Salt Sensitivity

Activation of Renal (Pro)Renin Receptor Contributes to High Fructose-Induced Salt Sensitivity

Chuanming Xu, Aihua Lu, Xiaohan Lu, Linlin Zhang, Hui Fang, Li Zhou, Tianxin Yang

Abstract—A high-fructose diet is shown to induce salt-sensitive hypertension, but the underlying mechanism largely remains unknown. The major goal of the present study was to test the role of renal (pro)renin receptor (PRR) in this model. In Sprague–Dawley rats, high-fructose intake increased renal expression of full-length PRR, which were attenuated by allopurinol. High-fructose intake also upregulated renal mRNA and protein expression of sodium/hydrogen exchanger 3 and Na/K/2Cl cotransporter, as well as in vivo Na/K/2Cl cotransporter activity, all of which were nearly completely blocked by a PRR decoy inhibitor PRO20 or allopurinol treatment. Parallel changes were observed for indices of intrarenal renin–angiotensin–system including renal and urinary renin and angiotensin II levels. Radiotelemetry demonstrated that high-fructose or a high-salt diet alone did not affect mean arterial pressure, but the combination of the 2 maneuvers induced a ≥10-mmHg increase of mean arterial pressure, which was blunted by PRO20 or allopurinol treatment. In cultured human kidney 2 cells, both fructose and uric acid increased protein expression of soluble PRR in a time- and dose-dependent manner; fructose-induced PRR upregulation was inhibited by allopurinol. Taken together, our data suggest that fructose via uric acid stimulates renal expression of PRR/soluble PRR that stimulate sodium/hydrogen exchanger 3 and Na/K/2Cl cotransporter expression and intrarenal renin–angiotensin system to induce salt-sensitive hypertension.

(Key Words: fructose diet • hypertension • renin-angiotensin system • uric acid)

Fructose consumption has increased dramatically during the past several decades, mostly as a result of the increased of fructose-based sweeteners. Epidemiological data indicate that this upward trend continued until recently. Excessive fructose intake has been linked to the epidemics of diabetes mellitus, obesity, renal failure, and hypertension. Many previous studies have reported that feeding rats with a high-fructose (HF) diet causes hypertension; whereas Brands et al and Bezerra et al showed that an HF diet alone did not increase blood pressure (BP) in rats but induced salt-sensitive hypertension in conjunction with a high-salt (HS) diet as assessed by using radiotelemetry. D’Angelo et al also pointed out that previous reports of HF-induced hypertension reflect a heightened response to restraint and thermal stress, leading to an increase in sympathetic output and contributing to the increase in BP, as monitored by using the tail-cuff method. HF intake is involved in enhancing sodium (Na) reabsorption in both the kidney and the intestine, which may contribute to the development of hypertension. Fructose stimulates sodium/hydrogen exchanger 3 (NHE3) activity in the proximal tubule (PT) and sensitizes it to angiotensin II (Ang II). All of these studies have shown a close relationship between HF intake, salt sensitivity, and hypertension. The intrinsic mechanisms, however, have not been well established.

(Pro)renin receptor (PRR) was first discovered by Nguyen et al in 2002 as a 39-kDa single transmembrane protein (full-length PRR [fPRR]) that binds both renin and prorenin. PRR is cleaved by furin or a disintegrin and metalloproteinase domain 19 to generate a 28-kDa N-terminal region, soluble PRR (sPRR), and a C-terminal transmembrane form (M8.9 complexed with V-ATPase). Both fPRR and sPRR are able to bind to renin and the inactive prorenin, thus enhancing the catalytic activity of renin, promoting the nonproteolytic activation of prorenin, and increasing the catalytic efficiency of local Ang II formation. sPRR can be not only secreted into plasma, urine, and cell culture medium but also retained inside the cell. Both renal PRR and plasma sPRR were shown to be increased during the development of hypertension. BP was increased in transgenic rats with human PRR overexpression in vascular smooth muscle cells, but not with ubiquitous overexpression of PRR. Knockdown of endogenous PRR in the supraoptic nucleus, brain, and neuron, as well as intracerebroventricular infusion of the PRR antagonist PRO20 (the first 20 amino acid residues of the prorenin prosegment, L`PTRATFERIPLKKPSVR`), attenuated the development of hypertension. Overall, this finding shows a potential role of local PRR in the regulation of BP, but the mechanisms involved remain largely elusive. Of note, the binding of prorenin to PRR, independent of Ang II, also stimulates a variety of intracellular signaling cascades.
Within the kidney, PRR is expressed in multiple structures including the PCTs, medullary thick ascending limbs, connecting tubules, and collecting ducts.25–29 Emerging evidence supports an important role of renal PRR in regulation of intrarenal renin–angiotensin system (RAS) and renal function.25–31 On the contrary, previous studies have documented interaction between fructose metabolism and the RAS. For example, chronic treatment of Sprague–Dawley (SD) rats with fructose for 16 weeks significantly increased renal Ang II type 1 receptor, renin, and Ang II protein and mRNA expression.32 Fructokinase, a rate-limiting enzyme for fructose metabolism, is highly expressed in the PCTs where fructokinase-dependent production of uric acid (UA) contributes to proinflammatory response.33,34 Furthermore, the PTs where fructokinase-dependent production of uric acid limiting enzyme for fructose metabolism, is highly expressed in the hypothesis that fructose-derived UA activates renal PRR and accounting to the decrease of Na+ and food-derived energy intake, whereas HF-induced changes in body weight and food intake show basic metabolism data. Of note, HF-induced increases in blood glucose and plasma insulin, indicating insulin resistance, which was improved by either PRO20 or allopurinol, whereas HF-induced changes in body weight and food intake were unaffected. HF-induced decreases in food intake, leading to the decrease of Na+ and food-derived energy intake, which were unaffected by PRO20 or allopurinol. HF-induced polydipsia, polyuria, and ≈50% energy from fructose solution were enhanced by allopurinol but unaffected by PRO20.

HF intake significantly increased 24-hour urinary UA excretion (Figure S1A), accompanied with increased renal fructokinase protein expression (Figure S1B–S1D), which were both attenuated by allopurinol treatment, whereas plasma UA concentration only exhibited a trend of reduction (Figure S1E).

We examined the regulation of renal PRR expression in SD rats during HF intake with or without allopurinol. Immunoblotting analysis demonstrated that HF consistently increased protein abundance of fPRR and renin in the renal cortex, outer and inner medulla (Figure 1A). Allopurinol treatment attenuated HF-induced renal protein (Figure 1A) and mRNA (Figure 1B) expression of PRR and renin in the renal cortex, outer medulla, and inner medulla. Immunofluorescence showed predicted predominant labeling of PRR in the intercalated cells of the collecting duct in normal animals. HF intake induced a widespread enhancement of the signal in most renal tubules contrasting to negative labeling in the glomerulus (Figure 1C and 1D). The specificity of the PRR labeling was validated by using the immunizing peptide (Figure 1E and 1F).

We examined renin levels in plasma and urine in control, HF, HF+allopurinol, and HF+PRO20 rats. HF intake significantly elevated urinary renin activity (Figure 2A), active renin content (Figure 2B), total renin content (Figure 2C), and prorenin content (Figure 2D), which were all attenuated by allopurinol or PRO20 treatment albeit in some varying degrees. In contrast, among the plasma renin parameters in HF rats, only plasma renin activity (Figure S2A) was elevated, contrasting to decreased plasma active renin concentration (Figure S2B) and no change in plasma total renin concentration (Figure S2C) and plasma prorenin concentration (Figure S2D). Similarly, urinary Ang II excretion (Figure 2E) was elevated by HF intake, and this increase was blunted by allopurinol and PRO20, whereas plasma Ang II (Figure S2E) remained constant among all groups. These results highlight the selective activation of the intrarenal versus systemic RAS during HF intake and also suggest an underlying mechanism involving PRR and the metabolism of fructose.

We also examined the electrolyte levels in urine and plasma in control, HF, HF+allopurinol, and HF+PRO20 rats at week 12. HF intake increased plasma Na+ (Figure S3A), Cl− (Figure S3B) concentrations, and osmolality (Figure S3C), but decreased urinary Na+ (Figure S3D) and Cl− (Figure S3E), all of which were inhibited by allopurinol or PRO20 treatment. Immunoblotting analysis and real-time quantitative PCR were performed to examine renal cortical and medullary expression of NHE3 and Na/K/2Cl cotransporter (NKCC2), in control, HF, HF+allopurinol, and HF+PRO20 rats. HF intake consistently increased NHE3 and NKCC2 protein and mRNA expression in both kidney regions, which was effectively blocked by allopurinol or PRO20 (Figure 4). The in vivo NKCC2 activity was determined by examining the acute diuretic and natriuretic responses to furosemide. Furosemide-induced increases in urine volume (Figure 5A), urinary Na+ (Figure 5B), K+ (Figure 5C), and Cl− (Figure 5D) excretion, as well as the fall in urine osmolality (Figure 5E) was significantly enhanced by HF intake, which was nearly completely blocked by allopurinol or PRO20.

Radiotelemetry was performed to measure BP in control, HF, HF+allopurinol, and HF+PRO20 animals at basal condition and after a HS diet. As expected, HF intake alone did not affect mean arterial pressure (MAP), systolic BP, or diastolic BP, but HF intake in combination with a HS diet consistently induced an increase of ≈10 mm Hg for each of these parameters. Remarkably, HF/HS-induced hypertension was abolished by allopurinol or PRO20 (Figure 6).

In Vitro Experiments Using Human Kidney 2 Cells

We conducted in vitro experiments to examine the direct effect of fructose or UA on PRR expression in cultured human kidney 2 (HK2) cells. After reaching confluence, the cells
were serum starved for 12 hours and then exposed to various concentrations of fructose ranging from 1 to 10 mmol/L for 72 hours. By immunoblotting, HF treatment stimulated the expression of fPRR and sPRR in a dose-dependent manner (Figure S3A). For time-course studies, HK2 cells were exposed to 5 mmol/L fructose for various times ranging from 12 to 72 hours. The increase in fPRR and sPRR expression was noticeable at 24 hours and sustained until 72 hours (Figure S3B). Allopurinol treatment completely blocked fructose-induced fPRR and sPRR protein expression and medium sPRR secretion (Figure S3C and S3D). After exposure to UA, sPRR but not fPRR protein expression in the HK2 cells as assessed by immunoblotting analysis was increased in a time-dependent manner (Figure S3E), corresponding to a remarkable increase in medium sPRR (Figure S3F).

Discussion

Our overall hypothesis was that chronic fructose intake via UA stimulates renal PRR and renin expression that activates intrarenal RAS and renal Na⁺ transporters NHE3 and NKCC2, leading to increased salt sensitivity. In vitro studies, we found that 5 mmol/L fructose increased fPRR and sPRR protein expression and medium sPRR secretion in HK2 cells in a dose- and time-dependent manner, so did UA; the stimulatory effect of HF on PRR was sensitive to allopurinol. In vivo studies, HF intake elevated renal PRR and renin expression in parallel with increased urinary renin and Ang II levels, predisposing salt-sensitive hypertension. All of these were attenuated by PRO20. Allopurinol treatment also attenuated HF-induced renal PRR and renin expression, as well as salt-sensitive hypertension. Both PRO20 and allopurinol inhibited HF-induced Na⁺ retention, as shown by the reduced plasma Na⁺ concentration and increased 24-hour urinary Na⁺ excretion associated with a reduction of NKCC2 and NHE3 expression and NKCC2 activity. These results suggest a novel role for PRR in the pathogenesis of salt-sensitive hypertension during HF intake, likely through the activation of the renal RAS and renal Na⁺ transporters.

During the past decade, there has been a paradigm shift in our understanding of local versus systemic RAS in BP control. Local RAS exists in multiple tissues including brain,37...
gastrointestinal tract, adipose tissue, cardiovascular system, and the kidney. In particular, the intrarenal RAS is shown to play an important role in pathogenesis of Ang II–induced hypertension. Ang II infusion upregulated urinary and renal medullary renin but downregulated plasma and renal cortical renin, indicating the independent relationship between intrarenal and systemic RAS. Previous studies have shown that feeding rats with a HF diet activates local RAS in aorta, kidney, and skeletal muscle, as reflected by the upregulation of Ang II type 1 receptor, renin/prorenin, and Ang II levels. Consistent with this notion, we present evidence for overactivation of the intrarenal RAS in HF-treated rats including robust and consistent increases in urinary renin activity, active and total renin content, and prorenin content, and Ang II excretion, in parallel with increased renal PRR expression and urinary sPRR excretion. Functional evidence demonstrated that PRR antagonism with PRO20 effectively suppressed the indices of intrarenal RAS in HF-treated rats. These results support the association of PRR and intrarenal RAS in the current experimental model. A series of our recent studies using both pharmacological and genetic approaches demonstrate that such an association exists during Ang II infusion and water deprivation.

When compared with the consistent changes in urinary RAS components as discussed above, circulating RAS components exhibited a different profile. HF intake increased plasma renin activity but decreased plasma active renin content, with unchanged plasma total renin content, prorenin, and Ang II. It is unclear whether the discordance of these data is related to differences in circulation angiotensinogen levels. Of note, Iyer et al reported that plasma Ang II levels of fructose-fed rats were significantly increased at the end of the second week and
returned to basal levels at the end of the fourth week of dietary treatment. The time-dependent regulation of circulating RAS by HF intake may explain why we observed no changes in plasma Ang II levels after prolonged HF treatment.

Salt sensitivity usually reflects changes in BP after a short-term change of dietary sodium intake. In the present study, salt sensitivity was determined by examining MAP response to a 1-week HS diet that contained 8% NaCl. As reported earlier, MAP was unaffected by HF alone but an increase of ≈10 mm Hg in MAP was induced by HS superimposed on HF. Although different criteria are used to evaluate salt sensitivity in clinical studies, a mean BP increase of 3 mm Hg between diets is considered as being salt sensitive. Therefore, the increase of MAP of 10 mm Hg in response to HS in our experimental model is of clinical relevance. This MAP increase was completely abolished by PRO20 treatment, supporting an important role of PRR in mediating the hypertensive response to HF/HS. Besides over-activation of intrarenal RAS, deranged Na+ reabsorption likely contributes to increased salt sensitivity during HF intake.

In the kidney, the PTs reabsorb 60% to 70% of the filtered Na+ and the thick ascending limb reabsors 25% of the total filtered Na+. In spontaneously hypertensive rats, NHE3 activity and expression were significantly increased in PT. By using in vivo stationary microperfusion, Queiroz-Leite et al and Cabral et al found that fructose acutely enhanced NHE3 activity. In extension of this observation, we for the first time demonstrated that chronic HF intake significantly upregulated renal NHE3 expression at both mRNA and protein. More importantly, we found that the upregulation of renal NHE3 expression by HF intake was inhibited by PRO20 treatment. This result suggests stimulatory effect of PRR in HF-induced renal NHE3 expression.

Of note, fructose enhanced the stimulatory effect of Ang II on

![Figure 3. Plasma Na+ concentration (A), plasma Cl− concentration (B), plasma osmolality (C), urinary Na+ excretion (D), urinary Cl− excretion (E), and urine osmolality (F) in control, high-fructose (HF), HF+allopurinol, or HF+PRO20 rats at week 12. n=5 to 10 per group. Data are mean±SEM. ***P<0.001 vs control, #P<0.05, ##P<0.01, and ###P<0.001 vs HF.](http://hyper.ahajournals.org/Downloaded-from.jpg)
NHE3 activity in isolated perfused rat PT. Moreover, oxidative stress leads to overstimulation of renal NHE3 by exaggerating Ang II signaling. Overall, renal PRR may mediate HF-induced NHE3 activity and expression via activation of intrarenal RAS. Enhanced NKCC2 activity has been linked to salt-sensitive hypertension in humans and Dahl and Milan salt-sensitive rats. To date, there is no previous report on NKCC2 regulation by fructose except the statement by Ares and Ortiz in a review article that acute exposure to fructose induces NKCC2 trafficking to the apical membrane in an in vitro preparation. Our in vivo study is the first to show that a long-term HF intake consistently increased renal NKCC2 mRNA and protein expression, and activity in rats. Moreover, we demonstrated that the activation of NKCC2 by fructose can be ascribed to PRR activation because it is sensitive to PRO20. The mechanism for the concurrent activation of NHE3 and NCCK2 by PPR during HF intake remains elusive. We speculate that PRR-dependent activation of intrarenal RAS may mediate the upregulation of the transporters. In support of this possibility, Ang II is shown to activate both NHE3 and NKCC2. The mechanism for the concurrent activation of NHE3 and NCCK2 by PPR during HF intake remains elusive. We speculate that PRR-dependent activation of intrarenal RAS may mediate the upregulation of the transporters. In support of this possibility, Ang II is shown to activate both NHE3 and NKCC2. Future studies are needed to determine the relative contribution of the individual transporters to HF-induced salt-sensitive hypertension.

UA plays an important pathogenic role in many disease processes, such as obesity, hypertension, kidney disease, and metabolic syndrome. Some of the actions of UA have been tied to its association with the RAS. In particular, it has been shown that UA stimulated local RAS leading to rat vascular smooth cell proliferation, human vascular endothelial cell dysfunction, and immortalized human mesangial cell proliferation. UA is one of the major metabolic end products of fructose. It has been shown that a primary mechanism of fructose-induced metabolic syndrome is related to the increase of intracellular UA, which can stimulate generation of intracellular reactive oxygen species via nicotinamide adenine dinucleotide phosphate oxidases–dependent mitochondrial oxidant system, or activate mitogen-activated protein kinase signaling to increase transforming growth factor levels.

Although some experimental studies showed HF intake caused serum UA elevation in rats, a clinical study showed that HF intake for 10 weeks does not raise circulating UA levels at normal levels of human consumption. Our results showed that after HF intake, plasma UA levels remained unchanged but urinary UA excretion was significantly elevated, which was partially attenuated by allopurinol. Plasma UA levels showed a trend of decrease. These results seem to suggest renal origin of increased UA production during HF intake at least in our current experimental model. Moreover, allopurinol treatment effectively blocked HF-induced renal PRR expression and indices of intrarenal RAS. In parallel, allopurinol also inhibited HF-induced Na+ retention, accompanied with suppressed
expression of NHE3 and NKCC2. Overall, these results suggest that UA may be a major mediator of HF-induced activation of intrarenal RAS and renal Na⁺ absorptive processes.

However, it is interesting to note that in cultured HK2 cells, UA induced PRR cleavage to increase sPRR production without affecting the total abundance of fPRR, and thus it did not fully mimic the effect of fructose. It seems reasonable to speculate that in addition to UA, other metabolites or pathways will likely be involved to elicit the full biological response to fructose. Along this line, we noticed difference in the effect of allopurinol and PRO20 on fluid homeostasis. Allopurinol induced polydipsia and polyuria and PRO20 did not, indicating that the changes in fluid balance because of inhibition of xanthine oxidase are not entirely dependent on PRR.

The present study has many limitations. The major one is the lack of the time-course studies on Na⁺ excretion and homeostasis. The physiological parameters with the use of metabolic cages were only collected at the end of the study when homeostasis had already been re-established. This was mostly because

Figure 5. Effect of allopurinol and PRO20 on in vivo Na/K/2Cl cotransporter (NKCC2) activity as reflected by rapid diuresis and natriuretic responses to furosemide. Control, high-fructose (HF), HF+allopurinol, and HF+PRO20 rats were all subjected to a single dose of furosemide treatment, followed by 1-h urine collection. A, One-hour urine volume. B, One-hour urinary Na⁺ excretion. C, One-hour urinary K⁺ excretion. D, One-hour urinary Cl⁻ excretion. E, Urine osmolality. n=10 to 20 per group. Data are mean±SEM. **P<0.01 and ***P<0.001 vs control; #P<0.05, ##P<0.01, and ###P<0.001 vs HF.
of the need to monitor BP in a stress-free environment. In addition, we provided strong functional data to support involvement of UA and PRR to HF-induced salt-sensitive hypertension, but the underlying mechanism about the relationship between these mediators remains incompletely understood. In particular, UA may target other pathways than PRR.

In summary, the present study examined the renal mechanism of HF-induced salt-sensitive hypertension in rats. The mechanism involves PRR-dependent activation of intrarenal RAS and stimulation of NHE3 and NKCC2 via UA. Targeting PRR or other components of the pathogenic cascades may offer a new intervention for management of HF-induced salt-sensitive hypertension.

**Perspectives**

The anti-RAS regimen causes high prorenin/renin state that has long been considered to be pathogenic. The present study demonstrates the pathogenic role of activation of PRR, the receptor of prorenin and renin during HF/HS-induced hypertension. This finding provides a rationale for complementing inhibition of RAS with a PRR inhibitor for treatment of hypertension. In addition, clinical studies demonstrate that allopurinol lowers BP in adolescence with prehypertension and in older adults with established hypertension. Our results call for clinical evaluation of antihypertensive property of allopurinol and PRO20 in patients with hypertension associated with HF and HS intake.

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**Disclosures**

None.

**References**

enhances urine concentration capability as a target of liver X receptor.
Novelty and Significance

What Is New?

- It is known that high fructose causes salt-sensitive hypertension, but the underlying mechanism largely remains elusive. Here, we for the first time demonstrate that fructose via uric acid stimulates renal expression of (pro)renin receptor that stimulates Na/H exchanger 3 and Na/K/2Cl cotransporter expression and intrarenal renin–angiotensin system to drive salt-sensitive hypertension.

What Is Relevant?

- Both PRO20 and allopurinol blocked high fructose–induced salt-sensitive hypertension, which may provide a new target for development of anti-hypertensive therapy.

Summary

The present study for the first time provides a novel mechanism of fructose-induced salt-sensitive hypertension, which involves (pro)renin receptor–dependent activation of intrarenal renin–angiotensin–system and stimulation of Na/H exchanger 3 and Na/K/2Cl cotransporter via uric acid. Targeting (pro)renin receptor or other components of the pathogenic cascades may offer a new intervention for management of high fructose–induced salt-sensitive hypertension.
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Activation of Renal (Pro)Renin Receptor Contributes to High Fuctose-Induced Salt Sensitivity

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Materials and Methods

Animal experiments

SD rats were fed a standard diet and randomized into 4 groups (n=5 / each group): Control, fructose (HF), fructose plus allopurinol (a xanthine oxidase inhibitor, Cat# A8003, Sigma, Louis, MO, USA) (HF + allopurinol), and fructose plus PRO20 (HF + PRO20). Fructose was given to the rats in the later two groups as a 20% solution in drinking water ad libitum for 12 weeks, whereas the control rats received normal drinking water. PRO20 was administered to the fructose plus PRO20 group at 700 μg/kg/day in 0.9% NaCl via subcutaneous injection three times a day (every 8 h) and the other three groups received a subcutaneous injection of 0.9% NaCl as a vehicle. Allopurinol was administered at 30 mg/kg/day in drinking water1. At week 12, rats were housed in metabolic cages to measure 24-h food and water intake. At the end of the experiment, all rats were sacrificed and blood and kidneys were harvested. After decapsulation, the kidneys were cut into cortex, outer medulla, and inner medulla. Urine and plasma electrolytes were determined with an automatic analyzer (9180 Electrolyte analyzer, Roche, Berlin, Germany). Urine and plasma uric acid (UA) was measured by using the QuantiChrom Uric Acid Assay Kit (Cat# D1UA-250, BioAssay Systems, Hayward, CA, USA) according to the manufacturer’s instructions.

Renin activity assay

Renin activity assay was performed as previously described2. Renin activity in plasma and urine was determined by using an ELISA kit and the delta value of Ang I generation after incubating the sample at 4 °C and 37 °C, respectively, for 1 h. Total renin content was measured with excess angiotensinogen (AGT) plus trypsinization and active renin content with excess AGT. Urine and plasma samples were spiked with the synthetic renin substrate tetradecapeptide (1 μM, Cat# R8129, Sigma-Aldrich, Saint Louis, MO, USA). Following incubation at 37 °C for 1 h, Ang I generation was assayed by using an Angiotensin-I EIA kit (Cat# S-1188, Peninsula Laboratories International, San Carlos, CA, USA) according to the manufacturer’s instructions. The values were expressed as nanograms per milliliter per hour of generated Ang I. For measurement of total renin content, trypsinization was performed to activate prorenin to renin. The samples were incubated with trypsin from bovine pancreas (100 g/L, Cat# T1426, Sigma-Aldrich, Saint Louis, MO, USA) at 37 °C for 6 h. The reaction was then terminated with soybean trypsin inhibitor (100 g/L, Cat# T6522, Sigma-Aldrich, Saint Louis, MO, USA) at 37 °C for 1 h.

qRT-PCR

Total RNA was isolated from snap-frozen renal samples by using TRIzol reagent (Cat# 15596018, Invitrogen, Carlsbad, CA, USA) and reverse transcribed to cDNA by using the Transcriptor First Strand cDNA Synthesis Kit (Cat# 04379012001, Roche, Berlin, Germany) according to the manufacturer’s instructions. Total RNA concentrations were determined using NANODROP 2000 Spectrophotometer
Quantitative real-time PCR was performed by using the ABI Prism StepOnePlus System (Applied Biosystems, Life Technologies, Singapore) and the FastStart Universal SYBR Green Master (ROX) (Cat# 04913914001, Roche, Berlin, Germany) according to the manufacturer’s instructions. Oligonucleotides were designed with Primer3 software (available at http://www.simgene.com/Primer3), their sequences are shown in Table S1. All reactions were run in duplicate. The data are shown as a relative value normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

**Immunoblot analysis**

The kidney samples from each animal were lysed and subsequently sonicated in RIPA buffer (Biocolors, Shanghai, China) with protease inhibitor cocktail (Roche, Berlin, Germany). Protein concentrations were determined with the Pierce BCA Protein Assay Kit (Cat# NCI3225CH, Thermo Scientific, Rockford, IL, USA) according to the manufacturer’s instructions. Thirty micrograms of protein for each sample was separated by SDS-PAGE and transferred onto polyvinylidene fluoride membrane (Immobilon-P, Millipore, Bedford, MA, USA). The membranes were blocked with 5% nonfat dry milk, followed by incubation with primary antibodies (PRR, HPA003156; Renin, AV41409; Na/K/2Cl cotransporter (NKCC2), SAB5200103; NHE3, SAB5200102; KHK, HPA007040; β-actin, A-2066; these primary antibodies were purchased from Sigma-Aldrich and were incubated for overnight at 4 °C and then with goat anti-rabbit/mouse horseradish peroxidase-conjugated secondary antibody (Thermo Scientific) for 1 h at room temperature, and visualized with enhanced chemiluminescence (Thermo Scientific). They were then processed for signal detection by using a Tanon 5200 Luminescent Imaging Workstation (Tanon, Shanghai, China) and quantitated using Image-Pro Plus 6.0. The expression of protein was calculated in relation to β-actin.

**Immunofluorescence**

The tissues were fixed in 10% neutral buffered formalin for 24 h and then embedded in paraffin. After deparaffinization, thin sections (4 μm) were processed for labeling with immunofluorescence. The slides were blocked in 1% BSA for 1 h and were then incubated with primary antibody (PRR, 1:200 dilution, ab40790, Abcam) or PRR antibody peptide (human ATP6IP2 peptide, 1:100 dilution, ab41522, Abcam) at 4°C for overnight. After washing off the primary antibody, sections were incubated for 1 h at room temperature with Donkey anti-Rabbit IgG-TRITC (1:100, Life Technologies). After washing off the secondary antibody, images were captured using a Leica DMI4000B fluorescence microscope (Wetzlar, Germany).

**ELISA assays for sPRR and Ang II**

sPRR levels, released into the culture medium, were determined by using a soluble (Pro)renin Receptor Assay kit (Cat# 27782, Immuno-Biological Laboratories, Gunma, Japan). Ang II levels in urine and plasma were determined by using the Ang II ELISA kit (Cat# ADI-900-204, Enzo Life Sciences Inc., Farmingdale, NY, USA)
according to the manufacturer’s instructions.

**In vivo NKCC2 activity**

To determine the effect of PRR on in vivo NKCC2 activity, we administered 0.8 mg/kg furosemide by the intraperitoneal route to all rats and removed food and water following this injection as previously described. One-hour urine samples were collected and urine electrolytes determined with an automatic analyzer.

**Blood pressure measurement**

After 10 weeks of treatment with Control, HF, HF + allopurinol, and HF + PRO20 as described above, the radiotelemetric device (Data Sciences Inc., St. Paul, MN, USA) was implanted via catheterization of carotid artery. Rats were allowed to recover for 1 week from surgery. The BP was then recorded for 4 hours from 9:30AM to 3:30PM before and after 1 week of a high salt diet (8% NaCl).

**Cell culture**

Human renal proximal tubule epithelial cells HK-2 were grown to 95% confluence in Dulbecco’s Modified Eagle medium/Ham's F-12 medium supplemented with 10% fetal bovine serum, 10,000 U/mL penicillin-streptomycin, 2 mM L-glutamine, insulin (10 mg/L), transferrin (5.5 mg/L), and sodium selenite (6.7 μg/L). To detect the direct effect of fructose on PRR expression, HK-2 cells were serum-starved for 12 h and then treated with 1, 3, 5, and 10 mM fructose for 72 h, or treated with 5 mM fructose for various time periods, or pretreated with allopurinol (100 μM) for 1 h and then treated with 5 mM fructose for 72 h. The cells were then harvested for PRR expression analysis, and the cell medium for sPRR ELISA assay.

To detect the direct effect of exogenous UA on PRR expression, HK-2 cells were serum-starved for 12 h and then treated with 10 mg/dl UA for various time periods, cells were then harvested for PRR expression analysis, and the cell medium for sPRR assay.

**References**

### Table S1. Sequences of oligonucleotides used for qRT-PCR.

<table>
<thead>
<tr>
<th>Gene</th>
<th>PubMed No.</th>
<th>Sequence (5'→3')</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRR</td>
<td>NM_001007091.1</td>
<td>(F)ATCCTTGAGACGAAACAAGA (R)AGCCAGTCATAATCCACAGT</td>
<td>109</td>
</tr>
<tr>
<td>Renin</td>
<td>NM_012642.4</td>
<td>(F)GATCACCATGAAGGGGTCTCTGT (R)GTTCCTGAAGGGATTCTTTTGCAC</td>
<td>274</td>
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<tr>
<td>NKCC2</td>
<td>NM_001270618.1</td>
<td>(F)CAGTGGTGCCAGTCTTTCC (R)TGGTGTGGTGGGCCAAGGT</td>
<td>272</td>
</tr>
<tr>
<td>NHE3</td>
<td>NM_012654.1</td>
<td>(F)ACTGCTTAATGACCGTGACTGT (R)AAAGACGAAGCCAGGCTGATG</td>
<td>223</td>
</tr>
<tr>
<td>GAPDH</td>
<td>NM_017008.4</td>
<td>(F)GTCTTCACTACCATGGAGAAGG (R)TCATGGATGACCTTGGCCAG</td>
<td>197</td>
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</table>

### Table S2. General physiological data in rats.
<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>HF</th>
<th>HF + Allopurinol</th>
<th>HF + PRO20</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔBody weight (g)</td>
<td>372.0±10.2</td>
<td>329.4±6.1∗</td>
<td>311.34±7.40∗</td>
<td>314.8±8.7∗</td>
</tr>
<tr>
<td>KW/BW (g/kg)</td>
<td>5.04±0.18</td>
<td>5.56±0.12∗</td>
<td>6.12±0.23 †‖</td>
<td>5.01±0.14‖§</td>
</tr>
<tr>
<td>Food intake (g/24h)</td>
<td>16.48±1.02</td>
<td>11.05±0.71†</td>
<td>11.50±1.63†</td>
<td>12.33±0.99†</td>
</tr>
<tr>
<td>Na⁺ intake (mmol/24h)</td>
<td>2.15±0.13</td>
<td>1.44±0.09†</td>
<td>1.50±0.21∗</td>
<td>1.61±0.13∗</td>
</tr>
<tr>
<td>Water intake (ml/24h)</td>
<td>22.95±1.31</td>
<td>46.84±4.04‡</td>
<td>71.08±3.75‡‖</td>
<td>45.05±4.66‡</td>
</tr>
<tr>
<td>Total energy intake</td>
<td>214.20±13.27</td>
<td>300.21±17.64‡</td>
<td>387.19±11.34‖‖</td>
<td>310.87±22.34‖</td>
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<tr>
<td>Food-derived energy</td>
<td>214.20±13.27</td>
<td>143.59±9.16‡</td>
<td>149.50±21.22∗</td>
<td>160.23±12.92∗</td>
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<tr>
<td>Drinking-derived energy</td>
<td>0</td>
<td>156.62±13.52‡</td>
<td>237.69±12.53‖‖</td>
<td>150.65±15.58‡</td>
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<tr>
<td>Urine volume (ml/24h)</td>
<td>12.62±1.22</td>
<td>27.84±3.05‡</td>
<td>48.38±4.47‡‖§</td>
<td>26.03±3.38∗</td>
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<tr>
<td>Blood glucose (mM)</td>
<td>3.63±0.16</td>
<td>5.49±0.07∗</td>
<td>4.50±0.26∗§</td>
<td>6.47±0.22‡§</td>
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<tr>
<td>Plasma insulin (pg/ml)</td>
<td>0.72±0.17</td>
<td>1.22±0.12∗</td>
<td>0.71±0.15†</td>
<td>0.79±0.10‡</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.50±0.01</td>
<td>0.48±0.01</td>
<td>0.49±0.01</td>
<td>0.47±0.01</td>
</tr>
</tbody>
</table>

ΔBody weight: change in body weight, KW/BW: Kidney Weight / Body Weight, ∗p < 0.05, †p < 0.01, and ‡p < 0.001 vs. Control; §p < 0.05, †p < 0.01, and ‡p < 0.001 vs. HF.

Figure S1
Figure S1. Effect of allopurinol on UA production in HF-fed normal rats. Analysis of urinary (A) and plasma (E) UA. KHK protein expression of kidney cortex (B) and outer medulla (C) was analyzed by immunoblotting and densitometric analysis (D). N = 5 per group. Data are mean ± SEM. ***p < 0.001 vs. control, ###p < 0.001 vs. HF.
Figure S2. Effect of allopurinol and PRO20 on plasma renin and Ang II levels in HF-fed SD rats. SD rats were randomly divided into the following 4 groups: Control, HF, HF + allopurinol, or HF + PRO20. The plasma samples were assayed for renin and AngII. (A) Plasma renin activity. (B) Plasma active renin concentration. (C) Plasma total renin concentration. (D) Plasma prorenin concentration. (E) Plasma Ang II concentration. N = 5-10 per group. Data are mean ± SEM. **p < 0.01 and ***p < 0.001 vs. control, ##p < 0.01 vs. HF.
Figure S3. Effect of fructose and UA on PRR expression in HK-2 cells. (A) Dose-response studies of fructose regulation of PRR expression. Cells were exposed to fructose at various concentrations for 48 h, followed by immunoblotting and densitometric analysis of PRR protein expression. The expression was normalized by β-actin. N = 5 per group. Data are mean ± SEM. *p < 0.05 and **p < 0.01 vs. control or 1 mM, #p < 0.05 vs. 3 mM. (B) Time-course studies of fructose regulation of PRR expression. Cells were exposed to 5 mM fructose for the indicated time periods and PRR protein expression was analyzed by immunoblotting and densitometric analysis. The expression was normalized by β-actin. N = 5 per group. Data are mean ± SEM. *p < 0.05 vs. control or 12 h. (C and D) Effect of allopurinol on HF-induced PRR expression and medium sPRR secretion in HK-2 cells. Cells were pretreated with 100 μM allopurinol and then treated with 5 mM fructose for 72 h. PRR protein expression was measured by ELISA and expressed as pg/mg of total cellular proteins. N = 5 per group. Data are mean ± SEM. *p < 0.05 and **p < 0.01 vs. control, #p < 0.05 and ##p < 0.01 vs. HF. (E, F) Time-course studies of UA regulation of PRR expression in HK-2 cells. Cells were exposed to 10 mg/dl UA for the indicated time periods and PRR protein expression analyzed by immunoblotting and densitometric analysis (E). The expression was normalized by β-actin. (F) ELISA analysis of medium sPRR and expressed as pg/mg of total cellular proteins. The cells were exposed to 10 mg/dl UA for 24 h. N = 6 per group. Data are mean ± SEM. **p < 0.01 vs. control or 6 h, ***p < 0.001 vs. control or 6h or 12 h.