in response to NS and HS in FBNs

As shown in Figure 1, systolic blood pressures were high in aged rats than in adult rats treated with NS diet. Systolic BP increased further in aged rats fed with HS diet. HS diet did not affect systolic BP in adult rats. There was no difference in the diastolic BP between adult and aged rats fed either with NS or with HS diet (data not shown).

Distribution of D1R, AT1R, and gp^{91phox} NADPH-oxidase mRNA and protein in the cortex and medulla

As shown in Figure 2(A), D1R mRNA levels were profoundly low in the medulla of aged rats fed with NS diet, which did not change with HS feeding in these rats. HS diet treatment increased D1R mRNA levels in cortex and medulla of adult but not aged rats. Contrary to mRNA, D1R protein levels increased in cortex and medulla with HS diet in only adult rats (Figure 2B). AT1R mRNA levels were much higher in both cortex and medulla of aged rats than in adult rats, which were not affected by HS feeding in these animals (Figure 3A). However, their protein levels were increased only in the medulla of aged rats in response to HS diet (Figure 3B). Furthermore, gp^{91phox} NADPH-oxidase mRNA levels were higher in both cortex and medulla of aged rats than in adult rats fed either with NS or with HS diet (Figure 4A). Its protein levels, however, were higher in cortex of aged rats than in adult rats irrespective of salt-feeding which increased only in medulla of aged rats with HS feeding (Figure 4B).

Distribution of NHE3 and Na, K ATPase in cortex and medulla

The levels of NHE3 were higher in the membranes from medulla of aged rats fed with NS diet, which did not change with HS feeding in these rats (Figure 5A). Contrary to this, HS feeding increased the levels of Na, K ATPase only in the membranes from medulla of aged rats but not adult rats (Figure 5B).

Renin, albumin, and KIM-1 levels

As shown in Table 1, plasma renin levels were similar in adult and old rats fed with either NS or HS diet. In cortex, renin levels were similar in adult and old rats irrespective of salt intake. In medulla, however, renin levels were low in old rats than in adult rats fed with NS diet and were not affected by HS feeding in either adult or old rats. Urinary protein, albumin, and KIM-1 levels were higher in old rats than in adult rats. KIM-1 but not protein and albumin levels in the urine increased with HS feeding in old rats but not in adult rats.

Discussion

Dopamine D1 and AT1Rs are two key renal factors that contribute to the regulation of blood pressure (32). Abnormal functions of these receptors have been implicated in the development of hypertension (33–35). We also have reported diminished D1R function and exaggerated AT1R function, in terms of sodium excretion, in the aging kidneys, which were found to be associated with age-related hypertension in the FBN rats (19).

Kidney cortex and medulla are endowed with specific functions related to sodium transporter function and blood flow (20). Therefore, we became interested in identifying association, if any, between cortico-medullary topology of these receptors and their impaired renal functions and high blood pressure as reported in the aging FBN rats (19). The effect of HS feeding on some of these parameters was also tested. Since *gp^{91phox}*-NADPH-oxidase and sodium transporters (NHE-3 and Na, K-ATPase) are implicated in the regulation of blood pressure (29,36), we also examined their cortico-medullary distribution on salt feeding. The rationale for undertaking such a study was to tease out the effect of normal aging (NS) and the effect of HS diet on cortico-medullary distribution of these molecules. These molecules are important renal components that have been implicated in the regulation of blood pressure.

We found that the increases in age-related and salt-sensitive BP in aging FBNs are small. A small increase in BP may have adverse cardiovascular outcome as a mere 5–6mmHg decrease in systolic BP shows beneficial cardiovascular outcomes in humans (37,38). Age-related hypertension (NS effect) and salt sensitivity (HS effect) in FBN rats seem to have unique relationship with cortico-medullary distribution of D1R, AT1R, *gp^{91phox}*-NADPH-oxidase, NHE-3, and Na, K-ATPase. Medullary D1R mRNA levels were much less in the aged rats than in adult FBNs. However, its protein levels were decreased in both cortex and medulla of aged rats that were fed with HS. It is likely that low D1R density in the medulla contributes to age-related hypertension as it has been previously linked to high BP in the spontaneously hypertensive rat model (22). And, failure of HS response in the aging kidneys in terms of increasing cortical and medullary D1R protein levels, compared with adult rats, might be contributing to salt sensitivity in aged FBN rats. The reason for the loss of HS response on the D1R in the aging kidneys is not known. Perhaps a micro-RNA mechanism is responsible in this process as miR-142-3p depletion is reported to increase D1R levels in the neuronal cells (39). Whether HS feeding causes miR-142-3p up-regulation resulting into D1R depletion in the aging kidneys is not known and warrants further investigation.

A much higher levels of AT1R mRNA in the cortex and medulla were found in aged rats fed either with NS or with HS diet. HS diet does not seem to affect the levels of AT1R mRNA in both adult and aged rats. Interestingly, the levels of AT1R proteins increased with HS feeding only in the medulla of aged rats. These data suggest that medullary AT1R is likely involved in salt sensitivity in aged rats. Furthermore, cortical *gp^{91phox}*-NADPH-oxidase seems to be associated with age-related hypertension (NS effect) and not salt sensitivity as similar levels of its mRNA and protein were found in the aged rats fed with NS or with HS diet. Whereas medullary *gp^{91phox}*-NADPH-oxidase seems to play a role in salt sensitivity as its mRNA and protein levels increased specifically in the medulla of aged FBN rats fed with

HS diet. This is in contrast to the findings in SHR and Dahl-salt-sensitive rats where salt sensitivity was linked to up-regulation of cortical *gp^{91phox}*-NADPH-oxidase (23). This discrepancy may be due in part to a different rat strain and age group used in our study. Our data also suggest that a parallel relationship between mRNA and protein does not always exist as we could not find this relationship for some of the molecules examined.

Additionally, NHE-3 and Na, K-ATPase sodium transporters were examined as surrogates of apical and basolateral membrane transporters, respectively. These transporters play critical roles in the regulation of blood pressure (29,36). However, their cortico-medullary distributions in response to salt manipulation in the aging FBN model have never been examined. There was no change whatsoever in the levels of either NHE-3 or Na, K-ATPase proteins in the homogenates of cortex and medulla of adult and aged rats fed with NS and with HS diet (data not shown). In the membranes (representing active forms of these transporters), however, a reciprocal relationship between NHE-3 (high) and Na, K-ATPase (low) in the medulla but not in the cortex of aged rats fed with NS diet was found. HS feeding does not seem to impact these transporters in the cortex. However, in the medulla, it did cause an increase in the levels of Na, K-ATPase but not of NHE-3 in the aged rats. These data suggest that perhaps medullary NHE-3 plays a role in age-related hypertension whereas medullary Na, K-ATPase contributes to salt sensitivity in the aging FBNs.

Finally, systemic renin levels were similar in adult and aged rats irrespective of salt treatment suggesting that systemic RAS does not contribute to either age-related hypertension or salt sensitivity in the aging FBN rats. This might be true as maintenance of hypertension is reported to be independent of circulating RAS with normal or low plasma renin activity (40). Cortical tissue levels of renin were much higher than in medulla of aged rats fed either with NS or with HS diet. However, this was similar to the levels as seen in adult rats and does explain contribution of cortical RAS in age-related hypertension in FBN rats. RAS is profoundly affected by salt intake (41); however, FBN rat strain does not seem to respond to HS diet in terms of renin levels. The reason for this is not known and warrants future examination. In future, a similar study, as above, can be designed in Fischer 344 rat strain, another aging model, which possesses diminished D1R function but normal AT1R function and does not develop age-related hypertension (19). This would serve good controls to compare the present findings in FBN rats. Furthermore, aging FBN rats seem to possess compromised kidney function as they showed proteinuria, micro-albuminuria, and elevated urinary KIM-1 levels. HS intake does not seem to affect proteinuria and micro-albuminuria but increase urinary KIM-1 levels in aged rats. These data suggest that KIM-1 is perhaps a better urinary marker than proteinuria and micro-albuminuria for kidney damage arising from an insult such as NS in aging.

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References

1. Wang X, Armando I, Upadhyay K, et al. The regulation of proximal tubular salt transport in hypertension: an update. *Curr Opin Nephrol Hypertens*. 2009; 18:412–20. [PubMed: 19654544]
2. Zeng C, Jose PA. Dopamine receptors: important antihypertensive counterbalance against hypertensive factors. *Hypertension*. 2011; 57:11–7. [PubMed: 21098313]
3. Asghar M, Tayebati SK, Lokhandwala MF, Hussain T. Potential dopamine-1 receptor stimulation in hypertension management. *Curr Hypertens Rep*. 2011; 13:294–302. [PubMed: 21633929]
4. Felder RA, Jose PA. Mechanisms of disease: the role of GRK4 in the etiology of essential hypertension and salt sensitivity. *Nat Clin Pract Nephrol*. 2006; 2:637–50. [PubMed: 17066056]
5. Wang X, Villar VA, Armando I, et al. Dopamine, kidney, and hypertension: studies in dopamine receptor knockout mice. *Pediatr Nephrol*. 2008; 23:2131–46. [PubMed: 18615257]
6. Zeng C, Armando I, Luo Y, et al. Dysregulation of dopamine-dependent mechanisms as a determinant of hypertension: studies in dopamine receptor knockout mice. *Am J Physiol Heart Circ Physiol*. 2008; 294:H551–69. [PubMed: 18083900]
7. Zeng C, Felder RA, Jose PA. A new approach for treatment of hypertension: modifying D1 dopamine receptor function. *Cardiovasc Hematol Agents Med Chem*. 2006; 4:369–77. [PubMed: 17073613]
8. Zeng C, Zhang M, Asico LD, et al. The dopaminergic system in hypertension. *Clin Sci (Lond)*. 2007; 112:583–97. [PubMed: 17492945]
9. Zeng C, Eisner GM, Felder RA, Jose PA. Dopamine receptor and hypertension. *Curr Med Chem Cardiovasc Hematol Agents*. 2005; 3:69–77. [PubMed: 15638746]
10. Zeng C, Sanada H, Watanabe H, et al. Functional genomics of the dopaminergic system in hypertension. *Physiol Genomics*. 2004; 19:233–46. [PubMed: 15548830]
11. Kobori H, Nangaku M, Navar LG, Nishiyama A. The intrarenal renin-angiotensin system: from physiology to the pathobiology of hypertension and kidney disease. *Pharmacol Rev*. 2007; 59:251–87. [PubMed: 17878513]
12. Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. *Am J Physiol Cell Physiol*. 2007; 292:C82–97. [PubMed: 16870827]
13. Burson JM, Aguilera G, Gross KW, Sigmund CD. Differential expression of angiotensin receptor 1A and 1B in mouse. *Am J Physiol*. 1994; 267:E260–7. [PubMed: 8074205]
14. Crowley SD, Tharaux PL, Audoly LP, Coffman TM. Exploring type I angiotensin (AT1) receptor functions through gene targeting. *Acta Physiol Scand*. 2004; 181:561–70. [PubMed: 15283771]
15. Cervenka L, Wang CT, Mitchell KD, Navar LG. Proximal tubular angiotensin II levels and renal functional responses to AT1 receptor blockade in nonclipped kidneys of Goldblatt hypertensive rats. *Hypertension*. 1999; 33:102–7. [PubMed: 9931089]
16. Crowley SD, Gurley SB, Herrera MJ, et al. Angiotensin II causes hypertension and cardiac hypertrophy through its receptors in the kidney. *Proc Natl Acad Sci USA*. 2006; 103:17985–90. [PubMed: 17090678]
17. Crowley SD, Zhang J, Herrera M, et al. Role of AT(1) receptor-mediated salt retention in angiotensin II-dependent hypertension. *Am J Physiol Renal Physiol*. 2011; 301:F1124–30. [PubMed: 21849491]
18. Herrera M, Coffman TM. The kidney and hypertension: novel insights from transgenic models. *Curr Opin Nephrol Hypertens*. 2012; 21:171–8. [PubMed: 22274801]
19. Chugh G, Lokhandwala MF, Asghar M. Altered functioning of both renal dopamine D1 and angiotensin II type 1 receptors causes hypertension in old rats. *Hypertension*. 2012; 59:1029–36. [PubMed: 22411927]
20. Arthur JM, Thongboonkerd V, Scherzer JA, et al. Differential expression of proteins in renal cortex and medulla: a proteomic approach. *Kidney Int*. 2002; 62:1314–21. [PubMed: 12234301]
21. Miyata N, Park F, Li XF, Cowley AW Jr. Distribution of angiotensin AT1 and AT2 receptor subtypes in the rat kidney. *Am J Physiol*. 1999; 277:F437–46. [PubMed: 10484527]

22. Sidhu A, Kumar U, Uh M, Patel S. Diminished expression of renal dopamine D1A receptors in the kidney inner medulla of the spontaneously hypertensive rat. *J Hypertens*. 1998; 16:601–8. [PubMed: 9797171]
23. Fujii S, Zhang L, Igarashi J, Kosaka H. L-Arginine reverses p47phox and gp91phox expression induced by high salt in Dahl rats. *Hypertension*. 2003; 42:1014–20. [PubMed: 14504257]
24. Kitiyakara C, Chabrashvili T, Chen Y, et al. Salt intake, oxidative stress, and renal expression of NADPH oxidase and superoxide dismutase. *J Am Soc Nephrol*. 2003; 14:2775–82. [PubMed: 14569087]
25. Epstein M. Aging and the kidney. *J Am Soc Nephrol*. 1996; 7:1106–22. [PubMed: 8866401]
26. Fardoun RZ, Asghar M, Lokhandwala M. Role of oxidative stress in defective renal dopamine D1 receptor-G protein coupling and function in old Fischer 344 rats. *Am J Physiol Renal Physiol*. 2006; 291:F945–51. [PubMed: 16757732]
27. Najjar SS, Scuteri A, Lakatta EG. Arterial aging: is it an immutable cardiovascular risk factor? *Hypertension*. 2005; 46:454–62. [PubMed: 16103272]
28. Pestana M. Hypertension in the elderly. *Int Urol Nephrol*. 2001; 33:563–9. [PubMed: 12230296]
29. Carey RM. Theodore Cooper lecture: renal dopamine system: paracrine regulator of sodium homeostasis and blood pressure. *Hypertension*. 2001; 38:297–302. [PubMed: 11566894]
30. Chugh G, Asghar M, Patki G, et al. A high-salt diet further impairs age-associated declines in cognitive, behavioral, and cardiovascular functions in male Fischer brown Norway rats. *J Nutr*. 2013; 143:1406–13. [PubMed: 23864508]
31. Pokkunuri ID, Chugh G, Asghar M. Human kidney-2 cells harbor functional dopamine D1 receptors that require G α 1 for Gq/11 α signaling. *Am J Physiol Renal Physiol*. 2013; 305:F560–7. [PubMed: 23698121]
32. Gildea JJ. Dopamine and angiotensin as renal counter regulatory systems controlling sodium balance. *Curr Opin Nephrol Hypertens*. 2009; 18:28–32. [PubMed: 19077686]
33. Baylis C, Corman B. The aging kidney: insights from experimental studies. *J Am Soc Nephrol*. 1998; 9:699–709. [PubMed: 9555673]
34. Beheray S, Kansra V, Hussain T, Lokhandwala MF. Diminished natriuretic response to dopamine in old rats is due to an impaired D1-like receptor-signaling pathway. *Kidney Int*. 2000; 58:712–20. [PubMed: 10916094]
35. Zemel MB, Sowers JR. Salt sensitivity and systemic hypertension in the elderly. *Am J Cardiol*. 1988; 61:7H–12.
36. Hall JE. Control of sodium excretion by angiotensin II: intrarenal mechanisms and blood pressure regulation. *Am J Physiol*. 1986; 250:R960–72. [PubMed: 3521337]
37. Whelton PK, He J, Appel LJ, et al. National High Blood Pressure Education Program Coordinating C. Primary prevention of hypertension: clinical and public health advisory from The National High Blood Pressure Education Program. *JAMA: J Am Med Assoc*. 2002; 288:1882–8.
38. Whelton SP, Chin A, Xin X, He J. Effect of aerobic exercise on blood pressure: a meta-analysis of randomized, controlled trials. *Ann Intern Med*. 2002; 136:493–503. [PubMed: 11926784]
39. Tobon KE, Chang D, Kuzhikandathil EV. MicroRNA 142-3p mediates post-transcriptional regulation of D1 dopamine receptor expression. *PLoS One*. 2012; 7:e49288. [PubMed: 23152889]
40. Arnold AC, Okamoto LE, Gamboa A, et al. Angiotensin II, independent of plasma renin activity, contributes to the hypertension of autonomic failure. *Hypertension*. 2012; 61:701–6. [PubMed: 23266540]
41. Poch E, Gonzalez D, Giner V, et al. Molecular basis of salt sensitivity in human hypertension. Evaluation of renin-angiotensin-aldosterone system gene polymorphisms. *Hypertension*. 2001; 38:1204–9. [PubMed: 11711524]

Figure 1.

Conscious BP in adult and aged FBNs during NS and HS treatments: BP was determined as described in the “Methods” section. Results are mean $\pm$ SEM. $n = 7-8$ rats. $p < 0.05$. Significantly different from corresponding adult rats (*) and old NS (¥).

Figure 2.

The levels of mRNA and protein of D1R in the kidney cortex and medulla of FBNs fed with NS and HS diet. (A) D1R mRNA levels were determined by RT-qPCR (see details in the Methods section). House-keeping gene 18S rRNA was used to normalize the data. (B) D1R protein levels were determined by Western blotting. Upper panel: bars are ratios of densities of D1R and β -actin protein bands. Lower panel: representative blot. Data are mean $\pm$ SEM ($n = 6$ rats). Significantly different from the corresponding adult rats fed with HS (***), (**), and (*) and adult rats fed with NS (###). ### $p < 0.0005$; ** $p < 0.005$; * $p < 0.05$.

Figure 3.

The levels of mRNA and protein of AT1R in the kidney cortex and medulla of FBNs fed with NS and HS diet. (A) AT1R mRNA levels were determined by RT-qPCR (see details in the Methods section). House-keeping gene 18S rRNA was used to normalize the data. (B) AT1R protein levels were determined by Western blotting. Upper panel: bars are ratios of densities of AT1R and β -actin protein bands. Lower panel: representative blot. Data are mean $\pm$ SEM ($n = 6$ rats). Significantly different from the corresponding adult rats fed with HS (***), (**), and adult rats fed with NS (###). ### $p < 0.0005$; ** $p < 0.005$.

Figure 4.

The levels of mRNA and protein of *gp91phox*-NADPH-oxidase in the kidney cortex and medulla of FBNs fed with NS and HS diet. (A) *gp91phox*-NADPH-oxidase mRNA levels were determined by RT-qPCR (see details in the Methods section). House-keeping gene 18S rRNA was used to normalize the data. (B) *gp91phox*-NADPH-oxidase protein levels were determined by Western blotting. Upper panel: bars are ratios of densities of *gp91phox*-NADPH-oxidase and β -actin protein bands. Lower panel: representative blot. Data are mean $\pm$ SEM ($n = 6$ rats). Significantly different from the corresponding adult rats fed with HS (***) and (*) and adult rats fed with NS (###), (##). ### $p < 0.0005$, ## $p < 0.005$, * $p < 0.05$.

Figure 5.

The levels of proteins of NHE3 (A) and Na/K ATPase in the membranes of cortex and medulla of FBNs fed with NS and HS diet. NHE3 and Na/K ATPase protein levels were determined by Western immunoblotting as described in the Methods section. Upper panel: bars are ratios of densities of D1R and β -actin protein bands. Lower panel: representative blot. Data are mean $\pm$ SEM ($n = 6$ rats). Significantly different from the corresponding adult rats fed with HS (*), and adult rats fed with NS (##), old rats fed with NS (**). * $p < 0.05$, **, ## $p < 0.005$.

Table 1

Biochemical parameters in urine and plasma.

Parameter	Adult HS	Old HS	Adult NS	Old NS
Urinary protein (µg/µl)	3.58 ± 0.63	8.95 ± 2.07*	3.2 ± 0.63	8.22 ± 0.82**
Urinary albumin (µg/ml)	280 419 ± 6702	349 115 ± 10 628*	272 724 ± 23 067	379 386 ± 30 924**
Urinary KIM-1 (ng/ml)	0.391 ± 0.05	0.524 ± 0.049*	0.362 ± 0.104	0.426 ± 0.035**
Plasma renin (ng/ml/h)	43.53 ± 1.45	48.45 ± 2.89	41.59 ± 6.01	46.32 ± 8.18
Tissue renin medulla	68.77 ± 3.88	52.77 ± 3.51	78.65 ± 6.01	46.32 ± 8.18
Cortex (ng/ml/h)	78.97 ± 7.75	82.25 ± 1.18#	73.46 ± 3.98	84.27 ± 4.53##

Urinary protein, albumin, KIM-1, plasma, and tissue renin levels of NS and HS fed adult and old FBN rats. Urinary protein was measured by the BCA method and the rest was by kit-based assays as described in the Methods section. Significantly different from corresponding HS fed adults (*), NS fed adults (**); significantly different from corresponding adults rats fed with HS (#), adults rats fed with NS (##) in the medulla. Results are mean ± SEM (*n* = 6 rats).

*, # *p* < 0.05;

**, ## *p* < 0.005.