Effects of salt intake and potassium supplementation on renalase expression in the kidneys of Dahl salt-sensitive rats

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Abstract

Renalase is currently the only known amine oxidase in the blood that can metabolize catecholamines and regulate sympathetic activity. High salt intake is associated with high blood pressure (BP), possibly through the modulation of renalase expression and secretion, whereas potassium can reverse the high salt-mediated increase in blood pressure. However, whether potassium could also modulate BP through renalase is unclear. In this study, we aim to investigate how salt intake and potassium supplementation affect the level of renalase in rats. Eighteen salt-sensitive (SS) and 18 SS-13BN rats were divided into six groups, receiving normal salt (0.3% NaCl), high salt (8% NaCl) and high salt/potassium (8% NaCl and 8% KCl) dietary intervention for four weeks. At the end of experiments, blood and kidneys were collected for analysis. mRNA level of renalase was measured by quantitative real-time PCR and protein level was determined by Western blot. We found that mRNA and protein levels of renalase in the kidneys of SS and SS-13BN rats were significantly decreased ($P < 0.05$) after high salt intervention, whereas dopamine in plasma was increased ($P < 0.05$) compared with rats received normal salt, suggesting that salt may induce salt-sensitive hypertension through inhibition of renalase expression. We also found increased mRNA level and protein level of renalase, decreased catecholamine levels in plasma, and decreased BP in SS rats treated with high salt/potassium, compared with that of the high salt SS group. Taken together, the salt-induced increase and potassium-induced decrease in BP could be mediated through renalase. More studies are needed to confirm our findings and understand the underlying mechanisms.

Keywords: Salt sensitivity, renalase, sodium, potassium

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Introduction

Experimental and clinical studies have demonstrated a positive relationship between salt intake and high blood pressure (BP).^{1,2} However, the effect of salt intake or restriction on BP varies among individuals, and such heterogeneity is classified as salt sensitivity. Extensive studies have shown that high salt intake is associated not only with serious target organ damage, but also with long-term hypertension, cardiovascular conditions and mortality. Therefore, it is important to investigate the underlying mechanisms on how salt affects BP.

Inappropriate and excessive activation of the sympathetic nervous system has been proposed as a main cause for hypertension. Sympathetic nerve activity triggered through neural and humoral mechanisms could induce the production of harmful metabolites, thus promoting

cardiovascular end-organ damage by increased BP.³ Salt has been shown to modulate the biosynthesis, release, absorption, and circulating level of catecholamine, a sympathetic neural transmitter involved in the regulation of BP. Dopamine, the most abundant catecholamine in human plasma, plays an important role in the peripheral sympathetic nervous system. Therefore, plasma dopamine has been regarded as a sensitive indicator of peripheral dopaminergic activity. It has been suggested that dopamine modulates the cardiovascular and endocrine functions and participates in the pathogenesis of certain types of hypertension, including salt-sensitive (SS) hypertension, by regulating renal sodium transport and interacting with vasoactive substances and humoral factors.⁴ High salt and angiotensin can contribute to the increased sympathetic nerve activity, which ultimately results in increased BP.^{5,6}

Epidemiological studies have revealed negative associations of BP with urinary potassium excretion, urinary potassium/ sodium ratio and potassium intake.^{2,7} In a study of teenagers, Osborn JL et al. found that increased intake of potassium/sodium can promote sodium excretion and reduce sodium retention, thereby lowering the number of salt-sensitive (SS) teenagers with high blood pressure.^{8,9} Dietary potassium supplementation might prevent the salt loading-induced elevation of BP by inhibition of sympathetic nerve excitability, salt-induced insulin resistance and generation of oxygen-reactive free radicals.^{10,11} Renalase, a flavin adenine dinucleotide-dependent amine oxidase, is secreted into the blood from kidney.¹²⁻¹⁴ The circulating level and activity of renalase vary in plasma and urine, possibly because it is a dimer in urine and it is present in the precursor form in plasma. In plasma, the precursor form could be transformed by catecholamine into the active enzyme to promote the secretion and synthesis of renalase.¹³ Currently, renalase is the only known amine oxidase that can degrade catecholamine and reduce BP to improve cardiac function.¹⁴ The hyper sympathetic nerve activity induces high secretion of nor-epinephrine, epinephrine, and dopamine, thereby leading to high blood pressure. It has been indicated that sodium may regulate the expression and secretion of renalase, thereby affecting sympathetic nerve activity to modulate BP.^{14,15}

In this study, we investigated the mRNA level and protein level of renalase, as well as the change of plasma dopamine in Dahl SS rats supplemented with salt and potassium.

Materials and methods

Animals

18 Dahl SS rats and 18 SS-13BN male rats at the age of eight weeks were purchased from Charles River Laboratories (Seattle, Washington DC, USA). Dahl SS rats were hereditary hypertensive rats bred by salt loading method from SD (Sprague-Dawley rats) closed group in 1960s. They were widely used as models of hypertension by domestic and foreign scholars since they could become hypertensive when given high-salt intervention in the growth process without surgery or drugs, whereas the consomic SS-13BN rats were 98% identical to the SS rats, differing only by a subset of genes present on chromosome 13 that were carried from the parental Brown Norway strain. They do not develop elevated blood pressure on a high-salt diet as SS rats do.¹⁶ All rats were fed ad libitum in the specific-pathogen-free animal room of Xi'an Jiaotong University at $25 \pm 3^\circ\text{C}$ with a 12-h light/12-h dark cycle. The experiments were carried out according to state regulations on the care of experimental animals and the implementation rules for the laboratory animal conditions of Shaanxi Province. The institutional ethics committee of Xi'an Jiaotong University Medical School approved the study protocol. All procedures were performed according to institutional guidelines.

Animal treatment

Eighteen Dahl SS rats were divided into three groups randomly ($N=6$) for dietary intervention for four weeks, including normal salt (0.3% NaCl), high salt (8% NaCl) and high salt/potassium (8% NaCl and 8% KCl). Eighteen SS-13BN rats were also divided and treated with exactly the same dietary intervention.

Measurement of blood pressure

The rats were placed in blood pressure meter holder for adaptation training for 20 min every week before BP measurement. Tail blood pressure was measured in a quiet position for 10–15 min before and at the end of the dietary treatment by model-229 animal noninvasive blood pressure analyzer. Upon detection of tail pulse wave (i.e. equal internal and external pressures), the systolic blood pressure and diastolic blood pressure were measured. The interface display was obtained as shown in Figure 1. Blood pressure was measured five times at 2–3-min intervals.

Blood and kidney collection

At the end of the experiment, all rats were sacrificed. 15 ml blood was collected into an anticoagulant tube and centrifuged at 3000 rpm for 10 min. Kidney was collected, stripped of fascia, rinsed with saline, and then frozen in liquid nitrogen. Plasma and liver were stored at -80°C .

mRNA level of renalase in kidney

Total RNA was extracted by RNAiso Plus according to the manufacturer's instructions (TAKARA, Japan). Gene expression was normalized using GAPDH as a reference gene. Each PCR reaction contained SYBR Premix Ex TaqTM II ($2 \times$) 10 ml, GAPDH/upstream primer of the purpose gene 0.8 μl , GAPDH/downstream primer of the purpose gene 0.8 μl , dH₂O (DEPC worked) 6.4 μl , Template cDNA 2 μl , making up a total of 20 μl . The conditions of PCR were as follows: early degeneration at 95°C for 10 s; amplification at 95°C for 5 s, 60°C for 15 s, 72°C for 10 s, 40 cycles; and extension at 60°C for 10 s, 71 cycles. The primers used in our

Figure 1 Systolic blood pressure (SBP) of rats before and after salt intervention. BN (13BN normal salt group); BH (13BN high salt group); BHP (13BN high salt with potassium group); SN (SS normal salt group); SH (SS high salt group); SHP (salt-sensitive high salt with potassium group). * $P < 0.05$ vs. corresponding baseline; # $P < 0.05$ vs. SH intervention group

study included: renalase, Forward: 5'-CCC AgC AgT TTg ACC TTg TC -3, Reverse: 5'-CAAATgggACggTggTATgg-3; GAPDH, Forward: 5'-TACCCACggCAAgTTCAACg-3, Reverse: 5'-CACCAgCATCACCCCATTTg-3. Relative mRNA expression level was calculated as followings: $\Delta Ct(DG) = Ct(PG) - Ct(IG)$, $\Delta Ct(CG) = Ct(PG) - Ct(IG)$, $\Delta\Delta Ct(DG) = \Delta Ct(DG) - \Delta Ct(CG)$; $\Delta Ct(CG) = Ct(PG) - Ct(IG)$, $\Delta Ct(\text{average CG}) = Ct(\text{average PG}) - Ct(\text{average IG})$, $\Delta\Delta Ct(CG) = \Delta Ct(CG) - \Delta Ct(\text{average CG})$.

Protein level of renalase in kidney

Protein was extracted from kidney using RIPA. Protein concentration was measured using a protein assay kit (Bio-Rad, Hercules, CA, USA). About 30 μ g protein was separated by SDS-PAGE and transferred to nitrocellulose membranes (Penzberg, Germany). The membranes were blocked with 5% nonfat dried milk in buffer for 1 h, and incubated with the primary antibodies (Rabbit anti-rat renalase) at 4°C with gentle shaking overnight. The membranes were incubated with secondary antibody (Rabbit IgG) for 1 h the next morning. Finally, the membranes were exposed to the mixing reagents of Genshare hypersensitivity chemiluminescent substrate detection kit and visualized using the BIO-RAD Molecular Imager ECL immobilon western chemiluminescent. Western blot experiments were performed in triplicate.

Measurement of the levels of dopamine in plasma

For dopamine level measurement, 200 μ l plasma was used. Briefly, blood sample was thawed at room temperature, mixed for 1 min, and 200 μ l 5% perchloric acid solution was added. The mixture was centrifuged for 10 min at 10,000 r/min, and re-centrifuged for 10 min at 10,000 r/min after removing supernatant. Finally, 10 μ l of supernatant was used for dopamine level measurement using the chromatographic method.¹⁷

Statistical analysis

Data are expressed as mean $\pm$ standard deviation (SD). Paired *t*-test analysis and one-way analysis of variance was used to compare the difference between and within groups. All statistical analysis was performed using SPSS, version 17.0 (Chicago, IL, USA). $P < 0.05$ was considered as statistically significant.

Result

BP in rats

There was no significant difference in BP and body weight between SS-13BN rats and SS rats before the intervention (Figure 1; $P > 0.05$). BP in SS rats was higher than that in SS-13BN rats after normal salt (185 ± 9.1 mmHg vs. 151 ± 6.4 mmHg, Figure 1; $P < 0.05$), BP in SS rats with high-salt-diet intervention was significantly higher than that of SS rats with normal salt (185 ± 9.1 mmHg vs. 151 ± 6.4 mmHg, Figure 1; $P < 0.05$); and BP in SS rats with the high salt/potassium diet was significantly lower

Figure 2 Relative expression of renalase gene of each group after intervention SBP of rats before and after salt intervention. * $P < 0.05$ vs. SH intervention group

than that of the high salt-diet rats (166 ± 13.2 mmHg vs. 185 ± 9.1 mmHg, Figure 1; $P < 0.05$).

mRNA level of renalase gene in kidney

In SS rats, mRNA level of renalase gene in high salt group was significantly lower than that in normal salt group (Figure 2; $P < 0.05$), and mRNA level in high salt/potassium group was significantly higher than that in high salt group (Figure 2; $P < 0.05$). After high salt intervention, mRNA level in SS group was significantly lower than that in SS-13BN group (Figure 2, $P < 0.05$).

Protein level of renalase in kidney

In SS rats, the protein level of renalase in kidney in high salt group was significantly lower than that in normal salt and high salt/potassium group (Figure 3; $P < 0.05$). After high salt intervention, protein level of renalase in SS group was significantly lower than that in SS-13BN group (Figure 3; $P < 0.05$).

Dopamine level in plasma

In SS rats, plasma dopamine level was significantly higher in high salt group compared with that in normal salt group (Figure 4; $P < 0.05$). After normal or high salt treatment, dopamine level in SS rats was higher than in SS-13BN rats (Figure 4; $P < 0.05$).

Discussion

We found that mRNA and protein levels of renalase in kidney were significantly decreased in SS and SS-13BN rats after high salt or high salt/potassium intervention, whereas dopamine in plasma was increased compared with rats received normal salt, suggesting that salt may induce SS hypertension through inhibition of renalase expression.

Our result is consistent with a previous report on the inhibition of renalase expression with high salt intake by Ghosh et al.¹⁸ Several scholars have found that kidney aminotransferase gene Rs2576178 GG and Rs2296545 CC are associated with essential hypertension, which suggests that patients with the abovementioned genes are more likely to suffer from high blood pressure.¹⁹ Salt loading

Figure 3 Expression of renalase protein of each group after intervention SBP of rats before and after salt intervention. * $P < 0.05$ vs. BH intervention group; # $P < 0.05$ vs. SH intervention group

Figure 4 Concentration of dopamine in plasma of each group after intervention. * $P < 0.05$ vs. BN intervention group; # $P < 0.05$ vs. SN intervention group

inhibited the renalase gene and protein expression of Dahl SS rats, thereby decreasing the degradation of catecholamines and increasing the activity of the sympathetic nerve endings, which caused high blood pressure and kidney damage.^{12,20,21} Taken together, high salt intake could be a risk factor for hypertension mediated through renalase. In addition to renalase, certain upstream substances of renalase might also be altered, resulting in changes in renalase gene expression.

In SS rats, we observed increased BP with high salt treatment and decreased BP with high salt/potassium treatment. The ratio of 24 h urinary potassium/sodium of the SS and SS-13BN rats that received high salt intervention decreased, whereas the same ratio significantly increased after potassium intervention, opposite to the change in blood pressure. Von Bunge et al. have observed that increased potassium intake can promote sodium excretion, and vice versa, a two-way effect between sodium and potassium. High potassium intake has been shown to exhibit antihypertension effect through inhibition of the pressor effect of sodium in rats. Morris et al. found that the BP of SS rats treated with high potassium dietary decreased

significantly, whereas BP of SS-13BN rats decreased slightly. In our previous study of 300 youngsters, we found that potassium (60 mmol based on 310 mmol high salt diet daily) could promote urinary sodium excretion to inhibit salt-induced high BP.^{8,21} This effect is similar to that of severe salt restriction (50 mmol / day).

In this study, we found that renalase gene and protein expressions in Dahl SS rats increased after potassium intervention, whereas catecholamine levels in plasma, blood pressure decreased. Supplying potassium induced the opposite effect on SS hypertension through the following mechanisms^{2,7,22-24}: (1) supplying potassium promoted urinary sodium excretion and reduced sodium retention in the body; (2) potassium inhibited the sympathetic nerve activity of the SS hypertension rats; (3) supplying potassium inhibited insulin resistance, which was induced by high salt diet; and (4) the generation of reactive oxygen species was reduced. Supplying potassium enhanced the expression of renalase gene and protein level of the SS rats, increased the degradation of plasma catecholamine, and decreased the plasma catecholamine levels, sympathetic activity, blood pressure, and kidney damage.

Conclusions

In summary, we found that after high salt or high salt/potassium intervention, mRNA and protein levels of renalase in kidneys of SS and SS-13BN rats were significantly decreased, whereas dopamine in plasma was increased compared with normal salt rats, suggesting that salt may induce SS hypertension through inhibition of renalase expression. We also found increased mRNA level and protein level of renalase, decreased catecholamine levels in plasma, and decreased BP in SS rats treated with high salt/potassium. Taken together, the salt- or potassium-induced changes in BP might be mediated through renalase. More studies are needed to confirm our findings and understand the underlying mechanisms.

Authors' contributions: W-LZ, JW and J-JM conceived and designed the experiments; W-LZ and JW performed the experiments; F-QL, YW, DW, K-YR, T-SG and H-YX analyzed the data; Z-YY contributed to the analysis tools; W-LZ and JW wrote the paper. All authors read and approved the final manuscript.

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