Superoxide dismutase and oxidative stress in Dahl salt-sensitive and -resistant rats

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Meng, Shumei, L. Jackson Roberts II, Garrick W. Cason, Travis S. Curry, and R. Davis Manning, Jr. Superoxide dismutase and oxidative stress in Dahl salt-sensitive and -resistant rats. Am J Physiol Regul Integr Comp Physiol 283: R732–R738, 2002; 10.1152/ajpregu.00346.2001.—The roles of oxidative stress and renal superoxide dismutase (SOD) levels and their association with renal damage were studied in Dahl salt-sensitive (S) and salt-resistant (R) Rapp strain rats during changes in Na intake. After 3 wk of a high (8%)-Na diet in S rats, renal medullary Cu/Zn SOD was 56% lower and Mn SOD was 81% lower than in R high Na-fed rats. After 1, 2, and 3 wk of high Na, urinary excretion of F₂-isoprostanes, an index of oxidative stress, was significantly greater in S rats compared with R rats. Plasma F₂-isoprostane concentration increased in the 2-wk S high Na-fed group. After 3 wk, renal cortical and medullary superoxide production was significantly increased in Dahl S rats on high Na intake, and urinary protein excretion, an index of renal damage, was 273 ± 32 mg/d in S high Na-fed rats and 35 ± 4 mg/d in R high Na-fed rats (P < 0.05). In conclusion, salt-sensitive hypertension in the S rat is accompanied by marked decreases in renal medullary SOD and greater renal oxidative stress and renal damage than in R rats.

urinary protein excretion; isoprostane; dietary sodium; renal damage

IN HUMAN HYPERTENSION, reactive oxygen species such as superoxide ions (O₂⁻), hydroxyl radicals, and hydrogen peroxide (H₂O₂) contribute to the pathogenesis of hypertension and exacerbate renal damage. O₂⁻ and H₂O₂ produced by leukocytes and plasma levels of lipid peroxides were increased in patients with uncontrolled hypertension (17). Blood pressure reduction in these patients decreased free radical production and lipid peroxides to normal (17).

Animal models of hypertension also have increased oxidative stress. The spontaneous hypertensive rat (SHR) is characterized by increased production of O₂⁻ production in the renal cortex and medulla and urine and plasma F₂-isoprostanes, and renal tissue Cu/Zn SOD and Mn SOD were determined to assess the level of this antioxidant enzyme in the kidney. Renal damage was assessed by measuring urinary protein excretion.

METHODS

Animal protocol. Experiments were conducted in 59 R and 61 S male rats, Rapp strain (Harlan, Indianapolis, IN) at an age of 7–8 wk. The project had the approval of the local...
Institutional Animal Committee. The rats were placed on either a low-Na (0.03%) or a high-Na (8%) diet for 1–3 wk. Rats were housed in a temperature-controlled room with a 12:12-h light-dark cycle.

The first group of R and S rats was subjected to the specified Na diet for 1, 2, or 3 wk, and a 24-h urine sample was collected for analysis of urine F2-isoprostanes and protein. The Bradford (BioRad, Richmond, CA) method was used to measure urine protein concentration. The next day, rats were anesthetized with isoflurane, a laparotomy was performed, and blood was withdrawn from the aorta for analysis of plasma F2-isoprostanes. All samples were stored at –80°C until processing. Urine and plasma F2-isoprostane concentrations were measured with a gas chromatography, negative ion-chemical ionization mass spectrometry method (16).

Tissue preparation and Western blotting. After 3 wk on the specified Na diet, a second group of R and S rats was anesthetized with isoflurane, and kidneys were perfused through the aorta with 0.1 M phosphate-buffered saline containing 2% heparin (1,000 U/ml). The renal cortex and medulla were dissected out and were snap-frozen in liquid nitrogen and stored at –80°C until processing. Renal cortical and medullary Cu/Zn SOD and Mn SOD protein were determined by Western blotting. There are three isoforms of SOD that localize in the cytosol (Cu/Zn SOD), the mitochondria (Mn SOD), or the extracellular space (ecSOD). Carlsson et al. (6) found that rat ecSOD is a dimer and has low affinity for heparin. Therefore, ecSOD content is very low in rat tissues (6), so the protein expression of this SOD was not measured in this study.

Frozen kidney sections were homogenized in 20 mM HEPES buffer (pH 7.5) containing protease inhibitors (100 μM pepstatin A, 1 mM phenanthroline, 100 μg/ml aprotinin, 100 μg/ml leupeptin, 1 mM E-64, and 10 mM EDTA). Protein concentration was determined with the Lowry method. Equal amounts of protein from each sample were electrophoresed with a 4% polyacrylamide stacking gel and a 15% resolving gel. Separated proteins were transferred to nitrocellulose membranes and were incubated with the sheep anti-SOD antibody (Biodesign, Saco, ME). Membranes were washed and incubated with peroxidase-conjugated donkey anti-sheep/goat IgG (Biodesign). SOD protein was detected by sheep anti-SOD antibody (Biodesign) and normalized relative to actin. Cross-gel comparisons were done using a common control.

Chemiluminescence measurement of O2•− production in renal tissues. Chemiluminescence of tissue in 5 μM lucigenin (bis-N-methylacridinium nitrate) was detected using a scintillation counter (Beckman LS 6500) in the out-of-coincidence mode with a single active photomultiplier tube (15). The validity of measuring oxygen-free radical production with 5 μM lucigenin has been confirmed with electron spin resonance methods (11, 21). Renal cortical and medullary tissues were dissected out on dry ice, homogenized in lysis buffer (PBS containing: 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM PMSF, and 10 U/ml aprotinin, 10 mM sodiumorthovanadate), and the mixture was centrifuged. The supernatant was aspirated and kept on ice for free radical measurement. After 5 min of dark adaptation, 20 counts of 0.5 min each were made and only the last five counts were averaged and blank values were subtracted. The amounts of protein in the renal tissues were quantified with the Lowry assay. The final readings were expressed as counts per minute per milligram protein.

Data analysis. Statistics were performed by first using a two-way analysis of variance and a Fisher least-significant difference test for post hoc analysis at each experimental time point. Data were considered to be statistically different if P < 0.05. All data are expressed as means ± SE.

RESULTS

Urine and plasma F2-isoprostane responses to a high- or low-sodium diet. Figure 1 indicates that urine F2-isoprostane excretion, an index of oxidative stress in multiple pathological conditions (16), significantly increased throughout the experiment in S rats on a high-Na diet compared with R rats on a high-Na diet. Also, throughout the experiment, urinary F2-isoprostane excretion significantly increased in R high Na-fed and S high Na-fed rats compared with low Na-fed rats of the same strain at the same experimental time. Except for week 1, urinary isoprostane excretion during low Na intake was not significantly different between R and S rats. At 3 wk, the urinary excretion of isoprostanes was 31.4 ± 7.4 ng/day in S high Na-fed rats compared with 22.0 ± 2.8 ng/day in R high Na-fed rats (P < 0.05). Urine concentrations of isoprostane (ng/ml) at 1, 2, and 3 wk were 0.36 ± 0.04, 0.31 ± 0.03, and 0.40 ± 0.05 for R high Na-fed rats; 0.86 ± 0.19, 0.89 ± 0.10, and 0.98 ± 0.14 for R low Na-fed rats; 0.36 ± 0.05, 0.36 ± 0.02, and 0.37 ± 0.35 for S high Na-fed rats; and

Fig. 1. Urine F2-isoprostane excretion in Dahl salt-sensitive (S) and salt-resistant (R) rats on low (A)– or high (B)–sodium intake for 1, 2, or 3 wk. †P < 0.05 compared with R rats on the same Na intake at the same experimental time.
0.81 ± 0.24, 0.88 ± 0.23, and 0.89 ± 0.12 for S low Na-fed rats.

Plasma F₂-isoprostanes, an index of oxidative stress, are shown for R and S rats in Fig. 2. The average value for plasma F₂-isoprostane concentration in the 2-wk S high Na-fed group was 0.65 ± 0.22 ng/ml, which was significantly greater than the value in the 2-wk R high Na-fed group (0.23 ± 0.03 ng/ml; P < 0.05).

Renal cortical and medullary O₂⁻ production responses to a high- or low-sodium diet. Figure 3A shows that renal cortical O₂⁻ production significantly increased in S rats after 3 wk of high-Na intake, and the value was 68.7 ± 4.9 cpm/mg protein. Figure 3B shows that renal medullary O₂⁻ production also significantly increased in S high Na-fed rats compared with R rats on either low- or high-Na intake. The highest renal medullary O₂⁻ production occurred in S rats on high-Na intake, and the value was 37.3 ± 1.3 cpm/mg protein.

Renal cortical and medullary Cu/Zn SOD and Mn SOD responses to a high- or low-sodium diet. Figure 4 shows that renal cortical Cu/Zn SOD were not significantly different in the R and S high and low Na-fed groups in samples taken 3 wk after initiation of the Na diet. However, renal medullary Cu/Zn SOD was significantly lower in both the S high Na-fed and S low Na-fed groups compared with the R high Na-fed group.

Urinary protein excretion responses to a high- or low-sodium diet. Figure 6 shows that urinary protein excretion increased significantly at 1, 2, and 3 wk in S high Na-fed rats compared with the R high Na-fed group. Also, throughout the experiment, S high Na-fed rats also demonstrated a greater protein excretion than the S low Na-fed group. At 3 wk, the maximum value of urinary protein excretion in the S high Na-fed rats was 273 ± 32 mg/day, which was greater than the value of 35 ± 4 mg/day in the R high Na-fed rats at 3 wk (P < 0.05).

**DISCUSSION**

A major new finding in this study is that during a 3-wk increase in dietary Na in the S rat, renal oxida-
Changes in oxidative stress in S rats in the present experiment may have preceded major changes in arterial pressure and urinary protein excretion. Urinary isoprostane excretion in high Na-fed S rats reached a maximum value after 7 days of high Na, but arterial pressure of high Na-fed S rats in another study from our laboratory (14) reached significance for the first time on that day and had a value of 104 ± 4 mmHg. The maximum arterial pressure of 140 ± 3 mmHg was not reached until day 21 of high Na. After 7 days of high Na in S rats in the present experiment, urinary protein excretion was 94 ± 11 mg/day but increased to 273 ± 32 mg/day after 3 wk of high Na. Therefore, urinary isoprostane excretion reached a maximum after only 1 wk of high Na, and arterial pressure and urinary protein excretion continued to increase during the 3-wk period of high-Na intake. In another study from our laboratory, the increases in MAP and urinary protein excretion in S rats on high-Na intake for 3 wk were significantly blunted by long-term intravenous infusion of Tempol (14). This suggests that oxidative stress may contribute to the temporal increases in arterial pressure and renal damage in S rats on high-Na intake.

Increased oxidative stress can be caused by an increase in $O_2^\cdot$ production due to increased oxidase ac-

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**Fig. 4.** Renal cortical (A) and medullary (B) Cu/Zn SOD expression in S and R rats on low- or high-sodium intake for 3 wk. Dens, densitometric. Insets show a representative Western blot of renal cortical and medullary copper/zinc (Cu/Zn) SOD, respectively. SOD protein shown in the insets corresponds to the groups immediately below in the bar graphs. †P < 0.05 compared with S low Na-fed rats in the same type of tissue. *P < 0.05 compared with S high Na-fed rats in the same type of tissue. N = 7 for each group.

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**Fig. 5.** Renal cortical (A) and medullary (B) Mn SOD expression in S and R rats on low- or high-sodium intake for 3 wk. Insets show a representative Western blot of renal cortical and medullary manganese (Mn) SOD, respectively. SOD protein shown in the inset corresponds to the groups immediately below in the bar graphs. †P < 0.05 compared with S low Na-fed rats in the same type of tissue. *P < 0.05 compared with S high Na-fed rats in the same type of tissue. N = 7 for each group.
Fig. 6. Urinary protein excretion in S and R rats on low (A-) or high (B)-sodium intake for 1, 2, or 3 wk. †P < 0.05 compared with R rats on the same Na intake at the same experimental time.

have a deficient production or stability in renal medullary Mn SOD and Cu/Zn SOD and that salt-induced hypertension is associated with a further decrease in medullary Mn SOD protein expression.

Mn SOD is found in the mitochondria, and mitochondrial respiration is very high in renal tubules because of active transport mechanisms. This may increase leakage of O$_2^-$, and because Mn SOD levels in the S high Na-fed rats are decreased in the renal medulla, O$_2^-$ levels could increase, thus inactivating nitric oxide (NO) (12). Cu/Zn SOD levels are also decreased in the renal medulla of S rats, which could also increase O$_2^-$ concentrations in the kidney. Thus these decreases in renal medullary SOD could lead to increased medullary O$_2^-$ production, which could exacerbate the hypertension in S rats on high-Na intake.

Oxidative stress may have significant effects in human and experimental hypertension. Serum thiols and ascorbic acid were reduced in patients with hypertension, indicating an increased consumption of these antioxidants. Administration of vitamin C or other antioxidants decreased arterial pressure in these patients (7, 8) and improved the attenuated endothelial-dependent vasodilation in patients with essential hypertension (25). The SHR has high oxidative stress as indicated by elevated O$_2^-$ production in mesenteric arterioles (23) and a reduction of arterial pressure in the SHR but not in the WKY rat using the SOD mimetic Tempol (20). The S rat may also have high oxidative stress as evidenced by increased O$_2^-$ production in the mesentry and a high plasma H$_2$O$_2$ concentration (24). Also, vitamin E administration improved renal and arterial injuries in S rats (1). These studies confirm our results in the present study that increased oxidative stress is associated with renal damage in the S rat.

Hayakawa and Raij (9) showed that in the S rat on a high-Na diet, there are parallel increases in urinary protein excretion, the glomerular injury score, and the tubulointerstitial injury score. Tubulointerstitial damage, which is highly correlated with the progression of renal disease, was more severe in the juxtamedullary and medullary regions (9). Therefore, although we only measured urinary protein excretion, which is due to glomerular damage, it is likely that medullary damage also occurred.

We measured arterial pressure in several groups of R and S rats, and the S rats but not R rats increase their MAP in a salt-sensitive fashion. Our studies showed that R rats remain normotensive on a high-Na diet (27) and S rats experience up to a 50-mmHg increase in MAP over 3 wk of a high-Na diet (14). During this period of time, the high Na-fed S rats also experienced an increase in glomerulosclerosis and glomerular cross-sectional area. These increases in MAP and glomerular damage in high Na-fed S rats were significantly blunted by long-term intravenous infusion of Tempol (14), an SOD mimic. This further supports our hypothesis that a lack of SOD in S rats contributes to renal damage and suggests that oxidative stress may contribute to the temporal increases in...
arterial pressure and renal damage in S rats on high-Na intake.

Several recent studies suggest that high-Na intake in normotensive rats can increase oxidative stress. Sprague-Dawley (SD) rats were fed a low- or high-Na diet, and arteriolar and venular O$_2^\cdot$ production increased in the high Na-fed group (10). The O$_2^\cdot$ production decreased in high Na-fed rats treated with Tempol + catalase or SOD + catalase (10). These rats did not develop hypertension during high Na feeding, indicating that the increased O$_2^\cdot$ production was due to the high-Na diet but not hypertension. Other studies have shown that increased O$_2^\cdot$ could inactivate NO in SD rats on a high-Na intake, because the vasodilatory responses to NO agonists were markedly decreased in pial arterioles (13) and skeletal muscle resistance arteries (12).

The above studies indicate that a high-Na diet even in normotensive rats can cause a release of O$_2^\cdot$ (2, 3, 10, 12, and 13) and that a further increase in O$_2^\cdot$ may occur if a high-Na diet causes hypertension (12). In fact, this agrees with the present study, because a high-Na diet given to the highly salt-resistant R rat caused an increase in urinary F$_2$-isoprostane excretion. However, neither renal medullary nor cortical O$_2^\cdot$ production increased in the R rat on high-Na intake compared with low Na-fed R rats. This suggests that the increase in urinary isoprostanes may be caused by extrarenal increases in oxidative stress. When the highly salt-sensitive S rat was given a high-Na diet in the present study, urinary F$_2$-isoprostane excretion increased even more than in the R rats. The decrease in renal SOD in the high Na-fed Dahl S rats could have contributed to this increased oxidative stress.

Release of O$_2^\cdot$ can have detrimental effects, including DNA destruction, protein aggregation, and lipid peroxidation, which can cause renal damage. F$_2$-isoprostanes are generated in a bound form when O$_2^\cdot$ react with arachidonic acid in phospholipids, and they are subsequently released in a free form that are excreted in the urine (18). F$_2$-isoprostanes have been used as a marker of oxidative stress in vivo (16). Elevated renal excretion of F$_2$-isoprostanes have been reported in renal ischemia-reperfusion injury (26) and during hypertension in the SHR (20). Treatment of the SHR with Tempol decreased the F$_2$-isoprostane excretion and arterial pressure significantly (20).

Urinary excretion of F$_2$-isoprostanes in the present experiment could have been increased in response to an increase in glomerular filtration rate (GFR) in the R and S high Na-fed rats. However, we previously showed that GFR is not different in high Na-fed R and S rats, and the increase in GFR in both groups compared with low Na-fed R and S rats is only 15% (27). Therefore, the increases in urinary F$_2$-isoprostane excretion in high Na-fed R and S rats are not likely due to an increase in GFR.

In conclusion, oxidative stress increased in S rats on high-Na intake as evidenced by increased renal cortical and medullary O$_2^\cdot$ production and increased plasma F$_2$-isoprostanes and urinary excretion of F$_2$-isoprostanes. After 3 wk on high Na, the S rats experienced an 81% reduction in Mn SOD levels and a 56% reduction in Cu/Zn SOD levels in the renal medulla compared with R rats. The Mn SOD levels of S rats were also decreased in the cortex. Therefore, the ability of SOD to inactivate O$_2^\cdot$ is likely decreased in the kidney. R rats on high-Na intake also experienced an increase in urinary F$_2$-isoprostane excretion but no increase in either renal cortical or medullary O$_2^\cdot$ production, which may reflect an increase in extrarenal oxidative stress. Compared with the R high Na-fed rats, the high Na-fed S rats showed an increase in renal O$_2^\cdot$ production, significantly greater increase in urinary F$_2$-isoprostane excretion, markedly lower renal SOD levels, and increased renal damage as evidenced by marked elevations in urinary protein excretion. A high-Na diet may increase renal oxidative stress in the S rat, which is accompanied by low renal SOD and is associated with severe renal damage.

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